UNIVERSITY OF GENOA

PhD in SCIENCE AND TECHNOLOGY OF CHEMISTRY AND MATERIALS

CURRICULUM PHARMACEUTICAL, NUTRITIONAL AND COSMETIC SCIENCES

XXX Cycle

SYNTHESIS OF QUINOLIZIDINE AND BENZOAZOLE DERIVATIVES WITH POTENTIAL PHARMACOLOGICAL ACTIVITY

Candidate : Mohammadreza Sadeghi

Supervisor : Prof. Bruno Tasso

Academic Year 2016/17

ABSTRACT

The aim of my thesis is twofold: the first research line consists in the synthesis of quinolizidine derivatives endowed with antiparasitic and anticholinesterase activity; the second study concerns the design and synthesis of benzotriazole derivatives with antitumor activity.

PART 1

I focused my attention on quinolizidine alkaloids, particularly lupinine and cytisine, for the preparation of compounds endowed with potential therapeutic interest, and particularly antiparasitic against *Leishmania tropica* and *Leishmania infantum*, and anticholinesterase activity; the quinolizidine nucleus, indeed, is a chemical moiety that has been widely used to synthesize new molecules (as described in literature), due to its well known biological activities; quinolizidine alkaloids are also very suitable for the linkage to other chemical moiety, such as carbo- and heterocyclic systems.

I synthesized new N-alkyl and N-alkylarylcytisine derivatives, new basic molecules and some corresponding quaternary ammonium salts.

All these compounds showed a weak antiparasitic activity but a very good anticholinesterasic and antibutyrrilcholinesterasic activities. I also synthesized new lupinine, aminolupinane and thiolupinine derivatives of xanthene-9-carboxylic acid and new cytisine derivatives of 1,4-naphthoquinone and anthraquinone.

PART 2

In parallel with this research line, I also developed an interest towards benzotriazolic derivatives: the benzotriazole nucleus is largely explored due to its broad spectrum of biological activities including antiviral, antimicrobial and anticancer properties. In addition, in our laboratory we recently synthesized benzotriazole-3,4,5-trimethoxybenzoate esters endowed with interesting antiproliferative activity (IC_{50} submicromolar). On this basis I synthesized two small series of 1-substituted benzoylbenzotriazoles (eight compounds) and of benzoic esters of 1-(2,3-hydroxyalkyl) benzotriazoles (28 compounds). Moreover, I also focused on another alkaloid, colchicine, well known for its antimitotic activity and its antitumoral potential (not yet exploited because of its disadvantages in therapy

such as a very high toxicity). I synthesized a series of eight novel colchicine derivatives characterized by substituted 1,2,3-benzotriazoles moieties. Since colchicine exerts its activity by binding to a site localized in β-tubulin and thus achieving a conformational change in the tubulin dimer, I carried out docking simulations on the synthesized compounds, in order to predict their linkage to the β-tubulin. In comparison with the x-ray structure of colchicine bound to tubulin, the new synthesized compounds showed a different orientation in the binding sites but the calculated K_i values appeared to be in the nanomolar or subnanomolar concentration range, and one of them in particular was endowed with a lower K_i value than colchicine.

Contents

List of Figures

List of Tables

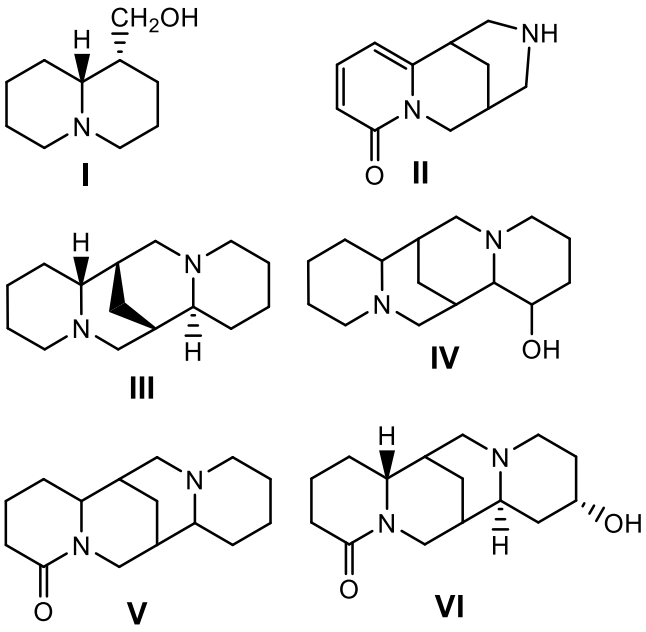
Section 1: Quinolizidine Derivatives

Background: Quinolizidine alkaloids historical background and chemistry

Alkaloids are a class of chemical compounds that, among all the natural substances, arouses a particular interest both for their peculiar chemical structures and their relevant bio-pharmacological properties. (Henry T. A., 1970)

A plenty of alkaloids are utilized in therapeutical situations; several other alkaloids, however, although endowed with considerable biological properties, have not been established themselves as terapeutic agents because of their drawbacks, such as an unsuitable pharmacokinetic profile, a low selectivity of action and a limited therapeutic index due to their toxicity.

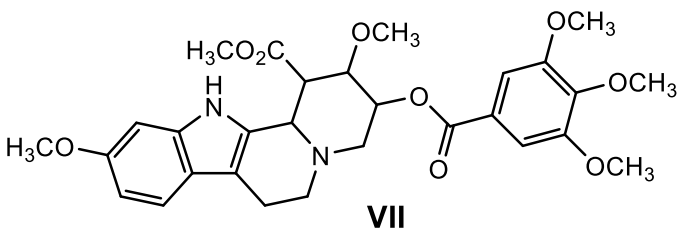
Nevertheless, a certain number of these alkaloids are characterized by chemical structures that could be modified in order to improve their pharmaco-toxicological profiles; this is the case of thebaine, convulsant without any therapeutic applications, that has been converted to naloxone and buprenorphine (Temgesic). The category of alkaloids suitable to be structurally modified could also include the alkaloids derived from the quinolizidine nucleus, widely represented in different species of *Lupinus*, *Genista*, *Cytisus* and other Leguminosae.(Leonard N. I., 1960; Bohlmann F., 1967) The class of the quinolizidinic alkaloids include lupinine (**I**), cytisine (**II**), sparteine (**III**), retamine (**IV**), lupanine (**V**) and hydroxylupanine (**VI**).



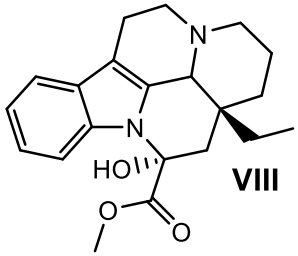
Among the alkaloids mentioned above, only sparteine (**III**) has been widely utilized in the Western countries for its ganglioplegic properties, that isolate the heart from the sympathetic and parasympathetic influences (Bovet O. et al., 1948; Hazard R., 1956); more recently, its specific antiarrhythmic activities have been highlighted (Raschack M.et al., 1974). In Latin American countries it has been used and it is still seldom used as a uterine stimulant (oxytocic drug) (Reynolds A. K., 1955).

The pharmacological profile of cytisine (**II**) has been extensively studied for its remarkable nicotine-like activities, but this drug has not therapeutic applications except in Russia, where it is reported in the Official Pharmacopoeia as a breathing stimulant (Zakusov V. V., 1960; Daqllemagne M. J. et al., 1955). Very recently, cytsine has aroused a renewed interest as a pharmacological agent, since it is one of the most potent ligands for central nicotinic receptors (Pabreza L. A. et al., 1991).

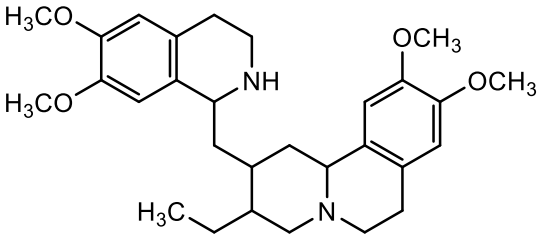
Regarding the other quinolizidine alkaloids mentioned above (and the others not reported here), it can be asserted that they have been subjected to very fragmentary pharmacological investigations, that have generally highlighted a common ganglioplegic and oxytocic activity. These alkaloids are generally quite known to confer toxic properties to the plants containing them, which therefore must be carefully avoided by their use as forage. However, peculiar activities have been observed in single alkaloids, such as (4-hydroxycinnamoyl)epi-lupinine exerts an acetylcholinesterase inhibition activity (Saito K.et al., 1995) and matrine is endowed with an anti-asthmatic effect (against the histamine- and acetylcholine-induced asthma) (Xie M.et al., 1984), a glutamate antagonist action at the neuromuscular junctions (Ishida M.et al., 1984), and an antitumor activity (Shibata S.et al., 1984). Moreover, it should be noted that the quinolizidine ring, characteristic of the “lupin alkaloids”, can be also found in the complex structures of other alkaloids (not bio-genetically linked with the previous ones) endowed with a remarkable therapeutical interest, such as reserpine (**VII**), vincamine (**VIII**), emetine (**IX**) and others. In these cases, it is obviously very hard to assess the contribution of the quinolizidine portion to the activity of the alkaloid.



VII



VIII

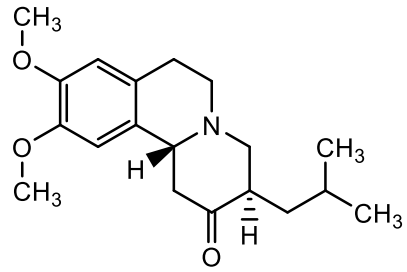


IX

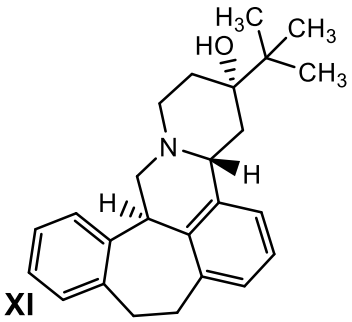
Based on these premises, since the late 30s, research has been carried out to modify the structure of the mentioned alkaloids, with the aim of obtaining compounds endowed with more concrete therapeutic interest.

In 1936 Ing and Patel (Ing H. R. et al., 1937) prepared cytisine derivatives, while Winterfeld and Hoffmann (Winterfeld K.et al., 1937; Jack W.et al., 1942) utilized lupanine to obtain aryl-dehydrosparteine derivatives. However, authors of Eastern Europe, especially Russian, carried out a fairly extensive study on lupinine (Knunyants I. L.et al., 1937; Chem. Abstr., 1938; Katznel'son M. M. et al., 1935) and cytisine (Pakudina Z. P. et al., 1957; Chem. Abstr., 1960; Luputiu G. et al., 1971) derivatives.

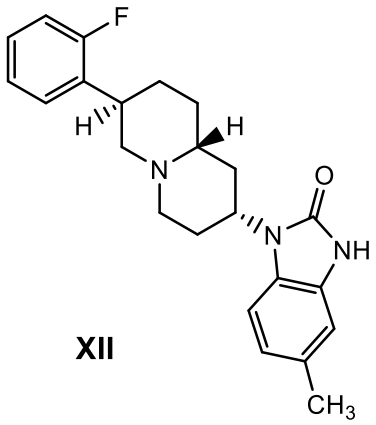
Unfortunately, in several cases, no pharmacological experiments have been performed or the results are not available. From the results available, the effects found are often similar to those exerted by the starting alkaloids, and this evidence can be explained by the limited structural changes accomplished on the starting substances. Some entirely synthetic molecules, namely not derived from the structural modification of alkaloids, contain the quinolizidine nucleus incorporated into complex structures that imitate the **VII-IX** alkaloids or other alkaloids with more or less similarity. This category of synthetic molecules includes tetrabenazine (**X**), (+)-butaclamol (**XI**) and the compound Ro14-8625 (**XII**), acting as dopamine depletors and antipsychotics (Ing H. R. et al., 1937) like reserpine. Nevertheless, the compound BRL20627 (**XIII**) exhibits remarkable gastrokinetic activity (by stimulation of the $5HT_4$ receptors), without any effect on the dopamine receptors (Handley M. S. et al., 1985). This latter compound, although clinically effective, has presented toxic aspects that have prevented its marketing.



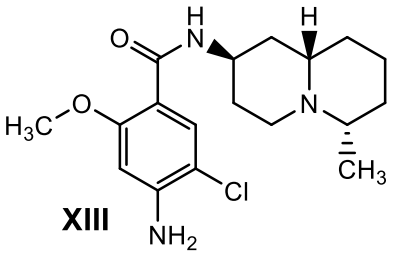
X



XI



XII



XIII

In order to utilize the quinolizidine alkaloids through their structural modification, it is noteworthy to point out that some byciclic structures, such as lupinine, are endowed with "dialkylaminoalkyl "or "cycloalkylaminocyclic" fragments (Fig.1), that are frequently represented also in drugs with fundamental therapeutic actions such as analgesic, local anesthetic, antipsychotic, antidepressant, antihistaminic, antiparkinsonian, chemotherapeutic etc.

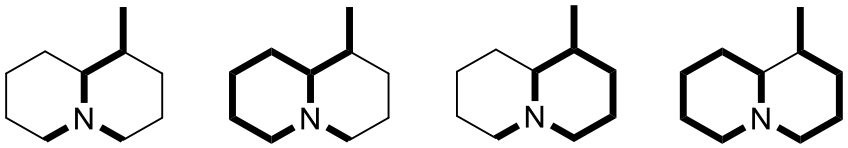


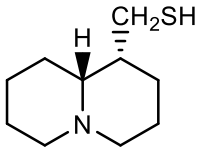
Fig.1 Dialkylaminoalkyl and cycloalkylaminocyclic fragments highlighted in the quinolizidine nucleus.

Therefore, the insertion of the opportune portion in the structure of these alkaloids could be a perspective to develop new molecules with different pharmacological activities, exploiting the analogy with the drugs currently used in therapy. Apparently, in the final products, the presence of the quinolizidine nucleus, may sometimes interfere with the receptor-drug interaction and thus prevent the manifestation of the expected biological effect.

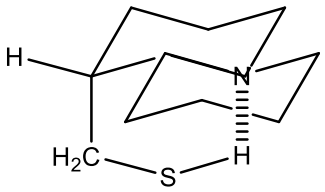
The quinolizidine nucleus, indeed, is endowed with peculiar characteristics such as its size and rigidity, and the unavoidable spatial relationships that it imposes

among the various molecular fragments merged. On the other hand, exactly these peculiarities may promote highly selective interactions with other receptors.

Based on these considerations, since 1959, a systematic study on structural modifications of quinolizidine alkaloids has been carried out by F. Sparatore and his Collaborators, starting from lupinine (**I**) (Sparatore F., 1962). Later, also a study on the structural modifications of cytisine started, (**II**) (1992), while the research on retamine (**IV**) (Savelli F. et al., 1975) and sparteine (**III**) (Boido V. et al., 1992), (Canu C., Boido V. et al., 1999) has had so far a limited development, in comparison to the studies carried out on lupinine.With regard to the present dissertation, the further possibility of preparing quinolizidine derivatives through the structural modification of lupinine, as determined by thiolupinine synthesis (quinolizidin-1-yl-metanthiol) (**XIV**), (**XIVa**: spatial structure of thiolupinine) (Novelli F., 1987;. Novelli F.et al., 1993), appears to be particularly relevant. Moreover, the thiol function facilitates the construction of molecules in which the distance between the quinolizidine nucleus and any “X” fragment can be freely changed, rather than extending the alkyl chain with complex procedures, even prohibitive above a certain length. This procedure benefits from the bioisosterism existing between the methylene and the sulfur atom.



XIV



XIVa

Chapter 1. Antileishmaniasis Quinolizidine Derivatives

Leishmaniasis background

The term leishmaniasis refers to a group of various clinical syndromes caused by obligate intracellular protozoa of the genus *Leishmania,* family *Trypanosomatidae* and order *Kinetoplastidae.* Leishmaniasis has become a major cause for concern and a serious problem worldwide; according to the World Health Organization (WHO) it is classified as the second leading tropical disease after malaria. It is endemic in several tropical and subtropical areas, from deserts to rainforests, from rural to suburban areas.

It is a tropical infection too often neglected even nowadays, and it is considered a disease of poverty (World Health Organization, 2014); it mainly affects, indeed, the poorest countries such as Southeast Asia, Africa and Latin America but it is also endemic in several Mediterranean Countries. On a global scale, approximately 350 million people live in areas characterized by the active transmission of *Leishmania*, in which 14 million people are directly affected by over twenty different species of *Leishmania.* (Pace D., 2014) Children under 15 years of age are the most susceptible individuals to this disease. The massive development of international transport and of travel has increased in recent years, leading to an increase in the incidence of leishmaniasis in non-endemic countries, and thus making crucial the recognition of this parasitic infection. (Field V. et al., 2010) In addition, the rise in number of individuals with immunosuppression (caused by the human immunodeficiency virus (HIV), chemotherapeutic agents or as a result of transplantation) has led to an increase in visceral leishmaniasis in Europe. (Okwor I. et al., 2013; Alvar J. et al., 2012)

Depending on the tropism, there are several types of leishmaniasis: cutaneous leishmaniasis (CL), mucosal leishmaniasis (MCL), visceral leishmaniasis (VL) and post-kala-azar cutaneous leishmaniasis (PCDL); each of these can lead to a different clinical spectrum ranging from an asymptomatic infection to a fatal form. (Croft S. L., 2003; Singh S. et al., 2004)

Kala azar, or visceral leishmaniasis (VL) caused by *L. donovani*, is the second most deadly parasitic disease in the world after malaria; it is spread by sand flies, afflicting hundreds of thousands of people, especially the poorest of the world in tropical countries. Cutaneous leishmaniasis (CL), caused by *L. major*, *L. mexicana*,

L. braziliensis and *L. panamensis*, resolves spontaneously in 3-18 months, leaving disfiguring scars. (Kheirandish F. et al., 2013; Singh N.et al., 2012; Yavar R.et al., 2013). MCL, caused by *L. braziliensis*, causes the destruction of the mucosa and cartilage of the mouth and pharynx. (Hussain H. et al., 2014)

Recently, in 2012, Alvar (Alvar J. et al., 2012) reported the most relevant statistical data on the spread and the mortality of *Leishmania* parasite in the world.

Annual incidence of VL is estimated to be approximately 0.2-0.4 million: over 90% of these cases occur in India, Bangladesh, Sudan, Ethiopia and Brazil, with an estimated mortality of 10%-20%, especially in poor areas. CL occurs mainly in the Mediterranean, in the Americas and in Western Asia; 75% of these cases are found in Brazil, Syria and Afghanistan. Around 35,000 cases of MCL are reported annually mainly in Brazil, Peru and Bolivia. (De Vries H. J. et al., 2015) In European countries, only two species of Leishmania are endemic: the first is *L. infantum*, widespread in the Mediterranean regions, which causes zoonotic skin diseases and finds its reservoir in the domestic dog; the second one is *L. Tropica*, which has caused sporadic cases of anthroponotic disease especially in Greece. An native transmission has been reported in Portugal, Spain, France, Italy, Greece, Malta, Cyprus, Croatia, Albania, Bulgaria and Turkey. VL is hyperendemic in the Mediterranean countries, where the cases reported account for 5% -6% of the global burden and do not necessarily lead to the clinical manifestation of the disease; most infections, indeed, remain asymptomatic, but malnutrition and immunosuppression, particularly the one caused by HIV, predispose to the onset of the clinical symptoms. (Okwor I. et al., 2013) Moreover, cases of co-infection by Leishmania/HIV have been reported in the Mediterranean region, especially in France, Italy, Portugal and Spain. (Monge-Maillo B., 2014) Since highly active antiretroviral therapy was introduced in 1997, a marked decrease in the number of co-infected patients have been observed in both the Mediterranean and Asia. (Singh S., 2014) However, VL infection is more rarely reported among patients who underwent organ transplantation. Despite a provisional estimate of mortality of 20,000–40,000 deaths for year, leishmaniasis belongs to the group of neglected tropical diseases. In 2007 the World Health Assembly approved a leishmaniasis control program. In 2010, the members of an Expert Committee determined that it is possible to perform an adequate control of leishmaniasis in the world, although there is still a political disengagement and a lack of international cooperation. (World Health Organization, 2014).

Regarding the parasite life cycle and transmission, these intracellular protozoa

have a complex digenetic life cycle, requiring a susceptible vertebrate host and a permissive insect vector, which allow their transmission.

Diniz (Diniz S. A. et al., 2008) stressed the importance of animal reservoirs in facilitating the transmission of VL in popoulous urban areas in Brazil, and recently the sexual and vertical transmission (mother-fetus) of VL in the canine populations have been reported to play an important role in the maintenance of this disease. (Turchetti A. P. et al., 2014) At least thirty species are possible vectors. The sandflies grow in moist and shady places, females need to feed on blood for egg maturation: they bite the host in hairless areas of their skin and feed on the blood coming from the wound. While these sandflies are feeding themselves, they regurgitate the parasites in flagellated promastigote form, beyond the mammalian host's skin-barrier.

The saliva of the *Lutzomya longipalpis* sandfly which transmits *Leishmania chagasi* contains a peptide (maxadilan) which causes and can enhance the infectivity of promastigotes, thus influencing the course of infection.

Once the promastigotes bind to the macrophage receptors, they are phagocytized and then differentiate, within the phagolysosomes, into a non-flagellated amastigote form that multiplies by binary fission. After the rupture of infected macrophages, the amastigotes are phagocytized by other macrophages. If ingested by other sandflies, the amastigotes are transformed back into promastigotes and, after at least 7 days, they become infectious.

In tropical areas, transmission can take place throughout the year. In temperate regions, the transmission mainly occurs in the summer: the lifespan of the sandflies is two months, but the infected ones live less and need many blood meals a day (4-6), since they have the trunk obstructed by a multitude of promastigotes of *Leishmania*; thus they bite more often, spreading the infection more extensively.

Leishmaniasis is maintained in the environment through a zoonotic cycle (Fig.2). The most important domestic reservoir is the dog, for which leishmaniasis is a chronic parasitic disease often incurable. An anthroponotic cycle is also possible: humans are usually accidental hosts, but in the case of *L. donovani* (in the Indian Sub-continent), they function just as proper reservoirs.

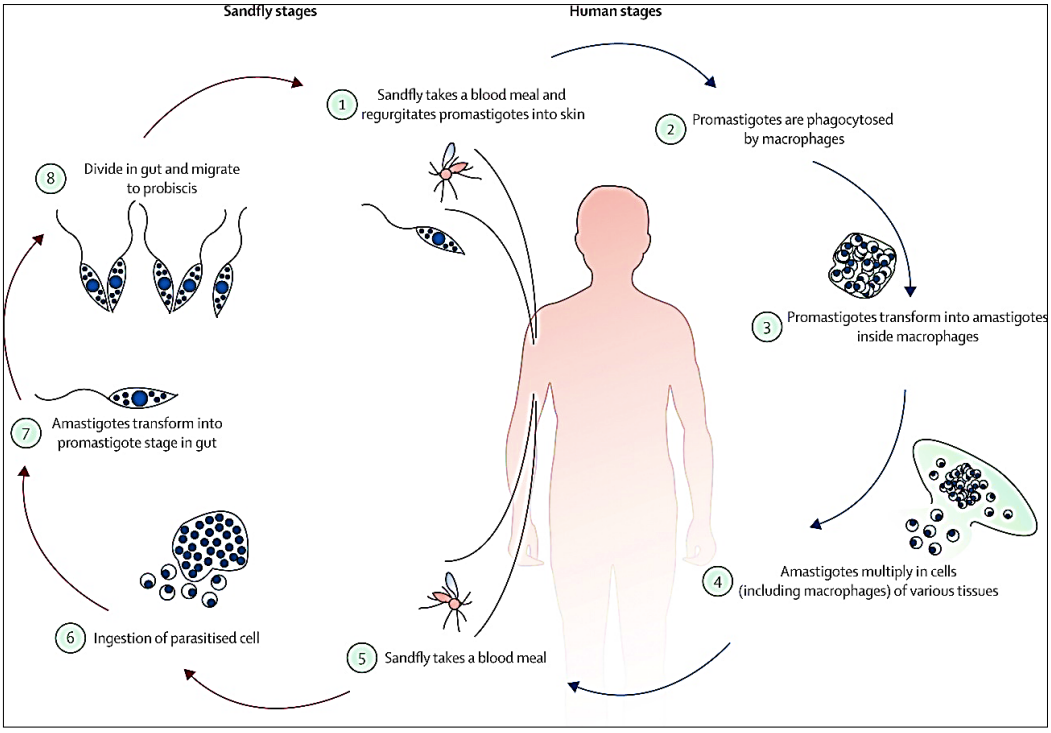


Fig.2 Leishmania's life cycle. Extracted by Reithinger et al., Elsevier, (2007)

As a result of a long host-parasitic co-evolutionary process, *Leishmania* protozoa has developed several immunomodulatory strategies that are essential for the infection progression. A recent study highlighted how the parasite can control the cell death and the immunitary responses in order to survive (Cecilio P. et al., 2014). The parasite, indeed, can alter the phagolysosome maturation process, modify the production of the cytokines and chemokines by the host cell, compromise cell function, enter host cells and successfully differentiate and infect. It is obvious that understanding the mechanisms associated with immunity and disease progression is essential for the development of new therapies and possible vaccine approaches. The next section deals with the therapies currently available in the treatment of this infection.

Leishmaniasis therapy

To date no licensed vaccine is available against human leishmaniasis, only a few drugs are available for the treatment and control of VL and CL. (Croft S. L. et al., 2003; Hussain H. et al., 2014) The disease severity depends on the infecting species of *Leishmania* and the host's immune response. Visceral disease that may

occur in post-kala-azar (PKDL) cutaneous leishmaniasis requires a systemic treatment, while cutaneous leishmaniasis is treated both by systemic and local therapy. (Hussain H. et al., 2014) (Fig. 3).

Therapies currently available for Leishmaniasis often have a limited efficacy and a high overall cost: therefore a preventive approach is primary, based on both the surveillance on insect vectors and infection reservoirs, and individual precautions (such as the personal protection from sandflies stings with shielding and/or repellent substances or by protective clothing).

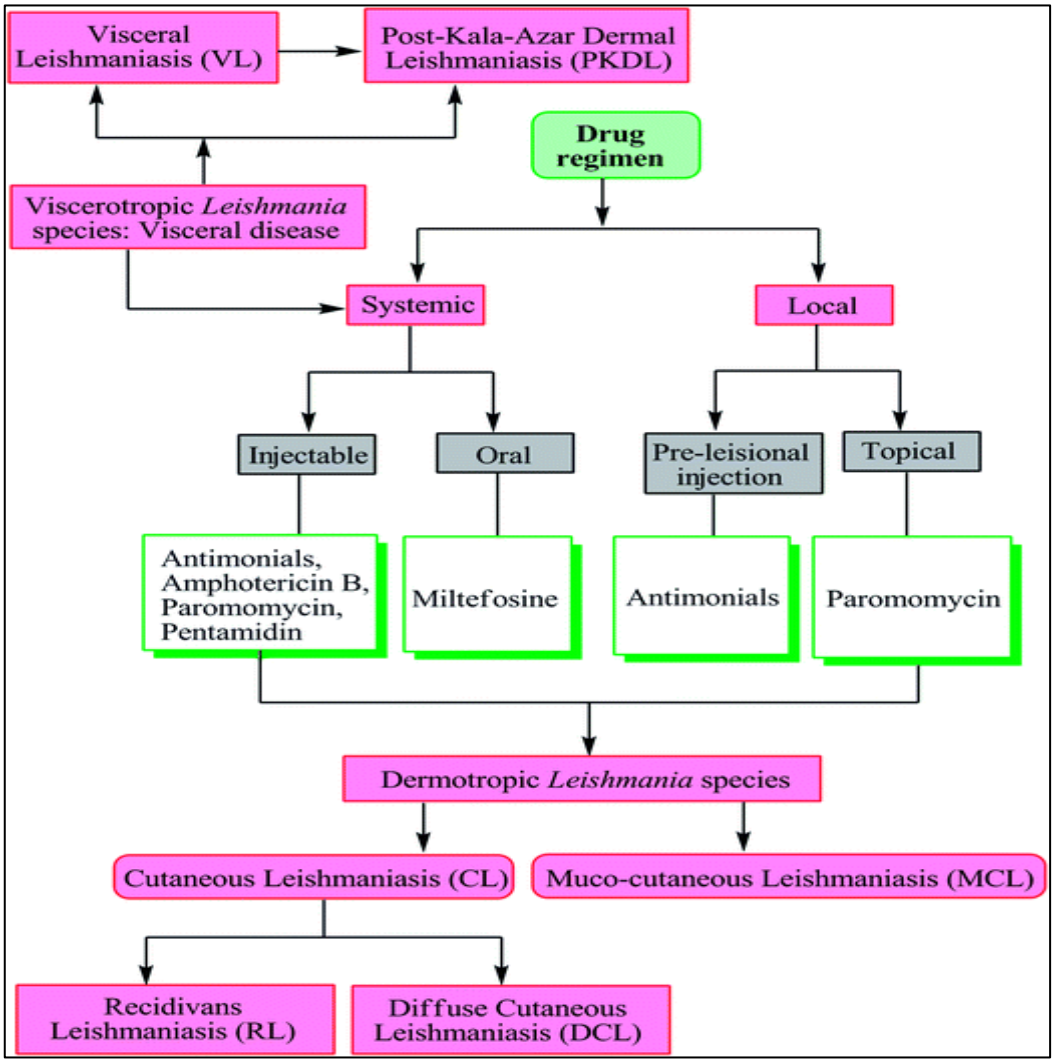


Fig.3 Leishmania species, corresponding clinical manifestations and related therapies. Extracted by Sangshetti J. N. et al., 2015.

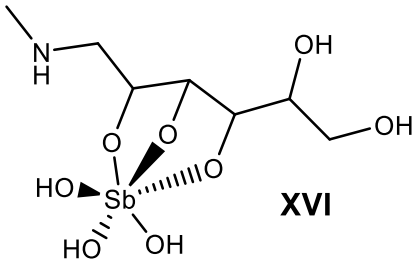
However, several therapeutic options are available; these are listed below and will be briefly discussed.

Pentavalent Antimonials

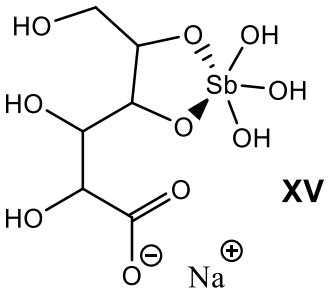
The Indian scientist Prof. Brahmchari, the Nobel Prize winner in 1929 for the discovery of an effective drug against *L. donovani*, synthesized urea stibamine (carbostibamide) in 1912 and saved many Indians' lives. Nevertheless, the drug administration had some side effects and was soon replaced by pentavalent antimonials that showed lower side effects. (Peter W., 1981)

Pentavalent antimonials were introduced into clinical practice in 1945 and were the most commonly used drugs in the next six decades. Their mechanism of action is not yet well known, but it has been reported that pentavalent antimony (Sb, V) is a prodrug that requires a biological reduction in its trivalent form (Sb, III) to exert its antileishmanial activity. (Roychoudhury J. et al., 2008 ; Ferreira C. S. et al., 2003) These drugs are utilized against both the major leishmaniasis forms (VL and CL), showing variable effectiveness, and have been the most recommended drugs in the first line treatment of the disease, until the development of drug resistance in some Indian areas has been noticed. (Hussain H. et al., 2014)

Pentavalent antimonials can be administered intramuscularly, intravenously and even via the endolymphatic route. (Freitas-Junior L. H. et al., 2012) The recommended dose is 20 mg/kg body weight for about 20-30 days, to reach a recovery of over 95%. Since the mid-40s, in the English-speaking countries of East Africa, stibogluconate has been used. (produced by GlaxoWellcome, London, UK, with the brand Pentostam T, PSM, containing sodium stibogluconate, **XV**). However, in the former French and Italian colonies in Africa the only drug used to treat VL and PKDL has been meglumine antimoniate (**XVI**, produced by Rhone-Poulenc-Rohrer, Paris, with the brand Glucantime T).



XVI



XV

Several causes have led to a decrease in the use of antimonials in therapy and in particular the onset of numerous and serious side effects. Indeed, cases of pancreatitis occurred during the treatment have been reported. Other side effects found are pancytopenia, reversible peripheral neuropathy, increase in serum aminotransferase levels, injection site pain, joint stiffness, gastrointestinal

problems, liver and kidney failure (nephrotoxicity). Cardiotoxicity may also occur, which can cause sudden death. Moreover, the long duration of treatment can cause drug accumulation in the liver and spleen tissues. (Hepburn N. C. et al., 2003; Zaghloul I. Y. et al., 2004)

Other factors have contributed to a decrease in antimonial drugs utilization: the parenteral administration (that is difficult to accomplish in some areas of Asiatic and African countries), the long-term treatment (up to 4 weeks), the variation in efficacy against VL and CL and finally the development of resistance. To overcome these problems, some formulation studies have been recently carried out; Fernandes *et al.*, 2013 indeed, reported a new oral administration strategy for the pentavalent antimonials in the treatment of visceral leishmaniasis. This strategy consists on the formation of an amphiphilic antimony (V) complex, through the reaction between antimony V and a N-alkyl-N-methylglucamide non-ionic tensioactive. Using this strategy, an improvement of the oral bioavailability and the pharmacokinetic profile has been achieved in mice, in comparison with the utilization of meglumine antimoniate. These amphiphilic complexes, administered orally, showed to be active in a murine model of visceral leishmaniasis. (Croft S. L. et al., 2003)

Amphotericin B

In the regions characterized by resistance to pentavalent antimonials, an antimycotic antibiotic such as amphotericin B (**XVII**) is used to treat the infection. In India this drug is recommended by the National Expert Comittee. (Bern C. et al., 2006; Saha A. K. et al., 1986; Thakur C. P. et al., 1999)

Amphotericin was discovered in 1956 in a bacteria, *Streptomyces nodosus*, taken from the banks of the Orinoco river in Venezuela. (Singh S. et al., 2004)

Amphotericin B, which is characterized by high affinity for the 24-substituted sterols, forms a complex with ergosterol (a main component of *Leishmania* cellular membrane), thus interfering with the ergosterol physiological pathway. This leads to the formation of aqueous pores, an increase in the permeability of the membrane, the release of cation and monovalent anions and small metabolites, that finally cause cell death (leishmanicidal activity). (Saha A. K. et al., 1986; Johnson R. H. et al., 2007; Ramos H. et al., 1996)

The currently available amphotericin B preparations include amphotericin B deoxycholate (Fungizone®) and various liposomal formulations (for example Ambisome ®). (Berman J. D. et al., 1998)

The amphotericin B liposomal formulations have been devised in order to improve its bioavailability and its pharmacokinetic properties; utilizing this method, indeed, amphotericin B is masked from susceptible tissues and its absorption by the endothelial reticular cells is facilitated, thus reducing the side effects and increasing its effectivenes. (Gangneux J. P. et al., 1996; Balana-Fouce R., 1998)

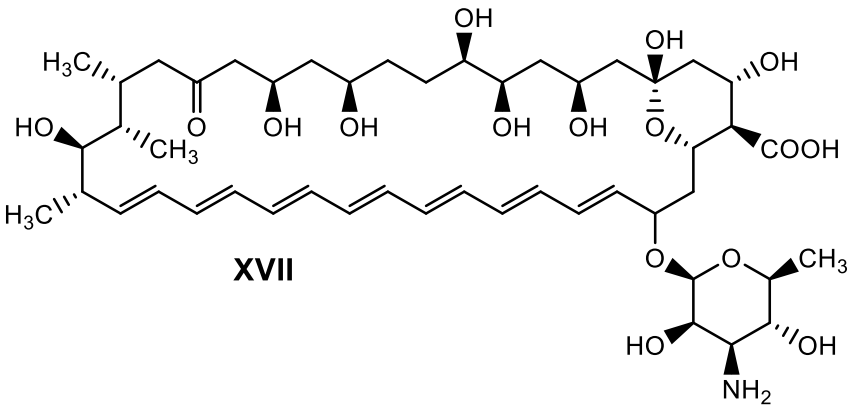
The liposomes have a very small size and stay longer in the blood than larger lipid particles that are quickly phagocytized by macrophages. Therefore, Ambisome can accumulate in the liver and reach its therapeutic concentration faster than antimonials. Because of its longer half-life, a better effect is achieved. (Berman J. D. et al., 1998; Yardley V. et al., 1997; New R. R. C. et al., 1981; Gradoni L. et al., 1993) Ambisome has been registered and approved for the VL treatment in various countries such as USA and in Europe and its utilization is recommended by WHO. It can also be used to treat CL and complex forms of CL (like PKDL), as well as PKDL itself. (Bern C. et al., 2006; Meheus F. et al., 2010; Meyerhoff A. et al., 1999)

Although having a higher toxicity profile than Ambisome, other formulations are also utilized, such as a concentrate for infusion (Albecet®) and a lyophilized powder for reconstitution (for intravenous administration), namely Amphocil®. (Bodhe P. V. et al., 1999; Sundar S. et al., 2004)

Despite some problems in administration and stability at high temperatures, liposomal amphotericin B has been shown to be much more effective than other formulations.

The side effects associated with its infusion are: nephrotoxicity, fever and chills, bone pain, hypotension, and rarely cardiac arrest.

The use of this medication requires a prolonged hospitalization and continuous monitoring. (Balana-Fouce R. et al., 1998)

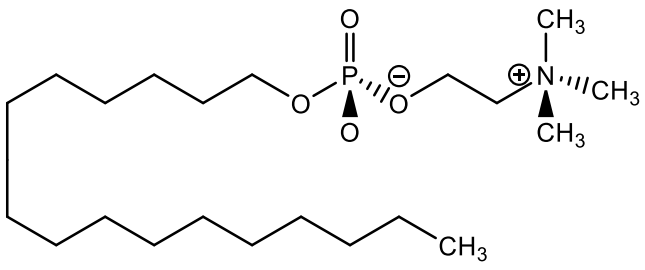


XVII

Miltefosine

Miltefosine (**XVIII**), chemically known as hexadecylphosphocholine, was originally prepared as an antineoplastic drug, used in the topical treatment of cutaneous metastases in breast cancer. Its use as an anti-leishmaniasis agent dates back to the mid-1980s, and was the first effective oral medication to treat this infection. In the 1990s, several studies demonstrated this molecule activity in patients affected by VL, including those who did not respond to antimonials.

The dose to be administered is 2.5 mg/kg/die of miltefosine for 28 days. Phase III clinical studies on the effectiveness of miltefosine indicated a 94% cure rate in patients affected by VL. The drug was the registered in 2002 and entered the Indian market. Two years later miltefosine was approved in Germany, showing its usefulness in the treatment of immunocompromised patients. Recently, miltefosine has been tested for the treatment of CL in Columbia, where *L. panamensis* is a diffused parasite, and a 91% cure rate was observed; however, in regions of Guatemale (Central America), where *L. braziliensis* and *L. mexicana* are more common, very lower cure rates were observed (53%) than the values achieved using antimonials (over 90%). (Hussain H. et al., 2014; Unger C. et al., 1990) Miltefosine has been administered to children suffering from cutaneous leishmaniasis (CL), and the results obtained were very similar to the administration of meglumine antimony; the difference consisted of a greater patients' tolerability to the oral administration. (Rubiano L. C. et al., 2012) The mechanism of action is still unclear, but it has been hypothesized that it acts in different ways: inhibiting phosphatidylcholine biosynthesis, altering phospholipids and sterol composition, inhibiting signal transduction and calcium homeostasis. (Cro S. L. et al., 2003; Sundar S. et al., 2003) The researchers also found out that miltefosine promotes apoptosis processes in *L. donovani*, but the mechanism is still unknown; it also stimulates the nitric oxide (NO) production, contributing to the parasite death within the macrophage.(Wadhone P. et al., 2009)



XVIII

It has a lower toxicity but a long half-life and it is a potential teratogenic drug, so its use must be avoided during pregnancy. Other adverse reactions include gastrointestinal disorders, hepatotoxicity and nephrotoxicity.

Unfortunately, some studies found that some species of Leishmania, such as *L. braziliensis, L. guyanensis,* and *L. mexicana* are resistant to miltefosine (Freitas-Junior L. H. et al., 2012; Perez-Victoria F. J. et al., 2003), so that a combined therapy of miltefosine and paromomycin or amphotericin B has been devised for the treatment of patients suffering from VL who are resistant to other treatments. (Seifert K. et al., 2006)

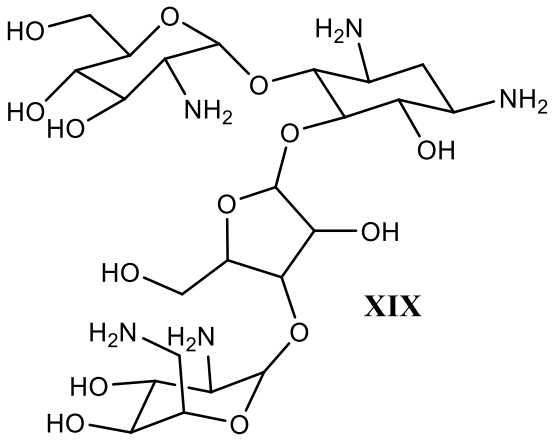
Paromomycin

Paromomycin (**XIX**), an aminoglycoside antibiotic, is a new broad spectrum antibiotic drug that has been used for the treatment of leishmaniasis. It can be utilized for the treatment of both the forms of leishmaniasis (VL e CL), but it is more effective against the cutaneous form. Its use is limited in the endemic regions due to its poor bioavailability. (Sundar S. et al., 2008; Sundar S. et al., 2009) It is available as an intramuscular preparation for parenteral administration in the treatment of systemic infections by VL, and as an ointment to treat local skin infections occurring in CL. (Hamilton J. G. et al., 2008; Grimaldi Jr G. et al., 1989)

Paromomycin is currently a non-patented drug and it has been recognized as an orphan drug by the FDA in the United States and by the EMA in the EU. (Maarouf M. et al., 1998) It is rather inexpensive and requires a dose of 16 mg/kg/die for about 21 days. (Sundar S. et al., 2007) Paromomycin is used also in the treatment of other diseases, but the mechanism of action against leishmanias has yet to be investigated and elucidated. However, both a reduction in magnesium levels and an inhibition of re-assembly of the ribosomal subunits in the cytoplasm and mitochondria of *L. donovani* have been observed; these events led to the inhibition of protozoal protein synthesis. (Maarouf M. et al., 1995)

Other mechanisms may consist in an alteration of lipid membrane fluidity and metabolism modifications. (Jhingran A. et al., 2009)

Some side effects observed after the treatment with paromomycin are: elevated transaminases levels, ototoxicity, injection site pain, nausea, abdominal cramps, and diarrhea. (Khan W. et al., 2011; Sundar S. et al., 2007)

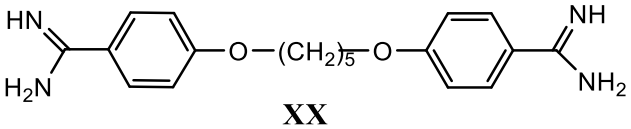


XIX

Pentamidine

Pentamidine (**XX**) is an aromatic diamidine, used as a treatment of antimonial-resistant cases. It was originally used intramuscularly in the therapy of VL, but, due to increasing resistance and toxicity, its utilization has been forbidden. (Patel T. A. et al., 2009)

Although its exact mechanism of action is still unknown, it is believed that the drug accumulates within the parasite, enters the promastigote through arginine and polyamines carriers, accumulates in mitochondria and finally inhibits the mitochondrial topoisomerase II. (Fries D. S. et al., 2003; Mishra J. et al., 2007; Singh N. et al., 2014) The treatment with pentamidine can cause myalgia, pain in the injection site, nausea, headache, and, less commonly, a feeling of burning and numbness, hypotension, insulin-dependent irreversible diabetes mellitus and eventually death. (Sundar S. et al., 2006)



XX

However, the current therapeutic armamentarium, consisting of the drugs listed above, is losing importance for some evident problems:

- not complete therapeutic coverage of all Leishmania strains
- Increased resistance from sensitive strains
- onset of numerous and severe side effects

For these reasons, in recent years, worldwide research has increased considerably in order to find new chemotherapeutic agents. It is also noteworthy that the spread

of disease in underdeveloped countries highlightes the necessity of targeting the research, if possible, towards the design of inexpensive medicines.

Vial H.J. et al., 1992 identified in the phospholipid metabolism, necessary for membrane biogenesis, a vital function for protozoal parasites, constituting the prerequisite for their development, growth and multiplication (Vial H.J. et al., 2003; Vial H.J. et al., 2005).

In details, phosphatidylcholine, the main constituent of the protozoan membranes, is synthesized by the parasitic enzyme apparatus starting from a polar head, the choline, whose carrier-mediated transport represents one of the limiting steps of the biosynthetic pathway (Ancelin M. L.,1989).

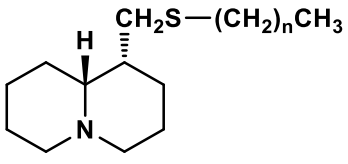
Compounds that, mimicking the structure of the choline, inhibit its carrier have shown a high antiprotozoal activity; numerous quaternary mono- and/or di-ammonium salts which showed a high choline antagonist action were thus synthesized.

Regarding this class of derivatives, SAR studies (Calas M. et al.,1997; Calas M. et al., 2000) have highlighted some essential parameters:

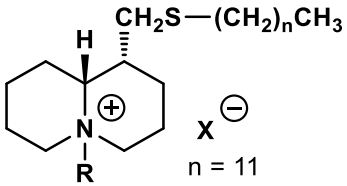
- the nature of the biological compound-target interaction, which involves one predominant ion interaction and an appropriate hydrophobic interaction;
- an adequate steric hindrance of the cationic head;
- the influence of the lipophilic chain length for quaternary mono-ammonium salts and of the linker length for the quaternary di-ammonium salts.

Chemistry discussion

On the basis of the data mentioned above (§pg.5), the thiolupinine quinolizidine moiety have been used as a starting molecule to design and synthesize both amine derivatives (**XXI**) and quaternary ammonium salts (**XXII**)(Santini F., Tesi di laurea, 2008).



XXI n = 5,7,11,15

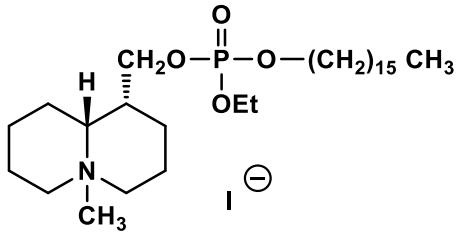


XXII

n = 11
R = CH_3, C_3H_7 X = I
R = $PhCH_2$, 4-ClPhCH_2 X = Cl

Some of these compounds were tested at the Pharmaceutical and Biomolecular Science Department of the University of Milan, to evaluate their *in vitro*-activity towards Leishmaniasis strains.

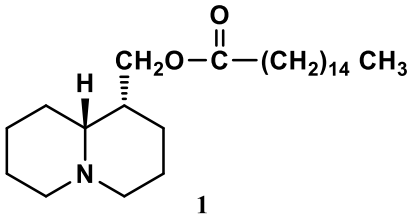
The quaternary ammonium salts (**XXII**) exhibited a moderate activity. In both cases the shortening of the lateral alkyl chain leads to an increase in activity, while the introduction of an arylalkyl (benzyl) residue into the cationic head causes a significant improvement of the activity obtaining the most active compound within this group of derivatives.



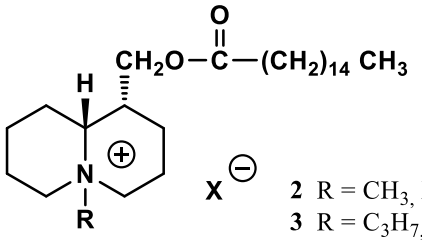
XXIII

Subsequently the compound **XXIII**, a miltefosine analogous, was synthesized, and its effects on *Leishmania tropica and infantum* were evaluated, resulting in a modest activity (IC_{50}> 30 μM).

In order to proceed with the study of the quinolizidine nucleus structural modulation, the palmitic ester of lupinine (**1**) and a small series of its quaternary ammonium salts in which the cationic head (**2-5**) is changed have been synthesized; these derivatives possess two structural characteristics in common with miltefosine: a chain of 16 carbon atoms and a cationic head.



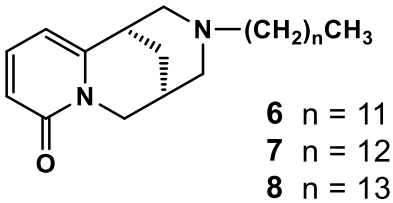
1



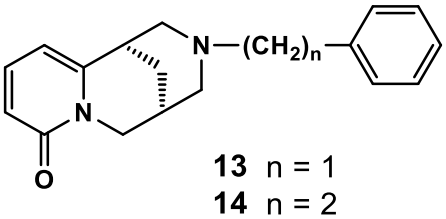
2 R = CH_3, X = I
3 R = C_3H_7, X = I
4 R = $PhCH_2$, X = Cl
5 R = 4-$ClPhCH_2$, X = Cl

Besides this mentioned series of compounds, a recent publication by Rakhimov et al., 2013 reports an antiparasitic and particularly anti-leishmaniasis activity exerted by N-benzylcytisine derivatives substituted on the phenyl ring; on this basis, a further series of quinolizidine derivatives starting from cytisine have been synthesized. In these compounds, to maintain the basic head, the secondary

nitrogen atom in 12- of cytisine has been replaced by alkyl chains of variable length (C12-C18) (**6**-**12**) or by alkyaryl portions (**13**-**14**).

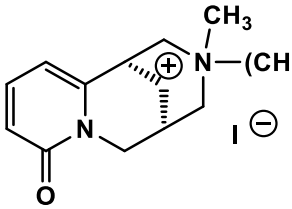


6 n = 11
7 n = 12
8 n = 13
9 n = 14
10 n = 15
11 n = 16
12 n = 17

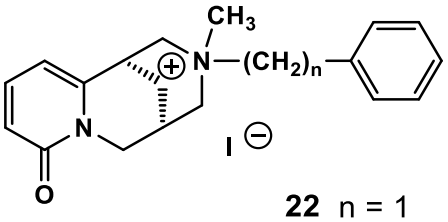


13 n = 1
14 n = 2

Starting from compounds **6**-**14**, the corresponding quaternary N-methyl ammonium salts have been prepared. (**15**-**23**)

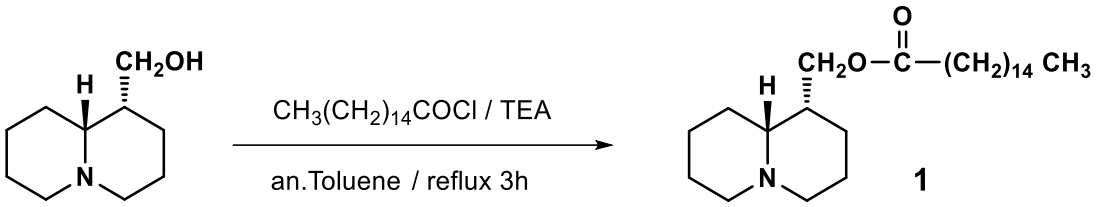


15 n = 11
16 n = 12
17 n = 13
18 n = 14
19 n = 15
20 n = 16
21 n = 17



22 n = 1
23 n = 2

To synthesize compound **1**, lupinine was reacted with palmitoyl chloride in anhydrous toluene under reflux for 3 hours in presence of triethylamine (Tlegenov R. T., 2011).

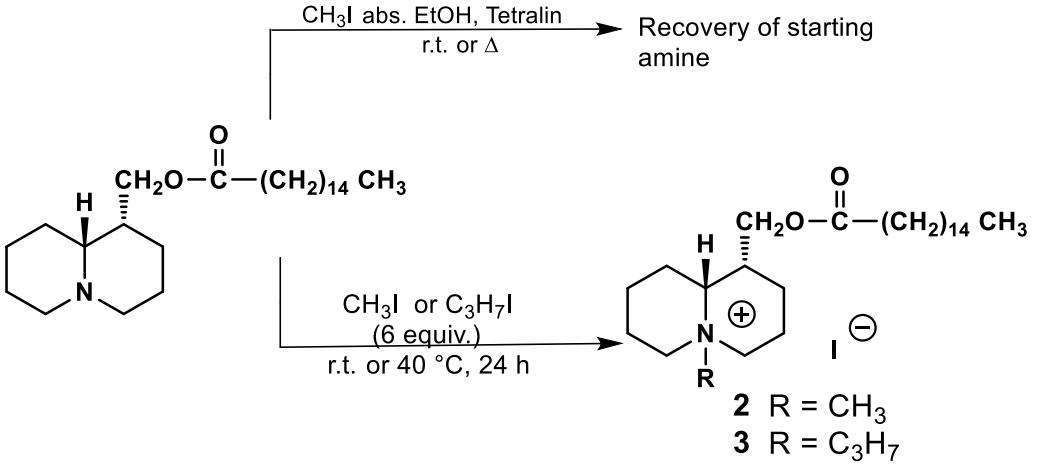


1

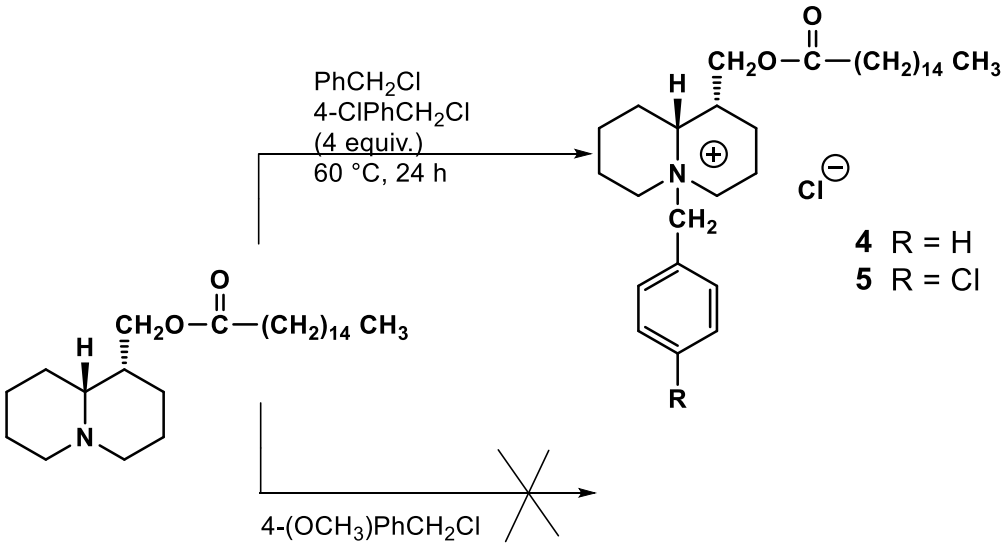
The reaction of quaternization to obtain the ammonium salts, although virtually simple, presented some difficulties; the reaction of the base with stoichiometric amounts of methyl iodide in various solvents (absolute ethanol, tetralin), with or without prolonged heating led to the quantitative recovery of the starting base, but not to the obtainment of the desired products.

The products were obtained by using a large excess of methyl or propyl iodide (6 equivalents), and stirring the reaction mixture in a closed tube. Compound **2** was

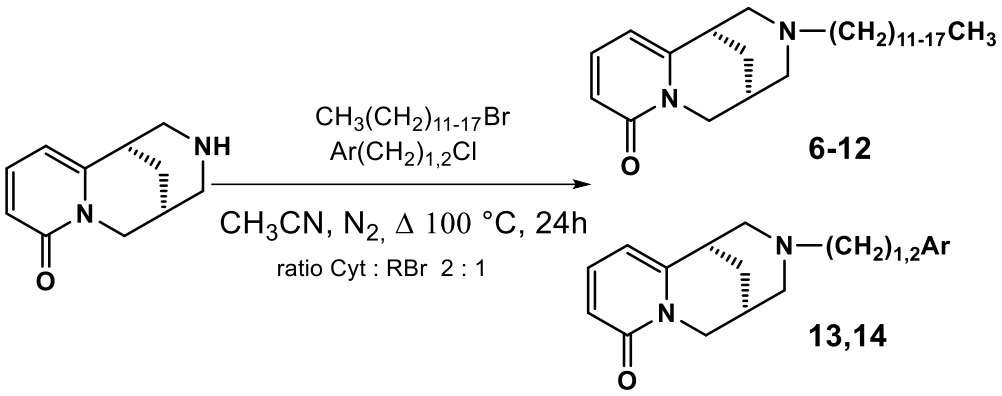
obtained maintaining the reaction mixture at RT for 24 hours; to obtain compound **3**, instead, the mixture was heated at 40°C for 24 hours.



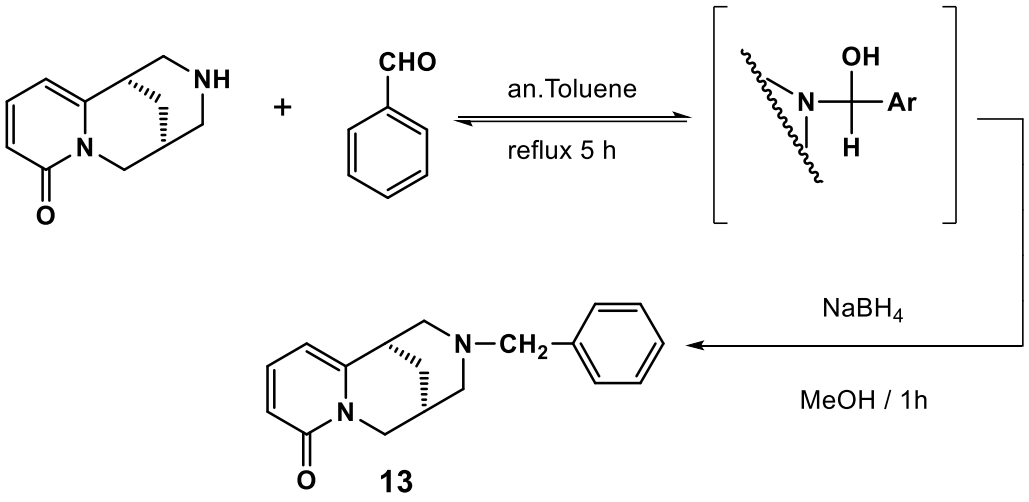
To obtain the benzyl derivatives **4** and **5**, the reaction was carried out using 4 equivalents of halide derivative, heating at 60°c for 24 hours. This reaction was also attempted using 4-methoxybenzyl chloride, but in this case a rapid darkening of the reaction mixture was observed and the desired product could not be isolated in adequate purity.



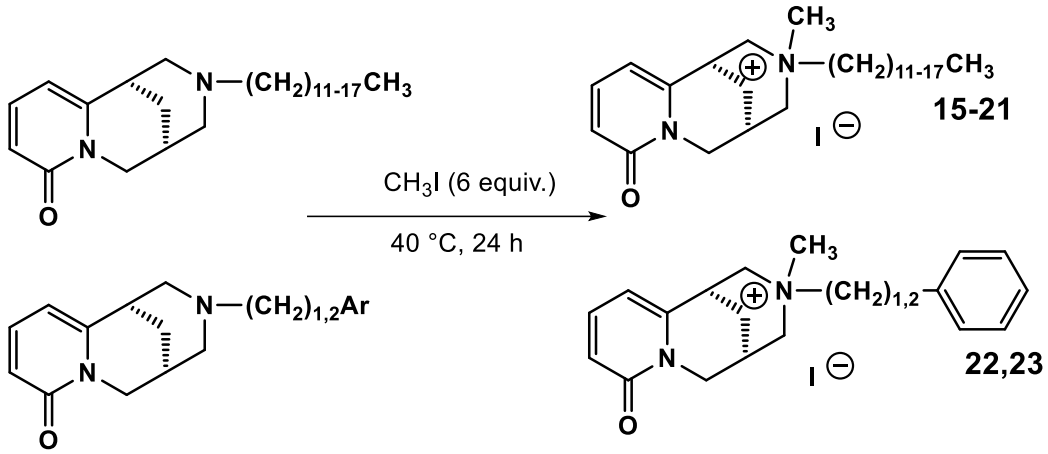
To provide the derivatives **6-14**, cytisine was reacted with the appropriate halide derivative, in the stoichiometric proportion 2:1, in acetonitrile, heated at 100°C for 24 hours in a closed tube under nitrogen atmosphere.



The benzyl derivative **13** was been obtained, in similar yields, also through the synthetic procedure described in the literature (Rakhimov S. B., 2013), changing only the used solvent (toluene instead of benzene); this method involved an initial reaction between cytisine and benzaldehyde in anhydrous toluene, and then a reduction reaction using $NaBH_4$ in methanol, in a typical reductive amination protocol.



The quaternization reaction of the cytisine bases have been carried out using 6 equivalents of methyl iodide and heating at 40°C for 24 hours.



The purification of the compounds with a chain up to 15 carbon atoms (**6-9**) was performed without any difficulty, while the hexadecyl derivative (**10**) and, with increasing difficulty, the heptadecyl (**11**) and octadecyl (**12**) derivatives showed problematic solubility with formation of emulsions. Reaction attempts performed using propyl iodide or benzyl chloride and N-dodecylcytisine, N-hexadecylcytisine and N-benzylcytisine led to the quantitative recovery of the free base, apparently owing to the excessive steric hindrance on the tertiary amine of the free bases.

In all quaternization reaction cases, at the end of the reaction a glassy solid is obtained, which is pulverized by repeated grinding processes in the presence of anhydrous ether.

Biological discussion

Some of these compounds (**1, 2, 4, 6, 11, 15, 17, 19**) have been tested at the Pharmacological and Biomolecular Science Department at the University of Milan and the results are reported in table 1.

Compound	IC_{50} (μM)[a]	
	L. tropica	***L. infantum***
1	> 30	> 30
2	> 30	> 30
4	8.48 ± 4.37	30.72 ± 7.22
6	> 30	> 30
11	> 30	> 30
15	> 30	27.89 ± 5.12
17	24.57 ± 5.48	18.96 ± 4.75
19	> 30	> 30
Miltefosine	43.26 ± 11.36	31.26 ± 10.43

Table. 1 Antileishmaniasis in vitro activity against *L. tropica* and *L. infantum* strains

The results are mean of at least three different experiment performed in duplicate

The results indicate a low activity of these compounds against the studied Leishmania strains. Nevertheless, although with the opportune caution given the small amount of data, analogies with the results of the previous series can be noticed. It is evident that, in the series of the lupinine derivatives, the benzyl cationic head improves the activity (**4** compared to **2**); in the series of the cytisine derivatives the optimal chain length appears to be 14 carbon atoms (**17**), while the shortest chain maintains a residual activity (comparison between **15** and **19**).

The result of the compound **4** may thus confirm the importance of the benzyl cationic head and the possible influence of substituents on the phenyl ring. In the second series the synthesis and the assays on compounds with shorter alkyl chains (C6-C10) could be useful to continue the investigation; moreover, the insertion of halide substituents on the aromatic ring of the compounds **13** and **14** could be performed in order to verify a greater activity, as the literature suggests (Rakhimov S. B.,2013).

Finally, it is noteworthy that all the compounds exert a low cytotoxicity (IC_{50} > 100 μM) on the HMEC-1 cell line.

Based on the few results available, a general low antileishmaniasis activity of these compounds can be confirmed. However some of these novel molecules could be

tested on *Plasmodium falciparum,* since other compounds previously synthesized have shown a higher activity against this kind of protozoa. Eventually, the first series of compounds may act as antitubercular agents, as in the literature lupinyl palmitate is reported to exert an antitubercular effect against *M. tuberculosis* $R_{37}RV$, causing a bacterial inhibition of 98% at the concentration of 6.25 μg/mL (Tlegenov R. T. ,2011).

Materials and Methods

Melting points were determined by capillary tube method using a Büchi melting point apparatus B-540 and they have not been corrected. The melting points of the quaternary ammonium salts have not been reported here, since they are not sharp and they don't possess a full reproducibility even using closed capillary tubes. Elemental analyses (C, H,N) were carried out at the Microanalysis Laboratory of the Department of Pharmacy, in the Pharmaceutical and Cosmetic Chemistry Section of University of Genoa. ^{1}H-NMR spectra were acquired on a Varian-Gemini 200 apparatus, using $CDCl_3$ or d_6-DMSO as solvents; J (expressed in Hz).

Synthetic processes

Lupinyl palmitate (1)

A solution of palmitoyl chloride (0.82 g; 3 mmol; d = 0.906; 0.91 mL) in 5 mL of anhydrous toluene is added dropwise to a solution of lupinine (0.50 g; 3 mmol) and triethylamine (0.30 g; 3 mmol; d = 0.726, 0.42 mL) in 15 mL of anhydrous toluene. The reaction mixture is heated under reflux for 3 hours. After cooling, the mixture is extracted three times with acidic water. The collected acqueous layers are alkalized with NaOH 2N and extracted with dichloromethane. The organic phase is dried over Na_2SO_4, filtered and evaporated. The oily residue is purified by crystallization from anhydrous Et_2O furnishing 1.03 g of white crystals with mp = 37-38 °C. (Yield = 84.4%).

The elemental analysis provided the following results:

	found:	C% 76.78	H% 12.16	N% 3.72
for $C_{26}H_{49}NO_2$	calc.:	C% 76.60	H% 12.11	N% 3.44

^{1}H-NMR ($CDCl_3$) δ: 4.61-4.42 (m, 2H); 3.40-3.14 (m, 2H); 2.92-2-62 (m, 6H); 2.62-1.98 (m, 6H); 1.98-1.37 (m, 23H); 1.37-1.04 (m, 6H).

Lupinyl palmitate N-methyl iodide (2)

120 mg (0.29 mmol) of lupinyl palmitate (**1**) and 0.25 g (1.76 mmol; 0.11 mL, d = 2.28) of methyl iodide are placed in a tube that is closed under nitrogen atmosphere, and the reaction mixture is stirred at rt for 24 hours. Upon completion of the reaction, the formation of a solid can be noticed. The reaction mixture is treated several times with anhydrous ether, grinding the insoluble solid each time and decanting the liquid, to obtain 113 mg of a white solid. (Yield = 71.1%)

The elemental analysis provided the following results:

	found:	C% 58.80	H% 9.56	N% 2.50
for $C_{27}H_{52}INO_2$	calc.:	C% 59.00	H% 9.54	N% 2.55

Lupinyl palmitate N-propyl iodide (3)

114 mg (0.28 mmol) of lupinyl palmitate (**1**) and 0.29 g (1.68 mmol; 0.16 mL, d = 1.743) of propyl iodide are placed in a tube that is closed under nitrogen atmosphere, and the stirring reaction mixture is heated at 40°C for 24 hours. Upon completion of the reaction, the formation of a solid is observed. The reaction mixture is treated several times with anhydrous ether, grinding the insoluble solid each time and decanting the liquid, to obtain 102 mg of a white solid. (Yield = 62.9%)

The elemental analysis provided the following results:

	found:	C% 60.19	H% 9.94	N% 2.46
for $C_{29}H_{56}INO_2$	calc.:	C% 60.30	H% 9.77	N% 2.42

Lupinyl palmitate N-benzyl chloride (4)

143 mg (0.35 mmol) of lupinyl palmitate (**1**) and 0.18 g (1.40 mmol; 0.16 mL, d = 1.1) of benzyl chloride are placed in a tube that is closed under nitrogen atmosphere, and the stirring reaction mixture is heated at 60°C for 24 hours. Upon completion of the reaction, the formation of a solid is observed. The reaction mixture is treated several times with anhydrous ether, grinding the insoluble solid each time and decanting the liquid, to obtain 96 mg of a white solid. (Yield = 51.3%)

The elemental analysis provided the following results:

	found:	C%	73.87	H%	10.79	N% 2.55
for $C_{33}H_{56}ClNO_2$	calc.:	C%	74.19	H%	10.57	N% 2.62

Lupinyl palmitate N-(4-chlorobenzyl) chloride (5)

138 mg (0.34 mmol) of lupinyl palmitate (**1**) and 0.22 g (1.35 mmol) of p-chlorobenzyl chloride are placed in a tube that is closed under nitrogen atmosphere, and the stirring reaction mixture is heated at 60°C for 24 hours. Upon completion of the reaction, the formation of a solid can be noticed. The reaction mixture is treated several times with anhydrous ether, grinding the insoluble solid each time and decanting the liquid, to obtain 85 mg of a white solid. (Yield = 44.1%)

The elemental analysis provided the following results:

	found:	C%	69.45	H%	9.92	N% 2.33
for $C_{33}H_{55}Cl_2NO_2$	calc.:	C%	69.69	H%	9.75	N% 2.46

N-Dodecyl cytisine (6)

0.76 g (4 mmol) of cytisine are dissolved in 6 mL of acetonitrile in a tube and 0.50 g (2 mmol; 0.48 mL, d = 1.038) of 1-bromododecane are added to the solution. The tube is closed under nitrogen atmosphere, and the stirring reaction mixture is heated at 100°C for 24 hours. After cooling, the formed solid is filtered: cytisine hydrobromide (0.49 g). The organic solution is dried and the residue is treated with acidic water. The acidic acqueous phase is first washed with ether and then alkalized and extracted with dichloromethane. The organic layer is dried over Na_2SO_4, filtered and evaporated, furnishing 0.72 g of a doughy compound that is purified by chromatography on Al_2O_3 (1:30) eluting with dichloromethane. A colorless oil is obtained (0.64 g), that is crystallized from anhydrous ether providing 0.57 g of white crystals with mp= 45-46 °C. (Yield = 79.2 %)

The elemental analysis provided the following results:

	found:	C%	77.28	H%	77.28	N% 7.99
for $C_{23}H_{38}N_2O$	calc.:	C%	77.04	H%	10.68	N% 7.81

^{1}H NMR *d*: 7.25 (dd, 1H, *J* = 9, 6.8, a-pyr); 6.40 (dd, 1H, *J* = 9, 1.2, a-pyr); 6.01 (dd, 1H, *J* = 6.8, 1.2, a-pyr); 4.04 (d, 1H, *J* = 15.6, bisp); 3.90 (dd, 1H, *J* = 15.6, 6.4, bisp); 3.01-2.84 (m, 3H, bisp); 2.58-2.16 (m, 5H, 3H, bisp + 2H, NCH_2); 2.01-1.75 (m, 2H, bisp); 1.43-0.97 (m, 20H); 0.92 (t, 3H, J = 6.4, CH_3).

N-Tridecyl cytisine (7)

0.76 g (4 mmol) of cytisine are dissolved in 6 mL of acetonitrile in a tube and 0.53 g (2 mmol; 0.52 mL, d = 1.03) of 1-bromotridecane are added to the solution. The tube is closed under nitrogen atmosphere, and the stirring reaction mixture is heated at 100° C for 24 hours. After cooling, the formed solid is filtered: cytisine hydrobromide (0.51 g). The organic solution is dried and the residue is treated with acidic water. The acidic acqueous phase is first washed with ether and then alkalized and extracted with dichloromethane. The organic layer is dried over Na_2SO_4, filtered and evaporated, furnishing 0.76 g of a doughy compound; this one is purified by chromatography on Al_2O_3 (1:30) eluting with dichloromethane and 0.69 g of a light yellow oil are obtained. The latter is finally crystallized from anhydrous ether, providing 0.62 g of white crystals with mp= 49-51 °C. (Yield = 82.7%)

The elemental analysis provided the following results:

	found:	C%	77.04	H%	11.08	N% 7.63
for $C_{24}H_{40}N_2O$	calc.:	C%	77.37	H%	10.82	N% 7.52

^{1}H NMR *d*: 7.26 (dd, 1H, *J* = 9, 7, a-pyr); 6.41 (dd, 1H, *J* = 9, 1.4, a-pyr); 5.99 (dd, 1H, *J* = 6.8, 1.4, a-pyr); 4.01 (d, 1H, *J* = 15.4, bisp); 3.89 (dd, 1H, *J* = 15.4, 6.6, bisp); 3.03-2.85 (m, 3H, bisp); 2.57-2.15 (m, 5H, 3H, bisp + 2H, NCH_2); 1.98-1.74 (m, 2H, bisp); 1.51-0.98 (m, 22H); 0.91 (t, 3H, J = 6.6, CH_3).

N-Tetradecyl cytisine (8)

0.76 g (4 mmol) of cytisine are dissolved in 6 mL of acetonitrile in a tube and 0.55 g (2 mmol; 0.59 mL, d = 0.932) of 1-bromotetradecane are added to the solution. The tube is closed under nitrogen atmosphere and the stirring reaction mixture is heated at 100° C for 24 hours. After cooling, the formed solid is filtered: cytisine hydrobromide (0.54 g). The organic solution is dried and the residue is treated with acidic water. The acidic acqueous phase is first washed with ether and then alkalized and extracted with dichloromethane. The organic layer is dried over Na_2SO_4, filtered and evaporated, furnishing 0.79 g of a doughy compound that is crystallized from acetone, providing 0.58 g of low-melting white crystals with mp= 52-54 °C. (Yield = 75.3%)

The elemental analysis provided the following results:

	found:	C%	77.58	H%	11.28	N% 7.46
for $C_{25}H_{42}N_2O$	calc.:	C%	77.67	H%	10.95	N% 7.25

^{1}H NMR *d*: 7.28 (dd, 1H, *J* = 9, 6.8, a-pyr); 6.44 (dd, 1H, *J* = 9, 1.4, a-pyr); 5.99 (dd, 1H, *J* = 7, 1.3, a-pyr); 4.05 (d, 1H, *J* = 15.4, bisp); 3.89 (dd, 1H, *J* = 15.4, 6.4, bisp); 3.01-2.82 (m, 3H, bisp); 2.53-2.14 (m, 5H, 3H, bisp + 2H, NCH_2); 1.98-1.72 (m, 2H, bisp); 1.44-0.98 (m, 24H); 0.90 (t, 3H, J = 6.2, CH_3).

N-Pentadecyl cytisine (9)

0.95 g (5 mmol) of cytisine are dissolved in 8 mL of acetonitrile in a tube and 0.73 g (2 mmol; 0.73 mL, d =1.005) of 1-bromopentadecane are added to the solution. The tube is closed under nitrogen atmosphere and the stirring reaction mixture is heated at 100° C for 24 hours. After cooling, the formed solid is filtered: cytisine hydrobromide (0.62 g). The organic solution is dried and the residue is treated with acidic water. The acidic acqueous phase is first washed with ether and then alkalized and extracted with dichloromethane. The organic layer is dried over Na_2SO_4, filtered and evaporated, furnishing 1.03 g of a solid compound that is crystallized from acetone, providing 0.80 g of low-melting white crystals with mp= 58-60 °C (Yield = 80%)

The elemental analysis provided the following results:

	found:	C%	77.71	H%	11.34	N% 7.31
for $C_{26}H_{44}N_2O$	calc.:	C%	77.94	H%	11.07	N% 6.99

^{1}H NMR *d*: 7.24 (dd, 1H, *J* = 9, 6.6, a-pyr); 6.42 (dd, 1H, *J* = 9, 1.2, a-pyr); 5.94 (dd, 1H, *J* = 6.8, 1.2, a-pyr); 4.01 (d, 1H, *J* = 15.6, bisp); 3.88 (dd, 1H, *J* = 15.6, 6.4, bisp); 3.02-2.78 (m, 3H, bisp); 2.55-2.12 (m, 5H, 3H, bisp + 2H, NCH_2); 2.00-1.73 (m, 2H, bisp); 1.56-0.97 (m, 26H); 0.92 (t, 3H, J = 6.2, CH_3).

N-Hexadecyl cytisine (10)

0.95 g (5 mmol) of cytisine are dissolved in 8 mL of acetonitrile in a tube and 0.76 g (2.5 mmol; 0.76 mL, d =0.999) of 1-bromohexadecane are added to the solution. The tube is closed under nitrogen atmosphere and the stirring reaction mixture is heated at 100° C for 24 hours. After cooling, the formed solid is filtered: cytisine

hydrobromide (0.61 g). The organic solution is dried and the residue is treated with acidic water. The acidic acqueous phase is first washed with ether and then alkalized, leading to the formation of a strong emulsion that is thoroughly extracted with dichloromethane and centrifuged each time. The organic layer is dried over Na_2SO_4, filtered and evaporated, furnishing 1.05 g of a doughy compound that is treated with water, filtered and washed with water two times. After being dried in a dryer, the white solid is crystallized from acetonitrile providing 0.79 g of low-melting white crystals with mp= 60-62°C (Yield = 76%)

The elemental analysis provided the following results:

	found:	C%	78.58	H%	11.10	N% 6.81
for $C_{27}H_{46}N_2O$	calc.:	C%	78.20	H%	11.18	N% 6.76

^{1}H NMR *d*: 7.26 (dd, 1H, *J* = 9, 6.4, a-pyr); 6.39 (dd, 1H, *J* = 9, 1.3, a-pyr); 5.99 (dd, 1H, *J* = 6.4, 1.4, a-pyr); 4.00 (d, 1H, *J* = 15.4, bisp); 3.89 (dd, 1H, *J* = 15.4, 6.4, bisp); 3.00-2.78 (m, 3H, bisp); 2.54-2.14 (m, 5H, 3H, bisp + 2H, NCH_2); 2.02-1.74 (m, 2H, bisp); 1.55-0.96 (m, 28H); 0.91 (t, 3H, J = 6, CH_3).

N-Heptadecyl cytisine (11)

0.95 g (5 mmol) of cytisine are dissolved in 8 mL of acetonitrile in a tube and 0.80 g (2.5 mmol) of 1-bromoheptadecane are added to the solution. The tube is closed under nitrogen atmosphere and the stirring reaction mixture is heated at 100° C for 24 hours. The cooling of the mixture leads to the formation of a pasty mass that is filtered obtaining a doughy solid; the latter is washed three times with hot acetonitrile (3 x 5 mL), furnishing a grayish solid : cytisine hydrobromide (0.63 g). The organic solution is dried and the residue is treated with acidic water. The acidic acqueous phase is first washed with ether and then alkalized, leading to the formation of a very strong emulsion, that is thoroughly extracted with dichloromethane and centrifuged each time to separate the two layers. The organic layer is dried over Na_2SO_4, filtered and evaporated, furnishing 1.12 g of a doughy compound that is treated with water, filtered and washed with water two times. After being dried in a dryer, the white solid is crystallized from acetonitrile providing 0.91 g of white crystals with mp= 66-68°C (Yield = 85 %)

The elemental analysis provided the following results:

	found:	C%	78.09	H%	11.64	N% 6.25
for $C_{28}H_{48}N_2O$	calc.:	C%	78.45	H%	11.29	N% 6.53

^{1}H NMR *d*: 7.25 (dd, 1H, *J* = 9, 6.4, a-pyr); 6.38 (dd, 1H, *J* = 9, 1.3, a-pyr); 5.99 (dd, 1H, *J* = 6.4, 1.4, a-pyr); 4.03 (d, 1H, *J* = 15.6, bisp); 3.92 (dd, 1H, *J* = 15.6, 6.4, bisp); 3.04-2.82 (m, 3H, bisp); 2.53-2.13 (m, 5H, 3H, bisp + 2H, NCH_2); 2.02-1.76 (m, 2H, bisp); 1.61-0.95 (m, 30H); 0.90 (t, 3H, J = 6.2, CH_3).

N-Octadecyl cytisine (12)

0.95 g (5 mmol) of cytisine are dissolved in 8 mL of acetonitrile in a tube and 0.83 g (2.5 mmol) of 1-bromooctadecane are added to the solution. The tube is closed under nitrogen atmosphere and the stirring reaction mixture is heated at 100° C for 24 hours. The cooling of the mixture leads to the formation of a pasty mass that is filtered obtaining a doughy solid; this one is washed four times with hot acetonitrile (4 x 5 mL), furnishing a grayish solid: cytisine hydrobromide (0.48 g). The organic solution is dried and the residue is treated with acidic water. The acidic acqueous phase is first washed with ether and then alkalized, leading to the formation of a very strong emulsion, that is thoroughly extracted with dichloromethane (8 x 25 mL) and centrifuged each time to separate the two layers. The organic layer is eventually dried over Na_2SO_4, filtered and evaporated, furnishing 1.17 g of a doughy compound that is treated with water, filtered and washed with water two times. After being dried in a dryer, the white solid is crystallized from acetonitrile providing 0.96 g of white crystals with mp= 72-73 °C (Yield = 86.5 %)

The elemental analysis provided the following results:

	found:	C%	78.50	H%	11.32	N% 6.70
for $C_{29}H_{50}N_2O$	calc.:	C%	78.68	H%	11.38	N% 6.33

^{1}H NMR *d*: 7.27 (dd, 1H, *J* = 9, 6.6, a-pyr); 6.39 (dd, 1H, *J* = 9, 1.3, a-pyr); 5.98 (dd, 1H, *J* = 6.6, 1.4, a-pyr); 4.02 (d, 1H, *J* = 15.4, bisp); 3.90 (dd, 1H, *J* = 15.4, 6.4, bisp); 3.03-2.82 (m, 3H, bisp); 2.55-2.13 (m, 5H, 3H, bisp + 2H, NCH_2); 2.03-1.75 (m, 2H, bisp); 1.66-0.93 (m, 32H); 0.91 (t, 3H, J = 6.2, CH_3).

N-Benzyl cytisine (13)

a) 0.76 g (4 mmol) of cytisine are dissolved in 6 mL of acetonitrile in a tube and 0.25 g (2 mmol; 0.23 mL, d = 1.1) of benzyl chloride recently distilled are added to the

solution. The tube is closed under nitrogen atmosphere and the stirring reaction mixture is heated at 100° C for 24 hours.

After cooling of the mixture the formed solid is filtered: cytisine hydrochloride (0.41 g). The organic solution is dried and the residue is treated with acidic water. The acidic acqueous phase is first washed with ether and the alkalized and extracted with dichloromethane. The organic phase is dried over Na_2SO_4, filtered and evaporated to obtain 0.53 g of grayish solid that is crystallized from acetone, furnishing 0.48 g of white crystals with mp =142-143 °C. (Yield = 85.7 %)

b) A solution of 0.38 g (2 mmol) of cytisine and 0.26 g (2.4 mmol; 0.25 mL, d = 1.04) of benzaldehyde in 15 mL di toluene is placed in a round flask. The reaction mixture is heated under reflux for 5 hours. After cooling of the mixture the solvent is removed and the residue is treated with 10 mL of methanol. The mixture is cooled in an ice bath and and a reduction reaction with 1 g di $NaBH_4$ is carried out for 1 hour. The solvent is removed and the residue is treated with 10 mL of water. The acqueous phase is thoroughly extracted with dichloromethane. The organic phase is dried over Na_2SO_4, filtered and evaporated to obtain 0.52 g of solid that is crystallized from acetone providing 0.46 g of white crystals with mp = 142-143 °C. (Yield = 82.1 %)

The elemental analysis provided the following results:

	found:	C%	77.32	H%	10.25	N% 6.96
for $C_{18}H_{20}N_2O$	calc.:	C%	77.11	H%	9.99	N% 7.19

^{1}H NMR d: 7.48-6.95 (m, 6H, 1H, a-pyr, 5H arom); 6.52 (dd, 1H, J = 9, 1.2, a-pyr); 5.94 (dd, 1H, J = 7, 1.2, a-pyr); 4.14 (d, 1H, J = 15.4, bisp); 3.91 (dd, 1H, J = 15.4, 6.4, bisp); 3.53-3.37 (m, sistema AB, 2H, Ar-CH_2); 3.01-2.27 (m, 3H, bisp); 2.53-2.21 (m, 3H, bisp); 2.02-1.73 (m, 2H, bisp).

N-(2-Phenylethyl) cytisine (14)

0.28 g (2 mmoli; 0.26 mL, d = 1.069) of 2-chloroethyl benzene are added to a tube containing a solution of 0.76 g (4 mmoli) of cytisine in 6 mL di acetonitrile. The tube is closed under nitrogen atmosphere, and the stirring reaction mixture is heated at 100° C for 24 hours.

After cooling of the mixture the formed solid is filtered: cytisine hydrochloride (0.40 g). The organic solution is dried and the residue is treated with acidic water. The acidic acqueous phase is first washed with ether and the alkalized and extracted with dichloromethane. The organic phase is dried over Na_2SO_4, filtered and evaporated

to obtain 0.55 g of a grayish solid that is crystallized from acetone, furnishing 0.48 g of white crystals with mp =140-142 °C. (Yield = 81.4 %)

The elemental analysis provided the following results:

	found:	C%	77.55	H%	9.57	N% 7.59
for $C_{19}H_{22}N_2O$	calc.:	C%	77.52	H%	9.52	N% 7.53

^{1}H NMR *d*: 7.36-6.77 (m, 6H, *1H* a-pyr, 5H arom); 6.39 (dd, 1H, *J* = 9, 1.2, a-pyr); 5.90 (dd, 1H, *J* = 6.8, 1.2, a-pyr); 3.93 (d, 1H, *J* = 15.4, bisp); 3.80 (dd, 1H, *J* = 15.4, 6.2, bisp); 3.00-2.74 (m, 2H, bisp); 2.55-2.02 (m, 5H, 3H, bisp + 2H, NCH_2); 1.90-1.46 (m, 5H, 3h, bisp + 2H CH_2Ar).

N-Dodecyl-N-methyl cytisine iodide (15)

116 mg (0.32 mmol) of N-dodecyl cytisine (**6**) and 0.28 g (1.94 mmol; 0.12 mL, d = 2.28) of methyl iodide are placed in a tube that is closed under nitrogen atmosphere, and the stirring reaction mixture is heated at 40° C for 24 hours. Upon completion of the reaction, the formation of a solid can be observed. The reaction mixture is treated several times with anhydrous ether, grinding the insoluble solid each time and decanting the liquid, to obtain 129 mg of a whitish solid. (Yield = 79.6%)

The elemental analysis provided the following results:

	found:	C%	57.42	H%	8.62	N% 5.49
for $C_{24}H_{41}IN_2O$	calc.:	C%	57.59	H%	8.26	N% 5.60

^{1}H NMR (d6-DMSO) d: 7.63-7.42 (m, 1H, a-pyr); 6.49-6.25 (m, 2H, a-pyr); 4.23-3.29 (m, 9H); 3.19-2.82 (m, 4H); 2.65 (s, 2H); 2.21-1.58 (m, 4H); 1.44-1.03 (m, 16H); 0.89 (t, J = 6.4, 3H).

N-Methyl-N-Tridecyl cytisine iodide (16)

114 mg (0.31 mmol) of N-tridecyl cytisine (**7**) and 0.26 g (1.83 mmol; 0.12 mL, d = 2.28) of methyl iodide are placed in a tube that is closed under nitrogen atmosphere, and the stirring reaction mixture is heated at 40° C for 24 hours. Upon completion of the reaction, the formation of a solid can be observed. The reaction mixture is treated several times with anhydrous ether, grinding the insoluble solid each time and decanting the liquid, to obtain 123 mg of a whitish solid (Yield= 78.3%)

The elemental analysis provided the following results:

	found:	C%	58.36	H%	8.42	N% 5.44
for $C_{25}H_{43}IN_2O$	calc.:	C%	58.69	H%	8.55	N% 5.21

N-Methyl-N-Tetradecyl cytisine iodide (17)

119 mg (0.33 mmol) of N-tetradecyl cytisine (**8**) and 0.28 g (1.97 mmol; 0.12 mL, d = 2.28) of methyl iodide are placed in a tube that is closed under nitrogen atmosphere, and the stirring reaction mixture is heated at 40° C for 24 hours. Upon completion of the reaction, the formation of a solid can be observed. The reaction mixture is treated several times with anhydrous ether, grinding the insoluble solid each time and decanting the liquid, to obtain 127 mg of a whitish solid (Yield = 77.9%)

The elemental analysis provided the following results:

	found:	C%	59.42	H%	8.47	N% 5.09
for $C_{26}H_{45}IN_2O$	calc.:	C%	59.08	H%	8.58	N% 5.30

N-Methyl-N-Pentadecyl cytisine iodide (18)

122 mg (0.30 mmol) of N-pentadecyl cytisine (**9**) and 0.26 g (1.83 mmol; 0.12 mL, d = 2.28) of methyl iodide are placed in a tube that is closed under nitrogen atmosphere, and the stirring reaction mixture is heated at 40° C for 24 hours. Upon completion of the reaction, the formation of a solid can be observed. The reaction mixture is treated several times with anhydrous ether, grinding the insoluble solid each time and decanting the liquid, to obtain 130 mg of a whitish solid. (Yield= 78.8%)

The elemental analysis provided the following results:

	found:	C%	59.64	H%	8.81	N% 5.40
for $C_{27}H_{47}IN_2O$	calc.:	C%	59.77	H%	8.73	N% 5.16

N-Methyl-N-Hexadecyl cytisine iodide (19)

121 mg (0.29 mmol) of N-hexadecyl cytisine (**10**) and 0.25 g (1.83 mmol; 0.11 mL, d = 2.28) of methyl iodide are placed in a tube that is closed under nitrogen atmosphere, and the stirring reaction mixture is heated at 40° C for 24 hours. Upon completion of the reaction, the formation of a solid can be observed. The reaction mixture is treated several times with anhydrous ether, grinding the insoluble solid

each time and decanting the liquid, to obtain 129 mg of a whitish solid. (Yield = 79.6%)

The elemental analysis provided the following results:

	found:	C%	60.45	H%	8.93	N% 4.84
for $C_{28}H_{49}IN_2O$	calc.:	C%	60.42	H%	8.87	N% 5.03

^{1}H NMR (d6-DMSO) d: 7.58-7.39 (m, 1H, a-pyr); 6.47-6.28 (m, 2H, a-pyr); 4.18-3.24 (m, 9H); 3.17-2.78 (m, 4H); 2.61 (s, 2H); 2.16-1.55 (m, 4H); 1.43-0.92 (m, 24H); 0.85 (t, J = 6.4, 3H).

N-Methyl-N-Heptadecyl cytisine iodide (20)

128 mg (0.30 mmol) of N-heptadecyl cytisine (**11**) and 0.25 g (1.79 mmol; 0.11 mL, d = 2.28) of methyl iodide are placed in a tube that is closed under nitrogen atmosphere, and the stirring reaction mixture is heated at 40° C for 24 hours. Upon completion of the reaction, the formation of a solid can be observed. The reaction mixture is treated several times with anhydrous ether, grinding the insoluble solid each time and decanting the liquid, to obtain 122 mg of a whitish solid (Yield = 71.8%)

The elemental analysis provided the following results:

	found:	C%	61.30	H%	9.13	N% 4.70
for $C_{29}H_{51}IN_2O$	calc.:	C%	61.04	H%	9.01	N% 4.91

N-Methyl-N-octadecyl cytisine iodide (21)

127 mg (0.29 mmol) of N-octadecyl cytisine (**12**) and 0.24 g (1.79 mmol; 0.11 mL, d = 2.28) of methyl iodide are placed in a tube that is closed under nitrogen atmosphere, and the stirring reaction mixture is heated at 40° C for 24 hours. Upon completion of the reaction, the formation of a solid can be observed. The reaction mixture is treated several times with anhydrous ether, grinding the insoluble solid each time and decanting the liquid, to obtain 115 mg of a whitish solid. (Yield = 68.4%)

The elemental analysis provided the following results:

	found:	C%	61.76	H%	9.47	N% 4.42
for $C_{30}H_{53}IN_2O$	calc.:	C%	61.63	H%	9.14	N% 4.79

N-Benzyl-N-Methyl cytisine chloride (22)

121 mg (0.43 mmol) of N-benzyl cytisine (**13**) and 0.36 g (2.6 mmol; 0.16 mL, d = 2.28) of methyl iodide are placed in a tube that is closed under nitrogen atmosphere, and the stirring reaction mixture is heated at 50° C for 24 hours. Upon completion of the reaction, the formation of a solid can be observed. The reaction mixture is treated several times with anhydrous ether, grinding the insoluble solid each time and decanting the liquid, to obtain 109 mg of a whitish solid. (Yield = 59.9%)

The elemental analysis provided the following results:

	found:	C%	51.13	H%	6.18	N% 6.11
for $C_{19}H_{23}IN_2O \bullet 1.5H_2O$	calc.:	C%	50.79	H%	5.83	N% 6.24

N-(2-phenylethyl)-N-Methyl cytisine chloride (23)

119 mg (0.40 mmol) of N-(2-phenylethyl) cytisine (**14**) and 0.34 g (2.4 mmol; 0.15 mL, d = 2.28) of methyl iodide are placed in a tube that is closed under nitrogen atmosphere, and the stirring reaction mixture is heated at 50° C for 24 hours. Upon completion of the reaction, the formation of a solid can be observed. The reaction mixture is treated several times with anhydrous ether, grinding the insoluble solid each time and decanting the liquid, to obtain 97 mg of whitish solid. (Yield = 55.4%)

The elemental analysis provided the following results:

	found:	C%	50.48	H%	6.51	N% 5.75
for $C_{20}H_{25}IN_2O \bullet 2H_2O$	calc.:	C%	50.85	H%	6.19	N% 5.93

Evaluation of anti-leishmanial activity

Promastigote stage of L. infantum strain MHOM/TN/80/IPT1 (kindly provided by Dr M. Gramiccia, ISS, Roma) and L. tropica (MHOM/IT/2012/ISS3130) were cultured in RPMI 1640 medium (EuroClone) supplemented with 10% heat-inactivated fetal calf serum (EuroClone), 20mM Hepes, and 2mM L-glutamine at 24 °C.

To estimate the 50% inhibitory concentration (IC_{50}), the MTT (3-[4.5-dimethylthiazol-2-yl]-2.5-diphenyltetrazolium bromide) method was used (Mosmann T. et al, 1993; Baiocco P. et al., 2010). Compounds were dissolved in DMSO and then diluted with medium to achieve the required concentrations.

Drugs were placed in 96 wells round-bottom microplates and seven serial dilutions made. Miltefosine was used as reference anti-Leishmania drug. Parasites were diluted in complete medium to $5x10^6$ parasites/mL and 100 µL of the suspension

was seeded into the plates, incubated at 24 °C for 72 h and then 20 µL of MTT solution (5 mg/mL) was added into each well for 3 h. The plates were then centrifuged at 1000xg for 8 min at r.t., the supernatants discarded and the resulting pellets dissolved in 100 µL of lysing buffer consisting of 20% (w/v) of a solution of SDS (Sigma), 40% of DMF (Merck) in H_2O. The absorbance was measured spectrophotometrically at a test wavelength of 550 nm and a reference wavelength of 650 nm. The results are expressed as IC_{50} which is the dose of compound necessary to inhibit parasite growth by 50%; each IC_{50} value is the mean of separate experiments performed in duplicate.

Chapter 2. Anticholinesterase quinolizidine derivatives

AD background

Alzheimer's disease (AD) is a neurodegenerative disorder that nowadays represents the most common form of dementia in the elderly (Anand R. et al, 2014) About 35 million people worldwide suffer from AD (Wimo A et al, 2013) and it has been estimated that by 2030 their number will increase to 70 million.(Dartigues J.F. et al, 2009) Although the differences in clinical manifestations among the individuals, some signs and symptoms are common: a progressive decline in cognitive function, particularly in short-term and remote memory, language, judgment, attention and executive functions such as planning and organization. These cognitive disorders progress during the course of the illness (Kawas C.H. et al., 2003) and are accompanied by behavioral changes and apathy in the early stages of the disease and then, in the later stages, by psychosis and agitation.(Mega M.S et al.,1996) Alterations to the motor and sensory systems are rather uncommon until the advanced course of the disease.(McKhann G. et al., 1984) The most evident neuropathological feature found in Alzheimer's patients consists of a neuronal and synaptic loss at various neurotransmitter systems, mainly at the cholinergic system, although major alterations are also observed at the neurotransmitter systems of noradrenaline, serotonin, glutamate, substance P and somatostatin. In the early 1980s, Bartus introduced the so-called "cholinergic hypothesis of the mnestic dysfunction in elderly": (Bartus R.T et al., 1982) post-mortem studies on the brain of AD patients found a loss of 60-90% of Choline acetyltransferase (ChAT), the enzyme responsible for the synthesis of ACh, and an equally significant reduction (30-90%) of cholinergic neurons.(Davies P. et al., 1976) Experimental studies in this field have investigated the action of inhibitors of acetylcholinesterase (AChE), the enzyme responsible for the catalytic hydrolysis of ACh, by analyzing the relationship between ACh cerebral levels and the capacity of animals in learning spatial skills: these studies revealed that learning ability improved with the activation of the cholinergic system.(Muthuraju S. et al., 2009) Another hallmark of the disease, besides the cholinergic hypofunction, is the diffused presence of extracellular senile plaques and intracellular neurofibrillary tangles, mainly formed by ß-amyloid peptide. The production of ß-amyloid peptide is considered crucial for the development of the AD, according to the so-called

“amyloid cascade hypothesis”. ß-amyloid peptide is generated by the incorrect cleavage of amyloid precursor protein (APP), a membrane protein expressed ubiquitously in the human body and whose function is still unclear nowadays, although it appears to be important in regulating the neurons survival, the neurites development and synaptic plasticity. APP is subject to a proteolytic cleavage by secretase enzymes leading to an amyloidogenic or non-amyloidogenic pathway (Fig. 4). Non-amyloidogenic pathway consists of APP cleavage by a α-secretase that produces a soluble N-terminal fragment (sAPPα) and a C-terminal fragment (C83) (Weidemann A., 1989 ; Haass C., 1992); this fragment remains attached to the membrane and is subject to the γ-secretase action, forming p3 fragment, released by the membrane. Amyloidogenic pathway, however, consists first of APP cleavage by ß-secretase, producing the soluble fragment sAPPß that is secreted, and the C99 fragment, anchored to the membrane, containing Aß sequence in its N-terminal part.(Seubert P. et al., 1993) C99, then, is subject to a second cleavage by γ-secretase, providing Aß peptide.(Anderson J.P.,1992) As a result, ß-amyloid fragments of 39-43 aminoacids are formed, among which the most plentiful are Aß(1-40) and Aß(1-42), that are secreted, and the APP Intracellular Domain (AICD), that is reputed to be crucial in calcium-mediated signaling regulation and, once in the nucleus, it may act as a transcription factor. (Mattson P.M. et al., 2004; Zheng H. et al., 2006; Beckett C. et al., 2012; Pardossi-Piquard R., et al., 2012)

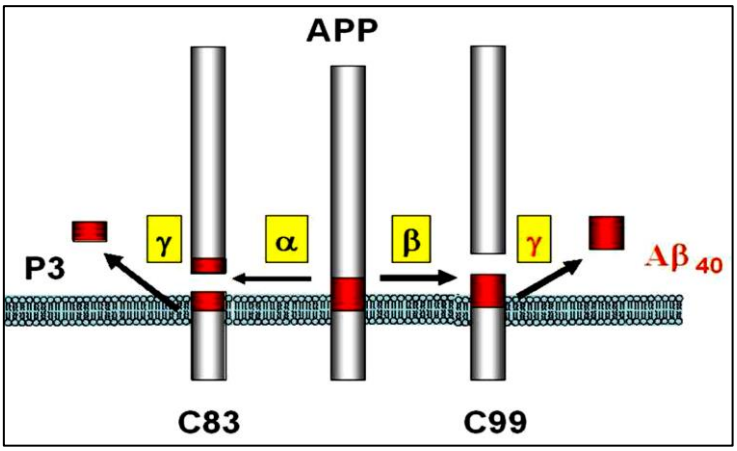


Fig.4 On the left the non-amyloidogenic pathway and on the right the amyloidogenic pathway, that forms the amyloid peptide due to the action of ß-secretase instead of α-secretase (Kulshreshtha A.,et al., 2016)

Although Aß (1-40) is more diffused than Aß(1-42), the latter, more hydrophobic, is considered to possess a higher amyloidogenic potential. However, both of them are able to assemble and form aggregates such as protofibrils, fibrils and eventually insoluble plaques. In healthy and young individuals, Aß peptides are

completely metabolized shortly after being secreted from the cells, thus preventing their accumulation; with advancing age, instead, the Aß peptides intensified production and reduced clearance can determine their deposition and subsequent aggregation, causing neuronal degeneration (Kirkitadze M.D, et al., 2005). Most cases of AD are sporadic, not hereditary; however, many genes, including the apolipoprotein E gene, are assumed to predispose to the disease. The apolipoprotein E gene has three alleles which codify for the isoforms 19E2, E3 and E4; individuals producing E4 have a high risk of developing the disease, since this isoform is reputed to intensify the amyloidogenic pathway of APP processing and, on the other hand, to promote the aggregation of Aβ peptides and reduce their clearance (Roses A.D. et al., 1997). These effects promote cerebrovascular disease, increase oxidative stress and the loss of synaptic plasticity. Until a few years ago, amyloid fibrils were commonly considered the main pathological agents of AD.

To support this hypothesis, several studies showed Aß aggregates *in vitro* toxicity,(Pike C.J. et al., 1993). that was attributed to the specific fibrillar morphology (Seilheimer B. et al., 1997) Co-localization of protein aggregates with degenerate tissues and the association of their presence with disease symptoms are a strong indication of the involvement of amyloid deposits in pathogenesis (Soto C., 2001). Recently, an alternative theory has emerged, disclosing that the soluble oligomeric intermediates represent the main toxic species instead of the insoluble aggregates. Studies on mice have shown that significant damages to the tissues and the clinical symptoms appear before any aggregate is identified; this fact implies the presence of an intermediate in the amyloidogenic pathway who might be the real cause of the pathogenicity (Zerovnik E. et al., 2002).

To confirm this evidence, amyloid plaques were also found in individuals not displaying clinical AD symptoms, whereas the severity of the disease is more related to the presence of soluble oligomers than the presence of plaques (Kayed R. et al., 2003).

Thus, it has been hypothesized that the protein aggregation into fibrils might be a mechanism to protect the cell from the toxic prefibrillar species (Roher A.E. et al., 2000); the fibrils, therefore, could represent stable and "harmless" reserves of such toxic forms.

ß-amyloid peptides possess, in physiological conditions, a non-structured native conformation, namely the monomers are not fold into a α-helix or ß-sheet structure, but they have mainly a random coil conformation. A fundamental step in the fibril

aggregation process is the partial misfolding of the native protein, that loses its rigid three-dimensional structure by exposing its hydrophobic regions to the solvent, thus promoting the "nucleation": the initial aggregation nuclei provide a kind of template capable of recruiting other partially folded molecules, thus increasing the size of the growing filaments and forming, in case, insoluble fibril aggregates. (Stefani M. et al., 2004) Finally, it has emerged that NMDAr-induced excitotoxicity represents a crucial pathogenic feature in AD.

AD therapy

Alzheimer's disease is nowadays, one hundred years after its discovery, the prevalent form of senile dementia in the world, as it affects about 2% of the population in industrialized countries. Longer life expectancy and aging of the population make it a serious public health problem for the future.

Over the last thirty years, many drugs have been approved to delay the progression of the disease but, to date, there are no preventive or curative treatments yet. Two classes of molecules are currently clinically available (Fig. 5): the inhibitors of acetylcholinesterase (AChEIs: donepezil (**XXIV)**, rivastigmine (**XXV)** and galantamine (**XXVI**) and the NMDA receptor antagonist memantine (**XXVII**).

AChEIs, although having individual characteristics and different pharmacokinetic profiles, share the same mechanism of action, essentially different from that of memantine. Neither of them is able to cure the disease, but they can slow down the illness course and improve the social and family integration for a certain period: this effect is achieved through the crucial improvement of some brain functions. The efficacy of memantine in treating moderate to severe Alzheimer's disease has been documented by several monotherapy studies, although the best results have been obtained and are obtained in association with AChEI and, in particular, with donepezil, due to the synergism and complementarity of the mechanism of action.(Tariot PN et al., 2004; Shaughnessy LW et al., 2008)

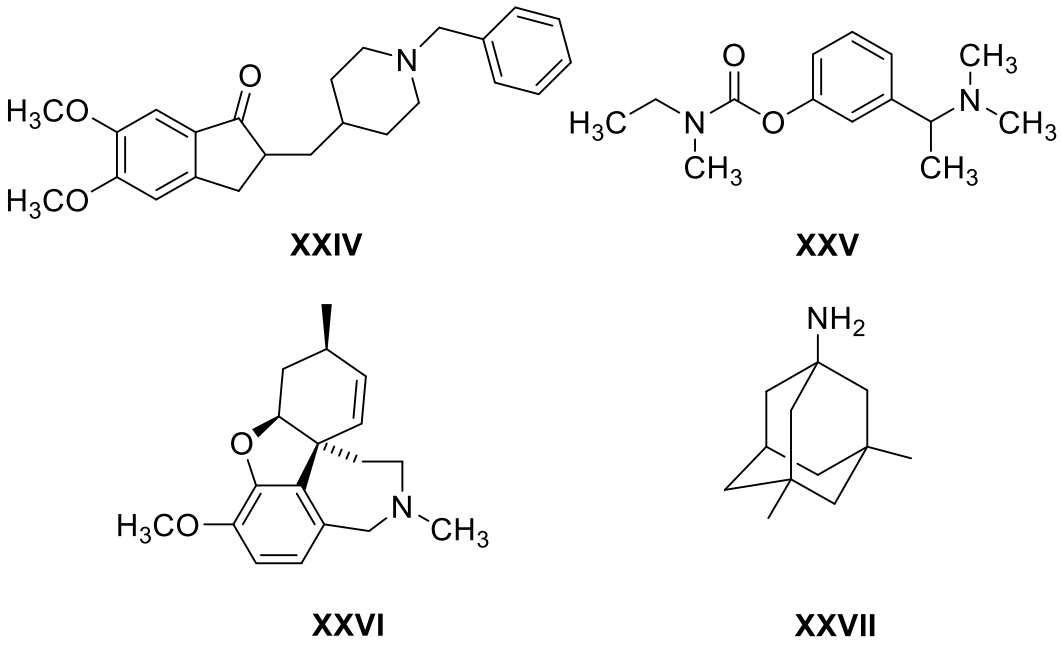


Fig.5 Structures of AChEIs and NMDA receptor antagonists used in AD therapy.

The combination of these two mechanisms of action has been accomplished with the one-daily dose formulation Namzaric® (Actavis [USA] and Adamas Pharmaceuticals [USA]), approved in 2014 for the treatment of moderate to severe dementia in AD; it assembles donepezil and memantine into a single molecule. A series of multitarget compound was synthesized, among which Memagal (Fig.6) has demonstrated to be the most promising. It combines the structures of galantamine and memantine and showed interesting *in vitro* properties (AchE and NMDAr inhibitor activity). The strategy of combining multiple action in a single formulation (multitarget drugs) has been widely developing over the last decades due to its advantages, such as the dosing facility and the improved patients' compliance; it appears also to be the most promising strategy to treat a disease with such a complex pathogenesis (Rosini M. et al., 2016). Regarding the inhibition of the acetylcholinesterase enzyme, it is also to be considered that AChE (Alvarez A. et al., 1995; Inestrosa NC. et al., 1996 ; De Ferrari GV. et al., 2001), within its peripheral anionic binding site (PAS), can promote the aggregation of Aß; therefore, the dual AChE binding site inhibitors may improve the cognitive and behavioural symptoms acting by both decreasing ACh hydrolisis and Aß aggregation. Moreover, Aß is reputed to bind non-phosphorilated tau protein, leading to its self-aggregation. In advanced AD, cerebral AChE levels decrease while those of butyrylcholinesterase (BChE) progressively increase. BChE is able to hydrolise ACh, but to a lesser extent. In order to increase cerebral ACh levels

and to reduce amyloid protein formation, BChE selective inhibitors have been identified (Giacobini E., 2001; Greig NH. et al.,2001; Guillozet A.L. et al., 1997).

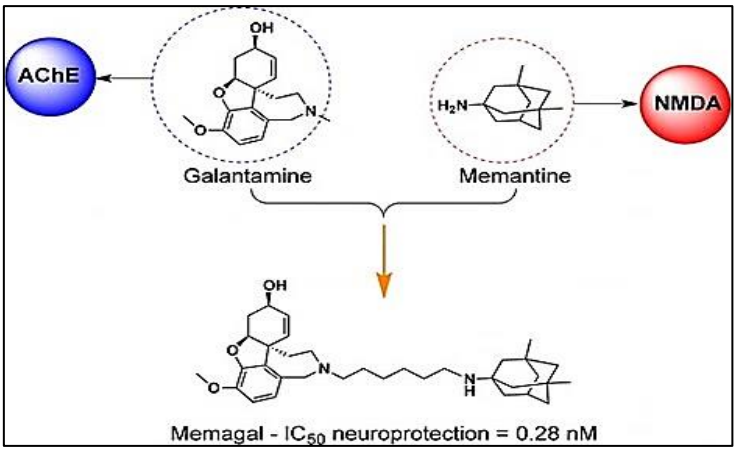


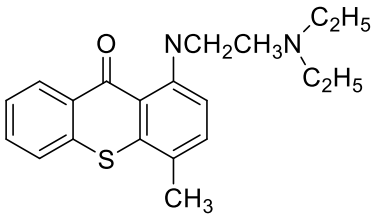
Fig.6 Memagal combines the structures of galantamine and memantine in one formulation, exerting a dual activity (anti-AChE and NMDA antagonism)

On the basis of the strong and selective BchE inhibition performed by ethopropazine and Astra 1397, the research lab where the present work has been accomplished recently preparared a group of compounds as potential dual (AChE/BChE) inhibitors or selective BChE inhibitors.(Tasso B. et al., 2011) These compounds, derived from phenothiazine and other related tricyclic systems, are characterized by different types of spacers between the tricyclic system and the quinolizidine nucleus. It is noteworthy that even more simple quinolizidine structures demonstrated a certain anticholinesterase activity. All the investigated compounds exhibit an activity toward both the ChEs, but BChE inhibitors are generally more powerful, with many of them showing submicromolar IC_{50} values.

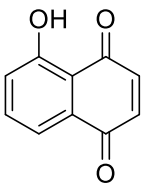
Also various naphto- and anthraquinones are able to influence relevant biological target.(Fig.7) Specifically, the compound E3330 (**XXVIII**) and other naphtoquinone analogues inhibit Ape-1 redox function (Nyland RL. et al., 2010), whereas another naphtoquinone, juglone (**XXIX**), blocks Aß oligomerization without interfering with its fibrillization (Necula M. et al., 2007). The oligomerization and fibrillization processes could be considered as independent pathways and the soluble amyloid low-molecular-weight oligomers could be reputed the main responsible of toxicity in the neurodegenerative diseases. The binding of naphtoquinone to the tryptophane amino group provides a strong inhibition (**XXX**) both of oligomerization and the fibrillization processes (Scherzer R. et al., 2010). Antitumor anthraquinone agents as the rubicins (**XXXI**), xantrones (i.e. mitoxantrone (**XXXII**) and pixantrone (**XXXIII**) (Bandiera T. et al., 1997) have emerged to be effective Aß oligomerization inhibitors, and moreover pixantrone is able to reduce Aß peptide cytotoxicity.

Polyhydroxy-anthraquinones (such as emodine (**XXXIV**), 1,2,5,8-tetrahydroxy-anthraquinone and the rubicins), are also able to inhibit protein tau aggregation and to dissolve preformed aggregates, preventing the formation of the neurofibrillary tangle characteristic of AD (Colombo R. et al., 2009).

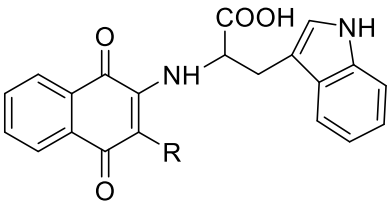
On the basis of these premises, novel quinolizidine derivatives have been devised, taking into consideration bi- and tri-cyclic quinone compounds (naphto- and anthraquinones), in order to obtain more effective ChEs inhibitors that, at the same time, would be able to inhibit Aß aggregation both acting on the PAS-induced aggregation and through a direct mechanism (Tonelli M. et al., 2015).



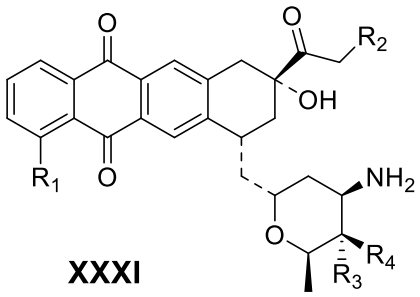
XXVIII



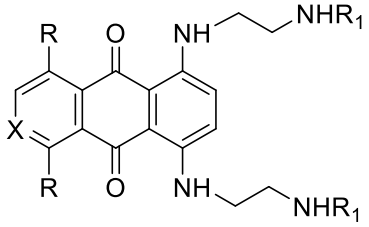
XXIX



XXX

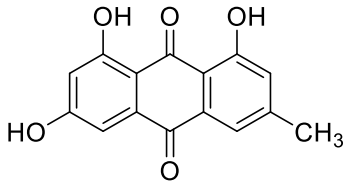


XXXI



XXXII : X= CH; R= OH; R_1= -$(CH_2)_2$OH

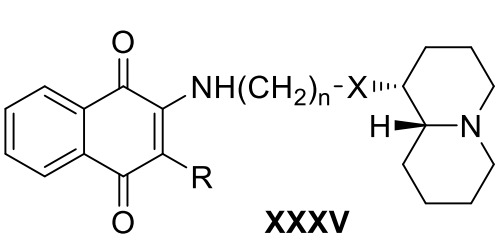
XXXIII : X= N; R=R_1= H



XXXIV

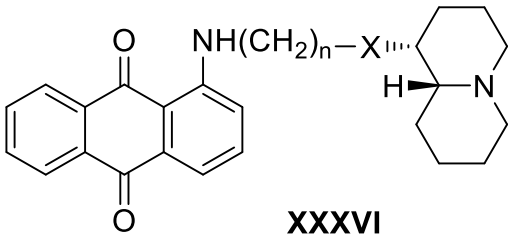
Fig.7 Structures of some naphto- and anthraquinones of biological interest

Among the naphthoquinones **XXXV** and the anthraquinones **XXXVI**, some compounds of remarkable interest have emerged especially as acetylcholinesterase inhibitors, with micromolar and, in some cases, submicromolar IC_{50}.



XXXV

n = 1-3
X = -SCH_2
R = H, Cl



XXXVI

n = 1-3
X = -SCH_2

Among many of these molecules the quinolizidine ring is linked to an aromatic aminic group through a polymethylene chain of variable length, with a possible insertion of a sulfur atom, isostere of methylene group. The effect of the linker length on the biological activity has been analyzed based on the information that the research group and other Authors (Feng Y. et al., 2012; Moreira P.I. et al., 2008; Fukuda M. et al., 2009; Sultana R. et al., 2011) gained from previous studies on homo- and hetero-dimer dual inhibitors of ChE binding sites.

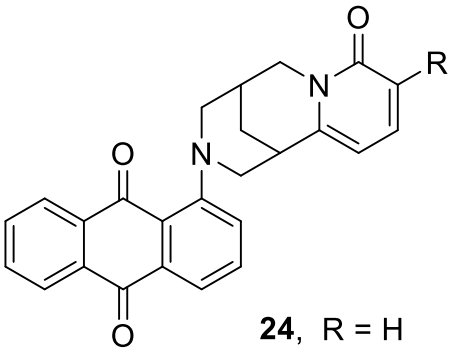
It is noteworthy that the naphtoquinone derivative **XXXV** (with R =Cl, n=3 e X = -), characterized by a long lateral chain, exerts a comparable or even higher activity (IC_{50} for AChE = 11 nM) than that exerted by donepezil (IC_{50}= 20 nM). Moreover, these naphtoquinone derivatives are dual inhibitors with greater affinity for AChE, even up to a 100-fold higher selectivity. Finally, the introduction of a chlorine atom on the naphtoquinone derivatives significantly increases the potency of cholinesterase inhibition.

Chemistry discussion

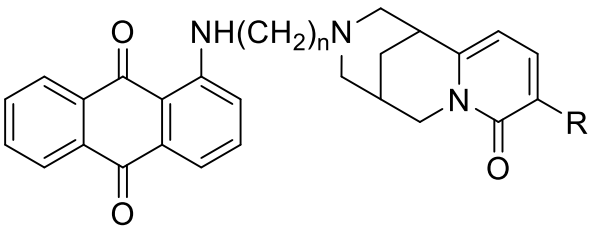
On the basis of the interesting results already obtained, novel anthraquinone (**24**-**31**) and naphtoquinone (**32**-**39**) derivatives have been synthesized; the basic nucleus has been varied, utilizing cytisine, another quinolizidine alkaloid, as the starting molecule. It is notable that the piridonic ring of cytisine presents a pseudo-aromatic character and also a hydrophilic character which markedly contrasts with the lipophilic nature of the entirely aliphatic lupinyl structure, present in the previously mentioned naphtoquinone derivatives **XXXV** and antraquinone derivatives **XXXVI**.

In the past, some studies verified that the replacement of the bulky basic head (from lupinine to cytisine) maintains a good biological activity (Rosini M. et al.,

2014. This finding was confirmed for cholinesterase inhibitors (phenothiazine derivatives of cytisine with submicromolar cholinesterase inhibition values, unpublished data), as well as for other pharmacological categories. These novel derivatives permit to investigate structural variations concerning the linker lenght and the substitution in position 3 of the cytisine nucleus.

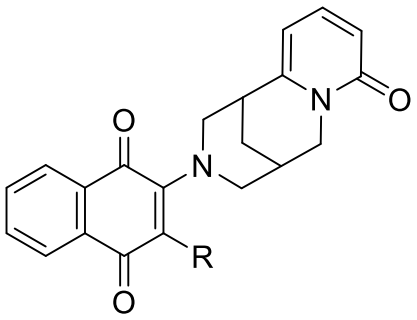


24, R = H
25, R = NO_2

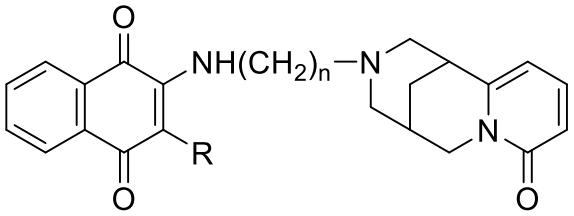


R = H,
n = 2: **26**
n = 3: **27**
n = 4: **28**

R = NO_2,
n = 2: **29**
n = 3: **30**
n = 4: **31**



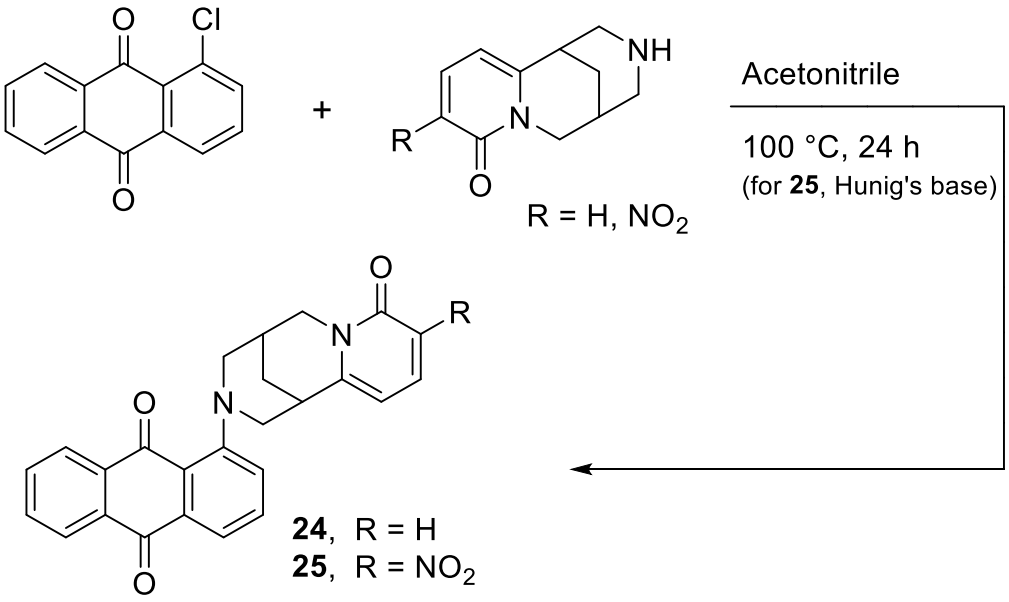
32: R = H
33: R = Cl



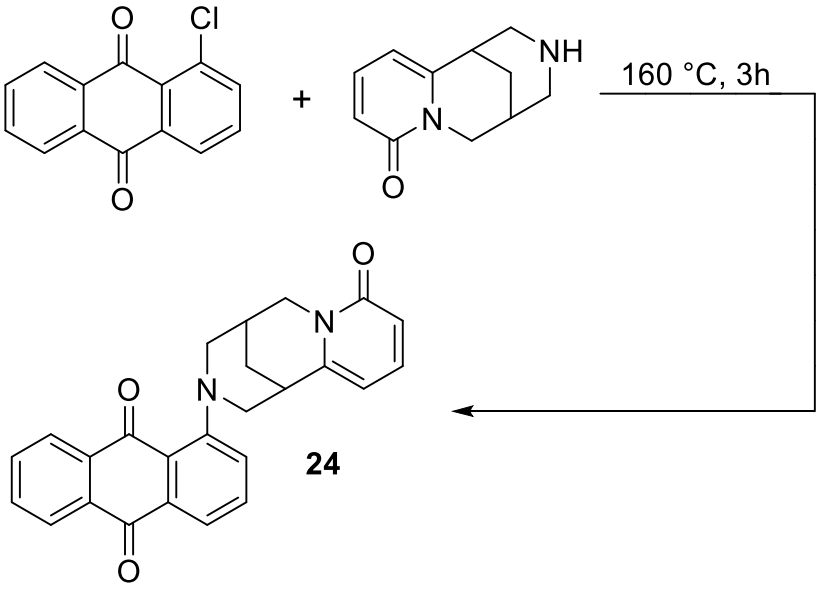
R = H,
n = 2: **34**
n = 3: **35**
n = 4: **36**

R = Cl,
n = 2: **37**
n = 3: **38**
n = 4: **39**

The synthesis of the compounds **24** and **25** consists in the direct reaction between 1-chloroanthraquinone and, respectively, cytisine and 3-nitrocytisine, carried out in a closed tube under nitrogen atmosphere, heated at 100°C for 24 hours.



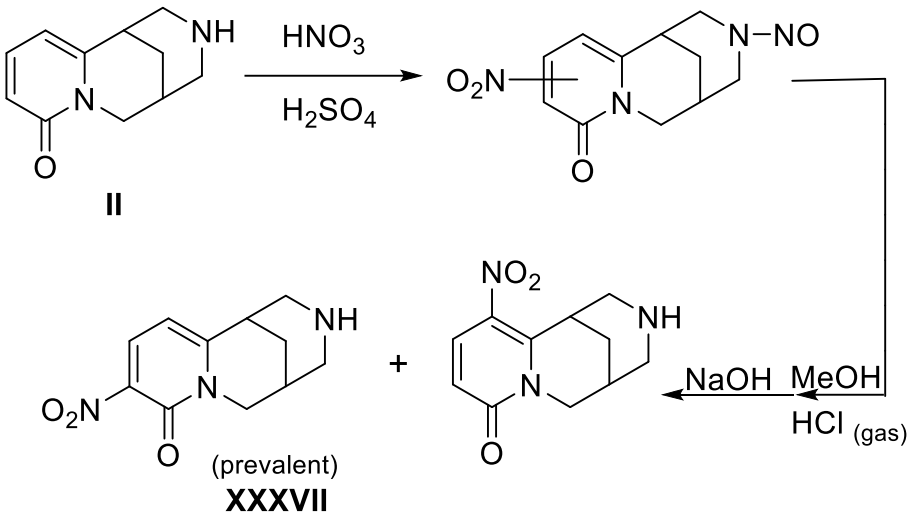
The derivative **24** has been obtained in lower yield, even heating the reactants in a closed tube at 160°C for 3 hours without using any solvent.



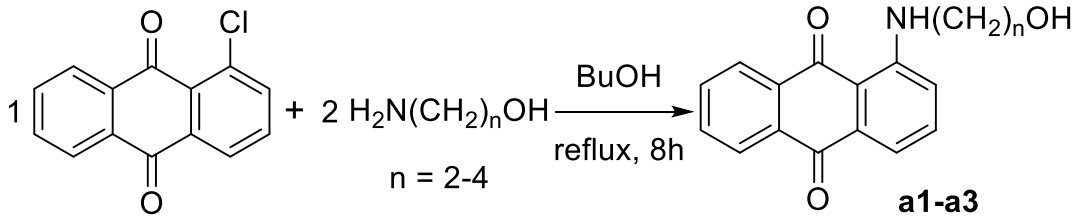
..

3-Nitrocytisine (Freund M., 2001) has been prepared according to the procedure described in the literature, through nitration of cytisine with fuming HNO_3/H_2SO_4 , leading to the mixture of 3-nitro and 5-nitroderivative.

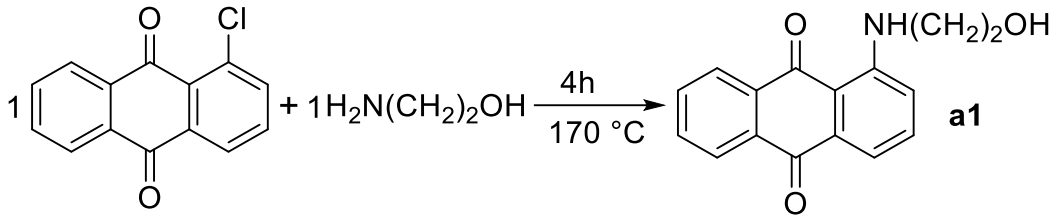
3-nitrocytisine (**XXXVII**), the prevalent isomer, has been purified through a crystallization from CH_2Cl_2, and then washed with a mixture of ether/ethanol.



To prepare the compounds **26-31**, first aminoalcohols **a1-a3** have been synthesized through a reaction between 1-chloroanthraquinone and the proper aminoalcohol, with stoichiometric ratio 1:2. The reaction was carried out in n-butanol, heating the mixture under reflux for 8 hours. The products obtained were purified in good yield through chromatography on Al_2O_3.

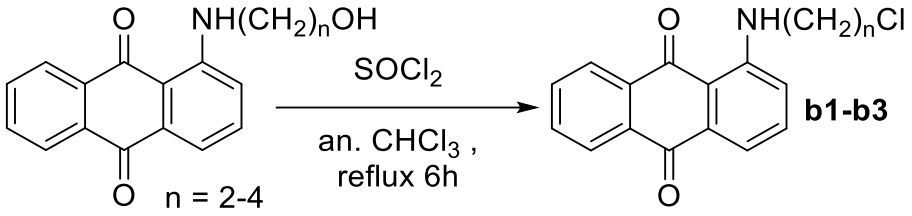


Alcohol **a1** was also prepared by melting 1-chloroanthraquinone and 4-aminoethanol with ratio 1:1 in a closed tube, heating at 160°C for 3 hours. Also in this case the yield resulted significantly lower than that obtained in the previous method.

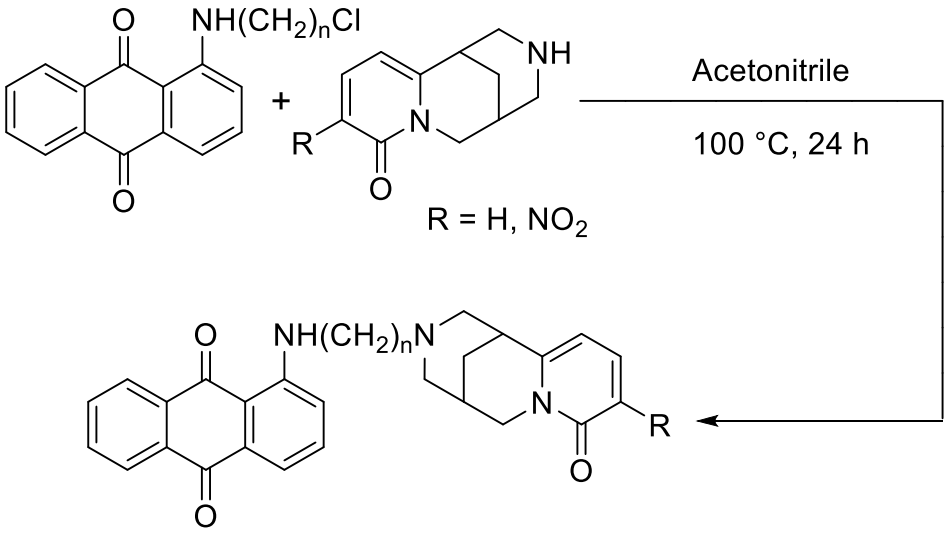


In the second phase, alcohols have been transformed in the corresponding alkyl chlorides **b1-b3**, reacting with an excess of thionyl chloride, in anhydrous $CHCl_3$. The excess of thionyl chloride has been removed washing the mixture with a cold sodium bicarbonate solution.

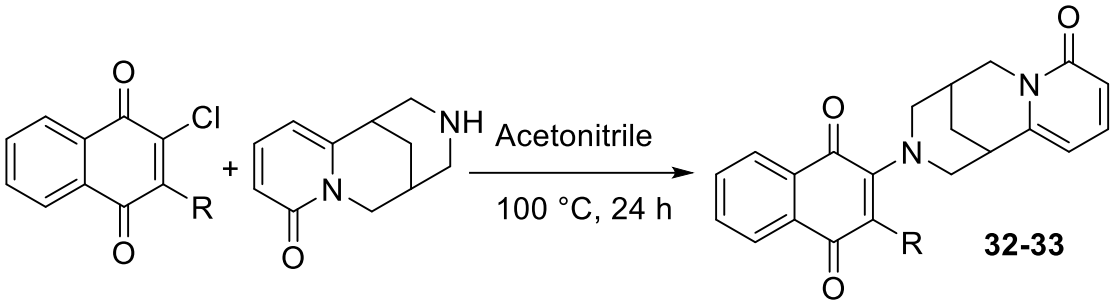
Also in this case the compounds have been obtained in high yield after purification through chromatography on Al_2O_3.



The reaction between the alkyl chlorides and cytisine has been performed to obtain the compounds **26-28**; cytisine was added in double quantity compared to the stoichiometric amount. The reaction conditions have already been described (in a closed tube, CH_3CN, heated at 100°C for 24 hours). In the synthesis of the compounds **29-31**, 3-nitrocytisine was added in stoichiometric amount in addition to an equivalent of a Hunig base (N,N-diisopropylethylamine).



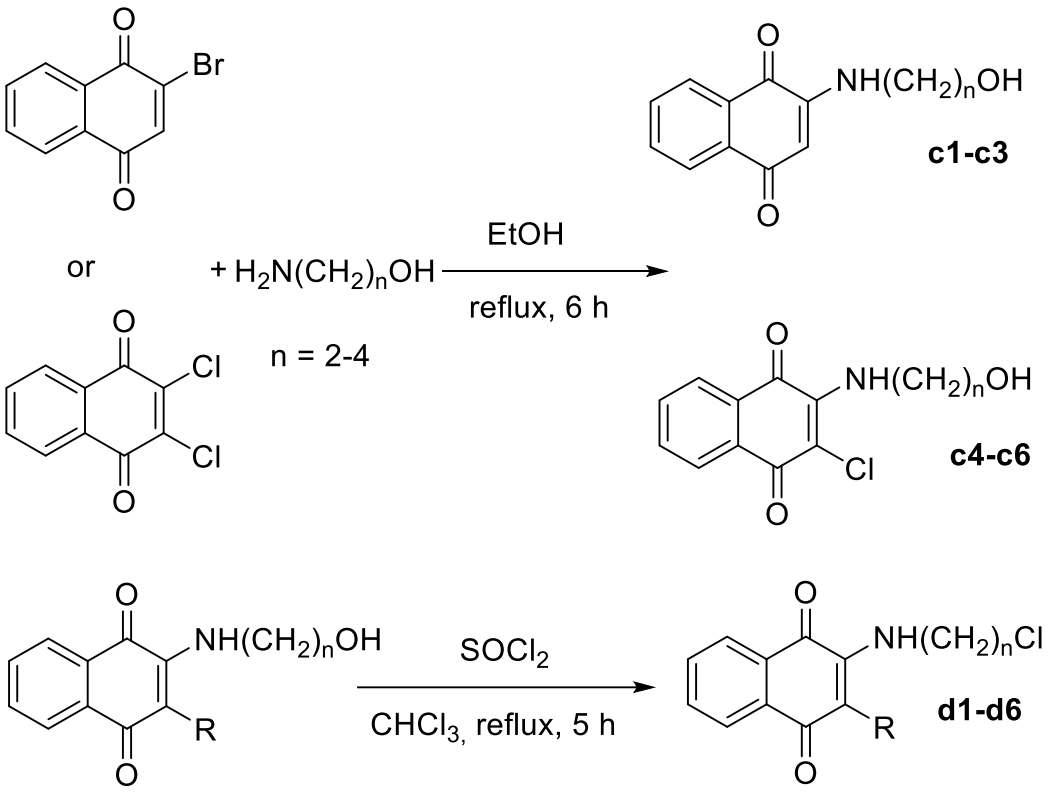
The synthesis of compounds **32** and **33** consists in the direct reaction between, respectively, 2-chloro-1,4-naphtoquinone and 2,3-dichloro-1,4-naphtoquinone and cytisine. The reaction was carried out in a closed tube under nitrogen atmosphere, in CH_3CN, heating at 100 °C for 24 hours.



R = H, Cl

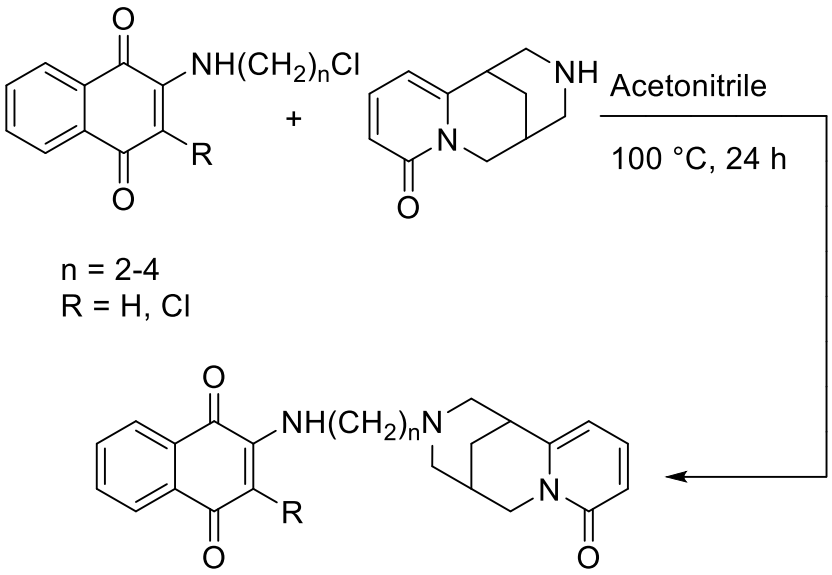
To prepare the compounds **34-39**, the aminoalcohols **c1-c6** are first synthesized through the reaction between 2,3-dichloro-1,4-naphtoquinone or 2-bromo-1,4-naphtoquinone and the suitable aminoalcohol in stoichiometric ratio of 1:2. The reaction is carried out in ethanolic solution, heating the mixture under reflux for 5-6 hours. The series of compounds obtained are purified in good yield through chromatography on Al_2O_3.

To obtain alcohol **c1** also a reaction between the mere 1,4-naphtoquinone and 2-aminoethanol (in ratio of 1:1) in ethanolic solution has been attempted. This method involved a reaction of addition to the activated double bond which was used in the past to obtain some aminolupinane derivatives of 1,4-naphtoquinone (Castellani R.J. et al., 2006). In this case, after repeated purification processes through chromatography on Al_2O_3 the desired alcohol has been obtained, but the yield was significantly lower than that obtained with the method previously described. In the second phase, alcohols have been transformed in the corresponding alkyl chlorides **d1-d6** through reaction with an excess of thionyl chloride in anhydrous $CHCl_3$. Also in this case the compounds have been obtained in high yield after been purified through chromatography on Al_2O_3.



R = H, Cl n = 2-4

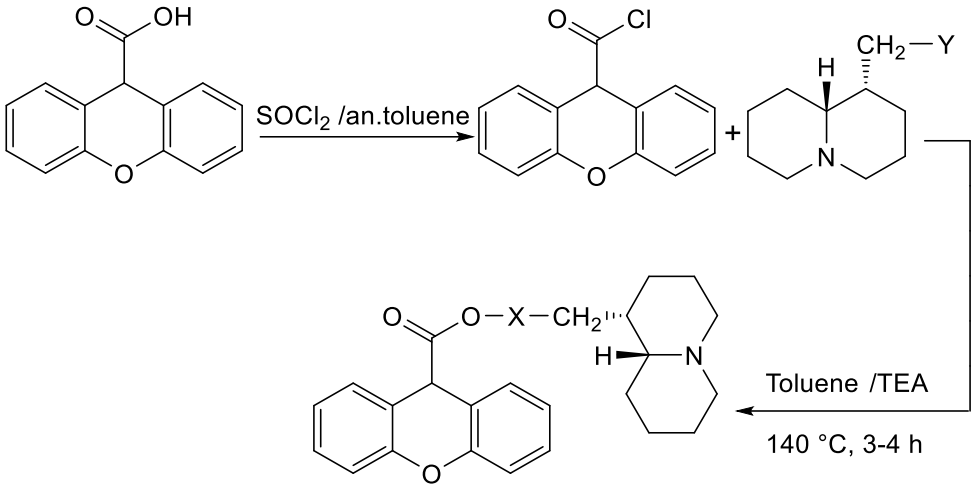
Finally, the reaction between alkyl chlorides and cytisine was carried out. Two stochiometric equivalents of cytisine was used and the reaction conditions utilized have been already described (in a closed tube, in CH_3CN, heating to 100°C for 24 hours).



R = H,		R = Cl,	
n = 2:	**34**	n = 2:	**37**
n = 3:	**35**	n = 3:	**38**
n = 4:	**36**	n = 4:	**39**

In order to examine the tricyclic xanthene system, I prepared the compounds **40** and **44**, already synthesized in the past. (Sparatore F., 1962) On the basis of the promising results of cholinesterase inhibition (**40**: IC_{50} = 1.59 ± 0.40 μM (AChE) and 0.140 ± 0.002 μM (BChE); **44**: IC_{50} = 49% at 10 μM (AChE) and 0.108 ± 0.006 μM (BChE)), the synthesis of the esters **41-43** and the amides **45-46** have been carried out; these compounds are characterized by a longer linker between the tricyclic system and the quinolizidine nucleus, which already in the past demonstrated to ameliorate the inhibitor activity. Furthermore I prepared the amides **47** and **48**, utilizing cytisine as quinolizidine alkaloid.

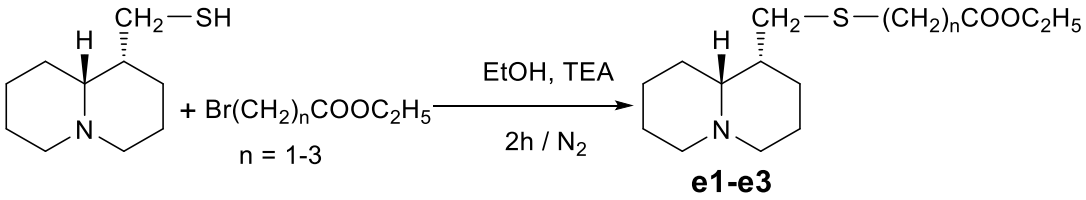
The synthesis of the esters **40** and **43** has been carried out quite easily, refluxing a solution of lupinine or its alcohol derivatives and the acyl chloride of xanthene-9-carboxylic acid in toluene, prepared by the reaction of the opportune carboxylic acid with thionyl chloride, in presence of triethylamine.



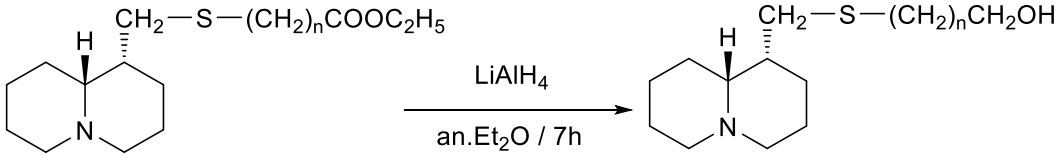
Y = OH	X = -	**40**
Y = SCH_2CH_2OH	X = CH_2CH_2S	**41**
Y = $SCH_2CH_2CH_2OH$	X = $CH_2CH_2CH_2S$	**42**
Y = $SCH_2CH_2CH_2OH$	X = $CH_2CH_2CH_2S$	**43**

..

To obtain the alcohols **f1-f3**, first the corresponding N-(lupinylthio)alkanoyl esters **e1-e3** have been prepared by reaction of thiolupinine with the ethyl ω-bromo alkanoate derivatives. The esters then have been reduced to alcohols through lithium aluminium hydride.



e1-e3

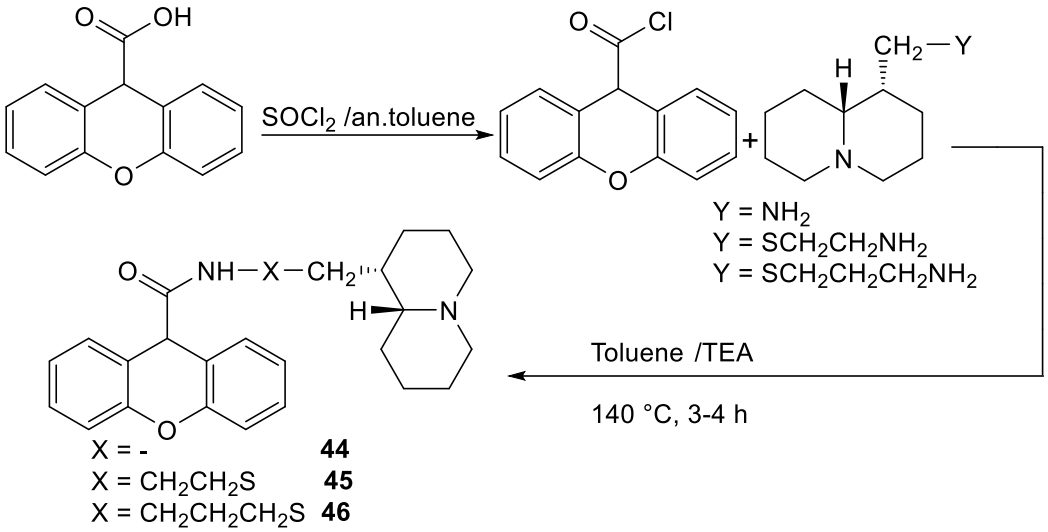


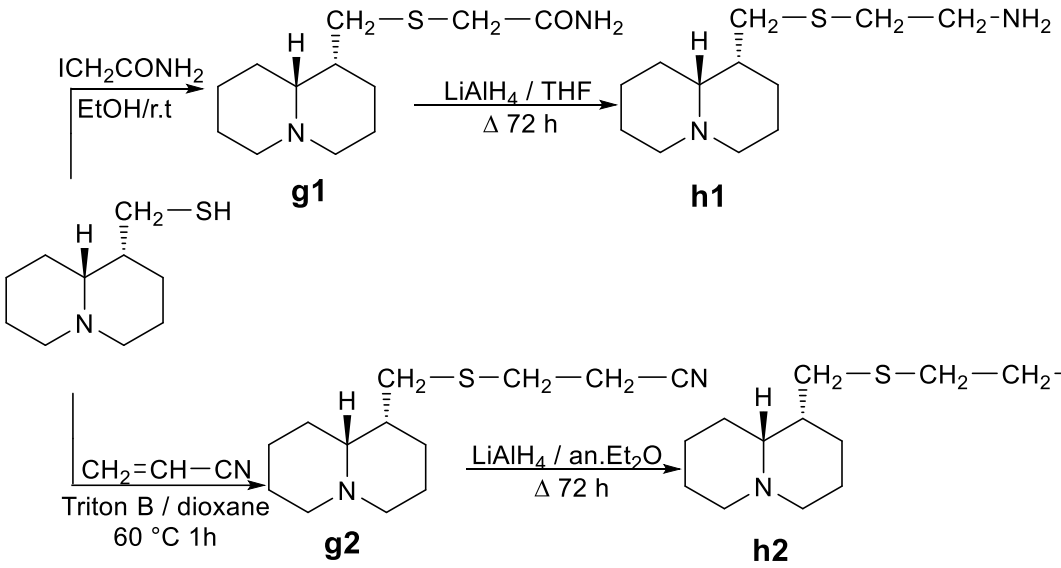
f1-f3

It is noteworthy that all esters **40-43** appear as pale yellow colored and very dense oils, sensitive to heat and light, tending to turn brown.

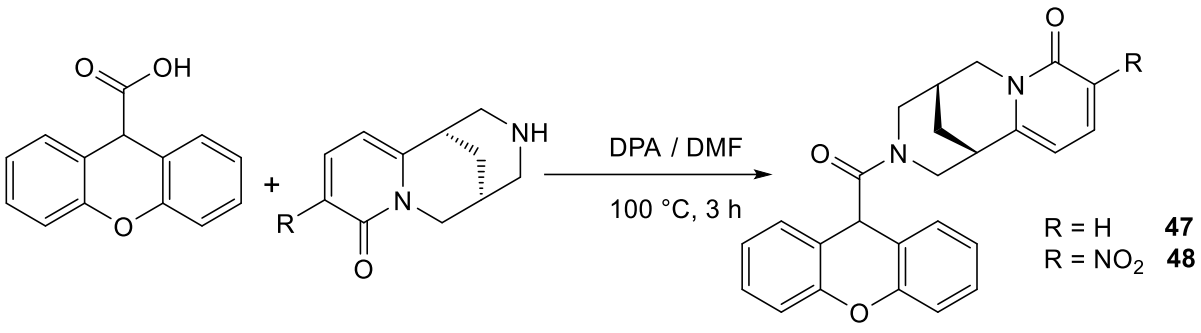
Also the synthesis of the amides **44-46** has been carried out in a similar way to that of the esters, replacing lupinine with aminolupinane, prepared as described by Sparatore for the compound **44**.(Sparatore F., 1962) Regarding the synthesis of the compounds **45** and **46**, first the ω-(lupinylthio)alkylamines **h1-h2** described by Tasso B. et al. in 2011 have been prepared. In this case thiolupinine reacted with

iodoacetamide or acrylonitrile (**g1-g2)**. The amide and the cyano derivative then have been reduced using $LiAlH_4$ to provide the opportune amines for the next step.





Finally, amides **47** and **48** have been obtained by the direct reaction between cytisine or 3-nitrocytisine with 9*H*-xanthene-9-carboxylic acid in presence of diphenyl-diphosphoryl azide in DMF.



Biological discussion

The results obtained by testing the AChe and BChe inhibition activities are reported in table 2. The assays were carried out by Dr. Marco Catto, at the Department of Drug Science at the University of Bari. The inhibition activities on AChE enzyme (from torpedo) and BChE enzyme (from equine serum) have been determined with the spectrophotometric Ellman method (Ellman G.L. et al., 1961).The results have been reported as IC_{50} (µM) for the most active compounds, while for the less active compounds (i.e.<50%) the values have been reported as inhibition percentage at 10 µM.

Given the limited amount of available data, it is not possible to correlate the structural characteristics and the inhibitory activity. However, all these novel compounds exhibit a moderate activity towards at least one enzyme.

Table.2 inhibitor activity towards AChE and BChE of compounds **24-31**

Compound	eeAChE		esBChE	
	% (10 μM)	IC_{50} (μM)	% (10 μM)	IC_{50} (μM)
24		3.38±0.45		6.97±0.63
25		4.07±0.42		4.73±0.52
26		2.81±0.01		2.58±0.29
27		1.79±0.19		1.91±0.21
28	(45±3%)			1.50±0.15
29		3.14±0.06	(47±1%)	
30	(48±3%)			0.733±0.072
31		4.70±0.13		1.30±0.42

All the values represent means of three independent experiments± SEM.

Compound	eeAChE		esBChE		Inib. Aggreg. Aβ (100 μM)
	% (10 μM)	IC50 (μM)	% (10 μM)	IC50 (μM)	
32		**5.57±0.02**	**(23±3%)**		
33		**6.97±0.68**		**8.35±0.24**	
34		**6.20±0.55**		**5.78±0.66**	
35		**10.4±0.4**		**4.24±1.00**	
36	**(43±3%)**		**(45±1%)**		
37		**5.13±0.75**		**4.55±0.55**	
38		**6.02±0.80**		**0.137±0.013**	**65±1%**
39		**5.85±0.06**		**7.32±0.47**	

Table.3 inhibitor activity toward AChE and BChE of compounds **32-39**

All the values represent means of three independent experiments± SEM.

In comparison with the previous anthraquinone analogues endowed with a basic head derived from lupinine or a diethylaminopropyl chain (range IC_{50} = 0.84 - 2.9 μM), these derivatives show a slight decrease in the inhibitor activity toward AChE, while the activity toward BChE slightly increases. This tendency confirms what has been already found for the cytisine derivatives of naphtoquinone. The novel compounds don't exhibit a remarkable selectivity toward one of the two enzymes, except for the compound **28** and especially the compound **30**, which showed a ratio IC_{50}AchE/IC_{50}BChE of about 15 and the highest activity with a submicromolar IC_{50} (0.733 μM).

The importance of the spacer lenght has been already highlighted in other derivatives. The linker elongation appears to improve the inhibitor activity toward BChE and, indeed, the results obtained indicate a higher activity toward BChE.
Derivatives **24** and **25** show that the steric hindrance due to the proximity of the anthraquinone and cytisine nuclei provides a low inhibitor activity. It is notable that the presence of the nitro group at the position 3 of the piridonic ring determines a slight decrease of the activity toward AChE, while it improves the activity toward BChE. In particular, compound **30**, bearing a linker of three carbon atoms and the nitro group in position 3, exhibits the highest activity toward BChE (0.733 μM). This result highlights the possibility to test other derivatives with substituents in that position.
Also all the novel naphtoquinone derivatives, except derivative **36**, show a moderate activity toward at least an enzyme. In comparison with the previous naphtoquinone analogues presenting the lupinine-derived basic head, these compounds exert a marked decrease of the inhibitor activity toward AChE but an improvement of the activity toward BChE. The chlorine atom in position 3 appears to have a positive influence, although to a limited extent. However, no significant selectivity toward own of the two enzymes has been observed, except the compound **38**, with a ratio IC_{50}AchE/IC_{50}BChE of about 44. This compound has also been tested to evaluate its ability in the inhibition of Aβ aggregation, showing a low/moderate effect. The linker elongation doesn't appear to influence the biological activity, although the importance of the linker length has been already highlighted in other derivatives. Nevertheless, the compound with n = 3 e R = Cl (**38**) appears to be endowed with the optimal distance for the interaction with the enzyme, while a further elongation seems to dramatically worsen IC_{50} values.
In conclusion, the novel anthraquinone derivatives maintain the activity showed by the previous compounds (**XXXV** and **XXXVI**), in certain cases improving the selectivity toward BChE, while the novel naphtoquinone derivatives don't seem to maintain the activity and selectivity exerted by the previous compounds. However, the importance of the presence of the chlorine atom in position 3 of the naphtoquinone nucleus has been confirmed for the naphtoquinone derivatives.
Finally, the cytisine derivatives, unlike the lupinyl derivatives, appear to benefit more from the conjugation with the anthraquinone tryciclic system than with the naphtoquinone byciclic nucleus.

Materials And Methods

Melting points were determined by capillary tube method using a Büchi melting point apparatus B-540 and they have not been corrected. The melting points of the quaternary ammonium salts have not been reported here, since they are not sharp and they don't possess a full reproducibility even using closed capillary tubes. Elemental analyses (C, H,N) were carried out at the Microanalysis Laboratory of the Department of Pharmacy, in the Pharmaceutical and Cosmetic Chemistry Section of University of Genoa. ^{1}H-NMR spectra were acquired on a Varian-Gemini 200 apparatus, using $CDCl_3$ or d_6-DMSO as solvents; J (expressed in Hz).

Synthetic processes

3-Nitro-N-nitrosocytisine

1 g (5.25 mmol) of cytisine is added in small amounts to a mixture of concentrated H_2SO_4 (2 mL) and fuming HNO_3 (2.4 mL) maintained at 0°C. The solution becomes colorless after about 20 hours at rt, then is diluted with 7 mL of cold H_2O and a yellow powder precipitates. The precipitate is filtered and long washed with H_2O, to obtain 1.2 g of yellow solid that is washed by decantation with CH_2Cl_2. The insoluble yellow residue (g 0.76) is the 3-nitro-N-nitrosocytisine with mp = 267-270 °C. (yield = 58%).

3-Nitrocytisine hydrochloride

0.76 g (2.8 mmol) of 3-nitro-N-nitrosocytisine are suspended in 20 mL of MeOH and treated at the bath temperature of 40 ° C with a dry gaseous HCl stream. The product passes gradually into solution; the solubilization process is complete when saturation with HCl (obtaining a clear and yellow solution) is achieved. The solvent is removed under vacuum with a bland heating and the solid remained is filtered and long washed with very cold MeOH, to obtain 0.52 g of pale-yellow crystals that melt at about 300 °C. (yield = 67%).

3-Nitrocytisine (XXXVII)

0.32 g (1.17 mmol) of 3-nitrocytisine • HCl are dissolved in H_2O, the solution is cautiously alkalized with NaOH 2N and thoroughly extracted with CH_2Cl_2. The solvent is dried over Na_2SO_4 and removed to obtain 0.26 g of yellow crystals that are further purified by crystallization from EtOH/Et_2O: 0.22 g of yellow crystals with mp = 186-189 °C. (yield = 79%).

1-Cytisinyl-anthraquinone (24)

a) to a solution of 0.57 g (3 mmol) of cytisine in 5 mL of acetonitrile, 0.29 g (1.5 mmol) of 1-chloroanthraquinone are added into a tube, that is closed under nitrogen atmosphere and heated to 100 °C for 24 hours. The solid formed (cytisine hydrochloride) is cold filtered and the organic solution is evaporated to dryness. The residue obtained is dissolved in chloroform and the organic phase is extracted first with acidic water, then with water, and finally dried over Na_2SO_4, filtered and evaporated to dryness. The product obtained as a red solid (0.73 g) is purified by chromatography on SiO_2 (1:20), eluting with $CHCl_3$, to furnish 0.41 g of red solid with mp= 225-227 °C. (yield = 69.5%).

The elemental analysis provided the following results:

	found:	C% 75.64	H%	5.47	N% 7.03
for $C_{25}H_{20}N_2O_3$	calc.:	C% 75.74	H%	5.08	N% 7.07

^{1}H-NMR ($CDCl_3$) δ: 8.32-8.08 (m, 2H, arom); 7.93-7.63 (m, 3H, arom); 7.52-7.23 (m, 2H, arom); 6.95 (d, 1H, J = 8.4, Pyr); 6.49 (dd, 1H, J= 9.2, 1.4, Pyr); 6.11 (dd, 1H, J= 6.8, 1.4, Pyr); 4.36 (d, 1H, J= 15.6, Q); 3.88 (dd, 1H, J= 15.6, 6.4, Q); 3.66-3.07 (m, 4H, Q); 2.60-2.44 (m, 1H, Q); 2.22-1.82 (m, 3H, Q).

b) 0.38 g (2 mmol) of cytisine and 0.48 g (2 mmol) of 1-chloroanthraquinone are placed in a tube that is closed under nitrogen atmosphere and is heated to 170 °C for 3 hours. The mixture is then cold taken up with chloroform and the organic solution is washed first with acidic water and then with water and finally dried over Na_2SO_4, filtered and evaporated to dryness. The product obtained as a red solid (0.66 g) is purified by chromatography on SiO_2 (1:20), eluting with $CHCl_3$, furnishing 0.33 g of red solid with mp = 225-227 °C. (yield = 41.3%).

1-(3-Nitrocytisinyl)-anthraquinone (25)

To a solution of 0.24 g (1 mmol) of 3-nitrocytisine in 5 mL of acetonitrile, 0.24 g (1 mmol) of 1-chloroanthraquinone and 0.13 g (d = 0.742; 0.18 mL) of N,N-diisopropylethylamine (Hünig's base) are added in a tube, that is closed under nitrogen atmosphere and heated to 100 °C for 24 hours. Then, after cooling, the suspension is evaporated to dryness. The residue obtained is dissolved in chloroform and the organic phase is extracted first with acidic water, then with water, and finally dried over Na_2SO_4, filtered and evaporated to dryness. The product obtained (0.43 g) as a red solid is purified by chromatography on SiO_2 (1:20) eluting with $CHCl_3$, to furnish 0.26 g of red solid with mp = 232-234 °C. (yield= 59.1%).

The elemental analysis provided the following results:

	found:	C% 68.33	H%	4.67	N% 9.28
for $C_{25}H_{19}N_3O_5$	calc.:	C% 68.02	H%	4.34	N% 9.52

^{1}H-NMR ($CDCl_3$) δ: 8.52-8.39 (m, 1H, arom); 8.21-7.86 (m, 2H, arom); 7.82-7.51 (m, 3H, arom); 7.40-7.21 (m, 2H, 1H arom, 1H Pyr); 6.26 (d, 1H, J = 8.2, Pyr); 4.68 (d, 1H, J= 16.0, Q); 4.08 (dd, 1H, J= 15.6, 6.6, Q); 3.59-3.23 (m, 4H, Q); 2.82-2.63 (m, 1H, Q); 2.27-1.78 (m, 3H, Q).

1-(2-Hydroxyethylamino)-anthraquinone (a1)

a) To a suspension of 1.21 g (5 mmol) of 1-chloroanthraquinone in 20 mL of butanol, 0.61 g (10 mmoL; d = 1.012; 0.60 mL) of 2-aminoethanol are added. The reaction mixture is heated under reflux for 8 hours, obtaining a dark red-coloured solution. After cooling, the solvent is evaporated to dryness and the solid obtained is taken up with $CHCl_3$. The organic solution is washed first with acidic water, then with water and finally dried over Na_2SO_4, filtered and evaporated to dryness, to obtain a red solid (1.24 g) that is crystallized from toluene. The final product (0.91 g) consists of dark red-coloured crystals with mp=168–170°C. (yield= 67.9%)

The elemental analysis provided the following results:

	found:	C% 71.71	H%	5.08	N% 5.38
for $C_{16}H_{13}NO_3$	calc.:	C% 71.90	H%	4.90	N% 5.24

^{1}H-NMR ($CDCl_3$) δ: 9.67 (s, 1H, NH); 8.38-8.16 (m, 2H, arom); 7.85-7.49 (m, 4H, arom); 7.44 (dd, J = 8.0, 1.8, 1H, arom); 4.02 (t, 2H, J= 5.2, OCH_2); 3.59 (t, 2H, J=

5.4, NCH_2).

b) 1.21 g (5 mmol) of 1-chloroanthraquinone and 0.30 g (5 mmol; d = 1.012; 0.30 mL) of 2-aminoethanol are placed in a tube that is heated to 170 °C for 4 ore. After cooling, the mixture is taken up with $CHCl_3$. The organic solution is washed one fold with acidic water, twice with water and the dried over Na_2SO_4, filtered and evaporated to dryness. The dark red-coloured solid obtained is first purified by chromatography on SiO_2 (1:15) eluting with $CHCl_3$ and then crystallized from toluene obtaining 0.58 g of dark red-coloured crystals with mp=168–170°C. (yield = 43.3%).

1-(3-Hydroxypropylamino)-anthraquinone (a2)

To a suspension of 1-chloroanthraquinone (1.21 g; 5 mmol) in 20 mL of butanol, 0.75 g (8 mmol; d = 0.982; 0.76 mL) of 3-amino-1-propanol are added. The reaction mixture is heated under reflux for 8 hours to obtain a dark red-coloured solution. After cooling, the solvent is evaporated to dryness and the solid obtained is taken up with $CHCl_3$. The organic solution is washed one fold with acidic water, twice with water and then dried on Na_2SO_4, filtered and evaporated to dryness, to obtain a red solid (1.31 g) that is crystallized from toluene. The final product consists in 1.04 g of dark red-coloured crystals with mp=178–179°C. (yield = 73.8%)

The elemental analysis provided the following results:

	found:	C% 72.55	H%	5.73	N% 4.69
for $C_{17}H_{15}NO_3$	calc.:	C% 72.58	H%	5.37	N% 4.98

^{1}H-NMR ($CDCl_3$) δ: 9.72 (s, 1H, NH); 8.39-8.21 (m, 2H, arom); 7.94-7.52 (m, 4H, arom); 7.31 (dd, J = 8.0, 1.8, 1H, arom); 3.91 (t, 2H, J= 6.0, OCH_2); 3.55 (t, 2H, J= 6.6, NCH_2); 2.06 (quintet, 2H, J = 6.0).

1-(4-Hydroxybutylamino)-anthraquinone (a3)

To a suspension of 1-chloroanthraquinone (1.21 g; 5 mmol) in 20 mL of butanol, 0.89 g (10 mmol; d = 0.967; 0.92 mL) of 4-amino-1-butanol are added. The mixture is heated under reflux for 8 hours to obtain a dark red-coloured solution. After cooling, the solvent is evaporated to dryness and the solid obtained is taken up with $CHCl_3$. The organic solution is washed one fold with acidic water, twice with

water and then dried on Na_2SO_4, filtered and evaporated to dryness, to obtain a red solid (1.44 g) that is purified by chromatography on SiO_2 (1:20), eluting with $CHCl_3$. The final product consists in 1.01 g of dark red-coloured solid with mp=130–131°C. (yield= 68.2%)

The elemental analysis provided the following results:

	found:	C% 73.00	H%	5.95	N% 4.60
for $C_{18}H_{17}NO_3$	calc.:	C% 73.20	H%	5.80	N% 4.74

^{1}H-NMR ($CDCl_3$) δ: 9.69 (s, 1H, NH); 8.38-8.20 (m, 2H, arom); 7.91-7.53 (m, 4H, arom); 7.18 (d, J = 8.0, 1H, arom); 3.79 (t, 2H, J= 6.2, OCH_2); 3.42 (t, 2H, J= 6.6, NCH_2); 2.08-1.74 (m, 4H, J = 6.0).

1-(2-Chloroethylamino)-anthraquinone (b1)

To a suspension of 1-(2-hydroxyethylamino)-anthraquinone (**a1**) (1.13 g; 4.2 mmol) in 25 mL of anhydrous $CHCl_3$, a solution of thionyl chloride (2.02 g; 17.0 mmol; d = 1.631; 1.24 mL) in 5 mL of anhydrous $CHCl_3$ is added dropwise. The mixture is heated under reflux for 6 hours, obtaining a dark red-coloured solution. After cooling, 15 mL of very cold 10% $NaHCO_3$ solution is added and the mixture is stirred for 5 minutes. The organic phase is separated in a separating funnel, then washed with very cold water and finally dried over Na_2SO_4, filtered and evaporated to dryness. The residue obtained as a red solid (1.22 g) is purified by chromatography on SiO_2 (1:20) eluting with chloroform, to furnish 1.04 g of dark red-coloured solid with mp=174–176°C. (yield= 86.7%).

The elemental analysis provided the following results:

	found:	C% 67.09	H%	4.58	N% 4.66
for $C_{16}H_{12}ClNO_2$	calc.:	C% 67.26	H%	4.23	N% 4.90

^{1}H-NMR ($CDCl_3$) δ: 10.02 (s, 1H, NH); 8.40-8.12 (m, 2H, arom); 7.92-7.57 (m, 4H, arom); 7.10 (dd, J = 8.2, 1,4, 1H, arom); 3.94-3.68 (m, 4H, NCH_2 e CH_2Cl).

1-(3-Chloropropylamino)-anthraquinone (b2)

To a suspension of 1-(3-hydroxypropylamino)-anthraquinone (**a2**) (1.16 g; 4.1 mmol) in 25 mL of anhydrous $CHCl_3$, a solution of thionyl chloride (2.02 g; 17.0 mmol; d = 1.631; 1.24 mL) in 5 mL of anhydrous $CHCl_3$ is added dropwise. The

mixture is heated under reflux for 6 hours obtaining a dark red-coloured solution. After cooling, 15 mL of very cold 10% $NaHCO_3$ solution is added and the mixture is stirred for 5 minutes. The organic phase is separated in a separating funnel, then washed with very cold water and finally dried over Na_2SO_4, filtered and evaporated to dryness. The red residue obtained (1.31 g) is purified by chromatography on SiO_2 (1:20), eluting with chloroform to furnish 1.12 g of dark red-coloured solid with mp=125–127°C. (yield = 91.1%).

The elemental analysis provided the following results:

	found:	C% 67.99	H%	4.58	N% 4.66
for $C_{17}H_{14}ClNO_2$	calc.:	C% 68.12	H%	4.71	N% 4.67

^{1}H-NMR ($CDCl_3$) δ: 9.81 (s, 1H, NH); 8.41-8.17 (m, 2H, arom); 7.96-7.51 (m, 4H, arom); 7.12 (dd, J = 8.0, 1,8, 1H, arom); 3.75 (t, J = 6.2, 2H, CH_2Cl); 3.59 (t, J = 6.2, 2H, NCH_2); 2.38-2.16 (quintet, J = 6.4, 2H, $CH_2CH_2CH_2$).

1-(4-Chlorobutylamino)-anthraquinone (b3)

To a suspension of 1-(4-hydroxybutylamino)-anthraquinone (**a3**) (0.91 g; 3.1 mmol) in 25 mL of anhydrous $CHCl_3$, a solution of thionyl chloride (1.47 g; 12.3 mmol; d = 1.631; 0.90 mL) in 5 mL of anhydrous $CHCl_3$ is added dropwise. The reaction mixture is heated under reflux for 6 hours to obtain a dark red-coloured solution. After cooling, 15 mL of very cold 10% $NaHCO_3$ solution is added and the mixture is stirred for 5 minutes. The organic phase is separated in a separating funnel, then washed with very cold water and finally dried over Na_2SO_4, filtered and evaporated to dryness. The red residue obtained (1.07 g) is purified by chromatography on SiO_2 (1:20), eluting with chloroform to furnish 0.82 g of a dark red-coloured solid with mp=89–90°C. (yield= 82.8%).

The elemental analysis provided the following results:

	found:	C% 68.73	H%	4.97	N% 4.29
for $C_{18}H_{16}ClNO_2$	calc.:	C% 68.90	H%	5.14	N% 4.46

^{1}H-NMR ($CDCl_3$) δ: 9.80 (s, 1H, NH); 8.38-8.21 (m, 2H, arom); 7.88-7.54 (m, 4H, arom); 7.08 (dd, J = 8.0, 2.0, 1H, arom); 3.66 (t, J = 6.2, 2H, CH_2Cl); 3.42 (t, J = 6.4, 2H, NCH_2); 2.15-1.86 (m, 4H, $CH_2CH_2CH_2CH_2$).

1-[2-(Cytisinyl)ethylamino]anthraquinone (26)

To a solution of cytisine (0.38 g; 2 mmol) in 5 mL di acetonitrile, 0.29 g (1 mmol) of 1-(2-chloroethylamino)-anthraquinone (**b26**) is added into a tube, that is closed and heated to 100 °C for 24 hours. The solid formed (cytisine hydrochloride), after cooling, is filtered and the organic solution is washed with NaOH 1 N, water, and finally dried over Na_2SO_4, filtered and evaporated to dryness. The residue obtained is purified by chromatography on SiO_2 (1:20), eluting with CH_2Cl_2, to obtain 0.31 g of red solid with mp = 202-203 °C. (yield = 70.5%).

The elemental analysis provided the following results:

	found:	C% 73.62	H%	5.84	N% 9.43
for $C_{27}H_{25}N_3O_3$	calc.:	C% 73.79	H%	5.73	N% 9.56

^{1}H-NMR ($CDCl_3$) δ: 9.68 (s, 1H, NH); 8.36-8.21 (m, 2H, arom); 7.84-7.61 (m, 3H, arom); 7.38-7.22 (m, 2H, arom); 7.00 (d, 1H, J = 9.4, Pyr); 6.44 (d, 1H, J= 9.2, Pyr); 6.02 (d, 1H, J= 7.0, Pyr); 4.10 (d, 1H, J= 15.6, Q); 3.90 (dd, 1H, J= 15.6, 6.4, Q); 3.32-2.84 (m, 4H, Q); 2.53-2.20 (m, 5H, 1H, Q, 4H CH_2CH_2); 2.01-1.58 (m, 3H, Q).

1-[3-(Cytisinyl)propylamino]anthraquinone (27)

To a solution of cytisine (0.38 g; 2 mmol) in 5 mL of acetonitrile, 0.30 g (1 mmol) of 1-(3-chloropropylamino)-anthraquinone (**b27**) are added in a tube that is closed under nitrogen atmosphere and heated to 100 °C for 24 hours. The solid formed (cytisine hydrochloride), after cooling, is filtered and the organic solution is washed with NaOH 1 N, washed with water, and finally dried over Na_2SO_4, filtered and evaporated to dryness. The residue obtained is purified by chromatography on SiO_2 (1:20), eluting with $CHCl_3$, to obtain 0.34 g of red solid with mp= 193-195 °C. (yield = 75.6%).

The elemental analysis provided the following results:

	found:	C% 74.12	H%	6.13	N% 9.20
for $C_{28}H_{27}N_3O_3$	calc.:	C% 74.15	H%	6.00	N% 9.27

^{1}H-NMR ($CDCl_3$) δ: 9.61 (s, 1H, NH); 8.34-8.18 (m, 2H, arom); 7.83-7.49 (m, 3H, arom); 7.34-7.18 (m, 2H, arom); 6.88 (d, 1H, J = 7.4, Pyr); 6.42 (d, 1H, J= 8.8, Pyr); 6.00 (d, 1H, J= 5.6, Pyr); 4.15 (d, 1H, J= 15.4, Q); 3.92 (dd, 1H, J= 15.4, 7, Q); 3.21-2.81 (m, 4H, Q); 2.58-2.26 (m, 5H, 1H, Q, 4H NCH_2 + CH_2N); 2.02-1.60 (m, 5H, 3H, Q, 2H $CH_2CH_2CH_2$).

1-[4-(Cytisinyl)butylamino]anthraquinone (28)

To a solution of cytisine (0.38 g; 2 mmol) in 5 mL of acetonitrile, 0.31 g (1 mmol) of 1-(4-chlorobutylamino)anthraquinone (**b28**) are added into a tube, that is closed under nitrogen atmosphere and heated to 100° C for 24 hours. The solid formed (cytisine hydrochloride), after cooling, is filtered and the organic solution is washed with NaOH 1 N, washed with water, and finally dried over Na_2SO_4, filtered and evaporated to dryness. The residue obtained is purified by chromatography on SiO_2 (1:20), eluting with $CHCl_3$, to obtain 0.29 g of red solid with mp= 234-235 °C. (yield = 61.7%).

The elemental analysis provided the following results:

	found:	C% 74.29	H%	6.47	N% 8.72
for $C_{29}H_{29}N_3O_3$	calc.:	C% 74.50	H%	6.25	N% 8.99

^{1}H-NMR ($CDCl_3$) δ: 9.62 (s, 1H, NH); 8.43-8.14 (m, 2H, arom); 7.92-7.42 (m, 3H, arom); 7.22-7.06 (m, 2H, arom); 6.93 (dd, 1H, J = 8.2, 1.6, Pyr); 6.32 (dd, 1H, J= 9.2, 1.4, Pyr); 5.94 (dd, 1H, J= 7.0, 1.4, Pyr); 4.20-3.95 (m, 2H, Q); 3.38-2.90 (m, 4H, Q); 2.77-2.38 (m, 5H, 1H, Q, 4H NCH_2 + CH_2N); 2.19-1.72 (m, 7H, 3H, Q, 4H $CH_2CH_2CH_2CH_2$).

1-[2-(3-Nitrocytisinyl)ethylamino]anthraquinone (29)

To a solution of 3-nitrocytisine (0.24 g; 1 mmol) in 5 mL of acetonitrile, 0.29 g (1 mmol) of 1-(2-chloroethylamino)anthraquinone (**b26**) and 0.13 g (1 mmol; d=0.742; 0.18 mL) of N,N- diisopropylethylamine (Hünig's base) are added into a tube that is closed under nitrogen atmosphere and heated to 100 °C for 24 hours. The suspension, after cooling, is evaporated to dryness. The residue obtained is dissolved in chloroform and the organic phase is extracted first with acidic water, then with water and finally it is dried over Na_2SO_4, filtered and evaporated to dryness. The product obtained as a red solid (0.44 g) is purified by chromatography on SiO_2 (1:20), eluting with $CHCl_3$, furnishing 0.28 g of a red solid with mp = 219-221 °C. (yield = 58.3%).

The elemental analysis provided the following results:

	found:	C% 67.21	H%	5.13	N% 11.35
for $C_{27}H_{24}N_4O_5$	calc.:	C% 66.93	H%	4.99	N% 11.56

^{1}H-NMR ($CDCl_3$) δ: 9.56 (s, 1H, NH); 8.32-8.04 (m, 3H, arom); 7.88-7.61 (m, 2H, arom); 7.59-7.35 (m, 2H, arom); 6.90 (dd, 1H, J = 8.0, 1.4, Pyr); 6.08 (d, 1H, J= 8.0, Pyr); 4.41-4.05 (m, 2H, Q); 3.42-3.01 (m, 5H, 1H, Q, 4H, NCH_2 e CH_2N); 2.98-2.38 (m, 5H, Q); 2.08-1.74 (m, 2H, Q).

1-[3-(3-Nitrocytisinyl)propylamino]anthraquinone (30)

To a solution of 3-nitrocytisine (0.24 g; 1 mmol) in 5 mL of acetonitrile, 0.30 g (1 mmol) of 1-(3-chloropropylamino)anthraquinone (**b27**) and 0.13 g (1 mmol; d = 0.742; 0.18 mL) of N,N- diisopropylethylamine (Hünig's base) are added into a tube that is closed under nitrogen atmosphere and heated to 100 °C for 24 hours. The suspension, after cooling, is evaporated to dryness. The residue obtained is dissolved in chloroform and the organic phase is extracted first with acidic water, then with water and finally it is dried over Na_2SO_4, filtered and evaporated to dryness. The product obtained as a red solid (0.46 g) is purified by chromatography on SiO_2 (1:20), eluting with $CHCl_3$, furnishing eventually 0.34 g of red solid with mp = 189-191 °C. (yield= 68.0%).

The elemental analysis provided the following results:

	found:	C% 67.14	H%	5.58	N% 11.38
for $C_{28}H_{26}N_4O_5$	calc.:	C% 67.46	H%	5.26	N% 11.24

^{1}H-NMR ($CDCl_3$) δ: 9.55 (s, 1H, NH); 8.36-8.13 (m, 3H, arom); 7.93-7.62 (m, 2H, arom); 7.62-7.40 (m, 2H, arom); 6.81 (dd, 1H, J = 8.0, 1.8, Pyr); 6.10 (d, 1H, J= 8.0, Pyr); 4.23 (d, 1H, J = 15.8; Q); 3.99 (dd, 1H, J = 16.0, 6.6, Q); 3.28-2.92 (m, 5H, 1H, Q, 4H, NCH_2 e CH_2N); 2.74-2.27 (m, 5H, Q); 2.06-1.64 (m, 4H, 2H, Q + 2H, $CH_2CH_2CH_2$).

1-[4-(3-Nitrocytisinyl)butylamino]anthraquinone (31)

To a solution of 3-nitrocytisine (0.24 g; 1 mmol) in 5 mL of acetonitrile, 0.31 g (1 mmol) of 1-(4-chlorobutylamino)anthraquinone (**b28**) and 0.13 g (1 mmol; d = 0.742; 0.18 mL) of N,N- diisopropylethylamine (Hünig's base) are added into a tube that is closed under nitrogen atmosphere and heated to 100 °C for 24 hours. The suspension, after cooling, is evaporated to dryness. The residue obtained is dissolved in chloroform and the organic phase is extracted first with acidic water,

then with water and finally it is dried over Na_2SO_4, filtered and evaporated to dryness. The product obtained as a red solid (0.42 g) is purified by chromatography on SiO_2 (1:20), eluting with $CHCl_3$, furnishing 0.27 g of red solid with mp = 169-172 °C. (yield = 52.9%).

The elemental analysis provided the following results:

	found:	C% 68.22	H%	5.83	N% 10.68
for $C_{29}H_{28}N_4O_5$	calc.:	C% 67.96	H%	5.51	N% 10.93

^{1}H-NMR ($CDCl_3$) δ: 9.59 (s, 1H, NH); 8.24-7.96 (m, 3H, arom); 7.82-7.51 (m, 2H, arom); 7.40-7.24 (m, 2H, arom); 6.47 (dd, 1H, J = 8.0, 1.8, Pyr); 6.26 (d, 1H, J= 8.0, Pyr); 4.67 (d, 1H, J = 16.0; Q); 4.07 (dd, 1H, J = 16.0, 6.6, Q); 3.57-3.22 (m, 5H, 1H, Q, 4H, NCH_2 e CH_2N); 2.94-2.62 (m, 5H, Q); 2.37-1.85 (m, 6H, 2H, Q + 4H, $CH_2CH_2CH_2CH_2$).

2-Cytisinyl-1,4-naphtoquinone (32)

To a solution of 0.57 g (3 mmol) of cytisine in 5 mL of acetonitrile, 0.36 g (1.5 mmol) of 2-bromo-1,4-naphtoquinone are added into a tube, that is closed under nitrogen atmosphere and heated to 100 °C for 24 hours. After cooling, the solid formed is filtered and the organic solution is evaporated to dryness. The residue obtained is dissolved in chloroform and the organic phase is washed first with acidic water, then with water and finally it is dried over Na_2SO_4, filtered and evaporated to dryness. The product obtained as a red solid (0.48 g) is purified by chromatography on Al_2O_3 (1:25), eluting with $CHCl_3$, to furnish 0.28 g of red solid with mp= 176-178 °C. (yield= 53.8%).

The elemental analysis provided the following results:

	found:	C% 72.56	H%	5.41	N% 8.36
for $C_{21}H_{18}N_2O_3$	calc.:	C% 72.82	H%	5.24	N% 8.09

^{1}H-NMR ($CDCl_3$) δ: 8.23-8.02 (m, 2H, arom); 7.82-7.58 (m, 2H, arom); 7.32-7.20 (m, 2H, 1H arom, 1H Pyr); 6.41 (dd, 1H, J= 9, 1.4, Pyr); 6.02 (dd, 1H, J= 7, 1.4, Pyr); 4.11 (d, 1H, J= 15.2, Q); 3.87 (dd, 1H, J= 15.6, 6.6, Q); 3.77-3.58 (m, 2H, Q); 3.02-2.83 (m, 2H, Q); 2.51-2.24 (m, 2H, Q); 2.01-1.76 (m, 2H, Q).

2-Cytisinyl-3-chloro-1,4-naphtoquinone (33)

To a solution of 0.57 g (3 mmol) of cytisine in 5 mL of acetonitrile, 0.34 g (1.5 mmol) of 2,3-dichloro-1,4-naphtoquinone are added into a tube, that is closed under nitrogen atmosphere and heated to 100 °C for 24 hours. After cooling, the solid formed is filtered and the organic solution is evaporated to dryness. The residue obtained is dissolved in chloroform and the organic phase is washed first with acidic water, then with water and finally it is dried over Na_2SO_4, filtered and evaporated to dryness. The product obtained as a red solid (0.64 g) is purified by chromatography on Al_2O_3 (1:25), eluting with $CHCl_3$, to furnish 0.38 g of red solid with mp = 194-195 °C. (yield = 66.7%).

The elemental analysis provided the following results:

	found:	C% 66.35	H%	4.70	N% 7.16
for $C_{21}H_{17}ClN_2O_3$	calc.:	C% 66.23	H%	4.50	N% 7.36

^{1}H-NMR ($CDCl_3$) δ: 8.16-7.92 (m, 2H, arom); 7.78-7.58 (m, 2H, arom); 7.41-7.20 (m, 1H, 1H Pyr); 6.17 (d, 1H, J= 9, Pyr); 6.17 (d, 1H, J= 6.6, Pyr); 4.65 (d, 1H, J= 15.6, Q); 3.84-3.48 (m, 4H, Q); 3.18 (s, 1H, Q); 2.59 (s, 1H, Q); 2.43-2.01 (m, 3H, Q).

2-(2-Hydroxyethylamino)-1,4-naphtoquinone (c1)

a) To s suspension of 2-bromo-1,4-naphtoquinone (0.95 g; 4 mmol) in 25 mL of ethanol, 0.49 g (8 mmol; d = 1.012; 0.49 mL) of 2-aminoethanol are added. The reaction mixture is heated under reflux for 6 hours to obtain a dark red-coloured solution. After cooling, the solvent is evaporated to dryness and the solid obtained is taken up with $CHCl_3$. The organic solution is washed twice with water and then dried over Na_2SO_4, filtered and evaporated to dryness to obtain a red solid (0.94 g) that is crystallized from ethanol/water. The final product consists of dark red-coloured crystals (0.66 g) with mp =157–159°C. (yield= 75.9%)

The elemental analysis provided the following results:

	found:	C% 66.16	H%	5.41	N% 6.59
for $C_{12}H_{11}NO_3$	calc.:	C% 66.35	H%	5.10	N% 6.45

^{1}H-NMR ($CDCl_3$) δ: 8.19-8.01 (m, 2H, arom); 7.83-7.58 (m, 2H, arom); 6.26 (s, broad, 1H, NH); 5.79 (s, 1H, arom); 3.96 (t, 2H, J= 5.2, OCH_2); 3.40 (q, 2H, J= 5.6, 10.8, NCH_2); 2.00 (s, broad, 1H, OH).

b) An ethanolic solution of 1,4-naphtoquinone (0.48 g; 3 mmol) and 2-aminoethanol (0.18 g; 3 mmol, d = 1.012, 0.18 mL) is heated under reflux for 5 hours, to obtain a dark brown-coloured solution. After cooling, the solvent is evaporated to dryness and the solid obtained is taken up with $CHCl_3$. The organic solution is washed twice with water and then dried over Na_2SO_4, filtered and evaporated to dryness to obtain a brown solid (0.57 g), which is first purified by chromatography on Al_2O_3 (1:25) eluting with $CHCl_3$ and then crystallized from ethanol/water. The final product consists of dark red-coloured crystals (0.31 g) with mp = 156–158°C. (yield = 47.7%)

2-(3-Hydroxypropylamino)-1,4-naphtoquinone (c2)

To a suspension of 2-bromo-1,4-naphtoquinone (0.95 g; 4 mmol) in 25 mL of ethanol, 0.60 g (8 mmol; d = 0.982; 0.62 mL) of 3-amino-1-propanol are added. The reaction mixture is heated under reflux for 6 hours to obtain a dark red-coloured solution. After cooling, the solvent is evaporated to dryness and the solid obtained is taken up with $CHCl_3$. The organic solution is washed twice with water and then dried over Na_2SO_4, filtered and evaporated to dryness to obtain a red solid (0.92 g) which is purified by chromatography on alumina (1:30), eluting with chloroform. The final product consists in 0.71 g of dark red-coloured solid with mp =133–134°C. (yield= 77.2%)

The elemental analysis provided the following results:

	found:	C% 67.14	H%	5.67	N% 6.06
for $C_{13}H_{13}NO_3$	calc.:	C% 67.52	H%	6.01	N% 5.97

^{1}H-NMR ($CDCl_3$) δ: 8.18-8.01 (m, 2H, arom); 7.82-7.56 (m, 2H, arom); 6.36 (s, broad, 1H, NH); 5.78 (s, 1H, arom); 3.87 (t, 2H, J= 5.6, OCH_2); 3.39 (q, 2H, J= 6.4, NCH_2); 2.07-1.92 (m, 2H, $CH_2CH_2CH_2$).

2-(4-Hydroxybutylamino)-1,4-naphtoquinone (c3)

To a suspension of 2-bromo-1,4-naphtoquinone (1.19 g; 5 mmol) in 30 mL of ethanol, 0.89 g (10 mmol; d = 0.967; 0.92 mL) of 4-amino-1-butanol are added. The reaction mixture is heated under reflux for 5 hours to obtain a dark red-coloured solution. After cooling, the solvent is evaporated to dryness and the solid obtained is taken up with $CHCl_3$. The organic solution is washed twice with water and then

dried over Na_2SO_4, filtered and evaporated to dryness to obtain a red solid (1.09 g) that is crystallized from ethanol. The final product consists of dark red-coloured crystals (0.89 g) with a mp = 138–140°C. (yield = 72.4%)

The elemental analysis provided the following results:

	found:	C% 68.49	H%	6.38	N% 5.89
for $C_{14}H_{15}NO_3$	calc.:	C% 68.56	H%	6.16	N% 5.71

^{1}H-NMR ($CDCl_3$) δ: 8.17-8.01 (m, 2H, arom); 7.82-7.57 (m, 2H, arom); 6.10 (s, broad, 1H, NH); 5.76 (s, 1H, arom); 3.87 (t, 2H, J= 5.6, OCH_2); 3.26 (q, 2H, J= 6.4, NCH_2); 1.94-1.62 (m, 4H, $CH_2CH_2CH_2CH_2$).

3-Chloro-2-(2-hydroxyethylamino)-1,4-naphtoquinone (c4)

To a suspension of 2,3-dichloro-1,4-naphtoquinone (1.13 g; 5 mmol) in 25 mL of ethanol, 0.61 g (10 mmol; d = 1.012; 0.6 mL) of 2-aminoethanol is added. The reaction mixture is heated under reflux for 6 hours to obtain a dark red-coloured solution. After cooling, the solvent is evaporated to dryness and the solid obtained is taken up with $CHCl_3$. The organic solution is washed twice with water and then dried over Na_2SO_4, filtered and evaporated to dryness to obtain a red solid (1.29 g) that is crystallized from toluene. The final product consists of dark red-coloured crystals (1.19 g) with a mp = 143–144°C. (yield = 92.2%)

The elemental analysis provided the following results:

	found:	C% 57.05	H%	4.07	N% 5.25
for $C_{12}H_{10}ClN_2O_3$	calc.:	C% 57.27	H%	4.01	N% 5.57

^{1}H-NMR ($CDCl_3$) δ: 8.23-7.94 (m, 2H, arom); 7.82-7.55 (m, 2H, arom); 6.21 (s, 1H, NH); 4.18-4.02 (m, 2H, CH_2O); 4.00-3.83 (m, 2H, NCH_2); 1.88 (s, broad, OH).

3-Chloro-2-(3-Hydroxypropylamino)-1,4-naphtoquinone (c5)

To a suspension of 2,3-dichloro-1,4-naphtoquinone (1.13 g; 5 mmol) in 25 mL of ethanol, 0.75 g (10 mmol; d = 0.982; 0.76 mL) of 3-amino-1-propanol are added. The reaction mixture is heated under reflux for 6 hours to obtain a dark red-coloured solution. After cooling, the solvent is evaporated to dryness and the solid obtained is taken up with $CHCl_3$. The organic solution is washed twice with water and then dried over Na_2SO_4, filtered and evaporated to dryness to obtain a red

solid (1.41 g) that is crystallized from toluene. The final product consists of dark red-coloured crystals (1.28 g) with a mp = 112–113°C. (yield= 96.2%)
The elemental analysis provided the following results:

	found:	C% 58.92	H%	4.54	N% 5.05
for $C_{13}H_{12}ClN_2O_3$	calc.:	C% 58.77	H%	4.55	N% 5.27

^{1}H-NMR ($CDCl_3$) δ: 8.24-7.94 (m, 2H, arom); 7.81-7.56 (m, 2H, arom); 6.28 (s, 1H, NH); 4.06 (t, 2H, J= 6.6, OCH_2); 3.89 (t, 2H, J= 5.8, NCH_2); 1.99 (quintet, 2H, $CH_2CH_2CH_2$ + s broad, 1H, OH).

3-Chloro-2-(4-hydroxybutylamino)-1,4-naphtoquinone (c6)

To a suspension of 2,3-dichloro-1,4-naphtoquinone (1.13 g; 5 mmol) in 25 mL of ethanol, 0.89 g (10 mmol; d = 0.967; 0.92 mL) of 4-amino-1-butanol are added. The reaction mixture is heated under reflux for 6 hours to obtain a dark red-coloured solution. After cooling, the solvent is evaporated to dryness and the solid obtained is taken up with $CHCl_3$. The organic solution is washed twice with water and then dried over Na_2SO_4, filtered and evaporated to dryness to obtain a red solid (1.45 g) which is purified by chromatography on alumina (1:30) eluting with chloroform. The final product consists of a red solid (1.09 g) with a mp = 99–100°C. (yield= 77.9%)
The elemental analysis provided the following results:

	found:	C% 60.12	H%	4.94	N% 4.83
for $C_{14}H_{14}ClN_2O_3$	calc.:	C% 60.11	H%	5.04	N% 5.01

^{1}H-NMR ($CDCl_3$) δ: 8.25-7.99 (m, 2H, arom); 7.84-7.58 (m, 2H, arom); 6.23 (s, broad, 1H, NH); 3.94 (t, 2H, J= 6.8, OCH_2); 3.76 (t, 2H, J= 6.2, NCH_2); 1.94-1.62 (m, 4H, $CH_2CH_2CH_2CH_2$ + s broad, 1H, OH).

2-(2-Chloroethylamino)-1,4-naphtoquinone (d1)

To a suspension of 2-(2-hydroxyethylamino)-1,4-naphtoquinone (**c1**) (0.54 g; 2.5 mmol) in 25 mL of anhydrous $CHCl_3$, a solution of thionyl chloride (1.49 g; 12.5 mmol; d = 1.631; 0.9 mL) in 5 mL of anhydrous $CHCl_3$ is added dropwise. The reaction mixture is heated under reflux for 4 hours to obtain a dark red-coloured solution. After cooling, 15 mL of very cold 10% $NaHCO_3$ solution are added. The

organic phase is separated in a separating funnel, washed with very cold water and then dried over Na_2SO_4, filtered and evaporated to dryness. The product obtained as a red foam (0.51 g) is purified by chromatography on alumina (1:30) eluting with chloroform, to furnish 0.29 g of a dark red-coloured solid with mp = 183–184°C. (yield= 49.2%). 0.14 g of the starting alcohol are also recovered.
The elemental analysis provided the following results:

	found:	C% 60.95	H%	4.62	N% 5.75
for $C_{12}H_{10}ClNO_2$	calc.:	C% 61.16	H%	4.28	N% 5.94

^{1}H-NMR ($CDCl_3$) δ: 8.23-8.05 (m, 2H, arom); 7.89-7.64 (m, 3H, arom); 6.37 (s, broad, 1H, NH); 4.23 (q, 2H, J= 5.8, NCH_2); 3.81 (t, 2H, J= 5.8, CH_2Cl).

2-(3-Chloropropylamino)-1,4-naphtoquinone (d2)

To a suspension of 2-(3-hydroxypropylamino)-1,4-naphtoquinone (**c2**) (0.64 g; 2.6 mmol) in 25 mL of anhydrous $CHCl_3$, a solution of thionyl chloride (1.52 g; 12.8 mmol; d = 1.631; 0.93 mL) in 5 mL of anhydrous $CHCl_3$ is added dropwise. The reaction mixture is heated under reflux for 5 hours to obtain a dark red-coloured solution. After cooling, 15 mL of very cold 10% $NaHCO_3$ solution are added. The organic phase is separated in a separating funnel, washed with very cold water and then dried over Na_2SO_4, filtered and evaporated to dryness. The product obtained as a red foam (0.57 g) is purified by chromatography on silica gel (1:30), eluting with chloroform, to furnish 0.42 g of dark red-coloured solid with mp = 158–160°C. (yield= 64.6%). 0.08 g of the starting alcohol are also recovered.
The elemental analysis provided the following results:

	found:	C% 62.47	H%	4.95	N% 5.84
for $C_{13}H_{12}ClNO_2$	calc.:	C% 62.53	H%	4.84	N% 5.61

^{1}H-NMR ($CDCl_3$) δ: 9.34 (s, broad, 1H, NH); 8.20-7.96 (m, 2H, arom); 7.82-7.56 (m, 3H, arom); 4.13 (q, 2H, J= 6.0, NCH_2); 3.79 (t, 2H, J= 6.2, CH_2Cl); 2.39 (quintet, 2H, J= 6.2, $CH_2CH_2CH_2$).

2-(4-Chlorobutylamino)-1,4-naphtoquinone (d3)

To a suspension of 2-(4-hydroxybutylamino)-1,4-naphtoquinone (**c3**) (0.86 g; 3.5 mmol) in 25 mL of anhydrous $CHCl_3$, a solution of thionyl chloride (2.09 g; 17.5

mmol; d = 1.631; 0.93 mL) in 5 mL of anhydrous $CHCl_3$ is added dropwise. The reaction mixture is heated under reflux for 5 hours to obtain a dark red-coloured solution. After cooling, 15 mL of very cold 10% $NaHCO_3$ solution are added. The organic phase is separated in a separating funnel, washed with very cold water and then dried over Na_2SO_4, filtered and evaporated to dryness. The product obtained as a red foam (0.57 g) is purified by chromatography on alumina (1:30) eluting with chloroform, to furnish 0.36 g of a dark red-coloured solid with a mp = 142-143°C. (yield = 39.1%). Moreover, 0.21 g of the starting alcohol are recovered.
The elemental analysis provided the following results:

	found:	C% 63.45	H%	5.58	N% 5.19
for $C_{14}H_{14}ClNO_2$	calc.:	C% 63.76	H%	5.35	N% 5.31

^{1}H-NMR ($CDCl_3$) δ: 9.29 (s, broad, 1H, NH); 8.20-7.93 (m, 2H, arom); 7.80-7.55 (m, 3H, arom); 3.95 (t, 2H, J= 6.4, NCH_2); 3.65 (q, 2H, J= 6.0, CH_2Cl); 2.16-1.84 (m, 4H, $CH_2CH_2CH_2CH_2$).

3-Chloro-2-(2-chloroehylamino)-1,4-naphtoquinone (d4)

To a suspension of 3-chloro-2-(2-hydroxyethylamino)-1,4-naphtoquinone (**c4**) (0.88 g; 3.5 mmol) in 25 mL of anhydrous $CHCl_3$, a solution of thionyl chloride (1.67 g; 14 mmol; d = 1.631; 1.02 mL) in 5 mL of anhydrous $CHCl_3$ is added dropwise. The reaction mixture is heated under reflux for 4 hours to obtain a dark red-coloured solution. After cooling, 15 mL of very cold 10% $NaHCO_3$ solution are added. The organic phase is separated in a separating funnel, washed with very cold water and then dried over Na_2SO_4, filtered and evaporated to dryness. The product obtained as a red foam (0.98 g) is purified by chromatography on alumina (1:30) eluting with chloroform, to furnish 0.84 g of a dark red-coloured solid with a mp =166–168°C. (yield= 88.4%).
The elemental analysis provided the following results:

	found:	C% 53.62	H%	3.73	N% 4.88
for $C_{12}H_9Cl_2NO_2$	calc.:	C% 53.36	H%	3.36	N% 5.19

^{1}H-NMR ($CDCl_3$) δ: 8.25-7.98 (m, 2H, arom); 7.83-7.56 (m, 2H, arom); 6.24 (s, broad, 1H, NH); 4.04 (q, 2H, J= 6.6, OCH_2); 3.67 (t, 2H, J= 6.2, NCH_2).

3-Chloro-2-(3-chloropropylamino)-1,4-naphtoquinone (d5)

To a suspension of 3-chloro-2-(3-hydroxypropylamino)-1,4-naphtoquinone (**c5**) (0.93 g; 3.5 mmol) in 25 mL of anhydrous $CHCl_3$, a solution of thionyl chloride (1.67 g; 14 mmol; d = 1.631; 1.02 mL) in 5 mL of anhydrous $CHCl_3$ is added dropwise. The reaction mixture is heated under reflux for 5 hours to obtain a dark red-coloured solution. After cooling, 15 mL of very cold 10% $NaHCO_3$ solution are added. The organic phase is separated in a separating funnel, washed with very cold water and then dried over Na_2SO_4, filtered and evaporated to dryness. The product obtained as a red foam (1.04 g) is purified by chromatography on silica gel (1:30) eluting with chloroform, to provide 0.91 g of a dark red-coloured solid with a mp = 134–135°C. (yield = 91.9%).

The elemental analysis provided the following results:

	found:	C% 55.06	H%	3.70	N% 4.90
for $C_{13}H_{11}Cl_2NO_2$	calc.:	C% 54.95	H%	3.90	N% 4.93

^{1}H-NMR ($CDCl_3$) δ: 8.26-7.98 (m, 2H, arom); 7.84-7.58 (m, 2H, arom); 6.19 (s, broad, 1H, NH); 4.06 (q, 2H, J= 6.8, OCH_2); 3.68 (t, 2H, J= 6.2, NCH_2); 2.30-2.08 (m, 2H, $CH_2CH_2CH_2$).

3-Chloro-2-(4-chlorobutylamino)-1,4-naphtoquinone (d6)

To a suspension of 3-chloro-2-(4-hydroxybutylamino)-1,4-naphtoquinone (**c6**) (0.9 g; 3.2 mmol) in 25 mL of anhydrous $CHCl_3$, a solution of thionyl chloride (1.52 g; 12.8 mmol; d = 1.631; 0.94 mL) in 5 mL of anhydrous $CHCl_3$ is added dropwise. The reaction mixture is heated under reflux for 5 hours to obtain a dark red-coloured solution. After cooling, 15 mL of very cold 10% $NaHCO_3$ solution are added. The organic phase is separated in a separating funnel, washed with very cold water and then dried over Na_2SO_4, filtered and evaporated to dryness. The product obtained as a red foam (0.91 g) is purified by chromatography on alumina (1:30) eluting with chloroform, to provide 0.79 g of a dark red-coloured solid with a mp = 125–126°C. (yield= 83.2%).

The elemental analysis provided the following results:

	found:	C% 56.44	H%	4.18	N% 4.40
for $C_{14}H_{13}Cl_2NO_2$	calc.:	C% 56.40	H%	4.39	N% 4.70

^{1}H-NMR ($CDCl_3$) δ: 8.25-7.98 (m, 2H, arom); 7.82-7.58 (m, 2H, arom); 6.11 (s, broad,

1H, NH); 3.92 (q, 2H, J= 6.8, OCH_2); 3.62 (t, 2H, J= 6.0, NCH_2); 2.04-1.78 (m, 4H, $CH_2CH_2CH_2CH_2$).

2-(2-Cytisinylethylamino)-1,4-naphtoquinone (34)

To a solution of cytisine (0.38 g; 2 mmol) in 5 mL of acetonitrile, 0.24 g (1 mmol) of 2-(2-chloroethylamino)-1,4-naphtoquinone (**b34**) are added into a tube, that is closed under nitrogen atmosphere and heated to 100 °C for 24 hours. After cooling, the solid formed is filtered and the organic solution is evaporated to dryness. The residue obtained is dissolved in chloroform and the organic phase is washed with acidic water, dried over Na_2SO_4, filtered and evaporated to dryness, to obtain 0.43 g of red solid which is purified by chromatography on Al_2O_3 (1:25), eluting with $CHCl_3$. The final product consists of 0.25 g of red solid with mp = 153-155 °C. (yield = 64.1%).

The elemental analysis provided the following results:

found: C% 71.22 H% 6.03 N% 10.47

for $C_{23}H_{23}N_3O_3$ calc.: C% 70.93 H% 5.95 N% 10.79

^{1}H-NMR ($CDCl_3$) δ: 8.17-7.92 (m, 2H, arom); 7.84-7.53 (m, 3H, arom); 7.23-7.12 (m, 1H Pyr); 6.43 (dd, 1H, J= 9, 1.2, Pyr); 6.29 (s, 1H, broad, NH); 5.95 (dd, 1H, J= 6,8, 1.2, Pyr); 4.18-3.78 (m, 4H, 2H Q + 2H CH_2N); 3.14-2.88 (m, 3H, 1H Q + 2H CH_2N); 2.71-2.34 (m, 5H, Q); 2.01-1.75 (m, 2H, Q).

2-(3-Cytisinylpropylamino)-1,4-naphtoquinone (35)

To a solution of cytisine (0.38 g; 2 mmol) in 5 mL of acetonitrile, 0.25 g (1 mmol) of 2-(3-chloropropylamino)-1,4-naphtoquinone (**b35**) are added into a tube, that is closed under nitrogen atmosphere and heated to 100 °C for 24 hours. After cooling, the solid formed is filtered and the organic solution is evaporated to dryness. The residue obtained is dissolved in chloroform and the organic phase is washed with acidic water, dried over Na_2SO_4, filtered and evaporated to dryness, to obtain 0.44 g of a red solid which is purified by chromatography on Al_2O_3 (1:25), eluting with $CHCl_3$. The final product consists of a red solid (0.29 g) with mp = 127-129 °C. (yield= 72.5%).

The elemental analysis provided the following results:

found: C% 71.28 H% 6.09 N% 10.19

for $C_{24}H_{25}N_3O_3$ calc.: C% 71.44 H% 6.25 N% 10.41

^{1}H-NMR ($CDCl_3$) δ: 9.16 (s, 1H, broad, NH); 8.16-7.96 (m, 2H, arom); 7.82-7.58 (m, 3H, arom); 7.25-7.16 (m, 1H Pyr); 6.31 (d, 1H, J= 9, Pyr); 5.95 (dd, 1H, J= 6,8, Pyr); 4.18-3.62 (m, 4H, 2H Q + 2H CH_2N); 3.13-2.92 (m, 3H, 1H Q + 2H CH_2N); 2.58-2.27 (m, 5H, Q); 2.01-1.77 (m, 4H, 2H, Q + 2H, $CH_2CH_2CH_2$).

2-(4-Cytisinylbutylamino)- 1,4-naphtoquinone (36)

To a solution of cytisine (0.38 g; 2 mmol) in 5 mL of acetonitrile, 0.26 g (1 mmol) of 2-(4-chlorobutylamino)-1,4-naphtoquinone (**b36**) are added into a tube, that is closed under nitrogen atmosphere and heated to 100 °C for 24 hours. After cooling, the solid formed is filtered and the organic solution is evaporated to dryness. The residue obtained is dissolved in chloroform and the organic phase is washed with acidic water, dried over Na_2SO_4, filtered and evaporated to dryness, to obtain 0.44 g of a red solid, which is purified by chromatography on Al_2O_3 (1:25) eluting with $CHCl_3$. The final product consists of a red solid (0.27 g) with a mp = 108-110 °C. (yield = 64.3%).

The elemental analysis provided the following results:

found: C% 71.74 H% 6.27 N% 9.92

for $C_{25}H_{27}N_3O_3$ calc.: C% 71.92 H% 6.52 N% 10.06

^{1}H-NMR ($CDCl_3$) δ: 9.22 (s, 1H, broad, NH); 8.19-7.95 (m, 2H, arom); 7.80-7.57 (m, 3H, arom); 7.28-7.16 (m, 1H Pyr); 6.37 (d, 1H, J= 7.6, Pyr); 5.99 (dd, 1H, J= 6,6, Pyr); 4.16-3.66 (m, 4H, 2H Q + 2H CH_2N); 3.07-2.84 (m, 3H, 1H Q + 2H CH_2N); 2.50-2.21 (m, 5H, Q); 1.98-1.42 (m, 6H, 2H, Q + 4H, $CH_2CH_2CH_2CH_2$).

2-(2-Cytisinylethylamino)-3-chloro-1,4-naphtoquinone (37)

To a solution of cytisine (0.38 g; 2 mmol) in 5 mL of acetonitrile, 0.27 g (1 mmol) of 3-chloro-2-(2-chloroethylamino)-1,4-naphtoquinone (**b37**) are added into a tube, that is closed under nitrogen atmosphere and heated to 100 °C for 24 hours. After cooling, the solid formed is filtered and the organic solution is evaporated to dryness. The residue obtained is dissolved in chloroform and the organic phase is washed with acidic water, dried over Na_2SO_4, filtered and evaporated to dryness, to obtain 0.47 g of red solid which is purified by chromatography on Al_2O_3 (1:25),

eluting with $CHCl_3$. The final product consists of a red solid (0.29 g) with a mp = 182-183 °C. (yield= 69.1%).

The elemental analysis provided the following results:

	found:	C% 65.02	H%	5.55	N% 9.64
for $C_{23}H_{22}ClN_3O_3$	calc.:	C% 65.17	H%	5.23	N% 9.91

^{1}H-NMR ($CDCl_3$) δ: 8.18-7.94 (m, 2H, arom); 7.79-7.51 (m, 2H, arom); 7.28-7.08 (m, 1H Pyr); 6.46 (dd, 1H, J= 9, 1.4, Pyr); 6.29 (s, 1H, broad, NH); 5.97 (dd, 1H, J= 6,8, 1.2, Pyr); 4.18-3.73 (m, 4H, 2H Q + 2H CH_2N); 3.19-2.85 (m, 3H, 1H Q + 2H CH_2N); 2.76-2.33 (m, 5H, Q); 2.02-1.69 (m, 2H, Q).

2-(2-Cytisinylpropylamino)-3-chloro-1,4-naphtoquinone (38)

To a solution of cytisine (0.38 g; 2 mmol) in 5 mL of acetonitrile, 0.28 g (1 mmol) of 3-chloro-2-(2-chloropropylamino)-1,4-naftoquinone (**b38**) are added into a tube, that is closed under nitrogen atmosphere and heated to 100 °C for 24 hours. After cooling, the solid formed is filtered and the organic solution is evaporated to dryness. The residue obtained is dissolved in chloroform and the organic phase is washed with acidic water, dried over Na_2SO_4, filtered and evaporated to dryness, to obtain 0.51 g of a red solid which is purified by chromatography on Al_2O_3 (1:25) eluting with $CHCl_3$. The final product obtained consists of a red solid (0.33 g) with a mp = 155-157 °C. (yield= 75.0%).

The elemental analysis provided the following results:

	found:	C% 65.90	H%	5.77	N% 9.23
for $C_{24}H_{24}ClN_3O_3$	calc.:	C% 65.82	H%	5.52	N% 9.60

^{1}H-NMR ($CDCl_3$) δ: 8.22-7.96 (m, 2H, arom); 7.81-7.58 (m, 2H, arom); 7.38-7.21 (m, 1H Pyr); 6.42 (dd, 1H, J= 9, 1.4, Pyr); 6.04 (dd, 1H, J= 7, 1.4, Pyr; overlayed s, 1H, broad, NH); 4.22-3.44 (m, 4H, 2H Q + 2H CH_2N); 3.19-2.84 (m, 3H, 1H Q + 2H CH_2N); 2.68-2.23 (m, 5H, Q); 2.18-1.68 (m, 4H, 2H, Q + 2H, $CH_2CH_2CH_2$).

2-(2-Cytisinylbutylamino)-3-chloro-1,4-naphtoquinone (39)

To a solution of cytisine (0.38 g; 2 mmol) in 5 mL di acetonitrile, 0.30 g (1 mmol) of 3-chloro-2-(2-chlorobutylamino)-1,4-naphtoquinone (**b39**) are added into a tube, that is closed under nitrogen atmosphere and heated to 100 °C for 24 hours. After

cooling, the solid formed is filtered and the organic solution is evaporated to dryness. The residue obtained is dissolved in chloroform and the organic phase is washed with acidic water, dried over Na_2SO_4, filtered and evaporated to dryness, to obtain 0.50 g of a red solid which is purified by chromatography on Al_2O_3 (1:25) eluting with $CHCl_3$. The final product consists of a red solid (0.31 g) with a mp = 129-131 °C. (yield= 68.9%).

The elemental analysis provided the following results:

	found:	C% 66.26	H% 6.01	N% 9.07
for $C_{25}H_{26}ClN_3O_3$	calc.:	C% 66.44	H% 5.80	N% 9.30

^{1}H-NMR ($CDCl_3$) δ: 8.15-7.91 (m, 2H, arom); 7.72-7.49 (m, 2H, arom); 7.28-7.12 (m, 1H Pyr); 6.35 (dd, 1H, J= 9, 1.2, Pyr); 5.96 (dd, 1H, J= 6.8, 1.2, Pyr; sovvrapposto s, 1H, broad, NH); 4.12-3.48 (m, 4H, 2H Q + 2H CH_2N); 3.01-2.77 (m, 3H, 1H Q + 2H CH_2N); 2.44-2.12 (m, 5H, Q); 1.94-1.62 (m, 2H, Q); 1-52-1.24 (m, 4H, $CH_2CH_2CH_2CH_2$).

Lupinyl 9*H*-xanthene-9-carboxylate (40)

To a solution of lupinine (0.34 g ; 2 mmol) dissolved in 10 mL of anhydrous toluene, first freshly distilled TEA is added (0.28 mL; 2 mmol; d=0.726; 0.2 g) and then, a solution of the acyl chloride of 9*H*-xanthene-9-carboxylic acid in anhydrous toluene is added dropwise in the opportune amount. The acyl chloride has been prepared by reaction of thionyl chloride with 0.45 g (2 mmol) of the corresponding carboxylic acid. The reaction mixture is heated under reflux for 3 hours. Then, the organic solution is resumed with very cold acidic water; the acidic acqueous solution is washed with ether and then cautiously alkalized with very cold diluted NaOH and eventually extracted with dichloromethane. The organic phase is dried over sodium sulphate and the solvent is evaporated to furnish a pale brown-colored oil that is purified by chromatography on alumina (1: 25) eluting with dichloromethane. The product obtained is a colorless oil (0.62 g). Yield=82.7%

The elemental analysis provided the following results:

	found:	C% 76.53	H% 7.37	N% 3.48
for $C_{24}H_{27}NO_3$	calc.:	C% 76.36	H% 7.21	N% 3.71

^{1}H-NMR ($CDCl_3$) δ: 7.42-7.20 (m, 4H, arom); 7.20-7.03 (m, 4H, arom); 5.03 (s, 1H, CHC(O)); 4.18 (t, 2H, J = 6.8, OCH_2); 2.96-2.77 (m, 2H); 2.63 (t, 2H, J = 6.8, CH_2S);

2.19-1.14 (m, 12H, chinolizidinico).

Ethyl 2-(lupinylthio)acetate (e1)

To a solution of thiolupinine (0.93 g; 5 mmol) and 0.7 mL of freshly distilled TEA (5 mmol; 0.51 g) in 5 mL of absolute ethanol, a solution of 2-bromo-ethylacetate (0.56 mL; 5 mmol; d=1.506; 0.84 g) in 2 mL of absolute ethanol is added dropwise. The solution is heated to 80°C for 2 hours under a slow nitrogen stream. After cooling, the solution is concentrated to dryness under vacuum and the residue is recovered with acidic water. The acidic acqueous solution is first washed with ether and then alkalized and extracted with ether. The organic layer is dried over sodium sulphate and evaporated, providing an oil that is purified by chromatography on alumina (1: 20), eluting with ether, to furnish a colorless oil (1.07 g) Yield: 78.7%.

Ethyl 3-(lupinylthio)propionate (e2)

To a solution of thiolupinine (0.93 g; 5 mmol) and 0.7 mL of freshly distilled TEA (5 mmol; 0.51 g) in 5 mL of absolute ethanol, a solution of ethyl 3-bromopropionate (0.64 mL; 5 mmol; d= 1.412; 0.91 g) in 3 mL of absolute ethanol si added dropwise. The solution is heated to 80° C for 2 hours under a slow nitrogen stream. After cooling, the solution is concentrated to dryness under vacuum and the residue is recovered with acidic water. The acidic acqueous solution is first washed with ether and then alkalized and extracted with ether. The organic layer is dried over sodium sulphate and evaporated, obtaining an oil that is purified by chromatography con alumina (1: 25), eluting with ether. The final product consists in a colorless oil (1.04 g). Yield=72.7%

Ethyl 4-(Lupinylthio)butyrate (e3)

To a solution of thiolupinine (0.93 g; 5 mmol) and 0.7 mL of freshly distilled TEA (5 mmol; 0.51 g) in 5 mL of absolute ethanol, a solution of ethyl 4-bromobutyrate (0.72 mL; 5 mmoli; d= 1.363; 0.98 g) in 3 mL of absolute ethanol is added dropwise. The solution is heated to 80° C for 2 hours under a slow nitrogen stream. After cooling, the solution is concentrated to dryness under vacuum and the residue is resumed with acidic water; the acidic acqueous solution is first washed with ether and then alkalized and extracted with ether. The organic phase is dried over sodium

sulphate and evaporated to obtain an oil that is purified by chromatography on alumina (1: 20), eluting with ether. The final product consists in a colorless oil (1.11 g). Yield= 74.1%

2-(Lupinylthio)ethanol (f1)

To a suspension of $LiAlH_4$ (0.58 g; 15.3 mmol) in 20 mL of anhydrous ether (recently distilled over lithium aluminium hydride), a solution of ethyl 2-(lupinylthio)acetate (**e1**) (1.04 g; 3.8 mmol) in 10 mL of anhydrous ether is added dropwise. During the addition the suspension is cooled externally. After the addition the suspension is heated under reflux for 7 hours, protecting the refrigerating apparatus with a calcium chloride drying tube. Then, the excess of lithium aluminium hydride is decomposed by adding, in this order, 3 mL of water, 3 mL of NaOH 15% and eventually 3 additional mL of water. The suspension formed is then filtered and the precipitate is extracted several times with ether and then filtered again. The filtered portions are reunited and the organic phase is separated, dried over sodium sulphate and evaporated under reduced pressure to obtain a yellow oil that is purified by chromatography on alumina (1: 20), eluting with ether. The final product consists in a colorless oil (0.74 g). Yield: 85.1% of the starting ester.

3-(Lupinylthio)propan-1-ol (f2)

To a suspension of $LiAlH_4$ (0.53 g; 14 mmol) in 20 mL of anhydrous ether (recently distilled over lithium aluminium hydride), a solution of ethyl 3-(lupinylthio)propionate (**e2**) (1 g; 3.5 mmol) in 10 mL of anhydrous ether is added dropwise. During the addition the suspension is cooled externally. After the addition the suspension is heated under reflux for 7 hours, protecting the refrigerating apparatus with a calcium chloride drying tube. Then, the excess of lithium aluminium hydride is decomposed by adding, in this order, 3 mL of water, 3 mL of NaOH 15% and eventually 3 additional mL of water. The suspension formed is then filtered and the precipitate is extracted several times with ether and then filtered again. The filtered portions are reunited and the organic phase is separated, dried over sodium sulphate and evaporated under reduced pressure to obtain a yellow oil that is purified by chromatography on alumina (1: 20), eluting with ether. The final product consists in a colorless oil (0.76 g). Yield= 89.4 of the

starting ester.

4-(Lupinylthio)butan-1-ol (f3)

To a suspension of $LiAlH_4$ (0.55 g; 14.4 mmol) in 20 mL of anhydrous ether (recently distilled over lithium aluminium hydride), a solution of ethyl 4-(lupinylthio)butyrate (**e3**) (1.07 g; 3.6 mmol) in 10 mL of anhydrous ether is added dropwise. During the addition the suspension is cooled externally; after the addition the suspension is heated under reflux for 7 hours protecting the refrigerating apparatus using a calcium chloride drying tube. Then, the excess of lithium aluminium hydride is decomposed by adding, in this order, 3 mL of water, 3 mL of NaOH 15% and finally 3 additional mL of water. The suspension formed is then filtered and the precipitate is extracted several times with ether and filtered again. The filtered portions are assembled and the organic phase is separated, dried over sodium sulphate and evaporated under reduced pressure obtaining a yellow oil that is purified by chromatography on alumina (1: 20) eluting with ether. The final product consists in a colorless oil (0.81 g). Yield= 87.1% of the starting ester.

Ethyl-2-(lupinylthio) 9*H*-xanthene-9-carboxylate (41)

To a solution of 2-(lupinylthio)ethanol (**f1**) (0.34 g; 1.5 mmol) in 10 mL of anhydrous toluene, first 0.21 mL (1.5 mmol; d=0.726; 0.15 g) of freshly distilled TEA are added and then a solution of the acyl chloride of 9*H*-xanthene-9-carboxylic acid in anhydrous toluene is added dropwise in the opportune amount. The acyl chloride has been prepared by the reaction of thionyl chloride and 0.34 (1.5 mmol) of the corresponding carboxylic acid. The mixture is heated under reflux for 4 hours, then the suspension is filtered to remove triethylammonium chloride and the organic solution is extracted with very cold acidic water. The clear solution is cooled, alkalized and extracted with dichloromethane. Then the organic phase is dried over sodium sulphate, the solvent is evaporated to provide a pale brown-colored oil that is purified by chromatography on alumina (1: 30) eluting with dichloromethane. The final product consists in a colorless oil (0.48 g). Yield= 73.8%

The elemental analysis provided the following results:

	found:	C% 71.42	H% 7.36	N% 3.09	S% 7.12
for $C_{26}H_{31}NO_3S$	calc.:	C% 71.36	H% 7.14	N% 3.20	S% 7.33

^{1}H-NMR ($CDCl_3$) δ: 7.43-7.22 (m, 4H, arom); 7.22-7.01 (m, 4H, arom); 5.05 (s, 1H, CHC(O)); 4.21 (t, 2H, J = 7.0, OCH_2); 2.96-2.77 (m, 4H); 2.63 (t, 2H, J = 7.0, CH_2S); 2.19-1.14 (m, 14H, chinolizidinico).

Propyl-3-(lupinylthio) 9*H*-xanthene-9-carboxylate (42)

To a solution of 3-lupinylthiopropanol (**f2**) (0.37 g; 1.5 mmol) dissolved in 10 mL of anhydrous toluene, first freshly distilled TEA is added (0.21 mL; 1.5 mmol; d=0.726; 0.15 g) and then a solution of the acyl chloride of 9*H*-xanthene-9-carboxylic acid in anhydrous toluene is added dropwise in the opportune amount. The acyl chloride has been prepared by reaction of thionyl chloride with 0.34 g (1.5 mmol) of the corresponding carboxylic acid. The reaction mixture is heated under reflux for 4 hours. Then, the suspension is filtered to remove triethylammonium chloride and the organic solution is extracted with very cold acidic water. The clear solution is cooled, alkalized and extracted with dichloromethane. The organic phase is dried over sodium sulphate and the solvent is evaporated furnishing a pale brown-colored oil that is purified by chromatography on alumina (1: 30) eluting with dichloromethane. The final product consists in a colorless oil (0.49 g). Yield= 73.1%
The elemental analysis provided the following results:

	found:	C% 71.89	H% 7.63	N% 2.99	S% 7.45
for $C_{27}H_{33}NO_3S$	calc.:	C% 71.81	H% 7.36	N% 3.10	S% 7.10

^{1}H-NMR ($CDCl_3$) δ: 7.42-7.21 (m, 4H, arom); 7.21-7.02 (m, 4H, arom); 5.01 (s, 1H, CHC(O)); 4.14 (t, 2H, J = 6.2, OCH_2); 2.95-2.51 (m, 4H); 2.32 (t, 2H, J = 6.2, CH_2S); 2.18-1.03 (m, 16H, chinolizidinico).

Butyl 4-(lupinylthio) 9*H*-xanthene-9-carboxylate (43)

To a solution of 3-(lupinylthio)butanol (**f3**) (0.39 g; 2.9 mmol) in 10 mL of anhydrous toluene, first 0.21 mL of freshly distilled TEA (1.5 mmoli; d=0.726; 0.15 g) are added and then a solution of the acyl chloride of 9*H*-xanthene-9-carboxylic acid is added dropwise in the opportune amount. The acyl chloride has been prepared by the reaction of thionyl chloride with 0.34 (1.5 mmol) of the corresponding carboxylic acid in anhydrous toluene. The mixture is heated under reflux for 4 hours, then the suspension is filtered to remove the triethylammonium chloride and the organic

solution is extracted with very cold acidic water. The clear solution is cooled, alkalized and extracted with dichloromethane; the organic layer is dried over sodium sulphate and evaporated to obtain a pale brown-colored oil that is purified by chromatography on alumina (1 : 30), eluting with dichloromethane. The final product consists in a colorless oil (0.54 g). Yield= 77.1%

The elemental analysis provided the following results:

	found:	C% 72.45	H% 7.38	N% 2.82	S% 7.18
for $C_{28}H_{35}NO_3S$	calc.:	C% 72.22	H% 7.58	N% 3.01	S% 6.89

^{1}H-NMR ($CDCl_3$) δ: 7.40-7.22 (m, 4H, arom); 7.22-7.05 (m, 4H, arom); 5.01 (s, 1H, CHC(O)); 4.05 (t, 2H, J = 6.4, OCH_2); 2.96-2.63 (m, 4H); 2.40 (t, 2H, J = 6.4, CH_2S); 2.18-1.10 (m, 18H, chinolizidinico).

N-Lupinyl xanthene-9-carboxylamide (44)

To a solution of aminolupinane (0.34 g; 2 mmol) dissolved in 10 mL of anhydrous toluene, first 0.28 mL (2 mmol; d=0.726; 0.2 g) of freshly distilled TEA are added and then a solution of the acyl chloride of 9*H*-xanthene-9-carboxylic acid in anhydrous toluene is added dropwise in the opportune amount. The acyl chloride has been prepared by reaction of thionyl chloride with 0.42 (2 mmol) of the corresponding carboxylic acid. The mixture is heated under reflux for 3 hours, then the organic solution is resumed with very cold acidic water. The acidic acqueous solution is washed with ether, alkalized with very cold NaOH and then extracted with dichloromethane.

The organic phase is dried over sodium sulphate and the solvent is evaporated to obtain a pale yellow-colored solid that is crystallized from EtOH. The final product consists in 0.67 g of white crystals with mp=156-158 °C. Yield=89.3%.

The elemental analysis provided the following results:

	found:	C% 76.26	H% 7.29	N% 7.24
for $C_{24}H_{28}N_2O_2$	calc.:	C% 76.56	H% 7.50	N% 7.44

^{1}H-NMR ($CDCl_3$) δ: 9.12 (s broad, 1H, NH); 7.45-7.23 (m, 4H, arom); 7.23-6.96 (m, 4H, arom); 4.98 (s, 1H, CHC(O)); 3.37 (t, 2H, J = 6.8, $NHCH_2$); 3.13-2.62 (m, 2H); 2.43 (t, 2H, J = 6.2, CH_2S); 2.15-1.07 (m, 12H, chinolizidinico).

2-(Lupinylthio)acetamide (g1)

0.93 g (5 mmol) of freshly distilled thiolupinine dissolved in 2 mL of EtOH are placed in a tube with 0.95 g (5 mmol) of iodoacetamide dissolved in 4 mL of EtOH. The tube is closed under nitrogen atmosphere and the solution is stirred at r.t. for 3 days. Then the solvent is eliminated and the mixture is resumed with Et_2O and HCl 1N. The ethereal solution is extracted three times with HCl 1N. The acidic acqueous layers are washed with ether and alkalized with NaOH 2N and the formation of milkiness can be observed. The mixture is extracted with dichloromethane and the organic solution is dried over sodium sulphate and evaporated to obtain a solid which is crystallized from anhydrous ether. The final product consists in 0.81 g of crystals with mp= 110-111° C. Yield= 66.9%.

3-(Lupinylthio)propionitrile (g2)

A solution of thiolupinine (0.93 g; 5 mmol) in 2 mL of anhydrous dioxane is placed in a tube with 0.07 mL of Triton B and 0.34 mL (5 mmol) of acrylonitrile in 2 mL of anhydrous dioxane. The tube is closed under nitrogen atmosphere and the mixture is stirred at 60° C for 1 hour. After cooling, the mixture is resumed with ether and extracted with acidic water. The acidic acqueous phase is filtered, markedly alkalized and then extracted repeatedly with ether. The ethereal phase is dried over sodium sulphate, filtered and evaporated to dryness, providing an oil which is purified by chromatography on basic Al_2O_3 eluting with anhydrous ether. The final product consists in an oil that slowly crystallizes furnishing 0.93 g of crystals. Yield = 72.7%

1-Amino-2-(lupinylthio)ethane (h1)

To a suspension of $LiAlH_4$ (0.62 g; 16.1 mmol) in 15 mL of anhydrous THF, a solution of amide (**g1**) (0.78 g; 3.22 mmol) in 20 mL of anhydrous THF is added dropwise. The mixture is maintained under reflux for 72 hours. Then, the excess of $LiAlH_4$ is decomposed with water and NaOH 15%. The doughy solid is filtered, the solution is dried over KOH and evaporated to dryness, providing a brown muddy oil which is distilled by oil vacuum pump: 0.63 g of a colorless oil are obtained at 130-140°C. Yield= 85.1%.

1-Amino-3-(lupinylthio)propane (h2)

To a suspension of $LiAlH_4$ (0.66 g; 17.5 mmol) in anhydrous ether, a solution of cyano derivative (**g2**) (0.9 g; 3.5 mmol) in anhydrous ether is added dropwise. The mixture is maintained under reflux for 72 hours. Then, the excess of $LiAlH_4$ is decomposed with water and NaOH 15%. The doughy solid is filtered, the solution is dried over KOH and evaporated to dryness, providing a yellow oil that is distilled by oil vacuum pump. 0.59 g of a colorless oil are obtained at 110°C. Yield=69.4%

N-[Ethyl-2-(lupinylthio)] 9*H*-xanthene-9-carboxamide (45)

To a solution of 1-amino-2-(lupinylthio)ethane (**h1**) (0.34 g; 1.5 mmol) in 10 mL of anhydrous toluene, first 0.21 mL (1.5 mmol; d=0.726; 0.15 g) of freshly distilled TEA are added and then a solution of the acyl chloride of 9*H*-xanthene-9-carboxylic acid in anhydrous toluene is added, dropwise, in the opportune amount. The acyl chloride has been prepared by reaction of thionyl chloride with the corresponding carboxylic acid (0.34 g; 1.5 mmol). The reaction mixture is heated under reflux for 6 hours; then, the organic solution is resumed with very cold acidic water. The acidic acqueous solution is washed with ether, alkalized with very cold NaOH and extracted with dichloromethane. The organic phase is then dried over sodium sulphate and the solvent is evaporated obtaining a solid which is crystallized from anhydrous ether. The final product consists of a yellow oil (0.46 g) which does not solidify. Yield = 70.8%

The elemental analysis provided the following results:

found: C% 69.05 H% 7.33 N% 5.92 S% 7.17

for $C_{26}H_{32}N_2O_2S \bullet H_2O$ calc.: C% 68.69 H% 7.54 N% 6.16 S% 7.05

^{1}H-NMR ($CDCl_3$) δ: 9.17 (s broad, 1H, NH); 7.46-7.19 (m, 4H, arom); 7.19-6.93 (m, 4H, arom); 4.94 (s, 1H, CHC(O)); 3.25 (t, 2H, J = 6.4, $NHCH_2$); 3.08-2.58 (m, 4H); 2.35 (t, 2H, J = 6.2, CH_2S); 2.21-1.23 (m, 14H, chinolizidinico).

N-[Propyl-3-(lupinylthio)] 9*H*-xanthene-9-carboxamide (46)

To a solution of 1-amino-3-(lupinylthio)propane (**h2**) (0.36 g; 2 mmol) in 10 mL of

anhydrous toluene, 0.21 mL (1.5 mmol; d=0.726; 0.15 g) of freshly distilled TEA are first added, and then a solution of the acyl chloride of xanthene-9-carboxylic acid in anhydrous toluene is added, dropwise, in the opportune amount. The acyl chloride has been prepared by reaction of thionyl chloride with the corresponding carboxylic acid (0.34 g; 1.5 mmol). The reaction mixture is heated under reflux for 6 hours. Then, the organic solution is resumed with very cold acidic water. The acidic acqueous solution is washed with ether, alkalized with very cold NaOH and then extracted with dichloromethane. The organic layer is dried over sodium sulphate and the solvent is evaporated, furnishing a solid that crystallizes from anhydrous ether. The final product consists of 0.42 g of a yellow oil that does not solidify. Yield= 62.7%

The elemental analysis provided the following results:

found: C% 68.15 H% 7.68 N% 5.77 S% 7.21

for $C_{27}H_{34}N_2O_2S \bullet 1.5H_2O$ calc.: C% 67.89 H% 7.81 N% 5.87 S% 6.71

^{1}H-NMR ($CDCl_3$) δ: 9.11 (s broad, 1H, NH); 7.48-7.18 (m, 4H, arom); 7.18-6.92 (m, 4H, arom); 4.89 (s, 1H, CHC(O)); 3.42 (t, 2H, J = 6.8, $NHCH_2$); 3.01-2.24 (m, 7H); 2.18-0.96 (m, 15H, chinolizidinico).

N-Cytisinyl 9*H*-xanthene-9-carboxamide (47)

To a solution of 9*H*-xanthene-9-carboxylic acid (0.45 g; 2 mmol) and diphenyl diphosphoryl azide (0.66 g; 2.4 mmol; d = 1.277; 0.52 mL) in 4 mL of anhydrous dimethylformamide, a solution of cytisine (0.76 g; 4 mmol) in 3 mL of anhydrous dimethylformamide is added dropwise. The mixture is heated to 100° C for 4 hours. After cooling, the organic solution is diluted with 20 mL of chloroform and extracted thoroughly (6 x 15 mL) with water. The organic solution is dried over Na_2SO_4, filtered and evaporated to dryness. The product obtained is purified by chromatography on SiO_2, eluting with chloroform, furnishing a solid that is crystallized from acetone. The final product consists in 0.71 g of white crystals with mp= 284-285° C. Yield= 88.8%.

The elemental analysis provided the following results:

found: C% 75.25 H% 5.62 N% 6.88

for $C_{25}H_{22}N_2O_3$ calc.: C% 75.36 H% 5.57 N% 7.03

^{1}H-NMR ($CDCl_3$) δ: 7.43-6.92 (m, 9H, 8 arom + 1H, Pyr); 6.84 (d, 1H, J = 7.6, Pyr); 6.42 (d, 1H, J = 9.0, Pyr); 5.37 (s, 1H, CHC(O)); 3.84-3.58 (m, 1H, cit); 3.03-2.74 (m, 3H, cit); 1.88-1.60 (m, 6H, cit).

N-(3-Nitrocytisinyl) 9*H*-xanthene-9-carboxamide (48)

To a solution of 9*H*-xanthene-9-carboxylic acid (0.23 g; 1 mmol) and diphenyl diphosphoryl azide (0.33 g; 1.2 mmol, d = 1.277; 0.26 mL) in 4 mL of anhydrous dimethylformamide, a solution of 3-nitrocytisine (0.47 g; 2 mmol) in 3 mL of anhydrous dimethylformamide is added dropwise. The mixture is heated to 100° C for 4 hours; then, after cooling, the organic solution is diluted with 20 mL of chloroform and extracted thoroughly (6 x 15 mL) with water. The organic layer is dried over Na_2SO_4, filtered and evaporated to dryness, obtaining a mixture that is purified by chromatography on SiO_2 , eluting with chloroform. The solid obtained is crystallized from acetone, providing 0.36 g of yellow crystals with mp > 300 °C. Yield= 81.8%.

The elemental analysis provided the following results:

found: C% 67.81 H% 4.91 N% 9.25

for $C_{25}H_{21}N_3O_5$ calc.: C% 67.71 H% 4.77 N% 9.48

Biological Materials and Methods

The synthesized compounds were tested to determine their IC_{50} values (half maximal inhibitory concentration), the concentration able to inhibit 50% of the measured activity. The essays were performed using 5-7 concentrations in duplicate, in a range between 100-0.01 μM, and then the value was calculated by a non-linear regression of a response /log(concentration) curve, using the GraphPad Prism® v. 5.01 software (La Jolla, CA, USA). The values were obtained as the average of three independent experiments.

Cholinesterase inhibition test

The *in vitro* inhibition tests on torpedo AChE (463 U/mg) and BChE obtained from equine serum (13 U/mg) were carried out using a phosphate buffer 0.1 M, pH 8.0. Acetyl- and butyryl-choline iodides were utilized, respectively, as substrates while 5,5'- dithiobis(2-nitrobenzoic acid) (DTNB) was used as a chromophore reagent. [45] The inhibition tests were performed using a spectrophotometer UV-visibile Agilent 8453 E.

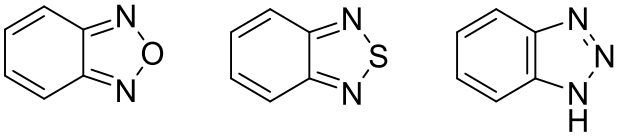
The solution of the tested compounds were prepared starting from standard solution 10 mM in DMSO, diluted with water to achieve a final amount of organic solvent lower than 1%. The inhibitory activity on AchE was determined using a reaction mixture of 100 µL of AChE solution (0.9 U/mL in phosphate buffer 0.1 M, pH 8.0), 100 µL of a 3.3 mM DTNB solution in phosphate buffer 0.1 M (pH 7.0) containing $NaHCO_3$ 6 mM, 100 µL of the inhibitor solution (six or seven concentration ranges between 1x10-8 and 1x10-4 M), and 600 µL of buffer solution. After incubation at 25°C for 20 minutes, acetylcholine iodide (100 µL of 5 mM acqueous solution) was added to the substrate, and the hydrolysis catalyzed by AChE was monitored by measuring the increase in absorbance at 412 nm for 5 minutes at 25°C. The inhibitory activity on BChE was evaluated in a similar way, using butyrylcholine iodide as substrate.

Section 2 : Benzotriazole Derivatives and Benzotriazole-Colchicine Hybrids

Background : Benzotriazoles historical background and chemistry

The search for original chemical structures and the the need of new and efficient syntheses to obtain them, has directed research towards heterocyclic structures. It has been observed, in particular, that nitrogen-based heterocycles suitably substituted exhibit numerous biological and pharmacological activities. A considerable attention has been directed to benzo-condensed azoles containing three heteroatoms, based on their significant activities and properties in different fields. Within this category, the most studied compounds are benzo[1,2-

c][1,2,5]oxadiazole (**XXXVIII**), benzo[1,2-c][1,2,5]thiadiazole (**XXXIX**) and 1*H*-benzo[d][1,2,3]triazole (**XL**). (Bhardwaj B. et al., 2013)



XXXVIII **XXXIX** **XL**

Among these heterocycles, benzotriazole has demonstrated several pharmacological activities, such as antitumor, antifungal, antibacterial, anti-tuberculosis, antiviral, antiparasitic and antioxidant effects (Ren Y. et al., 2014), in addition to the choleretic action (Paglietti G. et al., 1994), antiprotozoal activity (Lopez-Vallejo F. et al., 2011) and a regulator action on plant growth. (Davis D. et al., 1954; Sparatore F. et al., 1978) Benzotriazole, moreover, is characterized by a high stability, thus it can easily be inserted into other molecules through a variety of reactions during which it remains sufficiently stable; it is also inexpensive and devoid of high toxicity.(Bhardwaj B. et al., 2013)

The benzotriazole nucleus is characterized by two fused cycles and the 5-membered one can exist in 2 tautomeric forms, thus permitting to isolate the N-substituted derivatives of both the tautomers (Fig. 8). (Sease C., 1978)

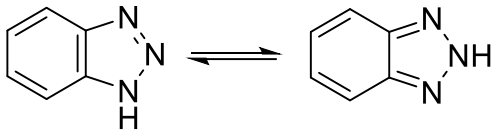
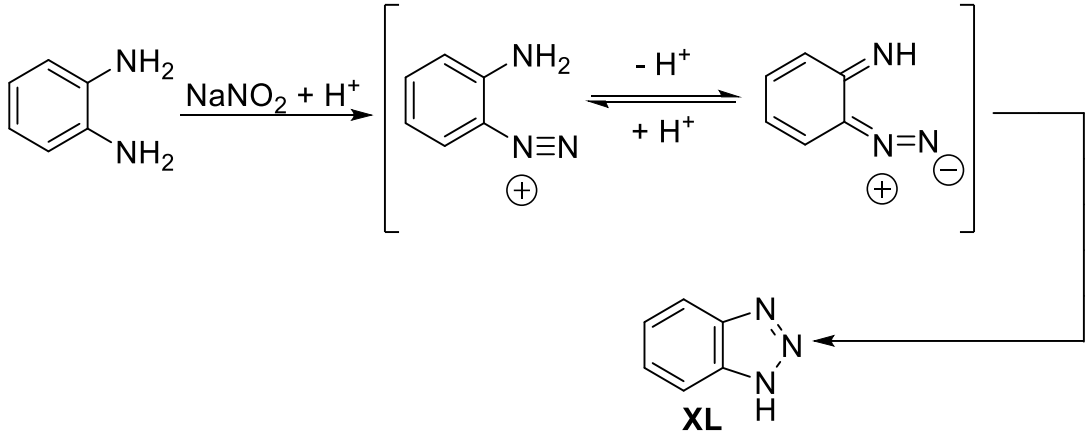


Fig.8 Tautomeric forms of benzotriazole nucleus.

The presence of a benzene ring fused with an aromatic heterocycle confers a conjugate system suitable to form π-π stacking interactions within the benzotriazole structure. Moreover, the nitrogen atoms facilitate the formation of hydrogen and coordination bonds, permitting benzotriazole and its derivatives to bind receptors and enzymes in biological systems through different non-covalent interactions that lead to interesting biological activities. Benzotriazole can also interact with metal ions allowing the formation of complexes that may act both as supramolecular agents and benzotriazole derivatives; such behavior could determine a dual mechanism of action able, for example, to circumvent pharmacological resistance. (Piccionello AP. et al., 2010)

For all these reasons, benzotriazole is studied and used for the development of potential innovative drugs. (Singh G. et al., 2013)

In the literature several synthetic pathways are described, but the benzotriazole nucleus can be easily obtained by the reaction between the opportune *o*-phenylenediamines, sodium nitrite and acetic acid performing a diazotization on one of the two aminic group. In this case, the reaction has been found to be promoted by low operating temperatures (5-10°C), possibly held for a short time in an ultrasound bath. (Robert A., 2002)



The benzotriazole structure, however, is extremely versatile and has demonstrated numerous applications. It is in fact used as a synthetic auxiliary (Kale R. et al., 2010; Katritzky A.R. et al., 2003) and as a good leaving group in the case of reaction with carbonyl groups. (Katritzky A.R. et al., 2000; Katritzky A.R. et al., 2003) In addition, N-acylbenzotriazoles can be used as easily manageable acylating agents, able to implement N-, O-, C- and S-acylations. (Paglietti G. et al., 1994) Moreover, benzotriazole acts as an electron-donor substitute and as a radicals and carbanions precursor. The benzotriazole moiety can be easily inserted in other chemical structures by reaction mechanisms of condensation, addition and alkylation. (Katritzky A.R et al., 1995; Katritzky A.R. et al., 2000)

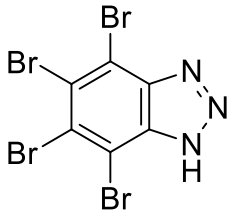
Due to this synthetic versatility, several benzotriazole derivatives endowed with important pharmacological activities, lower toxicity, few side effects, good water solubility and promising bioavailability have been synthesized and discovered. (Milosevic NP. et al., 2013).

Antiproliferative activity

As mentioned above, various benzotriazole derivatives have demonstrated an anticancer activity. Even the simple 1*H*-benzo[d][1,2,3]triazole showed a certain antiproliferative activity. (Briguglio I. et al., 2015)

Among the many substances studied, two benzotriazole compounds have entered clinical trials for some tumor forms:

- Vorozole, a competitive aromatase inhibitor
- 4,5,6,7-tetrabromobenzotriazole (TBB, **XLI**), a protein kinase CK2 inhibitor (Bhardwaj B. et al., 2013; Ren Y. et al., 2014)



XLI

MECHANISMS OF ACTION OF ANTITUMOR BENZOTRIAZOLES

The benzotriazole nucleus can be used as a scaffold for the production of derivatives that exert an antitumor activity. The mechanisms of action which have been studied so far are various, and are briefly mentioned, with some examples, in the following paragraphs.

BENZOTRIAZOLES AS PROTEIN KINASE CK2 INHIBITORS

The development of TBB and its remarkable CK2-mediated antitumor activity have led to the research of new benzotriazole molecules that target the inhibition of various kinases. Indeed, kinases represent a good biological target for the development of antitumor drugs, as they are involved in the cellular proliferation process. (Litchfield D.W. et al., 2003)

Casein kinase (CK2) is a highly preserved pleiotropic protein, a highly active serine/ threonine and tyrosine kinase, (Bian Y. et al., 2013) it is involved in several metabolic processes (Briguglio I. et al., 2015) and it is able to phosphorylate regulatory proteins; to date, about 450 different kinases localized in the cytoplasm and in the nucleus are known; (Ahmed K. et al. 2002) several of them are signaling proteins and 60 are transcriptional factors. (Chojnacki K. et al., 2017)

It is assumed that CK2 is involved in cell growth in both healthy cells and cancerous cells (Ghavidel A. et al., 2001), as well as in tRNA and mRNA synthesis (Murray B.W. et al., 2015), exerting a key role in maintaining normal cellular functions such

as cell growth, cell cycle control, proliferation, differentiation, migration, cell survival and apoptosis induction.

The importance of the kinases category is confirmed by the finding that the damages to specific kinase activities are related to approximately 400 human diseases with proliferative or inflammatory characteristics, such as cancer. (Pyerin W. et al., 2001)

In CK2 it is possible to identify two catalytic subunits (α and α') and two regulatory subunits (β and β'), which can join to form a tetrameric complex or exist as individual subunits; moreover, the tetrameric complexes can assemble to form more complex structures. (Pyerin W. et al., 2003; Meggio F. et al., 1990)

Many experimental evidences suggest that different molecular forms of CK2 may be involved in different cellular activities. Some substrates, for example, can only be phosphorylated by the tetrameric CK2 variant, while others may be phosphorylated only by the free catalytic subunits. (Bibby A.C. et al., 2005)

Moreover, proteins able to interact with and discriminate between the tetrameric form and the individual subunits have been identified. (Volodina L. et al. 2012)

It can be elicited from the scientific literature that this kinase exerts an anti-apoptotic activity; this may interfere with the different pathways of cell survival. This is demonstrated by the fact that CK2 determines a down regulation of various pro-apoptotic proteins, such as caspases. (Pinna L.A. et al., 1997) It has also been demonstrated that the cancer onset and the neoplastic evolution are directly related to CK2 activity. (Seldin D.C. et al., 1995) Moreover, CK2 emerged to be overexpressed within cancerous and leukemic cells. (Faust R.A. et al., 1996; Hessenauer A. et al., 2011)

CK2 has a remarkable expression in the tumor cells, where it exerts a role of protection from apoptosis, therefore the inhibition of CK2 induces a programmed cell death. For these reason this kinase represent a good target for new anti-cancer molecules. (Battistutta R.et al., 2001)

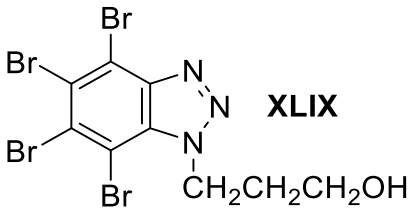
TBB exerts a selective inhibition against CK2, thereby leading to a pro-apoptotic effect on several tumor cell lines. (Szyszka R. et al., 1995; Pagano M. A. et al., 2008) This compound, indeed, forms a bond with its substrate in a different way than the other inhibitors. (Sarno S. et al., 2001) This mechanism was further confirmed by the fact that TBB, tested on 33 kinase proteins, was able to inhibit only three of them, two of which fall into the same subfamily. Moreover, within the same protein kinases family, TBB showed a higher selectivity for CK2 (K_i = 0,4 µM) than towards CK1 (K_i = 47 µM).(Battistutta R.et al., 2001) All this information

demonstrated that TBB exerts a selective inhibitor activity only towards CK2. (Briguglio I. et al., 2015)

The interaction between TBB and the α subunity of CK2 has also been studied. The selectivity of TBB towards this kinase is due to the presence of a small hydrophobic groove, where the TBB fits perfectly, within the active site next to the ATP/GTP binding site; the presence of the four bromine atoms permits the formation of the main hydrophobic interactions and the perfect insertion of the molecule in the cavity is allowed by the presence of halogens. (Szyszka R. et al., 1995) However, if the four bromine atoms are replaced with small atoms, such as chlorine atoms, the inhibitory power decreases. (Pagano M. A. et al., 2008)

The small size of the CK2 groove permits the formation of stable hydrophobic bonds; the formation of these stable bonds is prevented in the other kinases, where the hydrophobic pocket is larger; this fact could explain the selective inhibitor action towards CK2 exerted by TBB. The importance of bromine atoms was underlined as a result of the synthesis of all possible mono-, bi- and tribromuric isomers of benzotriazole; their chemico-physical properties have been then evaluated in acqueous medium and the analysis confirmed that hydrophobic and electrostatic interactions are predominant in completely halogenated benzotriazole derivatives. This characteristic promotes the selective inhibition of protein kinase CK2α. (Szyszka R. et al., 1995)

In order to increase inhibitory activity against CK2, TBB derivatives endowed with N-alkyl substituents were synthesized. This study demonstrated that the pharmacological activity depends on the lenght of the alkyl chain, and the propan-1-ol derivative (**XLIX**) showed the best activity (Wasik R. et al., 2012), while longer or shorter alkyl chains result in a drastic decrease in activity. (Briguglio I. et al., 2015)

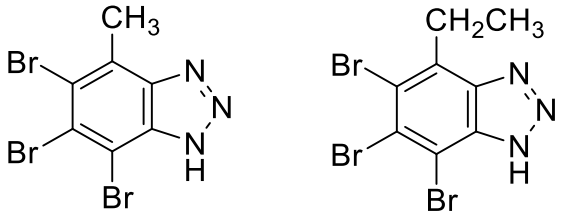


XLIX

Besides of the bromine atoms replacement with chlorine atoms, the substitution with methyl groups was also tested, leading to a lowering of the inhibitory activity against human and non-human CK2. (Briguglio I. et al., 2015)

Moreover, the bromine atoms in 4 and 5 positions were replaced with alkyl and aryl chains. Most of the compounds obtained did not show an increase of the activity

in comparison with 4,5,6,7-tetrabromobenzotriazole (IC_{50} = 0,46 µM); however, a slight increase of the activity could be observed for 2 derivatives: 5,6,7-tribromo-4-methyl-1*H*-benzotriazole (**L**) with an IC_{50} = 0,41 µM and 5,6,7-tribromo-4-ethyl-1*H*-benzotriazole (**LI**) with an IC_{50} = 0.16 µM. (Bretner M. et al., 2008)



L **LI**

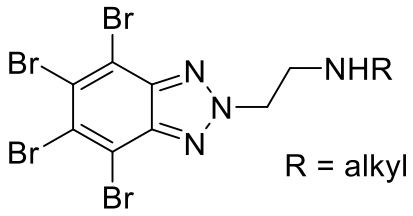
Most of the compounds, tested in assays to evaluate the antitumor activity of tetrabromobenzotriazoles and derivatives, showed to alter the cellular viability in cell line MCF-7 at a concentration of 50 µM.

By examining the effects of tetrabromobenzimidazole, tetrabromobenzotriazole and some their derivatives, it emerged that all the compounds at a concentration of 5 µM were able to inhibit PIM1 kinase and slightly also CK1and PKA, but none of them inhibited ERK1 kinase. In agreement with the experimental results, these analyses demonstrated that 4,5,6,7-tetrabromo-1*H*-benzotriazole inhibits fewer kinases than 4,5,6,7-tetrabromo-1*H*-benzimidazole, thus exerting a more selective action towards the 9 kinases tested. This fact could explain the higher cytotoxicity of the benzimidazole derivative. (Chojnacki K. et al., 2017)

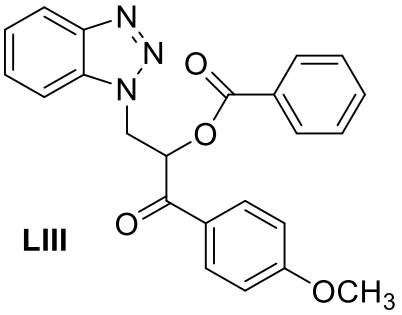
Other studies demonstrated that the introduction of a basic side chain on TBB structure reduces kinase inhibition activity, but maintains or even improves the cytotoxic activity, probably owing to a better permeability compared to the unsubstituted compound.

It was also found that the molecule permeability can be improved by the insertion of an aminoalkyl chain on TBB. Thus, although these compounds exhibit a loss of the CK2 inhibitor activity, some of them demonstrated *in vitro* a similar or higher cytotoxic activity compared to TBB toward leukemia and breast cancer cells. (compound **LII**).

However, some research confirmed that high CK2 expression and activity create a favorable environment for cancer development; this finding justifies the progress of new strategies to inhibit the abnormal CK2 activity and also with the aim to investigate deeply the physiological function of this enzyme. (Makowska M.et al., 2011)



LII



LIII

On the basis of all these results, although sometimes not fully consistent, the research of new benzotriazole derivatives has increased, in order to obtain compounds exerting a higher CK2 inhibitor activity. Among the compounds obtained, the halogen free derivative **LIII** is able to inhibit hepatocarcinoma BEL-7402 cell growth with an IC_{50} = 0,082 μM. (Swider R. et al., 2015)

BENZOTRIAZOLES AS HISTONE DEACETYLASE (HDAC) INHIBITORS

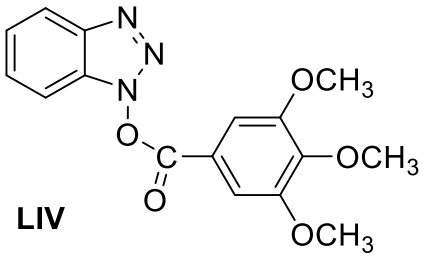
Histone deacetylases are a class of enzymes that catalyze the removal of an acetyl group from lysine residues; in particular they act on histones H3 and H4. (Zhang S. et al., 2008) This mechanism appears to be one of the key process that influences cellular function itself, besides of differentiation and proliferation processes. (Grunstein M. et al., 1997) It has been found that the abnormal HDAC functionality is effectively related to carcinogenesis; (Davie J.R. et al., 1998) for this reason small molecules which exert an inhibitor function on this enzyme could be used for their antitumor effects. (Mai A. et al., 2003)

18 human HDACs have been identified and classified into 4 classes depending on sequence homology to the yeast HDACs, subcellular localization and enzymatic activity. (Briguglio I. et al., 2015)

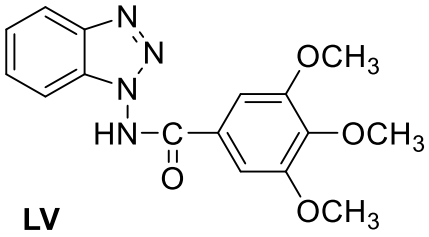
It is also possible to classify HDAC inhibitors into 5 groups, based on their chemical structures (Johnstone R.W. et al., 2002):

1. Hydroxamic acids
2. Cyclic tetrapeptides (apicidin)
3. Short-chain carboxylic acids like valproic acid
4. Benzamides
5. Ketoacids

Several studies detected that some benzotriazole derivatives possess a HDAC inhibitor activity that leads to a subsequent antiproliferative effect. The benzotriazole ring appears to be an essential structure to exert such antitumor activity. (Drummond D.C. et al., 2005) The most investigated derivatives are those substituted with a portion of benzoic acid. (Briguglio I. et al., 2015) Their antitumor action was tested on 3 different cell lines of human tumors, such as oral epidermoid carcinoma cells, non-small cell lung carcinoma H460 cells and stomach carcinoma MKN45 cells. (Ren Y. et al., 2014) The compounds exhibited a good antiproliferative activity (with IC_{50} from 1,2 to 750 nM) except 1*H*-benzo[*d*][1,2,3]triazol-1-il-3,4,5-trimethoxybenzoate (**LIV**) that showed an IC_{50} value between 1,2-2,4 nM, very close to that of the positive control (doxorubicin); (Briguglio I. et al., 2015) it also demonstrated a HDAC inhibitor activity (IC_{50} = 9,4 µg/mL. (Ren Y. et al., 2014)



LIV



LV

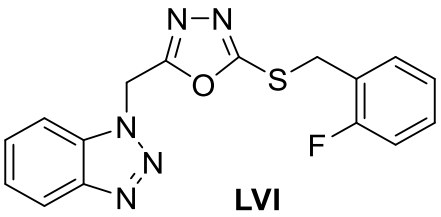
The modifications implemented on this molecule allowed to find out which fragments are essential to exert the activity. The replacement of the ester group with an amidic group led to a benzotriazole derivative (**LV**) with a lower antiproliferative activity. Moreover, through SAR studies, the importance of the OCH_3 groups on the antiproliferative activity has been highlighted. Confirming this finding, derivatives substituted with OCH_3 group at the 3-, 4-, 5-positions possess the highest activity; conversely, substitution with OCH_3 group on the benzotriazolic nucleus leads to a remarkable decrease in antiproliferative effect. (Drummond D.C. et al.,2005) Docking studies on the trimethoxy-derivative confirmed that two oxygen atoms of the ester group are able to form hydrogen bonds with the hydrogen atoms of aminic or amidic groups in His170 and Phe198 residues. Also π-stacking bonds were found between the two benzene rings and Tyr264. Moreover, further hydrophobic bonds between the benzene ring, the benzotriazolic ring and Phe141, Tyr196, Leu265, Lys267 e Tyr297, aminoacids localized in the enzyme binding groove, could form. The hydrogen bonds and the π-π interactions that form between the enzyme and the compound are pivotal and are influenced

by the different polarities and sizes of the substituents localized on the benzene and benzotriazolic rings. (Ren Y. et al., 2014)

BENZOTRIAZOLES AS FOCAL ADHESION KINASE (FAK) INHIBITORS

Focal adhesion kinase (FAK) is a non-receptor tyrosin kinase that exerts a crucial role in cellular proliferation, survival, motility, invasion, metastasization and in angiogenesis. FAK could be a good target for antitumor agents, since its activation can lead to uncontrolled proliferation, cellular survival and migration, namely the same effects characteristic of cancer development and progression. (Ren Y. et al., 2014)

Among the compounds that exhibited a FAK inhibitor activity, benzotriazoles containing 1,3,4-oxadiazole derivatives are included. In this category, 2-((1*H*-benzo[d][1,2,3]triazol-1-yl)methyl)-5-((2-fluorobenzyl)thio)-1,3,4-oxadiazole (**LVI**) showed the highest inhibitor activity toward MCF-7 cell lines with an IC_{50} = 5,68 µg/mL and HT29 cell lines with an IC_{50} = 10,21 µg/mL. (Fu J. et al., 2010) It also demonstrated a FAK inhibitor activity with an IC_{50} from 0,9 to 1,5 µmol/L. (Ren Y. et al., 2014)



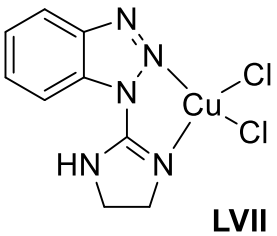
LVI

This compound effectively induced apoptosis in MCF-7 cells. Docking studies let to find out that this compound can interact with the FAK catalytic subunit through low-energy bonds. These research works, in combination with the biological results, suggest that this derivative could act as a FAK inhibitor. (Ren Y. et al., 2014)

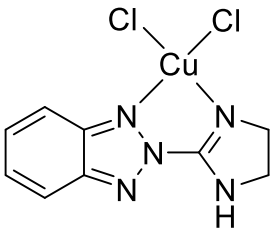
BENZOTRIAZOLES PARTICIPATING IN COORDINATION COMPLEXES

The complexes between transition metal ions and organic ligands could stabilize complexes formed between enzymes and DNA, thus influencing DNA replication and transcription in malignant tumor cells more effectively than the single organic ligand. (Zhang S. et al., 2013) Recently, within this research line, benzotriazole derivatives have been used to form metal complexes with potential antitumor activity. (Yu Ren et al., 2014) Copper complexes between bidentate chelating

ligands were synthesized, constituted by benzotriazoles 2-substituted. (Zhou CH. et al., 2010) Besides, other molecules have been synthesized to potentially imitate superoxide dismutase (SOD) activity; it is considered, indeed, that SOD activity in the cancerous cells is lower than in the healthy cells. (El-Asmy HA. et al., 2014)
These derivatives, that differ solely on the position of the benzotriazole ring, (**LVII**, **LVIII**), have demonstrated respectively a higher and a lower activity on SOD. Therefore, it can be deduced that the biological activity is extremely sensitive to small structural variations of the organic ligand taking part to the coordination complex. (Briguglio I. et al., 2015)

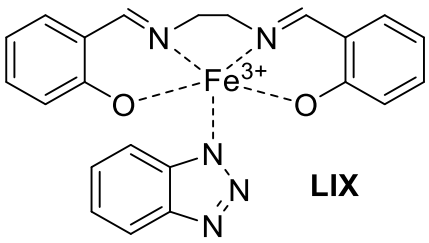


LVII



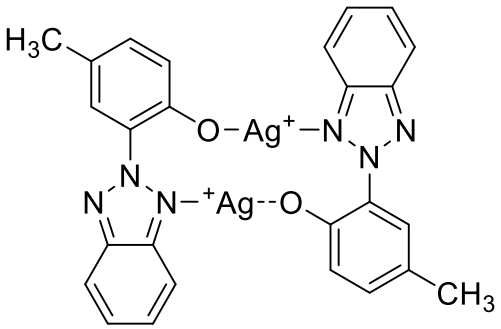
LVIII

The activity of these complexes has been tested on seven human tumor cell lines. The preliminary assays demonstrate that these compounds are able to inhibit the cellular growth on three tumor cell lines (5637, Kyse-520 e SISO) with an IC_{50} of approximately 20 µM. (Bhardwaj B. et al., 2013)
Besides, metal transition complexes often exert other biological activities, such as promoting DNA strand rupture. Finally, the combination of these complexes with benzotriazole derivatives able to form hydrogen bonds could lead to the constitution of polymer complexes exerting a powerful antitumor activity. (Ren Y. et al., 2014)
Compound **LIX**, for example, demonstrated a remarkable antitumor activity toward two human cell lines [chronic myeloid leukemia (K562) and breast adenocarcinoma (MCF7) with, respectively, IC_{50} = 10,9 µg/mL and IC_{50} = 16,9 µg/mL].



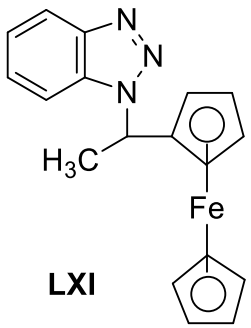
LIX

As a result of further investigations, it was found that this compound stimulates apoptosis by inducing a local imbalance in the hydrogen superoxide/peroxide levels. (Ren Y. et al., 2014)



LX

Benzotriazoles containing the structure of drometrizole and its derivatives are able to interact with metal ions and to exhibit powerful bioactivities. The novel structural silver complex (**LX**) showed an antitumor activity toward human breast cancer cell lines (MDA-MB231) and ovarian cancer cell lines (OVCAR-8) with values of IC_{50} = 14,13 µmol/L and IC_{50} = 13,54 µmol/L, respectively, considerably lower comparing to the reference drug, cisplatin (respectively IC_{50} = 32,0 µmol/L and IC_{50} = 30,86 µmol/L). It is noteworthy that this derivative has a very limited toxicity toward the breast and ovarian healthy cell lines. (Zhou CH., Zhang YY. et al., 2010) Finally, among the synthesized compounds, the ferrocenyl-benzotriazole derivative (**LXI**) showed a stronger inhibitory effect than the reference drug (cisplatin) on several human tumors, such as non-small cell lung cancer, endometrial cancer and esophageal cancer. (El-Asmy HA. et al., 2014) Through numerous studies it has been found that the benzotriazole fragment carries the lipophilic ferrocene, ensuring a good membrane permeability, thus contributing to its high bioactivity. Furthermore, the antitumor effect may be potentiated by the intercalation of the hydrophilic benzotriazole fragment between the DNA nitrogen base pairs and the subsequent formation of hydrogen bonds with the DNA phosphate groups.(Ren Y. et al., 2014)

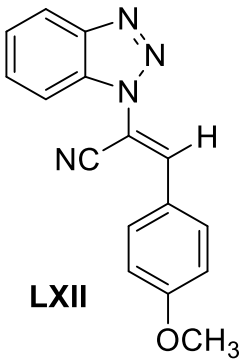


LXI

BENZOTRIAZOLES ACTING ON TUBULIN

Microtubules form the cytoskeleton filaments and therefore play a crucial role in regulating various processes such as cellular shape maintenance, chromosome segregation during mitosis, the membrane-bound organelles arrangement, and transport. For this reason, a strategy devised to inhibit cellular proliferation is to aim at the tubulin-microtubule system. However, the prolonged utilization of molecules that selectively target microtubules has developed as main drawback the drug resistance within the tumor cells. Therefore, the aim of the research has been to find compounds able to inhibit tubulin and at the same time circumvent the cellular drug resistance, thus improving, consequently, the clinical effectiveness of these derivatives. (Ren Y. et al., 2014)

Within a series of 3-aryl-2-(1*H*-benzotriazol-1-yl)acrylonitrile derivatives, synthesized and tested for antimicrobial and antitumor activities, compound **LXII** emerged to be the most active toward MT-4 cells, with a very low cytotoxicity. (Snegur LV. et al., 2008)



LXII

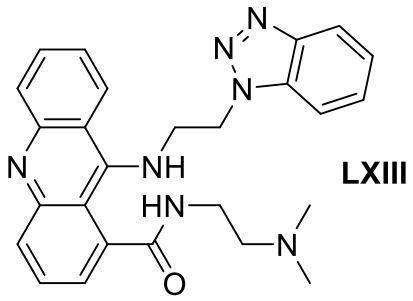
This N-(acrylonitrile)benzotriazole derivative showed remarkable antitumor activities compared to etoposide and a high potential if compared to 6-mercaptopurine. This derivative has been tested toward several human tumor cell lines, included splenic β-lymphoblastoid cells, acute β-lymphoblastic leukemia cells, melanoma cells and breast adenocarcinoma cells, with cytotoxic concentrations (calculated as CC_{50}) from 0,05 to 0,8 μmol/L. (Carta A. et al., 2002) Docking studies disclosed that this compound blocks cellular replication in metaphase, thus inhibiting tubulin polymerization. (Ren Y. et al., 2014)

BENZOTRIAZOLES AS DNA INTERCALATORS

One of the mechanisms of the antitumor drugs is the intercalation between the DNA nitrogen base pairs within the DNA major or minor groove. Even some

benzotriazole compounds are able to intercalate in the DNA. Based on amsacrine activity, a derivative in which the benzotriazole is bound to the aminoacridine nucleus through an opportune linker has been synthesized. (**LXIII**) (Ren Y. et al., 2014) This benzotriazole derivative exhibited a moderate antitumor activity toward a human leukemia cell line (HL60) with an IC_{50} = 23,4 µmol/L (Carta A. et al., 2011).

This compound may be able to bind to the DNA double strand or to inhibit topoisomerase II. However, the exact mechanism of action has yet to be clarified. (Ren Y. et al., 2014)



LXIII

Tumors background

Cancer is recognized as the second cause of death immediately after cardiovascular disease. (Jemal A. et al., 2011; Globocan, Cancer Fact Sheets., 2012) Worldwide, in 2015, 8.8 million deaths were recorded. According to World Health Organization statistics, the 5 globally deadliest types of cancer are:

- Lung cancer with an incidence of 1.69 million deaths
- Liver cancer with an incidence of 788.000 deaths
- Colorectal cancer with an incidence of 774.000 deaths
- Stomach cancer with an incidence of 754.000 deaths
- Breast cancer with an incidence of 571.000 deaths (http://www.who.int/mediacentre/factsheets/fs297/en/)

In Italy, however, 177.000 deaths attributable to cancer has been estimated for 2017.

Also in this case it is possible to identify the cancer types that lead to the greatest number of deaths:

- Lung cancer (20% of the overall cancer deaths)
- Colorectal cancer (11% of the overall cancer deaths)

- Breast cancer (8% of the overall cancer deaths)
- Stomach cancer (6% of the overall cancer deaths)
- Pancreatic cancer (6% of the overall cancer deaths)

Within the basic mechanisms of cancer development it is possible to detect a certain number of genetic modifications affecting somatic cells, which consequently stop responding to the regulatory mechanisms that normally control a healthy organism. The next stage consists in proliferation of these somatic cells and the formation of a neoplastic cell clone that has lost the proliferative control, thus becoming "immortal"; somatic cells, instead, have a normal replicative rate. Another event implicated in the tumor development is the inactivation of the density-dependent inhibition mechanism; the density-dependent inhibition mechanism allows cellular replication until achieving a certain cell density, which is then followed by a state of quiescence. (Briguglio I. et al., 2015) The abnormal proliferation leads the cells to grow beyond their borders, occupying the surrounding tissues; the invasion of other organs through a process called metastasization is one of the major causes of cancer death. (http://www.who.int/mediacentre/factsheets/fs297/en/.,2017)

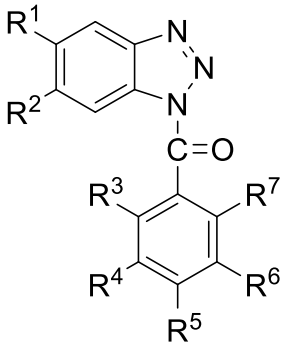
There therapeutic approaches in use are different: surgery, radiation, immunotherapy and chemotherapy, used alone or in combination, depending on the type of cancer and the stage of disease. (Chojnacki K. et al., 2017)

One of the main goals of chemotherapy is to obtain drugs that can selectively act on cancerous cells without acting on the healthy cells. (Lotfi-Jam K. et al., 2008) Nevertheless, anticancer drugs are not able to discriminate between the cancerous and the healthy cells because of their similarity and, therefore, also somatic healthy cells are exposed to the toxic effect of the chemotherapic drugs. The cells most affected by chemotherapic agents are those that are continually subjected to mitosis, such as the hair follicles, the intestinal epithelium and the bone marrow. (Tang YD. et al., 2012) Therefore, most anticancer drugs are often toxic to healthy tissues, causing numerous side effects that result in a limited efficacy of the treatment. Long-term efficacy is also limited by drug resistance and cumulative dose-dependent cardiotoxicity. (El Rashedy A.A. et al., 2013) For these reasons, the research for new molecules able to act selectively on cancerous cells is still under intensive development; nitrogen-containing heterocycles have been considered particularly interesting, since they are possible isosteres of structural components of natural nucleotides. (Husain A. et al., 2012; Yuan J. et al., 2013).

Chapter 3 : Benzotriazole Derivatives

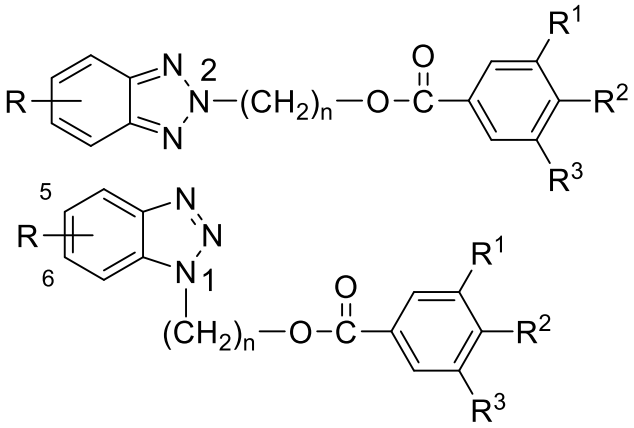
Chemistry discussion

On the basis of data of the literature on compounds LIV, LV and on some benzoic esters,(Tonelli M. et al., 2013) I attended my PhD synthesized two series of benzotriazole derivatives. In particular I synthesized a series of 1-benzoyl-1*H*-benzotriazoles (8 compounds, **49**-**56**) and opportune benzoic esters of 1-(2,3-hydroxyalkyl)-1*H*-benzotriazoles (32 compounds, **57**-**88**).



R^1, R^2 = H
49 R^5 =OCH_3; R^3, R^4, R^6, R^7 = H
50 R^7 = OCH_3; R^3, R^4, R^5, R^6 = H
51 R^3, R^7 = OCH_3; R^4, R^5, R^6 = H
52 R^4, R^6 = OCH_3; R^5, R^3, R^7 =H
53 R^4, R^6 = OCH_3; R^5 = OH, R^3, R^7 =H
54 R^4, R^5, R^6 = OCH_3; R^3, R^7 = H

R^1, R^2 = Cl
55 R^4, R^6 = OCH_3; R^3, R^5, R^7 = H
56 R^4, R^5, R^6 = OCH_3; R^3, R^7 = H



n=2

R=H

57 R^1,R^3,=OCH_3 ; R^2=H isomer 2

58 R^1,R^2,R^3=OCH_3 isomer 2

59 R^1,R^3,=OCH_3 ; R^2=H isomer 1

60 R^1,R^2,R^3=OCH_3 isomer 1

R=Cl

65 R^1,R^2,R^3=OCH_3 isomer 2

66 R^1,R^2,R^3=OCH_3 1,5-disubstituted

67 R^1,R^2,R^3=OCH_3 1,6-disubstituted

R=CF_3

71 R^1,R^2,R^3=OCH_3 isomer 2

72 R^1,R^2,R^3=OCH_3 1,5-disubstituted

73 R^1,R^2,R^3=OCH_3 1,6-disubstituted

R=OCH_3

77 R^1,R^2,R^3=OCH_3 isomer 2

78 R^1,R^2,R^3=OCH_3 1,5-disubstituted

79 R^1,R^2,R^3=OCH_3 1,6-disubstituted

R=(CO)Ph

83 R^1,R^2,R^3=OCH_3 isomer 2

84 R^1,R^2,R^3=OCH_3 1,5-disubstituted

85 R^1,R^2,R^3=OCH_3 1,6-disubstituted

n=3

R=H

61 R^1,R^3=OCH_3 ; R^2=H isomer 2

62 R^1,R^2,R^3=OCH_3 isomer 2

63 R^1,R^3,=OCH_3 ; R^2=H isomer 1

64 R^1,R^2,R^3=OCH_3 isomer 1

R=Cl

68 R^1,R^2,R^3=OCH_3 isomer 2

69 R^1,R^2,R^3=OCH_3 1,5-disubstituted

70 R^1,R^2,R^3=OCH_3 1,6-disubstituted

R=CF_3

74 R^1,R^2,R^3=OCH_3 isomer 2

75 R^1,R^2,R^3=OCH_3 1,5-disubstituted

76 R^1,R^2,R^3=OCH_3 1,6-disubstituted

R=OCH_3

80 R^1,R^2,R^3=OCH_3 isomer 2

81 R^1,R^2,R^3=OCH_3 1,5-disubstituted

82 R^1,R^2,R^3=OCH_3 1,6-disubstituted

R=(CO)Ph

86 R^1,R^2,R^3=OCH_3 isomer 2

87 R^1,R^2,R^3=OCH_3 1,5-disubstituted

88 R^1,R^2,R^3=OCH_3 1,6-disubstituted

The results of the antiproliferative activity of compounds **49**-**64** observed on three tumor cell lines (ovarian adenocarcinoma A2780, lung carcinoma A549, gastric carcinoma HGC-27) and performed by MTT assays showed for compound **64** IC_{50} values in the range 6-13.1 μM and for compound **58** values in the range 0.47-3.14 μM. None of the other compounds reached a 50% inhibition effect percentage at 20 μM concentration and therefore no further IC_{50} value was assessed. On the

basis of these data it has been hypothesized that two structural characteristics are essential to exert a good antiproliferative activity:

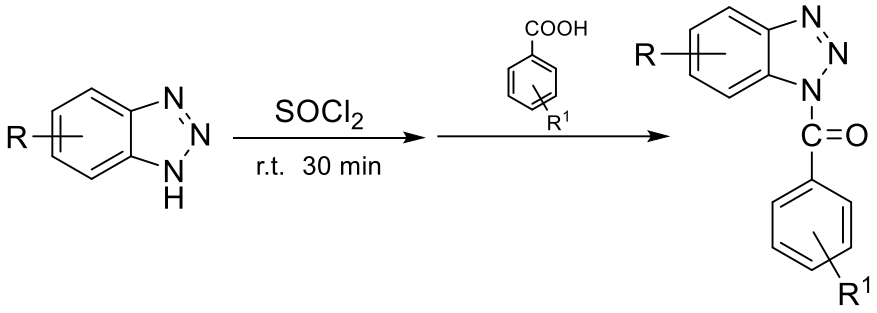
- a linker of two carbon atoms between the benzotriazole nucleus and the benzoic residue

- 3,4,5-trimethoxy substitution on the benzoic ring.

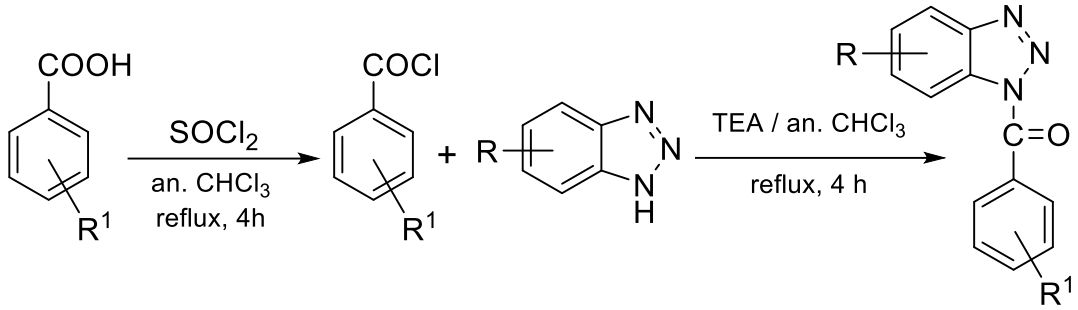
To confirm these preliminary hints and obtain further information, I synthesized other derivatives (**65**-**88**) in which the substituents present on the benzotriazole nucleus are introduced and modified.

In particular I synthesized two series of substituted 1-benzoylbenzotriazoles (8 compounds, **49**-**56**) and substituted benzoic esters of 1-(2,3-hydroxyalkyl)benzotriazoles (32 compounds, **57**-**88**).

The synthesis of compounds **49**-**56** was accomplished according Katritzky procedure (Kale R. et al.,2010) that involves first the reaction between a benzotriazole excess and thionyl chloride (ratio 4:1) in dichloromethane at RT and the subsequent addition of the appropriate carboxylic acid at RT.

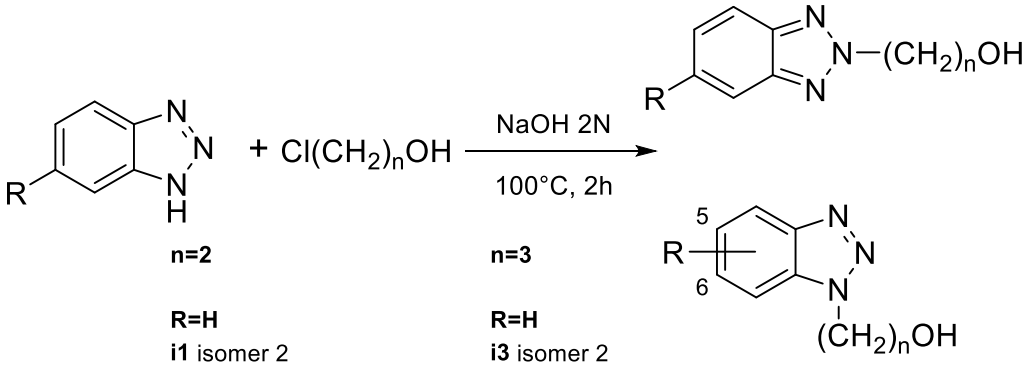


These reaction conditions are suitable for the derivatives of unsubstituted benzotriazole (**49-56**), while proved to be poorly effective for dichlorobenzotriazole, probably owing to dichlorobenzotriazole poor solubility at RT. Compounds **49,50,55,56** were also prepared by reaction between benzotriazole or dichlorobenzotriazole in anhydrous chloroform and the acyl chloride of the carboxylic acid, that in turn is prepared by reaction between the acid and thionyl chloride excess under reflux in anhydrous chloroform. Whereas for the derivatives **49** and **50** no advantages have been achieved, for the dichloroderivatives **55** and **56** the yield significantly increased.



As reported in the literature, in case of acylation of the benzotriazole nucleus only the formation of the 1-isomer occurs. It is noteworthy that ,utilizing the second procedure described, also the reaction between the 5-chloro and the 5-methylbenzotriazole with the acyl chloride of the trimethoxybenzoic acid has been carried out. The mixture of the two 1-isomers (ratio 65:35 calculated through ^{1}H-NMR) has been isolated but, to date, has not been purified, though various attempts of fractionated crystallization and chromatography were attempted. Neither the utilization of flash-chromatography was successfull.

To synthesize the compounds **57**-**88**, first the suitable alcohols **i1**-**i28** were prepared through reaction of the benzotriazole derivatives in alkaline solution with 2-chloroethanol or 3-chloropropanol (Piccionello AP. Et al.,2010). In all the cases of mono-substituted benzotriazoles [R = Cl, CF_3, OCH_3, C(O)Ar], the three possible isomers formed. The isomer 2 was purified through chromatography on alumina, eluting with chloroform, whereas the two 1-substituted isomers were separated through flash-chromatography (silica/ethyl acetate). The reaction with 5-nitrobenzotriazole led to remarkable solubility difficulties in the common organic solvents: the final esters were not synthesized, due to the low yield and the hard purification of the various isomers. All the alcohols have been characterized (melting points, elemental analysis and NMR), since most of them were unknown.



n=2

R=H
i1 isomer 2
i2 isomer 1

R=Cl
i5 isomer 2
i6 isomer 1,5-disubsituted
i7 isomer 1,6-disubsituted

R=CF_3
i11 isomer 2
i12 isomer 1,5-disubsituted
i13 isomer 1,6-disubsituted

R=OCH_3
i17 isomer 2
i18 isomer 1,5-disubsituted
i19 isomer 1,6-disubsituted

R=(CO)Ph
i23 isomer2
i24 isomer 1,5-disubsituted
i25 isomer 1,6-disubsitute

n=3

R=H
i3 isomer 2
i4 isomer 1

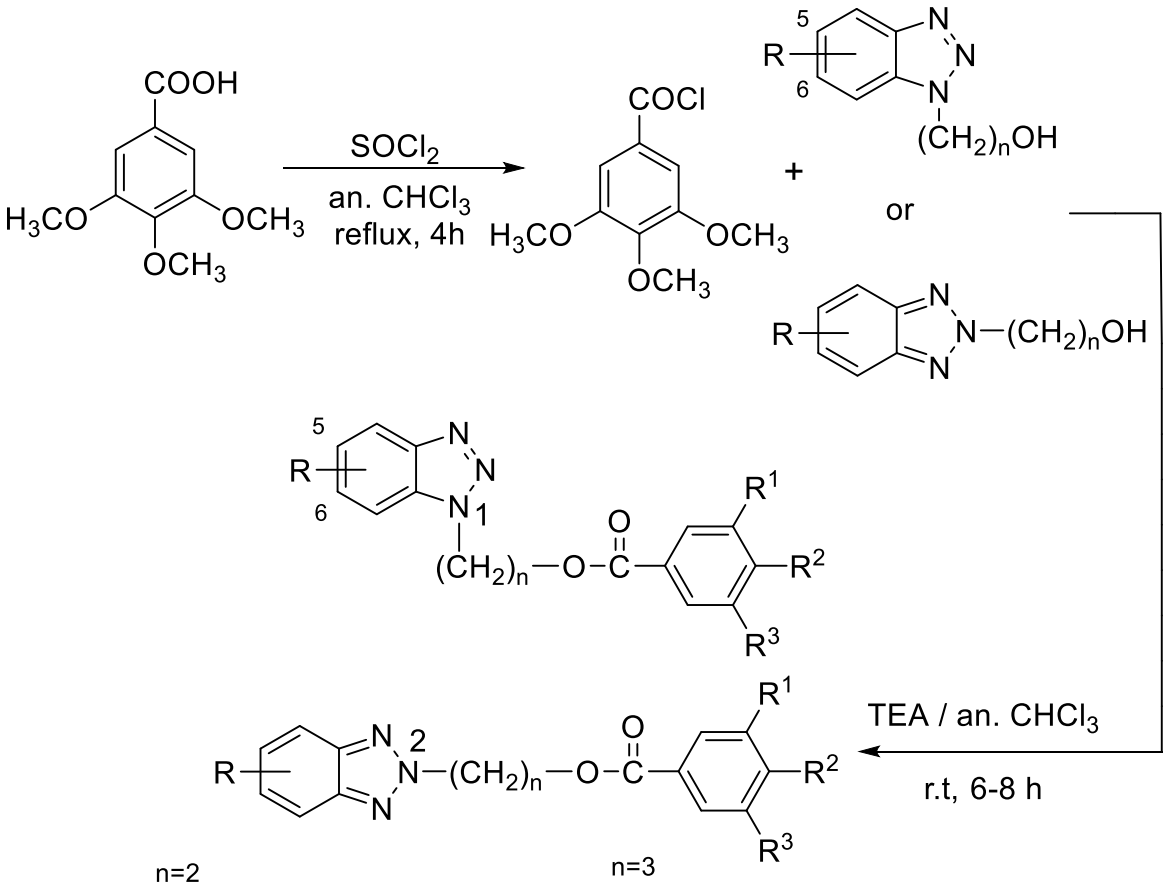
R=Cl
i8 isomer 2
i9 isomer 1,5-disubsituted
i10 isomer 1,6-disubsituted

R=CF_3
i14 isomer 2
i15 isomer 1,5-disubsitutedm
i16 isomer 1,6-disubsituted

R=OCH_3
i20 isomer 2
i21 isomer 1,5-disubsituted
i22 isomer 1,6-disubsituted

R=(CO)Ph
i26 isomer2
i27 isomer 1,5-disubsituted
i28 isomer 1,6-disubsituted

Finally, the alcohols reacted with the acyl chloride of the 3,4,5-trimethoxybenzoic acid, prepared by the reaction of the acid with thionyl chloride excess under reflux in anhydrous chloroform, to obtain the desired esters.



n=2

R=H

57 R^1,R^3,=OCH_3 ; R^2=H isomer 2

58 R^1,R^2,R^3=OCH_3 isomer 2

59 R^1,R^3,=OCH_3 ; R^2=H isomer 1

60 R^1,R^2,R^3=OCH_3 isomer 1

R=Cl

65 R^1,R^2,R^3=OCH_3 isomer 2

66 R^1,R^2,R^3=OCH_3 1,5-disubstituted

67 R^1,R^2,R^3=OCH_3 1,6-disubstituted

R=CF_3

71 R^1,R^2,R^3=OCH_3 isomer 2

72 R^1,R^2,R^3=OCH_3 1,5-disubstituted

73 R^1,R^2,R^3=OCH_3 1,6-disubstituted

R=OCH_3

77 R^1,R^2,R^3=OCH_3 isomer 2

78 R^1,R^2,R^3=OCH_3 1,5-disubstituted

79 R^1,R^2,R^3=OCH_3 1,6-disubstituted

R=(CO)Ph

83 R^1,R^2,R^3=OCH_3 isomer 2

84 R^1,R^2,R^3=OCH_3 1,5-disubstituted

85 R^1,R^2,R^3=OCH_3 1,6-disubstituted

n=3

R=H

61 R^1,R^3=OCH_3 ; R^2=H isomer 2

62 R^1,R^2,R^3=OCH_3 isomer 2

63 R^1,R^3,=OCH_3 ; R^2=H isomer 1

64 R^1,R^2,R^3=OCH_3 isomer 1

R=Cl

68 R^1,R^2,R^3=OCH_3 isomer 2

69 R^1,R^2,R^3=OCH_3 1,5-disubstituted

70 R^1,R^2,R^3=OCH_3 1,6-disubstituted

R=CF_3

74 R^1,R^2,R^3=OCH_3 isomer 2

75 R^1,R^2,R^3=OCH_3 1,5-disubstituted

76 R^1,R^2,R^3=OCH_3 1,6-disubstituted

R=OCH_3

80 R^1,R^2,R^3=OCH_3 isomer 2

81 R^1,R^2,R^3=OCH_3 1,5-disubstituted

82 R^1,R^2,R^3=OCH_3 1,6-disubstituted

R=(CO)Ph

86 R^1,R^2,R^3=OCH_3 isomer 2

87 R^1,R^2,R^3=OCH_3 1,5-disubstituted

88 R^1,R^2,R^3=OCH_3 1,6-disubstituted

Biological discussion

The evaluation of the cytotoxicity of the compounds synthesized has been carried out by Dr. Maurizio Viale and Dr. Marco Ponassi–IRCCS San Martino-.

A screening on three cell lines (A2780 – ovarian carcinoma; A549 – lung adenocarcinoma; HGC27 – gastrc carcinoma) was perfomed for compounds **49**-**64**. A pharmacologically significant antiproliferative activity was defined when the percent inhibition of cell proliferation at 30 μM concentration was > 70%. For the two compounds more active (**58** and **64**) was calculated the IC_{50} values. (Table 4)

Table.4
Antiproliferative activity (MTT assay) expressed as IC_{50} (μM)

Cpds	A2780	A549	HGC27
58	0.47 ± 0.11	3.14 ± 0.64	0.59 ± 0.15
64	6.0 ± 0.9	13.1 ± 1.7	7.3 ± 2.1

The compounds **65**–**76** were tested on two tumor cell lines (ovarian cancer SKOV3 and human breast adenocarcinoma MCF7). (Table 5)

Table.5 Antiproliferative activity expressed
as percent of survival at concentration 10 μM

Cpd	MCF-7	SKOV-3
65	74.94 ± 1.25	61.67 ± 2.33
66	91.93 ± 1.53	100.58 ± 5.18
67	88.34 ± 3.50	101.88 ± 7.83
68	113.39 ± 5.46	102.53 ± 6.76
69	105.22 ± 4.17	102.93 ± 3.45
70	105.61 ± 7.78	96.84 ± 8.10
71	93.93 ± 5.06	117.80 ± 2.36
72	89.44 ± 5.71	112.06 ± 3.20
73	88.99 ± 3.40	110.57 ± 3.34
74	96.35 ± 2.18	106.04 ± 5.70
75	110.38 ± 8.53	104.31 ± 8.28
76	103.66 ± 7.91	109.09 ± 10.28

The compound **65** exerted the better inhibition percentage at a concentration of 10 μM (61.67% on SKOV3 and 74.94% on MCF7). On both the cell lines, the IC_{50} value of this compound will be evaluated.

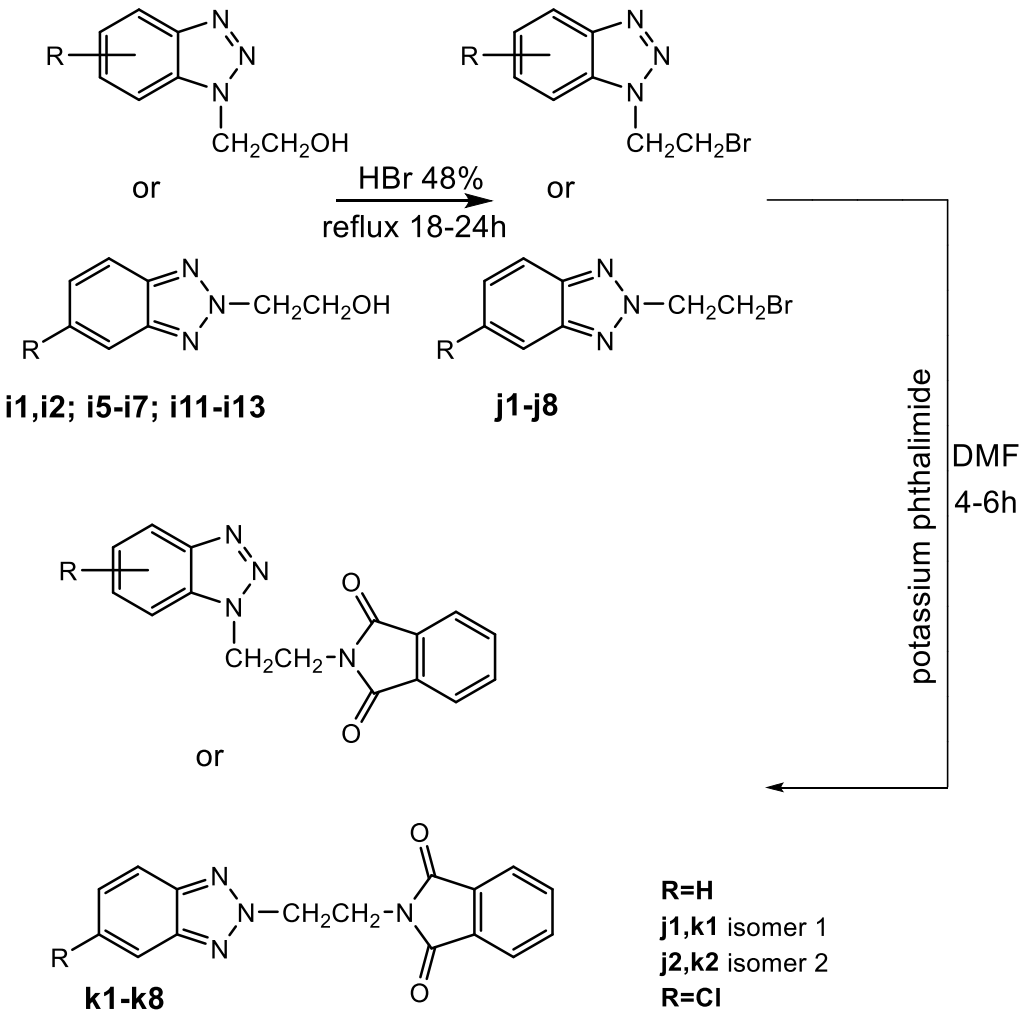
Despite the still limited results, it is confirmed that the linker of two carbon atoms between the benzotriazolic nucleus and the benzoic residue leads to a higher antiproliferative activity

It is noteworthy that the scarce number of active compounds could be determined by an extremely specific mechanism of action rather than an aspecifc cellular activity.
This research line, therefore, aims to identify an appropriate biological target by testing the three most active compounds. Then a rational design could individuate more detailed chemical requirements and thus lead to the synthesis of compounds with improved antiproliferative activity.

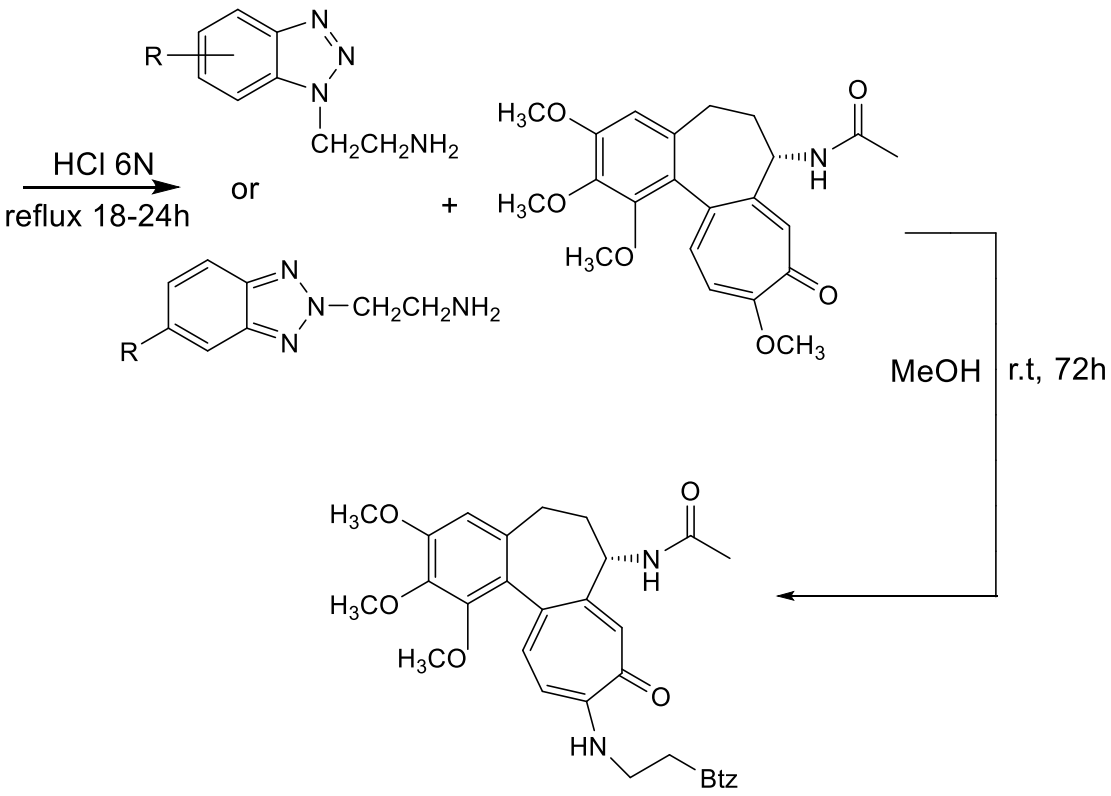
Chapter 4 : Benzotriazole-Colchicine Hybrids

Chemical discussion

In order to obtain compounds with a higher antitumor activity, novel colchicine derivatives characterized by opportunely substituted 1,2,3-benzotriazole portions (**89**-**96**) were devised and synthesized. The synthesis of the compounds **89**-**96** is described below. The alcohols (**i1,i2**, **i5,i6,i7,i12,i13,i11**) used to prepare some of the esters **57**-**88** and separated as previously described, have been treated with HBr 48% under reflux for 18-24 hours. The bromoderivatives obtained (**j1-j8**) have been purified through crystallization from anhydrous Et_2O or through chromatography ($SiO_2/CHCl_3$). Then the Gabriel synthesis was carried out to obtain the corresponding amines. The 2-bromoethyl-1/2 benzotriazoles were thus converted into phtalimide derivatives (**k1**-**k8**) in high yield (> 90%) and subsequently hydrolyzed under reflux by HCl 6N. The products, finally, were obtained through a reaction between the free bases and colchicine in methanol at RT for 72 hours and then purified through flash chromatography ($SiO_2/CHCl_3$: CH_3OH 90:10) (Yields: 50-70%). All the reaction intermediates and the products have been characterized through melting points (for the solid compounds), elemental analysis and ^1H NMR. ^{13}C NMR analysis was carried out only for the final products (**89**-**96**).

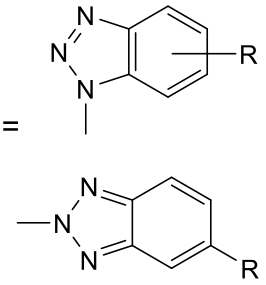


R=H
j1,k1 isomer 1
j2,k2 isomer 2
R=Cl
j3,k3 isomer 1,5-disubsituted
j4,k4 isomer 1,6-disubsituted
j5,k5 isomer 2
R=CF_3
j6,k6 isomer 1,5-disubsituted
j7,k7 isomer 1,6-disubsituted
j8,k8 isomer 2



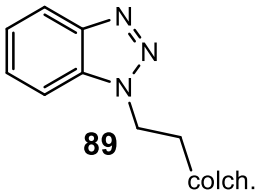
R=H
89 isomer 1
90 isomer 2
R=Cl
91 isomer 1,5-disubsituted
92 isomer 1,6-disubsituted
93 isomer 2
R=CF_3
94 isomer 1,5-disubsituted
95 isomer 1,6-disubsituted
96 isomer 2

Btz =

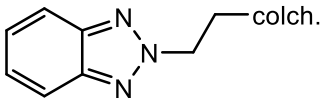


Computational discussion

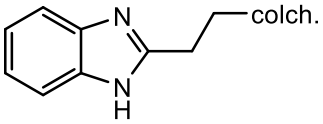
To rationalize SARs, docking studies were carried out on the benzotriazole compounds **89**-**90** and benzimidazole compounds **LVII**-**LVIII** The tubulin-colchicine complex was used as template structure.



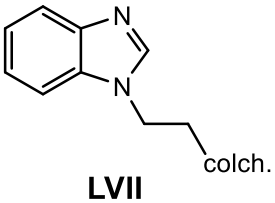
89



90



LVI



LVII

Initially colchicine K_i value was calculated using the docking protocol detailed in the experimental section. The calculation was able to reproduce the binding mode observed in the x-ray structure and the reference compound emerged to interact with tubilin dimer with an estimated K_i value of 9.51 nM. Docking studies were then carried out on benzotriazole derivatives **89** and **90** and benzimidazoles **LVI** and **LVII**. As summarized in Fig. 9, the insertion of a side chain on colchicine structure would cause a rotation in the docking pose, being the alkylated amino group of compounds **89-LVII** placed at equivalent position of the acetamido functionality in the colchicine molecule.

The analysis of the predicted K_i values (table 6) indicated that the modification of the colchicine molecule might forecast an increase of potency, being derivatives **89-LVII** 1.5-9.8-fold more potent than the reference compound. Furthermore, benzotriazole compound **90** was identified to be the most active molecule with an estimated K_i values in the picomolar range.

Cpd	Calculated K_i (nM)
89	6.06
90	0.97
LVI	5.01
LVII	2.01
Colchicine	9.51

Table.6 Predicted K_i values of compounds **89-LVII** and colchicine.

The complex is mainly stabilized by two hydrogen bonds involving the benzotriazole nitrogen atoms and Lys254 (β-tubulin) and Asn101 (α-tubulin) side chains (Fig. 9). The benzotriazole substructure would interact with Asn249, Ser178 and Tyr224; furthermore the ethyl linker would establish van der Waals interactions with Thr179, Ala180 and Glu183 side chains. Further stabilization to the complex is provided by the trimethoxyphenyl substructure that is in contact with Ala316,

Ile318, Val238, Cys241, Leu255 and Asn258.

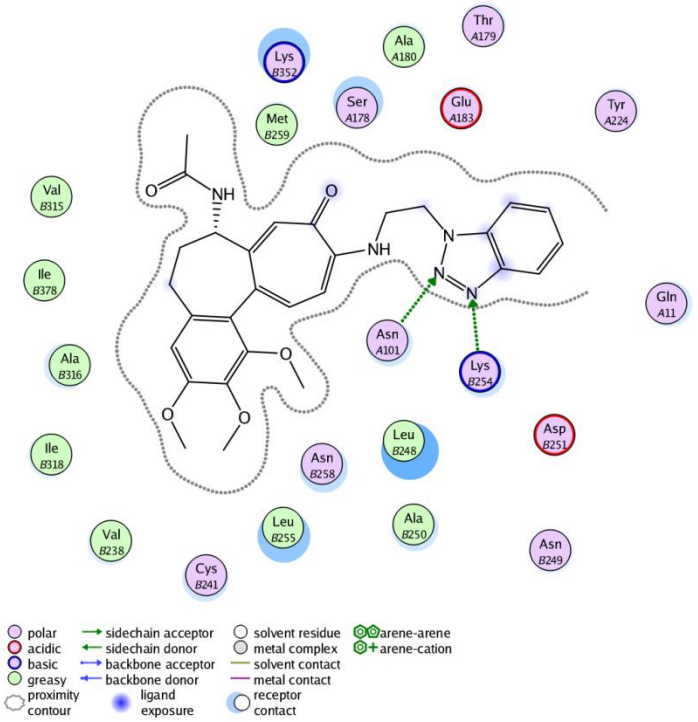
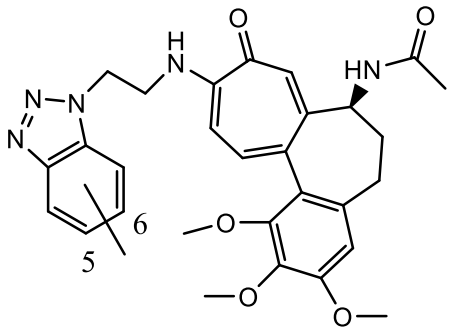


Fig.9 Ligplot of the 2-tubulin dimer complex. Residues of α-tubulin are indicated as A chain whereas β-tubulin is indicated as chain B.

These results indicated the colchicine-benzotriazole conjugates as a promising scaffold to develop novel and potent tubulin inhibitors. In an attempt to orientate the synthesis, a screening through Autodock has been carried out on benzotriazole derivatives bearing Cl (electron-withdrawing group) or OCH_3 (electron-donating group) at position 5 or 6 of the benzotriazole ring. As reported in table 7, for all the tested compounds nanomolar K_i value were calculated and derivative **92** was predicted as the most interesting compound of the series with a predicted K_i value of 0.41 nM.

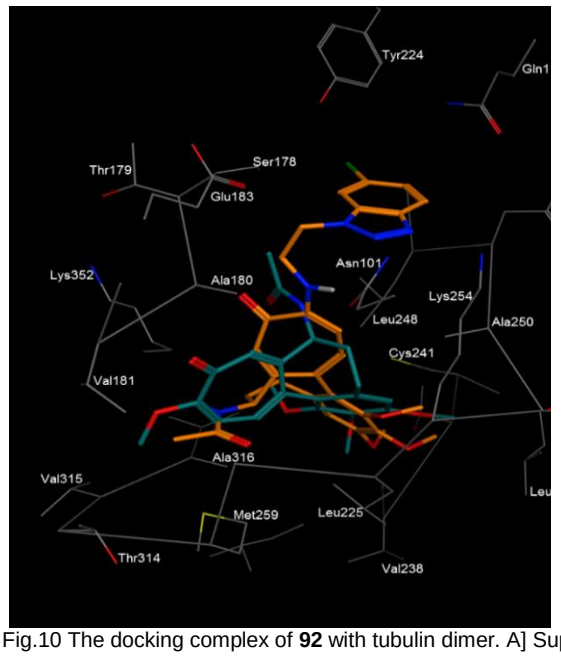


Cpd	R	Calculated K_i (nM)
91	5-Cl	0.59
n.s.	5-OMe	0.69
92	6-Cl	0.41
n.s.	6-OMe	0.62
Colchicine		9.51

Table.7 Predicted K_i values for derivatives **91**,**92**
n.s. the compounds were not synthesized

In the docking complex between tubulin dimer and **92**, the ligand would assume an orientation similar to that calculated for **89**. The complex is mainly stabilized by two hydrogen bonds involving Asn101 and Lys254 side chains with benzotriazole nitrogens (Fig. 10 A, B). Furthermore the chlorine atom would establish van der Waals interactions with Tyr224 and Ser178 side chains and is in contact with Gln247 backbone carbonyl. The Cl-O distance (3.29 Å) as well as the C-O···Cl (132°) and C-Cl···O (90°) angles are consistent with the formation of a halogen bond between the ligand and Gln247 amide carbonyl (Auffinger P.et al., 2004).

A



B

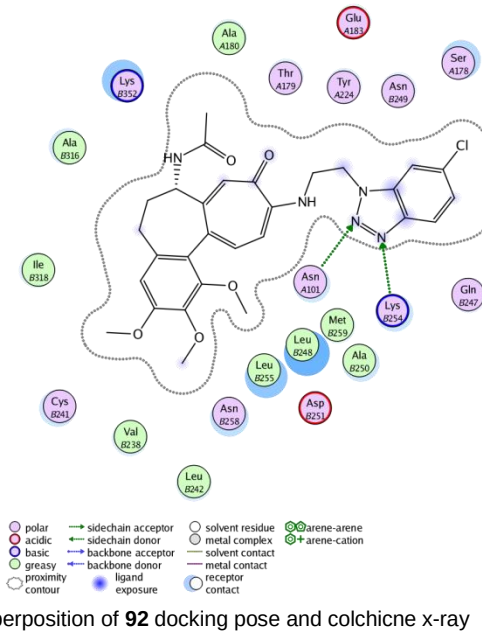


Fig.10 The docking complex of **92** with tubulin dimer. A] Superposition of **92** docking pose and colchicne x-ray structure (green) within tubulin dimer binding site. The ligands are shown as stick model. Colour code is the following: blue for nitrogen; red for oxygen, green for chlorine. Carbon atoms are coloured orang for **92** and green for colchicine. B] Ligplot of the interaction between **92** and tubulin dimer.

Materials And Methods

Melting points were determined by capillary tube method using a Büchi melting point apparatus B-540 and they have not been corrected. The melting points of the quaternary ammonium salts have not been reported here, since they are not sharp and they don't possess a full reproducibility even using closed capillary tubes. The flash chromatography were performed on Isolera One instruments (Biotage). Elemental analyses (C, H,N) were carried out at the Microanalysis Laboratory of the Department of Pharmacy, in the Pharmaceutical and Cosmetic Chemistry Section of University of Genoa. ^{1}H-NMR spectra were acquired on a Varian-Gemini 200 apparatus, using $CDCl_3$ or d_6-DMSO as solvents; J (expressed in Hz).

Benzotriazole derivatives

1-(4-Methoxybenzoyl)-1*H*-benzotriazole (49)

To a solution of 1*H*-benzotriazole (0.96 g; 8 mmol) in 12 mL of dichloromethane, 0.24 g (2 mmol; d = 1.631; 0.15 mL) of $SOCl_2$ are added. The solution is stirred at RT for 30 minutes. Then, 0.30 g (2 mmol) of 4-methoxybenzoic acid are added all at once, and the mixture is stirred another 2 hours at RT. The white precipitate formed is filtered and washed with dichloromethane (2 x 10 mL). The organic layers are combined, washed with NaOH 2N (3 x 10 mL), dried over Na_2SO_4, filtered and evaporated. The residue is purified by crystallization from anhydrous Et_2O to obtain 0.38 g of white crystals with mp = 109-111 °C. (yield = 76.0%).

The elemental analysis provided the following results:

	found:	C% 66.44	H% 4.36	N% 16.25
for $C_{14}H_{11}N_3O_2$	calc.:	C% 66.40	H% 4.38	N% 16.59

^{1}H-NMR ($CDCl_3$) δ: 8.41 (d, 2H, J = 8.2, arom); 8.39-8.26 (m, 2H, arom); 8.20 (d, 1H, J = 8.0, arom); 7.80-7.55 (m, 2H, arom); 7.10 (d, 1H, J = 8.4, arom); 3.96 (s, 3H, OCH_3).

1-(2-Methoxybenzoyl)-1*H*-benzotriazole (50)

To a solution of 1*H*-benzotriazole (0.96 g; 8 mmol) in 12 mL of dichloromethane, 0.24 g (2 mmol; d = 1.631; 0.15 mL) of $SOCl_2$ are added. The solution is stirred at

RT for 30 minutes. Then, 0.30 g (2 mmol) of 2-methoxybenzoic acid are added all at once, and the mixture is stirred another 2 hours at RT. The white precipitate formed is filtered and washed with dichloromethane (2 x 10 mL). The organic layers are combined, washed with NaOH 2N (3 x 10 mL), dried over Na_2SO_4, filtered and evaporated. The residue is purified by crystallization from anhydrous Et_2O to obtain 0.39 g of white crystals with mp = 110-112 °C. (yield = 78.0%).

The elemental analysis provided the following results:

	found:	C% 66.69	H% 4.44	N% 16.51
for $C_{14}H_{11}N_3O_2$	calc.:	C% 66.40	H% 4.38	N% 16.59

^{1}H-NMR ($CDCl_3$) δ: 8.43 (d, 1H, J = 8.2, arom); 8.17 (d, 1H, J = 8.2, arom); 7.78-7.54 (m, 4H, arom); 7.22-7.04 (m, 2H, arom); 3.81 (s, 3H, OCH_3).

1-(2,6-Dimethoxybenzoyl)-1*H*-benzotriazole (51)

To a solution of 1*H*-benzotriazole (0.96 g; 8 mmol) in 12 mL of dichloromethane, 0.24 g (2 mmol; d = 1.631; 0.15 mL) of $SOCl_2$ are added. The solution is stirred at RT for 30 minutes. Then, 0.36 g (2 mmol) of 2,6-dimethoxybenzoic acid are added all at once, and the mixture is stirred another 2 hours at RT. The white precipitate formed is filtered and washed with dichloromethane (2 x 10 mL). The organic layers are combined, washed with NaOH 2N (3 x 10 mL), dried over Na_2SO_4, filtered and evaporated. The residue is purified by crystallization from anhydrous Et_2O to obtain 0.40 g of white crystals with mp= 153-154 °C. (yield = 71.4).

The elemental analysis provided the following results:

	found:	C% 63.78	H% 4.66	N% 14.63
for $C_{15}H_{13}N_3O_3$	Calc.:	C% 63.60	H% 4.63	N% 14.83

1H-NMR ($CDCl_3$) δ: 8.40 (dd, 1H, J = 7.4, 0.8, arom); 8.20 (dd, 1H, J = 7.4, 0.8, arom); 7.82-7.66 (m, 1H, arom); 7.64-7.52 (m, 1H, arom); 7.37 (d, J = 2.2, 2H, arom); 6.80 (t, J = 2.2, 1H arom); 3.90 (s, 6H, $2OCH_3$).

1-(3,5-Dimethoxybenzoyl)-1*H*-benzotriazole (52)

To a solution of 1*H*-benzotriazole (0.96 g; 8 mmol) in 12 mL dichloromethane, 0.24 g (2 mmol; d = 1.631; 0.15 mL) of $SOCl_2$ are added. The solution is stirred at RT

for 30 minutes. Then, 0.36 g (2 mmol) of 3,5-dimethoxybenzoic acid are added all at once, and the mixture is stirred another 2 hours at RT. The white precipitate formed is filtered and washed with dichloromethane (2 x 10 mL). The organic layers are combined, washed with NaOH 2N (3 x 10 mL), dried over Na_2SO_4, filtered and evaporated. The residue is purified by crystallization from anhydrous Et_2O to obtain 0.39 g of white crystals with mp = 133-135 °C. (yield = 69.6).

The elemental analysis provided the following results:

	found:	C% 63.87	H% 4.65	N% 14.77
for $C_{15}H_{13}N_3O_3$	Calc.:	C% 63.60	H% 4.63	N% 14.83

^{1}H-NMR ($CDCl_3$) δ: 8.40 (dd, 1H, J = 7.4, 0.8, arom); 8.20 (dd, 1H, J = 7.4, 0.8, arom); 7.82-7.66 (m, 1H, arom); 7.64-7.52 (m, 1H, arom); 7.37 (d, J = 2.2, 2H, arom); 6.80 (t, J = 2.2, 1H arom); 3.90 (s, 6H, 2OCH_3).

1-(3,5-Dimethoxy-4-hydroxybenzoyl)-1*H*-benzotriazole (53)

To a solution of 1*H*-benzotriazole (0.96 g; 8 mmol) in 12 mL di dichloromethane, 0.24 g (2 mmol; d = 1.631; 0.15 mL) of $SOCl_2$ are added. The solution is stirred at RT for 30 minutes. Then, 0.40 g (2 mmol) of 3,5-dimethoxy-4-hydroxybenzoic acid are added all at once, and the mixture is stirred another 2 hours at RT. The white precipitate formed is filtered and washed with dichloromethane (2 x 10 mL). The organic layers are combined, washed with $NaHCO_3$ 10% (3 x 10 mL), dried on Na_2SO_4, filtered and evaporated. The residue is purified by crystallization from anhydrous Et_2O to obtain 0.40 g of white crystals with mp = 149-151 °C. (yield = 71.4).

The elemental analysis provided the following results:

	found:	C% 59.95	H% 4.74	N% 13.89
for $C_{15}H_{13}N_3O_4$	Calc.:	C% 60.20	H% 4.38	N% 14.04

^{1}H-NMR ($CDCl_3$) δ: 8.29 (d, 1H, J = 8.2, arom); 8.20 (d, 1H, J = 8.2, arom); 7.82-7.66 (m, 1H, arom); 7.69 (s, 2H, arom); 7.62-7.55 (m, 1H, arom); 6.19 (s, 1H, OH); 4.02 (s, 6H, 2OCH_3).

1-(3,4,5-Trimethoxybenzoyl)-1*H*-benzotriazole (54)

To a solution of 1*H*-benzotriazole (0.96 g; 8 mmol) in 12 mL of dichloromethane, 0.24 g (2 mmol; d = 1.631; 0.15 mL) of $SOCl_2$ are added. The solution is stirred at RT for 30 minutes. Then, 0.42 g (2 mmol) of 3,4,5-trimethoxybenzoic acid are added all at once, and the mixture is stirred another 2 hours at RT. The white precipitate formed is filtered and washed with dichloromethane (2 x 10 mL). The organic layers are combined, washed with NaOH 2N (3 x 10 mL), dried over Na_2SO_4, filtered and evaporated. The residue is purified by crystallization from anhydrous Et_2O to obtain 0.49 g of white crystals with mp= 129-130 °C. (yield= 79.0).

The elemental analysis provided the following results:

	found:	C% 61.30	H% 4.78	N% 13.39
for $C_{16}H_{15}N_3O_4$	Calc.:	C% 61.34	H% 4.83	N% 13.41

^{1}H-NMR ($CDCl_3$) δ: 8.40 (dd, 1H, J = 6.2, 1.0, arom); 8.21 (dd, 1H, J = 6.2, 1.0, arom); 7.82-7.67 (m, 1H, arom); 7.64-7.52 (m, 3H, arom); 4.02 (s, 3H, OCH_3); 3.98 (s, 6H, $2OCH_3$).

5,6-Dichloro-1*H*-benzotriazole

To a solution of 4,5-dichloro-o-phenylenediamine (3 g; 17 mmol) in 15 mL of very cold H_2O, 1.4 mL of concentrated HCl are added. The mixture is stirred at 0-5 °C for 10 minutes. Then, a solution of $NaNO_2$ (1.2 g; 17 mmol) in 5 mL of H_2O is added in two portions within 1 minute.

In order to fluidize the mixture, other 15 mL of H_2O are added. The mixture is stirred for 20 minutes at 5 °C and then for 30 minutes at RT.

Finally, the insoluble residue is filtered and washed with abundant water. The resulting material is dried on KOH in a dryer to obtain 3.17 g (99.4%) of a raw material of a hazelnut color with mp = 251-253 °C (dec) and U.V. with λ_{MAX} at 301(f), 292, 268, 261.

This raw product is utilized in the subsequent reactions without any further purification process.

1-(3,5-Dimethoxybenzoyl)-5,6-dichloro-1*H*-benzotriazole (55)

a) To a suspension of 5,6-dichloro-1*H*-benzotriazole (1.12 g; 6 mmol) in dichloromethane (12 mL), 0.18 g (2 mmol; d = 1.631; 0.11 mL) of $SOCl_2$ are added. The solution is stirred at RT for 30 minutes. Then, 0.27 g (1.5 mmol) of 3,5-dimethoxybenzoic acid are added all at once, and the mixture is stirred another 2 hours at RT. The white precipitate formed is filtered and washed with dichloromethane (2 x 10 mL). The organic layers are combined, washed with NaOH 2N (3 x 10 mL), dried over Na_2SO_4, filtered and evaporated. The residue is purified by chromatography on SiO_2 (1:20), eluting with chloroform and chloroform + 2% methanol, to obtain 0,09 g of light-grey solid with mp = 143-144 °C. (yield = 16.4%).

The elemental analysis provided the following results:

	found:	C% 51.28	H% 3.49	N% 11.77
for $C_{15}H_{11}Cl_2N_3O_3$	calc.:	C% 51.16	H% 3.15	N% 11.93

^{1}H-NMR ($CDCl_3$) δ: 8.56 (s, 1H, arom); 8.29 (d, 1H, J = 0.4, arom); 7.36 (d, 2H, J = 2.2, arom); 6.80 (t, 1H, J = 2.2, arom); 3.90 (s, 6H, $2OCH_3$).

b) To a suspension of 5,6-dichloro-1*H*-benzotriazole (0.56 g; 3 mmol) in 20 mL of anhydrous chloroform, 0.3 g (3 mmol; d = 0.726; 0.41 mL) of triethylamine and a solution of 3,4,5-trimethoxybenzoyl chloride in anhydrous chloroform (5 mL) are added. (3,4,5-Trimethoxybenzoyl chloride was synthesized starting from 3 mmol of the corresponding carboxylic acid and 12 mmol of $SOCl_2$, under reflux for 4 hours in anhydrous chloroform). The reaction mixture is refluxed for 5 hours. After being cooled, washed one time with very cold water and twice with a very cold 5% Na_2CO_3 solution, the organic solution is dried over Na_2SO_4, filtered and evaporated. The residue is purified by chromatography on Al_2O_3 (1.20), eluting with chloroform and chloroform + 2% methanol, to obtain 0.37 g of light-grey crystals with mp = 143-144 °C. (yield 35.2%).

1-(3,4,5-Trimethoxybenzoyl)-5,6-dichloro-1*H*-benzotriazole (56)

a) To a suspension of 5,6-dichloro-1*H*-benzotriazole (1.12 g; 6 mmol) in 20 mL of dichloromethane, 0.18 g (2 mmol; d = 1.631; 0.11 mL) of $SOCl_2$ are added. The solution is stirred at RT for 30 minutes. Then, 0.32 g (1.5 mmol) of 3,4,5-

trimethoxybenzoic acid are added all at once, and the mixture is stirred another 2 hours at RT. The white precipitate formed is filtered and washed with dichloromethane (2 x 10 mL). The organic layers are combined, washed with NaOH 2N (3 x 10 mL), dried over Na_2SO_4, filtered and evaporated. The residue is purified by crystallization from anhydrous Et_2O to obtain 0.13 g of white crystals with mp = 173-174 °C. (yield = 22.8%).

The elemental analysis provided the following results:

	found:	C% 50.45	H% 3.12	N% 10.98
for $C_{16}H_{13}Cl_2N_3O_4$	calc.:	C% 50.28	H% 3.43	N% 10.99

^{1}H-NMR ($CDCl_3$) δ: 8.58 (s, 1H, arom); 8.31 (d, 1H, J = 0.4, arom); 7.60 (s, 2H, arom); 4.03 (s, 3H, OCH_3); 3.99 (s, 6H, $2OCH_3$).

b) To a suspension of 5,6-dichloro-1*H*-benzotriazole (0.56 g; 3 mmol) in anhydrous chloroform (20 mL), 0.3 g (3 mmol; d = 0.726; 0.41 mL) of triethylamine and a solution of 3,4,5-trimethoxybenzoyl chloride in anhydrous chloroform (5 mL) are added. (3,4,5-Trimethoxybenzoyl chloride was synthesized starting from 3 mmol of the corresponding carboxylic acid and 12 mmol of $SOCl_2$, under reflux for 4 hours in anhydrous chloroform). The reaction mixture is refluxed for 5 hours. After being cooled, washed one time with very cold water and twice with a very cold 5% Na_2CO_3 solution, the organic solution is dried over Na_2SO_4, filtered and evaporated. The residue is purified by chromatography on Al_2O_3 (1.20), eluting with chloroform and chloroform + 2% methanol, obtaining 0.43 g of a light-grey solid with mp = 173-174 °C. (yield 37.7%).

2-(2-Hydroxyethyl)-2*H*-benzotriazole (i1)

1-(2-Hydroxyethyl)-1*H*-benzotriazole (i2)

To a solution of 1*H*-benzotriazole (2.38 g; 20 mmol) in 12 mL of NaOH 2N, 1.61 g (20 mmol; d = 1.201; 1.34 mL) of 2-chloroethanol are added. The reaction mixture is heated at 100 °C for 2 hours. After cooling, the alkaline solution is extracted three times with chloroform. The organic phase is dried on Na_2SO_4, filtered and evaporated to dryness, furnishing a grey residue (3.06 g) that is purified by chromatography on SiO_2 (1:20), eluting with dichloromethane.

The compounds were obtained with the following yields:

- 1.23 g of 2-(2-hydroxyethyl)-2*H*-benzotriazole (**i1**) with mp = 69-70 °C (yield= 37.7 %).

The elemental analysis provided the following results:

	found:	C%	59.08	H%	5.69	N% 25.46
for $C_8H_9N_3O$	calc.:	C%	58.89	H%	5.56	N% 25.75

^{1}H-NMR ($CDCl_3$) δ: 7.95-7-81 (m, 2H, arom); 7.50-7.36 (m, 2H, arom); 4.89 (t, 2H, J = 6.8, CH_2O); 4.27 (t, 2H, J = 6.8, NCH_2); 3.23 (s, 1H, OH).

- 1.58 g of 1-(2-hydroxyethyl)-1*H*-benzotriazole (**i2**) with mp = 90-91 °C (yield = 48.5 %).

The elemental analysis provided the results below:

	found:	C%	58.71	H%	5.43	N% 25.54
for $C_8H_9N_3O$	calc.:	C%	58.89	H%	5.56	N% 25.75

^{1}H-NMR ($CDCl_3$) δ: 7.94-7-72 (m, 1H, arom); 7.66-7.55 (m, 1H, arom); 7.55-7.39 (m, 1H, arom); 7.38-7.20 (m, 1H, arom); 4.92 (t, 2H, J = 6.8, CH_2O); 4.23 (t, 2H, J = 6.8, NCH_2); 3.64 (s, 1H, OH).

2-(3-hydroxypropyl)-2*H*-benzotriazole (i3)

1-(3-Hydroxypropyl)-1*H*-benzotriazole (i4)

To a solution of 1*H*-benzotriazole (2.38 g; 20 mmol) in 12 mL of NaOH 2N, 1.89 g (20 mmol; d = 1.131; 1.67 mL) of 3-chloropropan-1-ol are added. The reaction mixture is heated at 100 °C for 2 hours. . After cooling, the alkaline solution is extracted three times with chloroform. The organic phase is dried on Na_2SO_4, filtered and evaporated to dryness, furnishing a grey residue (3.13 g) that is purified by chromatography on SiO_2 (1:20), eluting with dichloromethane.

The compounds were obtained with the following yields:

- 1.24 g of 2-(3-hydroxypropyl)-2*H*-benzotriazole (**i3**) as a dense oil (yield= 35.2 %).

	found:	C%	60.82	H%	6.59	N% 23.44
for $C_9H_{11}N_3O$	calc.:	C%	61.00	H%	6.26	N% 23.71

^{1}H-NMR ($CDCl_3$) δ: 7.98-7-82 (m, 2H, arom); 7.50-7.36 (m, 2H, arom); 4.94 (t, 2H, J = 6.6, CH_2O); 3.68 (t, 2H, J = 6.6, NCH_2); 2.58 (s, 1H, OH); 2.34 (quintet, 2H, J = 6.8, $CH_2CH_2CH_2$).

- 1.55 g of 1-(3-hydroxypropyl)-1*H*-benzotriazole (**i4**) with mp = 70-71 °C (yield = 43.9 %).

The elemental analysis provided the following results:

	found:	C%	61.27	H%	6.45	N% 23.38
for $C_9H_{11}N_3O$	calc.:	C%	61.00	H%	6.26	N% 23.71

^{1}H-NMR ($CDCl_3$) δ: 8.03 (d, 1H, J = 7.2, arom); 7.68-7-24 (m, 3H, arom); 4.81 (t, 2H, J = 6.8, CH_2O); 3.66 (t, 2H, J = 6.8, NCH_2); 3.12 (s, 1H, OH); 2.25 (quintet, 2H, J = 5.6, $CH_2CH_2CH_2$).

2-(2*H*-Benzotriazol-2-yl)ethyl 3,5-dimethoxybenzoate (57)

To a solution of 2-(2-hydroxyethyl)-2*H*-benzotriazole (**i1**) (0.5 g; 3 mmol) in 15 mL of anhydrous toluene, 0.3 g (3 mmoli; d = 0.726; 0.41 mL) of triethylamine are first added and then, dropwise, a solution of 3,5-dimethoxybenzoyl chloride in anhydrous chloroform (5 mL) is added. (3,4,5-Trimethoxybenzoyl chloride was synthesized starting from 3 mmol of the corresponding carboxylic acid and 12 mmol of $SOCl_2$, under reflux for 4 hours in anhydrous chloroform). The solution is stirred at RT for 10 hours. The toluic solution is washed with very cold water and then evaporated to dryness and the residue is dissolved in chloroform. After being washed twice with a very cold 5% Na_2CO_3 solution and then with very cold water, the organic phase is eventually dried over Na_2SO_4, filtered and evaporated to dryness. The residue is purified by chromatography on SiO_2 (1:20), eluting with chloroform and chloroform + 2% methanol, to obtain 0.66 g of crystals with mp= 83-85 °C (yield = 67.3%).

The elemental analysis provided the following results:

	found:	C%	62.17	H%	5.42	N% 12.63
for $C_{17}H_{17}N_3O_4$	calc.:	C%	62.38	H%	5.23	N% 12.89

^{1}H-NMR ($CDCl_3$) δ: 7.88-7.64 (m, 2H, arom); 7.43-7.28 (m, 2H, arom); 7.16 (s, 2H, arom), 6.68 (s, 1H, arom); 4.91 (t, 2H, J = 6.0, CH_2O); 3.43 (t, 2H, J = 60, NCH_2); 3.81 (s, 6H, $2OCH_3$).

2-(2*H*-Benzotriazol-2-yl)ethyl 3,4,5-trimethoxybenzoate (58)

To a solution of 2-(2-hydroxyethyl)-2*H*-benzotriazole (**i1**) (0.5 g; 3 mmol) in 15 mL of anhydrous toluene, 0.3 g (3 mmol; d = 0.726; 0.41 mL) of triethylamine are first added and then, dropwise, a solution of 3,4,5-trimethoxybenzoyl chloride in anhydrous chloroform (5 mL) is added. (3,4,5-Trimethoxybenzoyl chloride was synthesized starting from 3 mmol of the corresponding carboxylic acid and 12 mmol of $SOCl_2$, under reflux for 4 hours in anhydrous chloroform). The solution is stirred at RT for 6 hours. The toluic solution is washed with very cold water and then evaporated to dryness and the residue is dissolved in chloroform. After being washed twice with a very cold 5% Na_2CO_3 solution and then with very cold water, the organic phase is eventually dried over Na_2SO_4, filtered and evaporated to dryness. The residue is eventually crystallized from anhydrous Et_2O to obtain 0.82 g of crystals with mp = 136-138 °C (yield = 76.6%).

The elemental analysis provided the following results:

	found:	C% 60.88	H% 5.57	N% 11.41
for $C_{18}H_{19}N_3O_5$	calc.:	C% 60.50	H% 5.36	N% 11.76

^{1}H-NMR ($CDCl_3$) δ: 7.96-7.82 (dd, 2H, J = 6.4, 3, arom); 7.51-7.36 (dd, 2H, J = 6.4, 3, arom); 7.16 (s, 2H, arom); 5.14 (t, 2H, J = 5.4, CH_2O); 4.96 (t, 2H, J = 5.4, NCH_2); 3.89 (s, 3H, OCH_3); 3.83 (s, 6H, $2OCH_3$).

2-(1*H*-Benzotriazol-1-yl)ethyl 3,5-dimethoxybenzoate (59)

To a solution of 1-(2-hydroxyethyl)-1*H*-benzotriazole (**i2**) (0.5 g; 3 mmol) in 15 mL of anhydrous toluene, 0.3 g (3 mmol; d = 0.726; 0.41 mL) of triethylamine are first added and then, dropwise, a solution of 3,5-dimethoxybenzoyl chloride in anhydrous chloroform (5 mL) is added. (3,4,5-Trimethoxybenzoyl chloride was synthesized starting from 3 mmol of the corresponding carboxylic acid and 12 mmol of $SOCl_2$, under reflux for 4 hours in anhydrous chloroform). The solution is stirred at RT for 10 hours. The toluic solution is washed with very cold water and then evaporated to dryness and the residue is dissolved in chloroform. After being washed twice with a very cold 5% Na_2CO_3 solution and then with very cold water, the organic phase is eventually dried over Na_2SO_4, filtered and evaporated to dryness. The residue is purified by chromatography on SiO_2 (1:20), eluting with

chloroform and chloroform + 2% methanol, to obtain 0.65 g of crystals with mp = 104-106 °C (yield = 66.3%).

The elemental analysis provided the following results:

	found:	C% 62.24	H% 5.30	N% 13.09
for $C_{17}H_{17}N_3O_4$	calc.:	C% 62.38	H% 5.23	N% 12.84

^{1}H-NMR ($CDCl_3$) δ: 8.03 (d, 1H, J = 7.2, arom); 7.68-7-28 (m, 4H, arom); 7.16 (s, 2H, arom); 4.93 (t, 2H, J = 6.8, CH_2O); 4.48 (t, 2H, J = 6.8, NCH_2); 3.93 (s, 6H, $2OCH_3$).

2-(1*H*-Benzotriazol-1-yl)ethyl 3,4,5-trimethoxybenzoate (60)

To a solution of 1-(2-hydroxyethyl)-1*H*-benzotriazole ((**i2**) 0.5 g; 3 mmol) in 15 mL of anidrous toluene, 0.3 g (3 mmol; d = 0.726; 0.41 mL) of triethylamine are first added and then, dropwise, a solution of 3,4,5-trimethoxybenzoyl chloride in anhydrous chloroform (5 mL) is added. (3,4,5-Trimethoxybenzoyl chloride was synthesized starting from 3 mmol of the corresponding carboxylic acid and 12 mmol of $SOCl_2$, under reflux for 4 hours in anhydrous chloroform). The solution is stirred at RT for 6 hours. The toluic solution is washed with very cold water and then evaporated to dryness and the residue is dissolved in chloroform. After being washed twice with a very cold 5% Na_2CO_3 solution and then with very cold water, the organic phase is dried over Na_2SO_4, filtered and evaporated to dryness. The residue is eventually crystallized from anhydrous Et_2O to obtain 0.69 g of crystals with mp = 126-127 °C (yield= 64.4%).

The elemental analysis provided the following results:

	found:	C% 60.68	H% 5.63	N% 11.48
for $C_{18}H_{19}N_3O_5$	calc.:	C% 60.50	H% 5.36	N% 11.76

^{1}H-NMR ($CDCl_3$) δ: 8.11 (d, 1H, J = 8.0, arom); 7.63-7-32 (m, 3H, arom); 7.09 (s, 2H, arom); 5.06 (t, 2H, J = 5.4, CH_2O); 4.84 (t, 2H, J = 5.4, NCH_2); 3.90 (s, 3H, OCH_3); 3.84 (s, 6H, $2OCH_3$).

3-(2*H*-Benzotriazol-2-yl)propyl 3,5-dimethoxybenzoate (61)

To a solution of 2-(3-hydroxypropyl)-2*H*-benzotriazole (**i3**) (0.5 g; 3 mmol) in 15 mL of anhydrous toluene, 0.3 g (3 mmoli; d = 0.726; 0.41 mL) of triethylamine are first

added and then, dropwise, a solution of 3,5-dimethoxybenzoyl chloride in anhydrous chloroform (5 mL) is added. (3,4,5-Trimethoxybenzoyl chloride was synthesized starting from 3 mmol of the corresponding carboxylic acid and 12 mmol of $SOCl_2$, under reflux for 4 hours in anhydrous chloroform). The solution is stirred at RT for 10 hours. The toluic solution is washed with very cold water and then evaporated to dryness and the residue is dissolved in chloroform. After being washed twice with a very cold 5% Na_2CO_3 solution and then with very cold water, the organic phase is eventually dried over Na_2SO_4, filtered and evaporated to dryness. The residue is purified by chromatography on SiO_2 (1:20), eluting with chloroform and chloroform + 2% methanol, to obtain 0.64 g of crystals with mp = 93-84 °C (yield = 62.7%).

The elemental analysis provided the following results:

	found:	C% 63.61	H% 5.74	N% 12.07
for $C_{18}H_{19}N_3O_4$	calc.:	C% 63.33	H% 5.61	N% 12.31

^{1}H-NMR ($CDCl_3$) δ: 7.94-7.73 (m, 2H, arom); 7.48-7.31 (m, 2H, arom); 7.14 (s, 2H, arom); 6.64 (s, 1H); 4.92 (t, 2H, J = 6.6, CH_2O); 4.42 (t, 2H, J = 6.6, NCH_2); 3.81 (s, 6H, $2OCH_3$); 2.61 (quintet, 2H, J = 56.4, $CH_2CH_2CH_2$).

3-(2*H*-Benzotriazol-2-yl)propyl 3,4,5-trimethoxybenzoate (62)

To a solution of 1-(3-hydroxypropyl)-2*H*-benzotriazole (**i3**) (0.53 g; 3 mmol) in 15 mL of anhydrous toluene, 0.3 g (3 mmol; d = 0.726; 0.41 mL) of triethylamine are first added and then, dropwise, a solution of 3,4,5-trimethoxybenzoyl chloride in anhydrous chloroform (5 mL) is added. (3,4,5-Trimethoxybenzoyl chloride was synthesized starting from 3 mmol of the corresponding carboxylic acid and 12 mmol of $SOCl_2$, under reflux for 4 hours in anhydrous chloroform). The solution is stirred at RT for 6 hours. The toluic solution is washed with very cold water and then evaporated to dryness and the residue is dissolved in chloroform. After being washed twice with a very cold 5% Na_2CO_3 solution and then with very cold water, the organic phase is eventually dried over Na_2SO_4, filtered and evaporated to dryness. The residue is purified by chromatography on SiO_2 (1:20), eluting with chloroform and chloroform + 2% methanol, to obtain 0.77 g of crystals with mp = 94-96 °C (yield= 69.4%).

The elemental analysis provided the following results:

	found:	C% 61.58	H% 6.04	N% 11.13

for $C_{19}H_{21}N_3O_5$	calc.:	C% 61.45	H% 5.70	N% 11.31

^{1}H-NMR ($CDCl_3$) δ: 7.93-7.78 (m, 2H, arom); 7.48-7.33 (m, 2H, arom); 7.26 (s, 2H, arom); 4.94 (t, 2H, J = 6.8, CH_2O); 4.46 (t, 2H, J = 6.8, NCH_2); 3.95 (s, 3H, OCH_3); 3.93 (s, 6H, $2OCH_3$); 2.65 (quintet, 2H, J = 6.4, $CH_2CH_2CH_2$).

3-(1*H*-Benzotriazol-1-yl)propyl 3,5-dimethoxybenzoate (63)

To a solution of 1-(3-hydroxypropyl)-1*H*-benzotriazole (**i4**) (0.53 g; 3 mmol) in 15 mL of anhydrous toluene, 0.3 g (3 mmoli; d = 0.726; 0.41 mL) of triethylamine are first added and then, dropwise, a solution of 3,5-dimethoxybenzoyl chloride in anhydrous chloroform (5 mL) is added. (3,4,5-Trimethoxybenzoyl chloride was synthesized starting from 3 mmol of the corresponding carboxylic acid and 12 mmol of $SOCl_2$, under reflux for 4 hours in anhydrous chloroform). The solution is stirred at RT for 10 hours. The toluic solution is washed with very cold water and then evaporated to dryness and the residue is dissolved in chloroform. After being washed twice with a very cold 5% Na_2CO_3 solution and then with very cold water, the organic phase is eventually dried over Na_2SO_4, filtered and evaporated to dryness. The residue is purified by chromatography on SiO_2 (1:20), eluting with dichloromethane to obtain 0.58 g of a dense colorless oil (yield = 56.9%).
The elemental analysis provided the following results:

	found:	C% 63.64	H% 5.98	N% 12.55
for $C_{18}H_{19}N_3O_4$	calc.:	C% 63.33	H% 5.61	N% 12.31

^{1}H-NMR ($CDCl_3$) δ: 8.07 (d, 1H, J = 5.6); 7.89-7-56 (m, 4H, arom); 7.14 (s, 2H, arom); 4.92 (t, 2H, J = 6.2, CH_2O); 4.42 (t, 2H, J = 6.4, NCH_2); 3.81 (s, 6H, $2OCH_3$); 2.59 (quintet, 2H, J = 6.4, $CH_2CH_2CH_2$).

3-(1*H*-Benzotriazol-1-yl)propyl 3,4,5-trimethoxybenzoate (64)

To a solution of 1-(3-hydroxypropyl)-1*H*-benzotriazole (**i4**) (0.53 g; 3 mmol) in 15 mL of anhydrous toluene, 0.3 g (3 mmol; d = 0.726; 0.41 mL) of triethylamine are first added and then, dropwise, a solution of 3,4,5-trimethoxybenzoyl chloride in anhydrous chloroform (5 mL) is added. (3,4,5-Trimethoxybenzoyl chloride was

synthesized starting from 3 mmol of the corresponding carboxylic acid and 12 mmol of $SOCl_2$, under reflux for 4 hours in anhydrous chloroform). The solution is stirred at RT for 6 hours. The toluic solution is washed with very cold water and then evaporated to dryness and the residue is dissolved in chloroform. After being washed twice with a very cold 5% Na_2CO_3 solution and then with very cold water, the organic phase is eventually dried over Na_2SO_4, filtered and evaporated to dryness. The residue is purified by chromatography on SiO_2 (1:20), eluting with chloroform and chloroform + 2% methanol, to obtain 0.79 g of a white solid with mp = 103-104 °C (yield = 71.2%).

The elemental analysis provided the following results:

	found:	C% 61.30	H% 5.78	N% 11.39
for $C_{19}H_{21}N_3O_5$	calc.:	C% 61.45	H% 5.70	N% 11.31

^{1}H-NMR ($CDCl_3$) δ: 8.07 (d, 1H, J = 8.6, arom); 7.62-7-22 (m, 3H, arom); 7.23 (s, 2H, arom); 4.84 (t, 2H, J = 6.8, CH_2O); 4.42 (t, 2H, J = 6.8, NCH_2); 3.98 (s, 3H, OCH_3); 3.95 (s, 6H, $2OCH_3$); 2.57 (quintet, 2H, J = 6.8, $CH_2CH_2CH_2$).

2-(2-Hydroxyethyl)-5-chloro-2*H*-benzotriazole (i5)

1-(2-hydroxyethyl)-5-chloro-1*H*-benzotriazole (i6)

1-(2-hydroxyethyl)-6-chloro-1*H*-benzotriazole (i7)

To a solution of 5-chloro-1*H*-benzotriazole (3.07 g ; 20 mmol) in 10 mL of NaOH 2N, 1.60 g (20 mmol; d = 1.201; 0.67 mL) of 2-chloroethanol are added. The reaction mixture is heated at 100 °C for 2 hours. After cooling of the mixture, the alkaline solution is extracted three times with chloroform and the organic phase is dried over Na_2SO_4, filtered and evaporated to dryness, to obtain a grey residue (3.71 g). The latter is purified by chromatography on SiO_2 (1:20) eluting with chloroform, to obtain in high purity the 2-substituted isomer (N2-5) and the mixture of the 1-substituted isomers (N1-5 and N1-6), that are then separated by flash chromatography eluting with ethyl acetate (overall yield of the three isomers = 85.8%).

The compounds were obtained with the following yields:

- 1.26 g of 2-(2-hydroxyethyl)-5-chloro-2*H*-benzotriazole (**i5**) with mp = 87-88 °C (yield = 31.9 %).

The elemental analysis provided the following results:

	found:	C% 48.49	H% 4.06	N% 21.18

for $C_8H_8ClN_3O$	calc.:	C% 48.46	H% 4.08	N% 21.26

^{1}H-NMR ($CDCl_3$) δ: 8.56 (dd, 1H, J = 0.8, 1.8, arom); 7.81 (dd, 1H, J = 0.8, 9, arom); 7.36 (dd, 1H, J = 1.8, 9, arom); 4.87 (m, 2H, CH_2O); 4.27 (m, 2H, NCH_2); 3.16 (t, J = 6.4, 1H, OH).

- 1.10 g of 1-(2-hydroxyethyl)-5-chloro-1*H*-benzotriazole (**i6**) with mp = 118-119 °C (yield= 27.8 %).

The elemental analysis provided the following results:

	found:	C% 48.52	H% 4.06	N% 21.05
for $C_8H_8ClN_3O$	calc.:	C% 48.46	H% 4.08	N% 21.26

^{1}H-NMR ($CDCl_3$) δ: 8.61 (dd, 1H, J = 0.8, 1.4, arom); 7.88 (dd, 1H, J = 0.8, 8.8, arom); 7.34 (dd, 1H, J = 1.4, 98.8 arom); 4.83 (m, 2H, CH_2O); 4.32 (m, 2H, NCH_2); 3.19 (t, J = 6.4, 1H, OH).

- 1.03 g of 1-(2-hydroxyethyl)-6-chloro-1*H*-benzotriazole (**i7**) with mp= 98-99 °C (yield = 26.1 %).

The elemental analysis provided the following results:

	found:	C% 48.55	H% 3.96	N% 21.12
for $C_8H_8ClN_3O$	calc.:	C% 48.46	H% 4.08	N% 21.26

^{1}H-NMR ($CDCl_3$) δ: 8.58 (dd, 1H, J = 1, 1.8, arom); 7.63 (dd, 1H, J = 0.8, 9, arom); 7.36 (dd, 1H, J = 1.8, 9, arom); 4.85 (m, 2H, CH_2O); 4.29 (m, 2H, NCH_2); 3.17 (t, J = 6.2, 1H, OH).

2-(3-Hydroxypropyl)-5-chloro-2*H*-benzotriazole (i8)

1-(3-hydroxypropyl)-5-chloro-1*H*-benzotriazole (i9)

1-(3-hydroxypropyl)-6-chloro-1*H*-benzotriazole (i10)

To a solution of 5-chloro-1*H*-benzotriazole (2.46 g ;16 mmol) in 10 mL of NaOH 2N, 1.51 g (16 mmol; d = 1.131; 1.34 mL) of 2-chloroethanol are added. The reaction mixture is heated at 100 °C for 2 hours. After cooling of the mixture, the alkaline solution is extracted three times with chloroform and the organic phase is dried over Na_2SO_4, filtered and evaporated to dryness, to obtain a grey residue (3.52 g). The latter is purified by chromatography on SiO_2 (1:20) eluting with

chloroform, to obtain in high purity the 2-substituted isomer (N2-5) and the mixture of the 1-substituted isomers (N1-5 and N1-6), that are then separated by flash chromatography eluting with ethyl acetate (overall yield of the three isomers = 82.3%).

The compounds were obtained with the following yields:

- 0.85 g of 2-(3-hydroxypropyl)-5-chloro-2*H*-benzotriazole (**i8**), colorless oil (yield = 30.6 %).

The elemental analysis provided the results below:

	found:	C% 51.07	H% 4.69	N% 19.73
for $C_9H_{10}ClN_3O$	calc.:	C% 51.07	H% 4.76	N% 19.85

^{1}H-NMR ($CDCl_3$) δ: 7.87 (dd, 1H, J = 1,8, 9, arom); 7.80 (d, 1H, J = 0.6, arom); 7.37 (dd, 1H, J = 1.8, 9.2, arom); 4.93 (t, 2H, J = 6.6, CH_2O); 3.70 (t, 2H, J = 5.6, CH_2N); 2.34 (q, 2H, J = 6.6, $CH_2\underline{CH_2}CH_2$); 2.19 (s, 1H, OH).

- 0.76 g of 1-(3-hydroxypropyl)-5-chloro-1*H*-benzotriazole (**i9**) with mp = 46-47 °C (yield = 27.3 %).

The elemental analysis provided the following results:

	found:	C% 51.05	H% 4.88	N% 19.95
for $C_9H_{10}ClN_3O$	calc.:	C% 51.07	H% 4.76	N% 19.85

^{1}H-NMR ($CDCl_3$) δ: 7.93 (d, 1H, J = 8.8, arom); 7.62 (d, 1H, J = 1.6, arom); 7.31 (dd, 1H, J = 1.2, 8.8, arom); 4.77 (t, 2H, J = 6.8, CH_2O); 3.66 (t, 2H, J = 5.8, NCH_2); 3.08 (s, 1H, OH); 2.24 (q, 2H, J = 6, $CH_2\underline{CH_2}CH_2$).

- 0.68 g of 1-(3-hydroxypropyl)-6-chloro-1*H*-benzotriazole (**i10**) with mp = 41-42 °C (yield = 24.3 %).

The elemental analysis provided the results below:

	found:	C% 51.31	H% 4.60	N% 19.52
for $C_9H_{10}ClN_3O$	calc.:	C% 51.07	H% 4.76	N% 19.85

^{1}H-NMR ($CDCl_3$) δ: 7.99 (d, 1H, J = 1.8, arom); 7.57 (d, 1H, J = 8.8, arom); 7.47 (dd, 1H, J = 1.8, 8.8, arom); 4.79 (t, 2H, J = 6.6, CH_2O); 3.66 (t, 2H, J = 5.6, NCH_2); 2.97 (s, 1H, OH); 2.24 (q, 2H, J = 6.2, $CH_2\underline{CH_2}CH_2$).

2-(5-Chloro-2*H*-benzotriazol-2-yl)ethyl 3,4,5-trimethoxybenzoate (65)

To a solution of 2-(2-hydroxyethyl)-5-chloro-2*H*-benzotriazole (**i5**) (0.3 g; 1.5 mmol) in 15 mL of anhydrous toluene, 0.15 g (1.5 mmol; d = 0.726; 0.21 mL) of triethylamine are first added and then, dropwise, a solution of 3,4,5-trimethoxybenzoyl chloride in 5 mL of anhydrous toluene. (3,4,5-Trimethoxybenzoyl chloride was prepared starting from 1.5 mmol of the corresponding carboxylic acid and 5.2 mmol of $SOCl_2$ under reflux for 4 hours in anhydrous choloroform). The solution is stirred at RT for 12 hours. The toluic solution, after being washed with very cold water, is evaporated and the residue is dissolved in chloroform. The organic phase is washed twice first with a very cold 5% Na_2CO_3 solution and then with very cold water and finally it is dried over Na_2SO_4, filtered and evaporated to dryness. The residue is crystallized from anhydrous Et_2O to obtain 0.51 g of crystals with mp = 145-146 °C (yield = 86.4%). The elemental analysis provided the following results:

	found:	C% 54.98	H% 4.30	N% 10.68
for $C_{18}H_{18}ClN_3O_5$	calc.:	C% 55.18	H% 4.63	N% 10.72

^{1}H-NMR ($CDCl_3$) δ: 8.03 (d, 1H, J = 8.8, arom); 7.58 (d, 1H, J = 1.2, arom); 7.36 (dd, 1H, J = 1.8, 9, arom); 7.12 (s, 2H, arom); 5.03 (t, 2H, J = 4.8, CH_2O); 4.83 (t, 2H, J = 5.4, NCH_2); 3.91 (s, 3H, OCH_3); 3.84 (s, 6H, $2OCH_3$).

2-(5-Chloro-1*H*-benzotriazol-1-yl)ethyl 3,4,5-trimethoxybenzoate (66)

To a solution of 1-(2-hydroxyethyl)-5-chloro-1*H*-benzotriazole (**i6**) (0.26 g;1.4 mmol) in 15 mL of anhydrous toluene, 0.14 g (1.4 mmol; d = 0.726; 0.19 mL) of triethylamine are first added, and then, dropwise, a solution of 3,4,5-trimethoxybenzoyl chloride in 5 mL of anhydrous toluene. (3,4,5-Trimethoxybenzoyl chloride was prepared starting from 1.5 mmol of the corresponding carboxylic acid and 5.2 mmol of $SOCl_2$ under reflux for 4 hours in anhydrous choloroform). The solution is stirred at RT for 12 hours. The toluic solution, after being washed with very cold water, is evaporated and the residue is dissolved in chloroform. The organic phase is washed twice first with a very cold 5% Na_2CO_3 solution and then with very cold water and finally it is dried over Na_2SO_4, filtered and evaporated to dryness. The residue is crystallized from anhydrous Et_2O to obtain 0.44 g of crystals with mp = 158-159 °C (yield = 80.2%). The elemental analysis provided the following results:

	found:	C%	54.98	H%	4.36	N% 10.62
for $C_{18}H_{18}ClN_3O_5$	calc.:	C%	55.18	H%	4.63	N% 10.72

^{1}H-NMR ($CDCl_3$) δ: 8.09 (s, 1H, arom); 7.81-7.43 (m, 2H, arom); 7.08 (s, 2H, arom); 5.05 (t, 2H, J = 5.2, CH_2O); 4.83 (t, 2H, J = 5.4, NCH_2); 3.91 (s, 3H, OCH_3); 3.83 (s, 6H, $2OCH_3$).

2-(6-Chloro-1*H*-benzotriazol-1-yl)ethyl 3,4,5-trimethoxybenzoate (67)

To a solution of 1-(2-hydroxyethyl)-6-chloro-1*H*-benzotriazole (**i7**) (0.26 g; 1.3 mmol) in 15 mL of anhydrous toluene, 0.13 g (1.3 mmol; d =0.726; 0.18 mL) of triethylamine are first added, and then, dropwise, a solution of 3,4,5-trimethoxybenzoyl chloride in 5 mL of anhydrous toluene. (3,4,5-Trimethoxybenzoyl chloride was prepared starting from 1.3 mmol of the corresponding carboxylic acid and 5.2 mmol of $SOCl_2$ under reflux for 4 hours in anhydrous choloroform). The solution is stirred at RT for 12 hours. The toluic solution, after being washed with very cold water, is evaporated and the residue is dissolved in chloroform. The organic phase is washed twice first with a very cold 5% Na_2CO_3 solution and then with very cold water and finally it is dried over Na_2SO_4, filtered and evaporated to dryness. The residue is crystallized from anhydrous Et_2O to obtain 0.41 g of crystals with mp = 110-111°C (yield = 80.4%). The elemental analysis provided the following results:

	found:	C%	55.26	H%	4.64	N% 10.34
for $C_{18}H_{18}ClN_3O_5$	calc.:	C%	55.18	H%	4.63	N% 10.72

^{1}H-NMR ($CDCl_3$) δ: 8.24 (s, 1H, arom); 8.01 (d, 1H, J = 9, arom); 7.62 (dd, 1H, J = 1.6, 9, arom); 5.20 (t, 2H, J = 5, CH_2O); 4.98 (t, 2H, J = 5.6, NCH_2); 3.91 (s, 3H, OCH_3); 3.84 (s, 6H, $2OCH_3$).

3-(5-Chloro-2*H*-benzotriazol-2-yl)propyl 3,4,5-trimethoxybenzoate (68)

To a solution of 2-(3-hydroxypropyl)-5-chloro-2*H*-benzotriazole (**i8**) (0.3 g; 1.4 mmol) in 15 mL of anhydrous toluene, 0.14 g (1.4 mmol; d =0.726; 0.19 mL) of triethylamine are first added, and then, dropwise, a solution of 3,4,5-trimethoxybenzoyl chloride in 5 mL of anhydrous toluene. (3,4,5-

Trimethoxybenzoyl chloride was prepared starting from 1.4 mmol of the corresponding carboxylic acid and 5.6 mmol of $SOCl_2$ under reflux for 4 hours in anhydrous choloroform). The solution is stirred at RT for 12 hours. The toluic solution, after being washed with very cold water, is evaporated and the residue is dissolved in chloroform. The organic phase is washed twice first with a very cold 5% Na_2CO_3 solution and then with very cold water and finally it is dried over Na_2SO_4, filtered and evaporated to dryness. The residue is purified by chromatography on Al_2O_3 eluting with dichloromethane to obtain 0.46 g of solid with mp = 145-146 °C (yield = 80.2%).

The elemental analysis provided the following results:

	found:	C% 56.11	H% 4.75	N% 10.64
for $C_{19}H_{20}ClN_3O_5$	calc.:	C% 56.23	H% 4.97	N% 10.35

^{1}H-NMR ($CDCl_3$) δ: 7.92-7.63 (m, 1H, arom); 7.41-7.22 (m, 2H, arom); 7.23 (s, 2H, arom); 4.92 (t, 2H, J = 6, OCH_2); 4.44 (t, 2H, J = 5.2, NCH_2); 3.93 (s, 3H, OCH_3); 3.91 (s, 6H, $2OCH_3$); 2.63 (q, 2H, J = 6.2, $CH_2\underline{CH_2}CH_2$).

3-(5-Chloro-1*H*-benzotriazol-1-yl)propyl 3,4,5-trimethoxybenzoate (69)

To a solution of 1-(3-hydroxypropyl)-5-chloro-1*H*-benzotriazole (**i9**) (0.32 g;1.5 mmol) in 15 mL of anhydrous toluene, 0.15 g (1.5 mmol; d = 0.726; 0.21 mL) of triethylamine are first added, and then, dropwise, a solution of 3,4,5-trimethoxybenzoyl chloride in 5 mL of anhydrous toluene. (3,4,5-Trimethoxybenzoyl chloride was prepared starting from 1.5 mmol of the corresponding carboxylic acid and 6 mmol of $SOCl_2$ under reflux for 4 hours in anhydrous choloroform). The solution is stirred at RT for 12 hours. The toluic solution, after being washed with very cold water, is evaporated and the residue is dissolved in chloroform. The organic phase is washed twice first with a very cold 5% Na_2CO_3 solution and then with very cold water and finally it is dried over Na_2SO_4, filtered and evaporated to dryness. The residue is purified by chromatography on Al_2O_3 eluting with dichloromethane to obtain 0.46 g of solid with mp = 158-159 °C (yield = 77.1%).

The elemental analysis provided the following results:

	found:	C% 56.58	H% 5.02	N% 10.23
for $C_{19}H_{20}ClN_3O_5$	calc.:	C% 56.23	H% 4.97	N% 10.35

^{1}H-NMR ($CDCl_3$) δ: 7.97 (dd, 1H, J = 0.6, 8.8); 7:56-7.25 (m, 2H, arom); 7.21 (s, 2H, arom); 4.79 (t, 2H, J = 6.8, OCH_2); 4.41 (t, 2H, J = 6, NCH_2); 3.94 (s, 3H, OCH_3); 3.90 (s, 6H, $2OCH_3$); 2.56 (q, 2H, J = 6.4, $CH_2\underline{CH_2}CH_2$).

3-(6-Chloro-1*H*-benzotriazol-1-yl)propyl 3,4,5-trimethoxybenzoate (70)

To a solution of 1-(3-hydroxypropyl)-6-chloro-1*H*-benzotriazole (**i10**) (0.30 g; 1.4 mmol) in 15 mL of anhydrous toluene, 0.14 g (1.4 mmol; d = 0.726; 0.19 mL) of triethylamine are first added, and then, dropwise, a solution of 3,4,5-trimethoxybenzoyl chloride in 5 mL of anhydrous toluene. (3,4,5-Trimethoxybenzoyl chloride was prepared starting from 1.4 mmol of the corresponding carboxylic acid and 5.6 mmol of $SOCl_2$ under reflux for 4 hours in anhydrous choloroform). The solution is stirred at RT for 12 hours. The toluic solution, after being washed with very cold water, is evaporated and the residue is dissolved in chloroform. The organic phase is washed twice first with a very cold 5% Na_2CO_3 solution and then with very cold water and finally it is dried over Na_2SO_4, filtered and evaporated to dryness. The residue is purified by chromatography on Al_2O_3 eluting with dichloromethane to obtain 0.45 g of solid with mp = 161-162 °C (yield = 78.9%).

The elemental analysis provided the following results:

	found:	C% 55.97	H% 4.81	N% 10.18
for $C_{19}H_{20}ClN_3O_5$	calc.:	C% 56.23	H% 4.97	N% 10.35

^{1}H-NMR ($CDCl_3$) δ: 7.55-7.36 (m, 3H, arom); 7.18 (s, 2H, arom); 4.82 (t, 2H, J = 6.6, OCH_2); 4.42 (t, 2H, J = 5.8, NCH_2); 3.95 (s, 3H, OCH_3); 3.93 (s, 6H, $2OCH_3$); 2.57 (q, 2H, J = 6.4, $CH_2CH_2CH_2$).

5-Trifluoromethyl-*o*-phenylenediamine

a) 2 g (9.7 mmol) of 4-trifluoromethyl-2-nitroaniline are dissolved in 10 mL of ethanol. To this solution, 2,54 g (38 mmol) of zinc powder and 2 mL of acetic acid diluted with 2 mL di water are added. The mixture is heated at 100 °C for 2 hours. After 30 minutes, 2 mL of acetic acid diluted with 2 mL of water are added. Finally, the zinc dust is filtered and the ethanol is removed under vacuum. The acqueous phase is alkalized and extracted with dichloromethane. After being dried over Na_2SO_4, filtered and evaporated to dryness, the organic phase forms an oil that

rapidly crystallizes. (1.44 g; yield 84.2%). On the TLC plate (Al_2O_3/CH_2Cl_2 + 5% MeOH) a single spot is visualized. The raw solid is used without any further purification in the subsequent reactions.

b) 2 g (9.7 mmol) of 4-trifluoromethyl-2-nitroaniline are dissolved in 5 mL of ethanol. To this solution 2.54 g (38 mmoli) of zinc powder and 3 mL of NaOH 4N are added. The mixture is heated at 100 °C for 6 hours. After 1 hour, 2 mL of NaOH 4N and 0,5 g of zinc powder are added. Finally, the zinc dust is filtered and the ethanol is removed under vacuum. The aqueous suspension is diluted with 15 mL of water and extracted with dichloromethane. After being dried over Na_2SO_4, filtered and evaporated, the organic phase forms an oil that rapidly crystallizes. (1.47 g; yield 85.9%). On the TLC plate (Al_2O_3/CH_2Cl_2 + 5% MeOH) a single spot is visualized. The raw solid is used without any further purification in the subsequent reactions.

5-Trifluoromethyl-1*H*-benzotriazole

To a mixture of acetic acid (5.1 mL) and water (15 mL), 4.18 g (~24 mmol) of 5-trifluoromethyl-*o*-phenylenediamine are added while heating. To this solution, cooled in water/ice bath (0-5 °C) and stirred, a solution of $NaNO_2$ (1.66 g; 24 mmol) in 4 mL of water is added within 2 minutes.

The mixture turns immediately dark green and after a few minutes a precipitate with a hazelnut colour forms. The mixture is stirred for another 30 minutes at 5 °C and another 30 minutes at RT. Finally, the suspension is filtered and the solid is washed with abundant water. The solid material is collected and dried on KOH in a dryer, to obtain 4.21 g (94.8%) of a hazelnut color solid with mp=125-126 °C. The raw solid is used in the subsequent reactions.

2-(2-Hydroxyethyl)-5-trifluoromethyl-2*H*-benzotriazole (i11)

1-(2-hydroxyethyl)-5-trifluoromethyl-1*H*-benzotriazole (i12)

1-(2-hydroxyethyl)-6-trifluoromethyl-1*H*-benzotriazole (i13)

To a solution of 5-trifluoromethyl-1*H*-benzotriazole (1.87 g; 10 mmol) in 10 mL of NaOH 2N, 0.80 g (10 mmol; d = 1.201; 0.67 mL) 2-chloroethanol are added. The reaction mixture is heated at 100 °C for 2 hours. After cooling, the alkaline solution is extracted three times with chloroform. The organic phase is dried over Na_2SO_4, filtered and evaporated to dryness, to obtain a grey residue (2.14 g). The latter is purified by chromatography on SiO_2 (1:20), eluting with dichloromethane to obtain in high purity the 2-substituted isomer (N2-5) and the mixture of the 1-substituted

isomers (N1-5 and N1-6), that are then separated by flash chromatography eluting with ethyl acetate (overall yield of the three isomers = 84.4%).

The compounds were obtained with the following yields:

- 0.72 g of 2-(2-hydroxyethyl)-5-trifluoromethyl-2*H*-benzotriazole (**i11**) with mp = 73-74 °C (yield = 31.2 %).

The elemental analysis provided the following results:

	found:	C% 47.04	H% 3.41	N% 18.50
for $C_9H_8F_3N_3O$	calc.:	C% 46.76	H% 3.49	N% 18.18

^{1}H-NMR ($CDCl_3$) δ: 8.24 (d, 1H, J = 1, arom); 8.01 (d, 1H, J = 9, arom); 7.62 (dd, 1H, J = 9, 1, arom); 4.96 (q, 2H, CH_2O); 4.32 (q, 2H, NCH_2); 3.01 (t, 1H, J = 6,.4, OH).

- 0.58 g of 1-(2-hydroxyethyl)-5-trifluoromethyl-1*H*-benzotriazole (**i12**) with mp = 104-105 °C (yield = 25.1 %).

The elemental analysis provided the following results:

	found:	C% 46.76	H% 3.35	N% 17.91
for $C_9H_8F_3N_3O$	calc.:	C% 46.76	H% 3.49	N% 18.18

^{1}H-NMR ($CDCl_3$) δ: 8.19 (d, 1H, J = 0.8, arom); 8.03 (d, 1H, J = 9, arom); 7.66 (dd, 1H, J = 0.8, 9, arom); 4.93 (q, 2H, CH_2O); 4.31 (q, 2H, NCH_2); 3.09 (t, 1H, J = 6,.2, OH).

- 0.65 g of 1-(2-hydroxyethyl)-6-trifluoromethyl-1*H*-benzotriazole (**i13**) with mp = 101-102 °C (yield = 28.1 %).

The elemental analysis provided the following results:

	found:	C% 46.52	H% 3.24	N% 17.86
for $C_9H_8F_3N_3O$	calc.:	C% 46.76	H% 3.49	N% 18.18

^{1}H-NMR ($CDCl_3$) δ: 8.23 (d, 1H, J = 0.8, arom); 8.04 (d, 1H, J = 8.8, arom); 7.69 (dd, 1H, J = 0.8, 8.8, arom); 4.92 (q, 2H, CH_2O); 4.35 (q, 2H, NCH_2); 3.07 (t, 1H, J = 6,.2, OH).

2-(3-Hydroxypropyl)-5-trifluoromethyl-2*H*-benzotriazole (i14)

1-(3-Hydroxypropyl)-5-trifluoromethyl-1*H*-benzotriazole (i15)

1-(3-hydroxypropyl)-6-trifluoromethyl-1*H*-benzotriazole (i16)

To a solution of 5-trifluoromethyl-1*H*-benzotriazole (1.87 g; 10 mmol) in 10 mL of NaOH 2N, 0.94 g (10 mmol; d = 1.131; 0.83 mL) of 3-chloropropan-1-ol are added. The reaction mixture is heated at 100 °C for 2 ore. After cooling, the alkaline solution is extracted three times with chloroform. The organic phase is dried over Na_2SO_4, filtered and evaporated to dryness, to obtain a grey residue (2.16 g) that is purified by chromatography on SiO_2 (1:20), eluting with dichloromethane (yield of the three isomers = 79.6%).

The compounds were obtained with the following yields:

- 0.72 g of 2-(3-hydroxypropyl)-5-trifluoromethyl-2*H*-benzotriazole (**i14**) with mp = 70-71 °C (yield = 29.4 %).

The elemental analysis provided the following results below:

	found:	C% 48.74	H% 4.21	N% 17.48
for $C_{10}H_{10}F_3N_3O$	calc.:	C% 48.98	H% 4.11	N% 17.14

^{1}H-NMR ($CDCl_3$) δ: 8.03 (d, 1H, J = 7.2, arom); 7.68-7-24 (m, 2H, arom); 4.81 (t, 2H, J = 6.8, CH_2O); 3.66 (t, 2H, J = 6.8, NCH_2); 3.12 (s, 1H, OH); 2.25 (quintet, 2H, J = 5.6, $CH_2\underline{CH_2}CH_2$).

- 0.65 g of 1-(3-hydroxypropyl)-5-trifluoromethyl-1*H*-benzotriazole (**i15**) with mp = 101-102 °C (yield = 26.5 %).

The elemental analysis provided the following results:

	found:	C% 48.87	H% 4.20	N% 17.37
for $C_{10}H_{10}F_3N_3O$	calc.:	C% 48.98	H% 4.11	N% 17.14

^{1}H-NMR ($CDCl_3$) δ: 7.98-7-82 (m, 1H, arom); 7.50-7.36 (m, 2H, arom); 4.94 (t, 2H, J = 6.6, CH_2O); 3.68 (t, 2H, J = 6.6, NCH_2); 2.58 (s, 1H, OH); 2.34 (quintet, 2H, J = 6.8, $CH_2\underline{CH_2}CH_2$).

- 0.58 g of 1-(3-hydroxypropyl)-6-trifluoromethyl-1*H*-benzotriazole (**i16**) with mp = 98-99 °C (yield = 23.7 %).

The elemental analysis provided the results below:

	found:	C% 48.77	H% 4.07	N% 17.25

for $C_{10}H_{10}F_3N_3O$ calc.: C% 48.98 H% 4.11 N% 17.14

^{1}H-NMR ($CDCl_3$) δ: 7.98-7-82 (m, 1H, arom); 7.50-7.36 (m, 2H, arom); 4.94 (t, 2H, J = 6.6, CH_2O); 3.68 (t, 2H, J = 6.6, NCH_2); 2.58 (s, 1H, OH); 2.34 (quintet, 2H, J = 6.8, $CH_2\underline{CH_2}CH_2$).

2-(5-Trifluoromethyl-2*H*-benzotriazol-2-yl)ethyl 3,4,5-trimethoxybenzoate (71)

To a solution of 2-(2-hydroxyethyl)-5-trifluoromethyl-2*H*-benzotriazole (**i11**) (0.3 g; 1.3 mmol) in 15 mL of anhydrous toluene, 0.13 g (1.3 mmol; d =0.726; 0.18 mL) of triethylamine are first added, and then, dropwise, a solution of 3,4,5-trimethoxybenzoyl chloride in 5 mL of anhydrous toluene. (3,4,5-Trimethoxybenzoyl chloride was prepared starting from 1.3 mmol of the corresponding carboxylic acid and 5.2 mmol of $SOCl_2$ under reflux for 4 hours in anhydrous choloroform). The solution is stirred at RT for 6 hours. The toluic solution, after being washed with very cold water, is evaporated to dryness and the residue is dissolved in chloroform. After being washed twice with a very cold 5% Na_2CO_3 solution and then with very cold water, the organic phase is dried over Na_2SO_4, filtered and evaporated to dryness. The residue is purified by chromatography on SiO_2 eluting with dichloromethane, to obtain an oil that crystallizes after being treated with anhydrous Et_2O: 0.48 g of crystals with mp = 91-92 °C (yield= 87.3%).

The elemental analysis provided the following results:

found: C% 53.39 H% 4.45 N% 9.85

for $C_{19}H_{18}F_3N_3O_5$ calc.: C% 53.65 H% 4.27 N% 9.88

^{1}H-NMR ($CDCl_3$) δ: 7.96-7.82 (dd, 1H, J = 6.4, 3, arom); 7.51-7.36 (dd, 2H, J = 6.4, 3, arom); 7.16 (s, 2H, arom); 5.14 (t, 2H, J = 5.4, CH_2O); 4.96 (t, 2H, J = 5.4, NCH_2); 3.89 (s, 3H, OCH_3); 3.83 (s, 6H, $2OCH_3$).

2-(5-Trifluoromethyl-1*H*-benzotriazol-1-yl)ethyl 3,4,5-trimethoxybenzoate (72)

To a solution of 1-(2-hydroxyethyl)-5-trifluoromethyl-1*H*-benzotriazole (**i12**) (0.3 g; 1.3 mmol) in 15 mL of anhydrous toluene, 0.13 g (1.3 mmol; d =0.726; 0.18 mL) of triethylamine are first added, and then, dropwise, a solution of 3,4,5-

trimethoxybenzoyl chloride in 5 mL of anhydrous toluene. (3,4,5-Trimethoxybenzoyl chloride was prepared starting from 1.3 mmol of the corresponding carboxylic acid and 5.2 mmol of $SOCl_2$ under reflux for 4 hours in anhydrous choloroform). The solution is stirred at RT for 6 hours. The toluic solution, after being washed with very cold water, is evaporated to dryness and the residue is dissolved in chloroform. After being washed twice with a very cold 5% Na_2CO_3 solution and then with very cold water, the organic phase is dried over Na_2SO_4, filtered and evaporated to dryness The residue is purified by chromatography on SiO2 eluting with dichloromethane, to obtain an oil that crystallizes after being treated with anhydrous Et_2O: 0.41 g of crystals with mp = 117-118 °C (yield = 74.5%).

The elemental analysis provided the following results:

	found:	C% 53.58	H% 4.42	N% 9.58
for $C_{19}H_{18}F_3N_3O_5$	calc.:	C% 53.65	H% 4.27	N% 9.88

^{1}H-NMR ($CDCl_3$) δ: 7.96-7.82 (dd, 1H, J = 6.4, 3, arom); 7.51-7.36 (dd, 2H, J = 6.4, 3, arom); 7.16 (s, 2H, arom); 5.14 (t, 2H, J = 5.4, CH_2O); 4.96 (t, 2H, J = 5.4, NCH_2); 3.89 (s, 3H, OCH_3); 3.83 (s, 6H, $2OCH_3$).

2-(6-Trifluoromethyl-1*H*-benzotriazol-1-yl)ethyl 3,4,5-trimethoxybenzoato (73)

To a solution of 1-(2-hydroxyethyl)-6-trifluoromethyl-1*H*-benzotriazole (**i13**) (0.3 g; 1.3 mmol) in 15 mL of anhydrous toluene, 0.13 g (1.3 mmol; d =0.726; 0.18 mL) of triethylamine are first added, and then, dropwise, a solution of 3,4,5-trimethoxybenzoyl chloride in 5 mL of anhydrous toluene. (3,4,5-Trimethoxybenzoyl chloride was prepared starting from 1.3 mmol of the corresponding carboxylic acid and 5.2 mmol of $SOCl_2$ under reflux for 4 hours in anhydrous choloroform). The solution is stirred at RT for 6 hours. The toluic solution, after being washed with very cold water, is evaporated to dryness and the residue is dissolved in chloroform. After being washed twice with a very cold 5% Na_2CO_3 solution and then with very cold water, the organic phase is dried over Na_2SO_4, filtered and evaporated to dryness. The residue is purified by chromatography on SiO2 eluting with dichloromethane, to obtain an oil that crystallizes after being treated with anhydrous Et_2O: 0.46 g of crystals with mp = 134-135 °C (yield = 83.6%).

The elemental analysis provided the following results:

	found:	C%	53.43	H%	4.23	N% 9.58
for $C_{19}H_{18}F_3N_3O_5$	calc.:	C%	53.65	H%	4.27	N% 9.88

^{1}H-NMR ($CDCl_3$) δ: 7.96-7.82 (dd, 1H, J = 6.4, 3, arom); 7.51-7.36 (dd, 2H, J = 6.4, 3, arom); 7.16 (s, 2H, arom); 5.14 (t, 2H, J = 5.4, CH_2O); 4.96 (t, 2H, J = 5.4, NCH_2); 3.89 (s, 3H, OCH_3); 3.83 (s, 6H, $2OCH_3$).

3-(5-Trifluoromethyl-2*H*-benzotriazol-2-yl)propyl 3,4,5-trimethoxybenzoate (74)

To a solution of 2-(3-hydroxypropyl)-5-trifluoromethyl-2*H*-benzotriazole (**i14**) (0.30 g; 1.4 mmol) in 15 mL of anhydrous toluene, 0.14 g (1.4 mmol; d =0.726; 0.19 mL) of triethylamine are first added, and then, dropwise, a solution of 3,4,5-trimethoxybenzoyl chloride in 5 mL of anhydrous toluene. (3,4,5-Trimethoxybenzoyl chloride was prepared starting from 1.4 mmol of the corresponding carboxylic acid and 5.6 mmol of $SOCl_2$ under reflux for 4 hours in anhydrous choloroform). The solution is stirred at RT for 18 hours. The toluic solution, after being washed with very cold water, is evaporated to dryness and the residue is dissolved in chloroform. After being washed twice with a very cold 5% Na_2CO_3 solution and then with very cold water, the organic phase is dried over Na_2SO_4, filtered and evaporated to dryness. The residue is purified by chromatography on Al_2O_3 eluting with dichloromethane to obtain 0.42 g of crystals with mp = 136-138 °C (yield = 72.4%).

The elemental analysis provided the following results:

	found:	C%	54.64	H%	4.76	N% 9.22
for $C_{20}H_{20}F_3N_3O_5$	calc.:	C%	54.67	H%	4.59	N% 9.56

^{1}H-NMR ($CDCl_3$) δ: 8.21 (s, 1H, arom); 7.98 (d, 1H, J = 9, arom); 7.59 (dd, 1H, J = 1.4, 8.8, arom); 7.26 (s, 2H, arom); 4.99 (t, 2H, J = 6.8, CH_2O); 4.46 (t, 2H, J = 6, NCH_2); 3.94 (s, 3H, OCH_3); 3.92 (s, 6H, $2OCH_3$); 2.67 (q, 2H, J = 6.6, $CH_2\underline{CH_2}CH_2$).

3-(5-Trifluoromethyl-1*H*-benzotriazol-1-yl)propyl 3,4,5-trimethoxybenzoate (75)

To a solution of 1-(3-hydroxypropyl)-5-trifluoromethyl-1*H*-benzotriazole (**i15**) (0.3 g; 1.2 mmol) in 15 mL of anhydrous toluene, 0.12 g (1.3 mmol; d = 0.726; 0.17 mL) of triethylamine are first added, and then, dropwise, a solution of 3,4,5-trimethoxybenzoyl chloride in 5 mL of anhydrous toluene. (3,4,5-Trimethoxybenzoyl chloride was prepared starting from 1.2 mmol of the corresponding carboxylic acid and 4.8 mmol of $SOCl_2$ under reflux for 4 hours in anhydrous choloroform). The solution is stirred at RT for 6 hours. The toluic solution, after being washed with very cold water, is evaporated to dryness and the residue is dissolved in chloroform. After being washed twice with a very cold 5% Na_2CO_3 solution and then with very cold water, the organic phase is dried over Na_2SO_4, filtered and evaporated to dryness. The residue is purified by chromatography on Al_2O_3 eluting with dichloromethane to obtain 0.37 g of crystals with mp = 136-138 °C (yield = 75.5%).

The elemental analysis provided the following results:

	found:	C% 54.56	H% 4.47	N% 9.32
for $C_{20}H_{20}F_3N_3O_5$	calc.:	C% 54.67	H% 4.59	N% 9.56

^{1}H-NMR ($CDCl_3$) δ: 8.17 (dd, 1H, J = 0.6, 8.6, arom); 7.85 (d, 1H, J = 0.8, arom); 7.58 (dd, 1H, J = 1.6, 8.8, arom); 7.18 (s, 2H, arom); 4.89 (t, 2H, J = 6.8, CH_2O); 4.43 (t, 2H, J = 6.2, NCH_2); 3.95 (s, 3H, OCH_3); 3.91 (s, 6H, $2OCH_3$); 2.61 (q, 2H, J = 6.2, $CH_2\underline{CH_2}CH_2$).

3-(6-Trifluoromethyl-1*H*-benzotriazol-1-yl)propyl 3,4,5-trimethoxybenzoate (76)

To a solution of 1-(3-hydroxypropyl)-6-trifluoromethyl-1*H*-benzotriazole (**i16**) (0.3 g; 1.2 mmol) in 15 mL of anhydrous toluene, 0.12 g (1.3 mmol; d = 0.726; 0.17 mL) of triethylamine are first added, and then, dropwise, a solution of 3,4,5-trimethoxybenzoyl chloride in 5 mL of anhydrous toluene. (3,4,5-Trimethoxybenzoyl chloride was prepared starting from 1.2 mmol of the corresponding carboxylic acid and 4.8 mmol of $SOCl_2$ under reflux for 4 hours in anhydrous choloroform). The solution is stirred at RT for 6 hours. The toluic solution, after being washed with very cold water, is evaporated to dryness and the residue is dissolved in chloroform. After being washed twice with a very cold 5%

Na_2CO_3 solution and then with very cold water, the organic phase is dried over Na_2SO_4, filtered and evaporated to dryness. The residue is purified by chromatography on Al_2O_3 eluting with dichloromethane to obtain 0.35 g of crystals with mp = 136-138 °C (yield = 71.4%).

The elemental analysis provided the following results:

	found:	C%	54.78	H%	4.78	N% 9.30
for $C_{20}H_{20}F_3N_3O_5$	calc.:	C%	54.67	H%	4.59	N% 9.56

^{1}H-NMR ($CDCl_3$) δ: 8.36 (d, 1H, J = 0.8, arom); 7.68 (m, 2H, arom); 7.18 (s, 2H, arom); 4.88 (t, 2H, J = 6.8, CH_2O); 4.43 (t, 2H, J = 6, NCH_2); 3.96 (s, 3H. OCH_3); 3.93 (s, 6H, $2OCH_3$); 2.60 (q, 2H, J = 6.2, $CH_2\underline{CH_2}CH_2$).

2-(2-Hydroxyethyl)-5-methoxy-2*H*-benzotriazole (i17)

1-(2-hydroxyethyl)-5-methoxy-1*H*-benzotriazole (i18)

1-(2-hydroxyethyl)-6-methoxy-1*H*-benzotriazole (i19)

To a solution of 5-methoxy-1*H*-benzotriazole (2.24 g; 15 mmol) in 10 mL of NaOH 2N, 1.20 g (15 mmol; d = 1.201; 1.00 mL) of 2-chloroethanol are added. The reaction mixture is heated at 100 °C for 2 hours. After cooling of the mixture, the alkaline solution is extracted three times with chloroform and the organic phase is dried over Na_2SO_4, filtered and evaporated to dryness, to obtain a grey residue (2.44 g). The latter is purified by chromatography on SiO_2 (1:20) eluting with chloroform, to obtain in high purity the 2-substituted isomer (N2-5) and the mixture of the 1-substituted isomers (N1-5 and N1-6), that are then separated by flash chromatography eluting with ethyl acetate (overall yield of the three isomers = 84.7%).

The compounds were obtained with the following yields:

- 0.82 g of 2-(2-hydroxyethyl)-5-methoxy-2*H*-benzotriazole (**i17**) with mp = 76-77 °C (yield = 30.8 %).

The elemental analysis provided the following results:

	found:	C%	61.27	H%	6.19	N% 23.58
for $C_9H_{11}N_3O$	calc.:	C%	61.00	H%	6.26	N% 23.71

^{1}H-NMR ($CDCl_3$) δ: 7.73 (dd, 1H, J = 1.2, 9.0, arom); 7.17-6.94 (m, 2H arom); 4.83 (t, 2H, J = 4.8, CH_2O); 4.25 (t, 2H, J = 4.8, NCH_2); 3.91 (s, 3H, OCH_3); 3.29 (s, 1H, OH).

- 0.74 g of 1-(2-hydroxyethyl)-5-methoxy-1*H*-benzotriazole (**i18**) with mp = 90-91 °C (yield= 27.9 %).

The elemental analysis provided the following results:

	found:	C% 61.09	H% 6.42	N% 23.64
for $C_9H_{11}N_3O$	calc.:	C% 61.00	H% 6.26	N% 23.71

^{1}H-NMR ($CDCl_3$) δ: 7.48 (dd, 1H, J = 0.6, 9.0, arom); 7.16-7.03 (m, 1H, arom); 6.93-6.82 (m, 1H, arom); 4.67 (t, 2H, J = 4.4, CH_2O); 4.25 (t, 2H, J = 4.6, NCH_2); 3.81 (s, 3H, OCH_3); 3.22 (s, 1H, OH).

- 0.69 g of 1-(2-hydroxyethyl)-6-methoxy-1*H*-benzotriazole (**i19**) with mp = 88-89 °C (yield = 26.0 %).

The elemental analysis provided the following results:

	found:	C% 61.17	H% 6.54	N% 23.48
for $C_9H_{11}N_3O$	calc.:	C% 61.00	H% 6.26	N% 23.71

^{1}H-NMR ($CDCl_3$) δ: 7.50 (dd, 1H, J = 0.6, 9.2, arom); 7.15-7.04 (m, 1H, arom); 6.98-6.85 (m, 1H, arom); 4.67 (t, 2H, J = 4.4, CH_2O); 4.25 (t, 2H, J = 5.0, NCH_2); 3.82 (s, 3H, OCH_3); 3.10 (s, 1H, OH).

2-(3-Hydroxypropyl)-5-methoxy-2*H*-benzotriazole (i20)

1-(3-hydroxypropyl)-5-methoxy-1*H*-benzotriazole (i21)

1-(3-hydroxypropyl)-6-methoxy-1*H*-benzotriazole (i22)

To a solution of 5-methoxy-1*H*-benzotriazole (2.46 g; 16 mmol) in 10 mL of NaOH 2N, 1.51 g (16 mmol; d = 1.131; 1.34 mL) of 3-chloropropan-1-ol are added. The reaction mixture is heated at 100 °C for 2 hours. After cooling of the mixture, the alkaline solution is extracted three times with chloroform and the organic phase is dried over Na_2SO_4, filtered and evaporated to dryness, to obtain a grey residue (2.83 g). The latter is purified by chromatography on SiO_2 (1:20) eluting with chloroform, to obtain in high purity the 2-substituted isomer (N2-5) and the mixture of the 1-substituted isomers (N1-5 and N1-6), that are then separated by flash chromatography eluting with ethyl acetate (overall yield of the three isomers = 81.6%).

The compounds were obtained with the following yields:

- 0.93 g of 2-(3-hydroxypropyl)-5-methoxy-2*H*-benzotriazole (**i20**), colorless oil (yield = 30.4 %).

The elemental analysis provided the results below:

	found:	C%	62.77	H%	6.67	N% 21.73
for $C_{10}H_{13}N_3O$	calc.:	C%	62.81	H%	6.85	N% 21.97

^{1}H-NMR ($CDCl_3$) δ: 7.77 (dd, 1H, J = 1,2, 9, arom); 7.23-7.17 (m, 1H, arom); 7.09-7.01 (m, 1H, arom); 4.82 (t, 2H, J = 4.6, CH_2O); 3.87 (t, 2H, J = 4.6, CH_2N); 2.41 (q, 2H, J = 4.8, $CH_2\underline{CH_2}CH_2$); 2.33 (s, 1H, OH).

- 0.82 g of 1-(3-hydroxypropyl)-5-methoxy-1*H*-benzotriazole (**i21**), colorless oil (yield = 26.8 %).

The elemental analysis provided the following results:

	found:	C%	62.55	H%	7.03	N% 22.12
for $C_{10}H_{13}N_3O$	calc.:	C%	62.81	H%	6.85	N% 21.97

^{1}H-NMR ($CDCl_3$) δ: 7.88 (d, 1H, J = 4.8, arom); 7.52-7.38 (m, 1H, arom); 7.29-7.18 (m, 1H, arom); 4.74 (t, 2H, J = 4.8, CH_2O); 3.72 (t, 2H, J = 5.8, NCH_2); 3.09 (s, 1H, OH); 2.22 (q, 2H, J = 6, $CH_2\underline{CH_2}CH_2$).

- 0.75 g of 1-(3-hydroxypropyl)-6-methoxy-1*H*-benzotriazole (**i22**), colorless oil (yield = 24.5 %).

The elemental analysis provided the results below:

	found:	C%	62.47	H%	7.07	N% 21.84
for $C_{10}H_{13}N_3O$	calc.:	C%	62.81	H%	6.85	N% 21.97

^{1}H-NMR ($CDCl_3$) δ: 7.89 (d, 1H, J = 4.6, arom); 7.55-7.39 (m, 1H, arom); 7.28-7.16 (m, 1H, arom); 4.77 (t, 2H, J = 5.6, CH_2O); 3.62 (t, 2H, J = 5.6, NCH_2); 2.97 (s, 1H, OH); 2.18 (q, 2H, J = 6.2, $CH_2\underline{CH_2}CH_2$).

2-(5-Methoxy-2*H*-benzotriazol-2-yl)ethyl 3,4,5-trimethoxybenzoate (77)

To a solution of 2-(2-hydroxyethyl)-5-methoxy-2*H*-benzotriazole (**i17**) (0.44 g; 2.5 mmol) in 15 mL of anhydrous toluene, 0.25 g (2.5 mmol; d = 0.726; 0.35 mL) of triethylamine are first added and then, dropwise, a solution of 3,4,5-trimethoxybenzoyl chloride in 5 mL of anhydrous toluene. (3,4,5-

Trimethoxybenzoyl chloride was prepared starting from 2.5 mmol of the corresponding carboxylic acid and 6 mmol of $SOCl_2$ under reflux for 4 hours in anhydrous choloroform). The solution is stirred at RT for 12 hours. The toluic solution, after being washed with very cold water, is evaporated and the residue is dissolved in chloroform. The organic phase is washed twice first with a very cold 5% Na_2CO_3 solution and then with very cold water and finally it is dried over Na_2SO_4, filtered and evaporated to dryness. The residue is purified by chromatography on SiO_2 eluting with dichloromethane, to obtain 0.81 g of whitw solid with mp = 117-118 °C (yield= 83.4%).

The elemental analysis provided the following results:

	found:	C% 59.04	H% 5.31	N% 10.62
for $C_{19}H_{21}N_3O_6$	calc.:	C% 58.91	H% 5.46	N% 10.85

^{1}H-NMR ($CDCl_3$) δ: 7.74 (d, 1H, J = 10.1, 1H); 7.32- 7.09 (m, 2H, arom); 7.07 (s, 2H, arom); 5.07 (t, 2H, J = 5.2, CH_2O); 4.95 (t, 2H, J = 5.4, NCH_2); 3.90 (s, 3H, OCH_3); 3.88 (s, 6H, $2OCH_3$); 3.84 (s, 3H, OCH_3).

2-(5-Methoxy-1*H*-benzotriazol-1-yl)ethyl 3,4,5-trimethoxybenzoate (78)

To a solution of 1-(2-hydroxyethyl)-5-methoxy-1*H*-benzotriazole (**i18**) (0.44 g; 2.5 mmol) in 15 mL of anhydrous toluene, 0.25 g (2.5 mmol; d = 0.726; 0.35 mL) of triethylamine are first added, and then, dropwise, a solution of 3,4,5-trimethoxybenzoyl chloride in 5 mL of anhydrous toluene. (3,4,5-Trimethoxybenzoyl chloride was prepared starting from 2.5 mmol of the corresponding carboxylic acid and 6 mmol of $SOCl_2$ under reflux for 4 hours in anhydrous choloroform). The solution is stirred at RT for 12 hours. The toluic solution, after being washed with very cold water, is evaporated and the residue is dissolved in chloroform. The organic phase is washed twice first with a very cold 5% Na_2CO_3 solution and then with very cold water and finally it is dried over Na_2SO_4, filtered and evaporated to dryness. The residue is purified by chromatography on SiO_2 eluting with dichloromethane, to obtain 0.83 g of whitw solid with mp = 117-118 °C (yield= 85.1%).

The elemental analysis provided the following results:

	found:	C% 59.16	H% 5.58	N% 10.73
for $C_{19}H_{21}N_3O_6$	calc.:	C% 58.91	H% 5.46	N% 10.85

^{1}H-NMR ($CDCl_3$) δ: 7.94 (d, 1H, J = 9, 1H); 7.10 (s, 2H, arom); 7.09- 6.76 (m, 2H, arom); 4.98 (t, 2H, J = 5.6, CH_2O); 4.85 (t, 2H, J = 5.2, NCH_2); 3.92 (s, 3H, OCH_3); 3.88 (s, 6H, $2OCH_3$); 3.73 (s, 3H, OCH_3).

2-(6-Methoxy-1*H*-benzotriazol-1-yl)ethyl 3,4,5-trimethoxybenzoate (79)

To a solution of 1-(2-hydroxyethyl)-6-methoxy-1*H*-benzotriazole (**i19**) (0.44 g; 2.5 mmol) in 15 mL of anhydrous toluene, 0.25 g (2.5 mmol; d = 0.726; 0.35 mL) of triethylamine are first added, and then, dropwise, a solution of 3,4,5-trimethoxybenzoyl chloride in 5 mL of anhydrous toluene. (3,4,5-Trimethoxybenzoyl chloride was prepared starting from 2.5 mmol of the corresponding carboxylic acid and 6 mmol of $SOCl_2$ under reflux for 4 hours in anhydrous choloroform). The solution is stirred at RT for 12 hours. The toluic solution, after being washed with very cold water, is evaporated and the residue is dissolved in chloroform. The organic phase is washed twice first with a very cold 5% Na_2CO_3 solution and then with very cold water and finally it is dried over Na_2SO_4, filtered and evaporated to dryness. The residue is crystallized from anhydrous Et_2O to obtain 0.41 g of crystals with mp = 125-126°C (yield = 80.9%). The elemental analysis provided the following results:

	found:	C% 58.73	H% 5.67	N% 10.59
for $C_{19}H_{21}N_3O_6$	calc.:	C% 58.91	H% 5.46	N% 10.85

^{1}H-NMR ($CDCl_3$) δ: 7.58-7.18 (m, 3H, arom); 7.10 (s, 2H, arom); 5.01 (t, 2H, J = 5.4, CH_2O); 4.84 (t, 2H, J = 5.6, NCH_2); 3.92 (s, 3H, OCH_3); 3.89 (s, 6H, $2OCH_3$); 3.79 (s, 3H, OCH_3).

3-(5-Methoxy-2*H*-benzotriazol-2-yl)propyl 3,4,5-trimethoxybenzoate (80)

To a solution of 2-(3-hydroxypropyl)-5-methoxy-2*H*-benzotriazole (**i20**) (0.38 g; 2 mmol) in 15 mL of anhydrous toluene, 0.20 g (2 mmol; d =0.726; 0.28 mL) of triethylamine are first added, and then, dropwise, a solution of 3,4,5-trimethoxybenzoyl chloride in 5 mL of anhydrous toluene. (3,4,5-Trimethoxybenzoyl chloride was prepared starting from 2 mmol of the corresponding carboxylic acid and 6 mmol of $SOCl_2$ under reflux for 4 hours in anhydrous choloroform). The solution is stirred at RT for 12 hours. The toluic solution, after being washed with very cold water, is evaporated and the residue is dissolved in chloroform. The organic phase is washed twice first with a very cold

5% Na_2CO_3 solution and then with very cold water and finally it is dried over Na_2SO_4, filtered and evaporated to dryness. The residue is purified by chromatography on Al_2O_3 eluting with dichloromethane to obtain 0.62 g of solid with mp = 117-118 °C (yield = 77.4%).

The elemental analysis provided the following results:

	found:	C% 59.71	H% 5.85	N% 10.69
for $C_{20}H_{23}N_3O_6$	calc.:	C% 59.84	H% 5.78	N% 10.47

^{1}H-NMR ($CDCl_3$) δ: 7.78 (m, 1H, arom); 7.41- 7.12 (m, 2H, arom); 7.09 (s, 2H, arom); 5.08 (t, 2H, J = 5.4, CH_2O); 4.96 (t, 2H, J = 5.4, NCH_2); 3.91 (s, 3H, OCH_3); 3.89 (s, 6H, $2OCH_3$); 3.84 (s, 3H, OCH_3); 2.66 (q, 2H, J = 6, $CH_2\underline{CH_2}CH_2$).

3-(5-Methoxy-1*H*-benzotriazol-1-yl)propyl 3,4,5-trimethoxybenzoate (81)

To a solution of 1-(3-hydroxypropyl)-5-methoxy-1*H*-benzotriazole (**i21**) (0.38 g; 2 mmol) in 15 mL of anhydrous toluene, 0.20 g (2 mmol; d = 0.726; 0.25 mL) of triethylamine are first added, and then, dropwise, a solution of 3,4,5-trimethoxybenzoyl chloride in 5 mL of anhydrous toluene. (3,4,5-Trimethoxybenzoyl chloride was prepared starting from 2 mmol of the corresponding carboxylic acid and 6 mmol of $SOCl_2$ under reflux for 4 hours in anhydrous choloroform). The solution is stirred at RT for 12 hours. The toluic solution, after being washed with very cold water, is evaporated and the residue is dissolved in chloroform. The organic phase is washed twice first with a very cold 5% Na_2CO_3 solution and then with very cold water and finally it is dried over Na_2SO_4, filtered and evaporated to dryness. The residue is purified by chromatography on Al_2O_3 eluting with dichloromethane to obtain 0.63 g of solid with mp = 156-157 °C (yield = 79.3%).

The elemental analysis provided the following results:

	found:	C% 59.88	H% 5.44	N% 10.71
for $C_{20}H_{23}N_3O_6$	calc.:	C% 59.84	H% 5.78	N% 10.47

^{1}H-NMR ($CDCl_3$) δ: 7.96 (d, 1H, J = 9.4, 1H); 7.55-7-22 (m, 2H, arom); 7.12 (s, 2H, arom); 4.86 (t, 2H, J = 5.6, CH_2O); 4.65 (t, 2H, J = 5.2, NCH_2); 3.94 (s, 3H, OCH_3); 3.89 (s, 6H, $2OCH_3$); 3.77 (s, 3H, OCH_3); 2.55 (q, 2H, J = 6.2, $CH_2\underline{CH_2}CH_2$).

3-(6-Methoxy-1*H*-benzotriazol-1-yl)propyl 3,4,5-trimethoxybenzoate (82)

To a solution of 1-(3-hydroxypropyl)-6-methoxy-1*H*-benzotriazole (**i22**) (0.38 g; 2 mmol) in 15 mL of anhydrous toluene, 0.20 g (2 mmol; d = 0.726; 0.25 mL) of triethylamine are first added, and then, dropwise, a solution of 3,4,5-trimethoxybenzoyl chloride in 5 mL of anhydrous toluene. (3,4,5-Trimethoxybenzoyl chloride was prepared starting from 2 mmol of the corresponding carboxylic acid and 6 mmol of $SOCl_2$ under reflux for 4 hours in anhydrous choloroform). The solution is stirred at RT for 12 hours. The toluic solution, after being washed with very cold water, is evaporated and the residue is dissolved in chloroform. The organic phase is washed twice first with a very cold 5% Na_2CO_3 solution and then with very cold water and finally it is dried over Na_2SO_4, filtered and evaporated to dryness. The residue is purified by chromatography on Al_2O_3 eluting with dichloromethane to obtain 0.6 g of solid with mp = 167-169 °C (yield = 75.2%).

The elemental analysis provided the following results:

	found:	C% 60.05	H% 5.93	N% 10.32
for $C_{20}H_{23}N_3O_6$	calc.:	C% 59.84	H% 5.78	N% 10.47

^{1}H-NMR ($CDCl_3$) δ: 7.57-7.21 (m, 3H, arom); 7.13 (s, 2H, arom); 4.96 (t, 2H, J = 6.2, OCH_2); 4.78 (t, 2H, J = 5.8, NCH_2); 3.95 (s, 3H, OCH_3); 3.92 (s, 6H, $2OCH_3$); 3.78 (s, 3H, OCH_3); 2.5 (q, 2H, J = 6.2, $CH_2CH_2CH_2$).

2-(2-Hydroxyethyl)-5-benzoyl-2*H*-benzotriazole (i23)

1-(2-hydroxyethyl)-5-benzoyl-1*H*-benzotriazole (i24)

1-(2-hydroxyethyl)-6-benzoyl-1*H*-benzotriazole (i25)

To a solution of 5-benzoyl-1*H*-benzotriazole (2.23 g; 10 mmol) in 10 mL of NaOH 2N, 0.80 g (10 mmol; d = 1.201; 1.00 mL) of 2-chloroethanol are added. The reaction mixture is heated at 100 °C for 2 hours. After cooling of the mixture, the alkaline solution is extracted three times with chloroform and the organic phase is dried over Na_2SO_4, filtered and evaporated to dryness, to obtain a grey residue (2.51 g). The latter is purified by chromatography on SiO_2 (1:20) eluting with chloroform, to obtain in high purity the 2-substituted isomer (N2-5) and the mixture of the 1-substituted isomers (N1-5 and N1-6), that are then separated by flash

chromatography eluting with ethyl acetate (overall yield of the three isomers = 86.2%).

The compounds were obtained with the following yields:

- 0.83 g of 2-(2-hydroxyethyl)-5-benzoyl-2*H*-benzotriazole (**i23**) with mp = 104-105 °C (yield = 31.2 %).

The elemental analysis provided the following results:

	found:	C% 67.33	H% 4.90	N% 15.50
for $C_{15}H_{13}N_3O_2$	calc.:	C% 67.40	H% 4.90	N% 15.72

^{1}H-NMR ($CDCl_3$) δ: 8.32 (d, 1H, J = 1, arom); 8.04-7.79 (m, 3H, arom); 7.72-7.46 (m, 4H arom); 4.96 (t, 2H, J = 4.8, CH_2O); 4.31 (t, 2H, J = 5.0, NCH_2); 3.01 (s, 1H, OH).

- 0.76 g of 1-(2-hydroxyethyl)-5-benzoyl-1*H*-benzotriazole (**i24**) with mp = 123-124 °C (yield= 28.5 %).

The elemental analysis provided the following results:

	found:	C% 67.45	H% 4.80	N% 15.42
for $C_{15}H_{13}N_3O_2$	calc.:	C% 67.40	H% 4.90	N% 15.72

^{1}H-NMR ($CDCl_3$) δ: 8.18-8.02 (m, 1H, arom); 7.88-7.21 (m, 7H, arom); 4.83 (t, 2H, J = 5.2, CH_2O); 4.26 (t, 2H, J = 4.8, NCH_2); 3.81 (s, 3H, OCH_3); 3.91 (s, 1H, OH).

- 071 g of 1-(2-hydroxyethyl)-6-benzoyl-1*H*-benzotriazole (**i25**) with mp = 99-101 °C (yield = 26.5 %).

The elemental analysis provided the following results:

	found:	C% 67.69	H% 4.89	N% 15.97
for $C_{15}H_{13}N_3O_2$	calc.:	C% 67.40	H% 4.90	N% 15.72

^{1}H-NMR ($CDCl_3$) δ: 8.10 (dd, 1H, J = 1.4, 8.6, arom); 7.94-7.38 (m, 7H, arom); 4.84 (t, 2H, J = 4.8, CH_2O); 4.31 (t, 2H, J = 5.4, NCH_2); 2.60 (s, 1H, OH).

2-(3-Hydroxypropyl)-5-benzoyl-2*H*-benzotriazole (i26)

1-(3-hydroxypropyl)-5-benzoyl-1*H*-benzotriazole (i27)

1-(3-hydroxypropyl)-6-benzoyl-1*H*-benzotriazole (i28)

To a solution of 5-benzoyl-1*H*-benzotriazole (2.23 g; 10 mmol) in 10 mL of NaOH 2N, 0.94 g (10 mmol; d = 1.131; 1.34 mL) of 3-chloropropan-1-ol are added. The reaction mixture is heated at 100 °C for 2 hours. After cooling of the mixture, the alkaline solution is extracted three times with chloroform and the organic phase is dried over Na_2SO_4, filtered and evaporated to dryness, to obtain a grey residue (2.83 g). The latter is purified by chromatography on SiO_2 (1:20) eluting with chloroform, to obtain in high purity the 2-substituted isomer (N2-5) and the mixture of the 1-substituted isomers (N1-5 and N1-6), that are then separated by flash chromatography eluting with ethyl acetate (overall yield of the three isomers = 82.2%).

The compounds were obtained with the following yields:

- 0.85 g of 2-(3-hydroxypropyl)-5-benzoyl-2*H*-benzotriazole (**i26**) with mp = 93-94°C (yield = 30.1 %).

The elemental analysis provided the results below:

	found:	C% 68.56	H% 5.71	N% 15.22
for $C_{16}H_{15}N_3O_2$	calc.:	C% 68.31	H% 5.37	N% 14.94

^{1}H-NMR ($CDCl_3$) δ: 8.19 (dd, 1H, J = 1,2, 9, arom); 8.02-7.38 (m, 7H); 4.87 (t, 2H, J = 4.8, CH_2O); 3.91 (t, 2H, J = 4.8, CH_2N); 3.27 (s, 1H, OH); 2.44 (q, 2H, J = 4.8, $CH_2\underline{CH_2}CH_2$).

- 0.79 g of 1-(3-hydroxypropyl)-5-benzoyl-1*H*-benzotriazole (**i27**) with mp = 132-133°C (yield = 28.2 %).

The elemental analysis provided the following results:

	found:	C% 68.45	H% 5.23	N% 14.81
for $C_{16}H_{15}N_3O_2$	calc.:	C% 68.31	H% 5.37	N% 14.94

^{1}H-NMR ($CDCl_3$) δ: 8.21-8.04 (m, 1H, arom); 7.86-7.27 (m, 7H, arom); 4.79 (t, 2H, J = 4.8, CH_2O); 3.66 (t, 2H, J = 5.8, NCH_2); 3.34 (s, 1H, OH); 2.18 (q, 2H, J = 6, $CH_2\underline{CH_2}CH_2$).

- 0.67 g of 1-(3-hydroxypropyl)-6-benzoyl-1*H*-benzotriazole (**i28**) with mp = 106-107°C (yield = 23.8 %).

The elemental analysis provided the results below:

	found:	C%	68.05	H%	5.44	N% 14.75
for $C_{16}H_{15}N_3O_2$	calc.:	C%	68.31	H%	5.37	N% 14.94

^{1}H-NMR ($CDCl_3$) δ: 8.08 (dd; 1H, J = 1.2, 8.8, arom); 7.82-7.31 (m, 7H, arom); 4.84 (t, 2H, J = 5.4, CH_2O); 3.65 (t, 2H, J = 5.6, NCH_2); 2.73 (s, 1H, OH); 2.25 (q, 2H, J = 6.2, $CH_2\underline{CH_2}CH_2$).

2-(5-Benzoyl-2*H*-benzotriazol-2-yl)ethyl 3,4,5-trimethoxybenzoate (83)

To a solution of 2-(2-hydroxyethyl)-5-benzoyl-2*H*-benzotriazole (**i23**) (0.43 g; 1.6 mmol) in 15 mL of anhydrous toluene, 0.16 g (1.6 mmol; d = 0.726; 0.22 mL) of triethylamine are first added and then, dropwise, a solution of 3,4,5-trimethoxybenzoyl chloride in 5 mL of anhydrous toluene. (3,4,5-Trimethoxybenzoyl chloride was prepared starting from 1.6 mmol of the corresponding carboxylic acid and 6 mmol of $SOCl_2$ under reflux for 4 hours in anhydrous choloroform). The solution is stirred at RT for 12 hours. The toluic solution, after being washed with very cold water, is evaporated and the residue is dissolved in chloroform. The organic phase is washed twice first with a very cold 5% Na_2CO_3 solution and then with very cold water and finally it is dried over Na_2SO_4, filtered and evaporated to dryness. The residue is purified by chromatography on SiO_2 eluting with dichloromethane, to obtain 0.58 g of solid with mp = 105-106 °C (yield= 78.7%).

The elemental analysis provided the following results:

	found:	C%	65.38	H%	5.27	N% 9.02
for $C_{25}H_{23}N_3O_6$	calc.:	C%	65.07	H%	5.02	N% 9.11

^{1}H-NMR ($CDCl_3$) δ: 8.31 (s, 1H, arom); 8.03-7.41 (m, 7H, arom); 7.20 (s, 2H, arom); 5.19 (t, 2H, J = 5, CH_2O); 4.98 (t, 2H, J = 5.4, NCH_2); 3.91 (s, 3H, OCH_3); 3.85 (s, 6H, $2OCH_3$).

2-(5-Benzoyl-1*H*-benzotriazol-1-yl)ethyl 3,4,5-trimethoxybenzoate (84)

To a solution of 1-(2-hydroxyethyl)-5-benzoyl-1*H*-benzotriazole (**i24**) (0.4 g; 1.5 mmol) in 15 mL of anhydrous toluene, 0.15 g (1.5 mmol; d = 0.726; 0.21 mL) of triethylamine are first added, and then, dropwise, a solution of 3,4,5-

trimethoxybenzoyl chloride in 5 mL of anhydrous toluene. (3,4,5-Trimethoxybenzoyl chloride was prepared starting from 1.5 mmol of the corresponding carboxylic acid and 6 mmol of $SOCl_2$ under reflux for 4 hours in anhydrous choloroform). The solution is stirred at RT for 12 hours. The toluic solution, after being washed with very cold water, is evaporated and the residue is dissolved in chloroform. The organic phase is washed twice first with a very cold 5% Na_2CO_3 solution and then with very cold water and finally it is dried over Na_2SO_4, filtered and evaporated to dryness. The residue is purified by chromatography on SiO_2 eluting with dichloromethane, to obtain 0.54 g of whitw solid with mp = 161-162 °C (yield= 77.7%).

The elemental analysis provided the following results:

	found:	C%	65.29	H%	5.16	N% 9.33
for $C_{25}H_{23}N_3O_6$	calc.:	C%	65.07	H%	5.02	N% 9.11

^{1}H-NMR ($CDCl_3$) δ: 7.99 (d, 1H, J = 9.4, 1H); 7.94-7.27 (m, 7H, arom); 7.16 (s, 2H, arom); 5.12 (t, 2H, J = 5.2, CH_2O); 4.92 (t, 2H, J = 5.2, NCH_2); 3.92 (s, 3H, OCH_3); 3.87 (s, 6H, $2OCH_3$).

2-(6-Benzoyl-1*H*-benzotriazol-1-yl)ethyl 3,4,5-trimethoxybenzoate (85)

To a solution of 1-(2-hydroxyethyl)-6-benzoyl-1*H*-benzotriazole (**i25**) (0.37 g; 1.4 mmol) in 15 mL of anhydrous toluene, 0.20 g (1.4 mmol; d = 0.726; 0.20 mL) of triethylamine are first added, and then, dropwise, a solution of 3,4,5-trimethoxybenzoyl chloride in 5 mL of anhydrous toluene. (3,4,5-Trimethoxybenzoyl chloride was prepared starting from 1.4 mmol of the corresponding carboxylic acid and 5.6 mmol of $SOCl_2$ under reflux for 4 hours in anhydrous choloroform). The solution is stirred at RT for 12 hours. The toluic solution, after being washed with very cold water, is evaporated and the residue is dissolved in chloroform. The organic phase is washed twice first with a very cold 5% Na_2CO_3 solution and then with very cold water and finally it is dried over Na_2SO_4, filtered and evaporated to dryness. The residue is purified by chromatography on SiO_2 eluting with dichloromethane, to obtain 0.48 g of solid with mp = 157-158 °C (yield= 74.3%).

The elemental analysis provided the following results:

	found:	C%	64.99	H%	5.07	N% 9.26
for $C_{25}H_{23}N_3O_6$	calc.:	C%	65.07	H%	5.02	N% 9.11

^{1}H-NMR ($CDCl_3$) δ: 8.10 (dd, 1H, J = 1.4, 8.8, arom); 7:92-7.43 (m, 7H, arom); 7.13 (s, 2H, arom); 5.13 (t, 2H, J = 5, CH_2O); 4.87 (t, 2H, J = 5.4, NCH_2); 3.91 (s, 3H, OCH_3); 3.84 (s, 6H, $2OCH_3$).

3-(5-Benzoyl-2*H*-benzotriazol-2-yl)propyl 3,4,5-trimethoxybenzoate (86)

To a solution of 2-(3-hydroxypropyl)-5-benzoyl-2*H*-benzotriazole (**i26**) (0.40 g; 1.4 mmol) in 15 mL of anhydrous toluene, 0.14 g (1.4 mmol; d = 0.726; 0.20 mL) of triethylamine are first added, and then, dropwise, a solution of 3,4,5-trimethoxybenzoyl chloride in 5 mL of anhydrous toluene. (3,4,5-Trimethoxybenzoyl chloride was prepared starting from 1.4 mmol of the corresponding carboxylic acid and 5.6 mmol of $SOCl_2$ under reflux for 4 hours in anhydrous choloroform). The solution is stirred at RT for 12 hours. The toluic solution, after being washed with very cold water, is evaporated and the residue is dissolved in chloroform. The organic phase is washed twice first with a very cold 5% Na_2CO_3 solution and then with very cold water and finally it is dried over Na_2SO_4, filtered and evaporated to dryness. The residue is purified by chromatography on Al_2O_3 eluting with dichloromethane to obtain 0.49 g of solid with mp = 109-110 °C (yield = 73.7%).

The elemental analysis provided the following results:

	found:	C% 65.94	H% 5.55	N% 9.04
for $C_{26}H_{25}N_3O_6$	calc.:	C% 65.68	H% 5.30	N% 8.84

^{1}H-NMR ($CDCl_3$) δ: 8.29 (s, 1H, arom); 8.01-7.38 (m, 7H, arom); 7.19 (s, 2H, arom); 5.18 (t, 2H, J = 5.2, CH_2O); 4.98 (t, 2H, J = 5.4, NCH_2); 3.93 (s, 3H, OCH_3); 3.84 (s, 6H, $2OCH_3$); 2.72 (q, 2H, J = 6, $CH_2\underline{CH_2}CH_2$).

3-(5-Benzoyl-1*H*-benzotriazol-1-yl)propyl 3,4,5-trimethoxybenzoate (87)

To a solution of 1-(3-hydroxypropyl)-5-benzoyl-1*H*-benzotriazole (**i27**) (0.37 g; 1.3 mmol) in 15 mL of anhydrous toluene, 0.13 g (1.3 mmol; d = 0.726; 0.19 mL) of triethylamine are first added, and then, dropwise, a solution of 3,4,5-trimethoxybenzoyl chloride in 5 mL of anhydrous toluene. (3,4,5-Trimethoxybenzoyl chloride was prepared starting from 1.3 mmol of the corresponding carboxylic acid and 5.2 mmol of $SOCl_2$ under reflux for 4 hours in

anhydrous choloroform). The solution is stirred at RT for 12 hours. The toluic solution, after being washed with very cold water, is evaporated and the residue is dissolved in chloroform. The organic phase is washed twice first with a very cold 5% Na_2CO_3 solution and then with very cold water and finally it is dried over Na_2SO_4, filtered and evaporated to dryness. The residue is purified by chromatography on Al_2O_3 eluting with dichloromethane to obtain 0.43 g of solid with mp = 159-160 °C (yield = 69.5%).

The elemental analysis provided the following results:

	found:	C% 65.64	H% 5.58	N% 8.79
for $C_{26}H_{25}N_3O_6$	calc.:	C% 65.68	H% 5.30	N% 8.84

^{1}H-NMR ($CDCl_3$) δ: 7.97 (d, 1H, J = 9.2, 1H); 7.88-7.25 (m, 7H, arom); 7.15 (s, 2H, arom); 5.11 (t, 2H, J = 5.2, CH_2O); 4.92 (t, 2H, J = 5.2, NCH_2); 3.91 (s, 3H, OCH_3); 3.86 (s, 6H, $2OCH_3$); 2.61 (q, 2H, J = 6.2, $CH_2\underline{CH_2}CH_2$).

3-(6-Benzoyl-1*H*-benzotriazol-1-yl)propyl 3,4,5-trimethoxybenzoate (88)

To a solution of 1-(3-hydroxypropyl)-6-benzoyl-1*H*-benzotriazole (**i28**) (0.37 g; 1.3 mmol) in 15 mL of anhydrous toluene, 0.13 g (1.3 mmol; d = 0.726; 0.19 mL) of triethylamine are first added, and then, dropwise, a solution of 3,4,5-trimethoxybenzoyl chloride in 5 mL of anhydrous toluene. (3,4,5-Trimethoxybenzoyl chloride was prepared starting from 1.3 mmol of the corresponding carboxylic acid and 5.2 mmol of $SOCl_2$ under reflux for 4 hours in anhydrous choloroform). The solution is stirred at RT for 12 hours. The toluic solution, after being washed with very cold water, is evaporated and the residue is dissolved in chloroform. The organic phase is washed twice first with a very cold 5% Na_2CO_3 solution and then with very cold water and finally it is dried over Na_2SO_4, filtered and evaporated to dryness. The residue is purified by chromatography on Al_2O_3 eluting with dichloromethane to obtain 0.44 g of solid with mp = 159-160 °C (yield = 70.3%).

The elemental analysis provided the following results:

	found:	C% 65.49	H% 5.33	N% 8.91
for $C_{26}H_{25}N_3O_6$	calc.:	C% 65.68	H% 5.30	N% 8.84

^{1}H-NMR ($CDCl_3$) δ: 8.12 (dd, 1H, J = 1.2, 9, arom); 7:89-7.36 (m, 7H, arom); 7.14 (s, 2H, arom); 5.13 (t, 2H, J = 5, CH_2O); 4.89 (t, 2H, J = 5.2, NCH_2); 3.94 (s, 3H, OCH_3); 3.86 (s, 6H, $2OCH_3$); 2.52 (q, 2H, J = 6, $CH_2CH_2CH_2$).

benzotriazole-colchicine hybrids

2-(2-Bromoethyl)-2*H*-benzotriazole (j1)

A suspension of 0.73 (4.5 mmoles) of 2-(2-hydroxyethyl)-2*H*-benzotriazole (**i1**) in 8 mL of HBr 48% is heated at 100 °C for 16 hours. After cooling, the mixture is alkalized and extracted with chloroform. The organic phase is dried over Na_2SO_4, filtered and evaporated to dryness, to obtain a solid residue.

The residue is purified by chromatography on SiO_2 eluting with chloroform, to obtain 0.9 g of white solid with mp = 61-62 °C (yield= 88.2%).

The elemental analysis provided the following results:

	found:	C% 42.54	H% 3.51	N% 18.68
for $C_8H_8BrN_3$	calc.:	C% 42.50	H% 3.57	N% 18.59

^{1}H-NMR ($CDCl_3$) δ: 7.99-7.83 (m, 2H, arom); 7.52-7.37 (m, 2H, arom); 5.14 (t, 2H, J = 6.8, CH_2Br); 4.01 (t, 2H, J = 6.6, CH_2N).

1-(2-Bromoethyl)-1*H*-benzotriazole (j2)

A suspension of 0.46 (2.8 mmoles) of 1-(2-hydroxyethyl)-1*H*-benzotriazole (**i2**) in 6 mL of HBr 48% is heated at 100 °C for 16 hours. After cooling, the mixture is alkalized and extracted with chloroform. The organic phase is dried over Na_2SO_4, filtered and evaporated to dryness, to obtain a solid residue.

The residue is purified by chromatography on SiO_2 eluting with chloroform, to obtain 0.54 g of white solid with mp = 117-118 °C (yield= 85.7%).

The elemental analysis provided the following results:

	found:	C% 42.84	H% 3.61	N% 18.30

for $C_8H_8BrN_3$	calc.:	C%	42.50	H%	3.57	N% 18.59

^{1}H-NMR ($CDCl_3$) δ: 8.13 (dd, 1H, 1, 8.2, arom); 7.68-7.34 (m, 3H); 5.06 (t, 2H, J = 6.6, CH_2Br); 3.91 (t, 2H, J = 6.6, CH_2N).

2-(2-Bromoethyl)-5-chloro-2*H*-benzotriazole (j3)

A suspension of 0.55 (2.8 mmoles) of 2-(2-hydroxyethyl)-5-chloro-2*H*-benzotriazole (**i5**) in 8 mL of HBr 48% is heated at 100 °C for 24 hours. After cooling, the mixture is alkalized and extracted with chloroform. The organic phase is dried over Na_2SO_4, filtered and evaporated to dryness, to obtain a solid residue. The residue is purified by chromatography on SiO_2 eluting with chloroform, to obtain 0.52 g of white solid with mp = 66-67 °C (yield= 71.2%).

The elemental analysis provided the following results:

	found:	C%	37.01	H%	2.66	N% 16.13
for $C_8H_7BrClN_3$	calc.:	C%	36.88	H%	2.71	N% 16.13

^{1}H-NMR ($CDCl_3$) δ: 7.96-7.79 (m, 2H, arom); 7.39 (dd, 1H, J = 1.8, 9, arom); 5.12 (t, 2H, J = 6.8, CH_2Br); 4.0 (t, 2H, J = 6.6, CH_2N).

1-(2-Bromoethyl)-5-chloro-1*H*-benzotriazole (j4)

A suspension of 0.5 (2.5 mmoles) of 1-(2-hydroxyethyl)-5-chloro-1*H*-benzotriazole (**i6**) in 8 mL of HBr 48% is heated at 100 °C for 24 hours. After cooling, the mixture is alkalized and extracted with chloroform. The organic phase is dried over Na_2SO_4, filtered and evaporated to dryness, to obtain a solid residue. The residue is purified by chromatography on SiO_2 eluting with chloroform, to obtain 0.48 g of white solid with mp = 91-92 °C (yield= 71.2%).

The elemental analysis provided the following results:

	found:	C%	37.07	H%	2.62	N% 16.02
for $C_8H_7BrClN_3$	calc.:	C%	36.88	H%	2.71	N% 16.13

^{1}H-NMR ($CDCl_3$) δ: 8.03 (dd, 1H, J = 0.8, 9, arom); 7.63 (dd, 1H, J = 0.8, 1.8, arom); 7.39 (dd, 1H, J = 1.8, 8.8, arom); 5.02 (t, 2H, J = 6.4, CH_2Br); 3.90 (t, 2H, J = 6.4, CH_2N).

1-(2-Bromoethyl)-6-chloro-1*H*-benzotriazole (j5)

A suspension of 0.5 (2.5 mmoles) of 1-(2-hydroxyethil)-6-chloro-1*H*-benzotriazole (**i7**)in 8 mL of HBr 48% is heated at 100 °C for 24 hours. After cooling, the mixture is alkalized and extracted with chloroform. The organic phase is dried over Na_2SO_4, filtered and evaporated to dryness, to obtain a solid residue. The residue is purified by chromatography on SiO_2 eluting with chloroform, to obtain 0.44 g of white solid with mp = 90-91 °C (yield= 66.7%).

The elemental analysis provided the following results:

	found:	C% 36.96	H% 2.69	N% 16.48
for $C_8H_7BrClN_3$	calc.:	C% 36.88	H% 2.71	N% 16.13

^{1}H-NMR ($CDCl_3$) δ: 8.08 (dd, 1H, J = 0.8, 1.6, arom); 7.65-7-43 (m, 2H, arom); 5.04 (t, 2H, J = 6.4, CH_2Br); 3.90 (t, 2H, J = 6.4, CH_2N).

2-(2-Bromoethyl)-5-trifluoromethyl-2*H*-benzotriazole (j6)

A suspension of 0.51 (2.2 mmoles) of 2-(2-hydroxyethil)-5-trifluoromethyl-2*H*-benzotriazole (**i11**) in 8 mL of HBr 48% is heated at 100 °C for 24 hours. After cooling, the mixture is alkalized and extracted with chloroform. The organic phase is dried over Na_2SO_4, filtered and evaporated to dryness, to obtain a solid residue. The residue is purified by chromatography on SiO_2 eluting with chloroform, to obtain 0.51 g of white solid with mp = 72-73 °C (yield= 78.5%).

The elemental analysis provided the following results:

	found:	C% 36.65	H% 2.41	N% 14.36
for $C_9H_7BrF_3N_3$	calc.:	C% 36.76	H% 2.40	N% 14.29

^{1}H-NMR ($CDCl_3$) δ: 8.27 (s, 1H, arom); 8.04 (d, 1H, J = 9, arom); 7.63 (d, 1H, J = 9, arom), 5.19 (t, 2H, J = 6.4, CH_2Br); 4.03 (t, 2H, J = 6.4, CH_2N).

1-(2-Bromoethyl)-5-trifluoromethyl-1*H*-benzotriazole (j7)

A suspension of 0.5 (2.5 mmoles) of 1-(2-hydroxyethil)-5-trifluoromethyl-1*H*-benzotriazole (**i12**) in 8 mL of HBr 48% is heated at 100 °C for 24 hours. After cooling, the mixture is alkalized and extracted with chloroform. The organic phase is dried over Na_2SO_4, filtered and evaporated to dryness, to obtain a solid residue. The residue is purified by chromatography on SiO_2 eluting with chloroform, to obtain 0.43 g of white solid with mp = 74-75 °C (yield= 66.2%).

The elemental analysis provided the following results:

	found:	C% 36.98	H% 2.31	N% 14.59
for $C_9H_7BrF_3N_3$	calc.:	C% 36.76	H% 2.40	N% 14.29

^{1}H-NMR ($CDCl_3$) δ: 8.03 (dd, 1H, J = 0.8, 9, arom); 7.63 (dd, 1H, J = 0.8, 1.8, arom); 7.39 (dd, 1H, J = 1.8, 8.8, arom); 5.02 (t, 2H, J = 6.4, CH_2Br); 3.90 (t, 2H, J = 6.4, CH_2N).

1-(2-Bromoethyl)-6-trifluoromethyl-1*H*-benzotriazole (j8)

A suspension of 0.5 (2.5 mmoles) of 1-(2-hydroxyethil)-6-trifluoromethyl-1*H*-benzotriazole (**i13**) in 8 mL of HBr 48% is heated at 100 °C for 24 hours. After cooling, the mixture is alkalized and extracted with chloroform. The organic phase is dried over Na_2SO_4, filtered and evaporated to dryness, to obtain a solid residue. The residue is purified by chromatography on SiO_2 eluting with chloroform, to obtain 0.39 g of white solid with mp = 45-46 °C (yield= 60.0%).

The elemental analysis provided the following results:

	found:	C% 36.69	H% 2.29	N% 14.54
for $C_9H_7BrF_3N_3$	calc.:	C% 36.76	H% 2.40	N% 14.29

^{1}H-NMR ($CDCl_3$) δ: 8.43 (d, 1H, J = 0.6, arom); 7.83-7-63 (m, 2H, arom); 5.11 (t, 2H, J = 6.4, CH_2Br); 3.94 (t, 2H, J = 6.2, CH_2N).

2-(2-Phtalimido)-2*H*-benzotriazole (k1)

To a solution of 0.38 g (1.7 mmoles) of 2-(2-bromoethyl)-2*H*-benzotriazole (**j1**) in 4 mL of dimethylformamide 0.32 g (1.7 mmoles) of potassium phtalimide are added. The mixture are heated at 100 °C for 6 hours. After cooling, the solved are removed in vacuum and the residue is purified by crystallization from EtOH obtaining 0.42 g of white crystal with mp = 189-190 °C (yield= 85.7%).

The elemental analysis provided the following results:

	found:	C% 65.72	H% 4.38	N% 18.93
for $C_{16}H_{12}N_4O_2$	calc.:	C% 65.75	H% 4.14	N% 19.17

^{1}H-NMR ($CDCl_3$) δ: 8.12-7.59 (m, 6H, arom); 7.48-7.21 (m, 2H, arom); 5.06 (t, 2H, J = 5.6); 4.38 (t, 2H, J = 5.2,).

1-(2-Phtalimido)-1*H*-benzotriazole (k2)

To a solution of 0.43 g (1.9 mmoles) of 1-(2-bromoethyl)-1*H*-benzotriazole (**j2**) in 4 mL of dimethylformamide 0.35 g (1.9 mmoles) of potassium phtalimide are added. The mixture are heated at 100 °C for 6 hours. After cooling, the solved are removed in vacuum and the residue is purified by crystallization from EtOH obtaining 0.42 g of white crystal with mp = 183-184 °C (yield= 83.6%).

The elemental analysis provided the following results:

	found:	C% 65.88	H% 4.43	N% 19.14
for $C_{16}H_{12}N_4O_2$	calc.:	C% 65.75	H% 4.14	N% 19.17

^{1}H-NMR ($CDCl_3$) δ: 8.08 (d, 1H, J = 8, arom); 7.89-7.64 (m, 4H, arom); 7.62-7.28 (m, 3H); 4.99 (t, 2H, J = 6); 4.27 (t, 2H, J = 6.2).

2-(2-Phatlimido)-5-chloro-2*H*-benzotriazole (k3)

To a solution of 0.43 g (1.6 mmoles) of 2-(2-bromoethyl)-5-chloro-2*H*-benzotriazole (**j3**) in 4 mL of dimethylformamide 0.30 g (1.6 mmoles) of potassium phtalimide are added. The mixture are heated at 100 °C for 6 hours. After cooling, the solved are removed in vacuum and the residue is purified by crystallization from EtOH obtaining 0.35 g of white crystal with mp = 204-205 °C (yield= 86.5%).
The elemental analysis provided the following results:

	found:	C% 59.02	H% 3.61	N% 17.04
for $C_{16}H_{11}ClN_4O_2$	calc.:	C% 58.82	H% 3.39	N% 17.15

^{1}H-NMR ($CDCl_3$) δ: 8.01-7.59 (m, 5H, arom); 7.41-7.22 (m, 2H, arom); 5.03 (t, 2H, J = 5.4); 4.36 (t, 2H, J = 5.8).

1-(2-Phtalimido)-5-chloro-1*H*-benzotriazole (k4)

To a solution of 0.55 g (2.1 mmoles) of 1-(2-bromoethyl)-5-chloro-1*H*-benzotriazole (**j4**) in 6 mL of dimethylformamide 0.39 g (1.6 mmoles) of potassium phtalimide are added. The mixture are heated at 100 °C for 6 hours. After cooling, the solved are removed in vacuum and the residue is purified by crystallization from EtOH obtaining 0.60 g of white crystal with mp = 168-169 °C (yield= 86.9%).
The elemental analysis provided the following results:

	found:	C% 58.94	H% 3.37	N% 17.15
for $C_{16}H_{11}ClN_4O_2$	calc.:	C% 58.82	H% 3.39	N% 17.15

^{1}H-NMR ($CDCl_3$) δ: 7.98 (d, 1H, J = 8.8); 7.92-7.68 (m, 4H, arom); 7. 57 (s, 1H, arom); 7.38- 7.26 (m, 2H, arom); 4.95 (t, 2H, J = 6); 4.24 (t, 2H, J = 5.8).

1-(2-Phtalimido)-6-chloro-1*H*-benzotriazole (k5)

To a solution of 0.31 g (1.2 mmoles) of 1-(2-bromoethyl)-6-chloro-1*H*-benzotriazole (**j5**) in 4 mL of dimethylformamide 0.22 g (1.2 mmoles) of potassium phtalimide are added. The mixture are heated at 100 °C for 6 hours. After cooling, the solved are

removed in vacuum and the residue is purified by crystallization from EtOH obtaining 0.35 g of white crystal with mp = 166-167 °C (yield= 89.7%).
The elemental analysis provided the following results:

	found:	C% 59.11	H% 3.54	N% 16.92
for $C_{16}H_{11}ClN_4O_2$	calc.:	C% 58.82	H% 3.39	N% 17.15

^{1}H-NMR ($CDCl_3$) δ: 8.06 (s, 1H, arom); 7.92-7.64 (m, 4H, arom); 7.58-7.37 (m, 2H, arom); 4.98 (t, 2H, J = 6.4); 4.26 (t, 2H, J = 5.2).

2-(2-Phatlimido)-5-trifluoromethyl-2*H*-benzotriazole (k6)

To a solution of 0.38 g (1.3 mmoles) of 2-(2-bromoethyl)-5-trifluoromethyl-2*H*-benzotriazole (**j6**) in 4 mL of dimethylformamide 0.24 g (1.3 mmoles) of potassium phtalimide are added. The mixture are heated at 100 °C for 6 hours. After cooling, the solved are removed in vacuum and the residue is purified by crystallization from EtOH obtaining 0.39 g of white crystal with mp = 173-174 °C (yield= 82.9%).
The elemental analysis provided the following results:

	found:	C% 56.91	H% 3.33	N% 15.32
for $C_{17}H_{11}F_3N_4O_2$	calc.:	C% 56.67	H% 3.08	N% 15.55

^{1}H-NMR ($CDCl_3$) δ: 8.05-7.62 (m, 5H, arom); 7.54-7.28 (m, 2H, arom); 5.08 (t, 2H, J = 5.6); 4.35 (t, 2H, J = 5.6).

1-(2-Phtalimido)-5-trifluoromethyl-1*H*-benzotriazole (k7)

To a solution of 0.41 g (1.4 mmoles) of 1-(2-bromoethyl)-5-trifluoromethyl-*H*-benzotriazole (**j7**) in 4 mL of dimethylformamide 0.26 g (1.4 mmoles) of potassium phtalimide are added. The mixture are heated at 100 °C for 6 hours. After cooling, the solved are removed in vacuum and the residue is purified by crystallization from EtOH obtaining 0.41 g of white crystal with mp = 162-164 °C (yield= 82.0%).
The elemental analysis provided the following results:

	found:	C% 56.73	H% 3.26	N% 15.58
for $C_{17}H_{11}F_3N_4O_2$	calc.:	C% 56.67	H% 3.08	N% 15.55

^{1}H-NMR ($CDCl_3$) δ: 8.19 (d. 1H, J = 8.8, arom); 7.98-7.61 (m, 5H, arom); 7.59 (d, 1H, J = 8.8, arom); 5.06 (t, 2H, J = 5.6); 4.28 (t, 2H, J = 5.8).

1-(2-Phtalimido)-6-trifluoromethyl-1*H*-benzotriazole (k8)

To a solution of 0.41 g (1.4 mmoles) of 1-(2-bromoethyl)-6-trifluoromethyl-*H*-benzotriazole (**j8**) in 4 mL of dimethylformamide 0.26 g (1.4 mmoles) of potassium phtalimide are added. The mixture are heated at 100 °C for 6 hours. After cooling, the solved are removed in vacuum and the residue is purified by crystallization from EtOH obtaining 0.42 g of white crystal with mp = 167-168 °C (yield= 84.0%). The elemental analysis provided the following results:

	found:	C% 56.49	H% 3.21	N% 15.37
for $C_{17}H_{11}F_3N_4O_2$	calc.:	C% 56.67	H% 3.08	N% 15.55

^{1}H-NMR ($CDCl_3$) δ: 8. 39 (s, 1H, arom); 7.94-7.58 (m, 6H, arom); 5.03 (t, 2H, J = 6); 4.28 (t, 2H, J = 6.2).

2-(2-Aminoethyl)-2*H*-benzotriazole (l1)

A suspension of 0.38 g (1.3 mmoles) of 2-(2-phtalimido)-2*H*-benzotriazole (**k1**) in 6 mL of HCl 6N is heated to 100 °C for 24 hours. After cooling, the formed solid is filtered. The acidic solution is alkalized and extracted with ether. The organic phase is dried on Na_2SO_4, filtered and evaporated.

The oily residue (0.19 g) (one spot in TLC: SiO_2/$CHCl_3$ + 5% MeOH) is used without further purification.

1-(2-Aminoethyl)-1*H*-benzotriazole (l2)

A suspension of 0.42 g (1.4 mmoles) of 1-(2-phtalimido)-1*H*-benzotriazole (**k2**) in 6 mL of HCl 6N is heated to 100 °C for 24 hours. After cooling, the formed solid is

filtered. The acidic solution is alkalized and extracted with ether. The organic phase is dried on Na_2SO_4, filtered and evaporated.
The oily residue (0.20 g) (one spot in TLC: $SiO_2/CHCl_3$ + 5% MeOH) is used without further purification.

2-(2-Aminoethyl)-5-chloro-2*H*-benzotriazole (l3)

A suspension of 0.40 g (1.2 mmoles) of 2-(2-phtalimido)-5-chloro--2*H*-benzotriazole (**k3**) in 6 mL of HCl 6N is heated to 100 °C for 24 hours. After cooling, the formed solid is filtered. The acidic solution is alkalized and extracted with ether. The organic phase is dried on Na_2SO_4, filtered and evaporated.
The oily residue (0.20 g) (one spot in TLC: $SiO_2/CHCl_3$ + 5% MeOH) is used without further purification.

1-(2-Aminoethyl)-5-chloro-1*H*-benzotriazole (l4)

A suspension of 0.40 g (1.2 mmoles) of 1-(2-phtalimido)-5-chloro--1*H*-benzotriazole (**k4**) in 6 mL of HCl 6N is heated to 100 °C for 24 hours. After cooling, the formed solid is filtered. The acidic solution is alkalized and extracted with ether. The organic phase is dried on Na_2SO_4, filtered and evaporated.
The oily residue (0.21 g) (one spot in TLC: $SiO_2/CHCl_3$ + 5% MeOH) is used without further purification.

1-(2-Aminoethyl)-6-chloro-1*H*-benzotriazole (l5)

A suspension of 0.31 g (1.0 mmoles) of 1-(2-phtalimido)-6-chloro--1*H*-benzotriazole (**k5**) in 6 mL of HCl 6N is heated to 100 °C for 24 hours. After cooling, the formed solid is filtered. The acidic solution is alkalized and extracted with ether. The organic phase is dried on Na_2SO_4, filtered and evaporated.
The oily residue (0.18 g) (one spot in TLC: $SiO_2/CHCl_3$ + 5% MeOH) is used without further purification.

2-(2-Aminoethyl)-5-trifluoromethyl-2*H*-benzotriazole (l6)

A suspension of 0.35 g (1.0 mmoles) of 2-(2-phtalimido)-5-trifluoromethyl--2*H*-benzotriazole (**k6**) in 6 mL of HCl 6N is heated to 100 °C for 24 hours. After cooling,

the formed solid is filtered. The acidic solution is alkalized and extracted with ether. The organic phase is dried on Na_2SO_4, filtered and evaporated.
The oily residue (0.19 g) (one spot in TLC: $SiO_2/CHCl_3$ + 5% MeOH) is used without further purification.

1-(2-Aminoethyl)-5-trifluoromethyl-1*H*-benzotriazole (l7)

A suspension of 0.37 g (1.0 mmoles) of 1-(2-phtalimido)-5-trifluoromethyl--1*H*-benzotriazole (**k7**) in 6 mL of HCl 6N is heated to 100 °C for 24 hours. After cooling, the formed solid is filtered. The acidic solution is alkalized and extracted with ether. The organic phase is dried on Na_2SO_4, filtered and evaporated.
The oily residue (0.20 g) (one spot in TLC: $SiO_2/CHCl_3$ + 5% MeOH) is used without further purification.

1-(2-Aminoethyl)-6-trifluoromethyl-1*H*-benzotriazole (l8)

A suspension of 0.38 g (1.1 mmoles) of 1-(2-phtalimido)-6-trifluoromethyl--1*H*-benzotriazole (**k8**) in 6 mL of HCl 6N is heated to 100 °C for 24 hours. After cooling, the formed solid is filtered. The acidic solution is alkalized and extracted with ether. The organic phase is dried on Na_2SO_4, filtered and evaporated.
The oily residue (0.22 g) (one spot in TLC: $SiO_2/CHCl_3$ + 5% MeOH) is used without further purification.

N-[(7S)-1,2,3-Trimethoxy-9-oxo-10-(2*H*-benzotriazol-2-ethylamino)-5,6,7,9tetrahydrobenzo[a] heptalen-7-yl]acetamide (89)

To a solution of colchicine (0.16 g, 0.39 mmol) in MeOH (3 mL) were added 2-(2-Aminoethyl)-2*H*-benzotriazole (**l1**) (0.19 g, 1.17 mmol). The reaction mixture was stirred at r.t. for 72 h, and then the solvent is evaporated. The residue is purified by flash chromatography (SiO_2, $CHCl_3$:MeOH 9:1), obtaining 148 mg of amorphous solid. Yield = 71.5%
The elemental analysis provided the following results:

	found:	C% 66.04	H% 5.83	N% 13.01
for $C_{29}H_{31}N_5O_5$	calc.:	C% 65.77	H% 5.90	N% 13.22

^{1}H-NMR ($CDCl_3$) δ: 8.21-8.08 (m, 1H, arom); 7.99-7.74 (m, 2H, arom); 7.76-7-24 (m, 5H, arom); 5.12 (t, 2H, J = 5.4); 4.72 (t, 2H, J = 5.6); 4.28-4.06 (m, 1H); 3.96 (s, 3H); 3.91 (s, 3H); 3.64 (s, 3H); 2.67-2.18 (m, 3H); 2.11-1.75 (m, 4H).

N-[(7S)-1,2,3-Trimethoxy-9-oxo-10-(1*H*-benzotriazol-2-ethylamino)-5,6,7,9tetrahydrobenzo[a] heptalen-7-yl]acetamide (90)

To a solution of colchicine (0.16 g, 0.41 mmol) in MeOH (3 mL) were added 1-(2-Aminoethyl)-1*H*-benzotriazole (**I2**) (0.20 g, 1.23 mmol). The reaction mixture was stirred at r.t. for 72 h, and then the solvent is evaporated. The residue is purified by flash chromatography (SiO_2, $CHCl_3$:MeOH 9:1), obtaining 152 mg of amorphous solid. Yield = 70.0%

The elemental analysis provided the following results:

	found:	C% 65.95	H% 6.16	N% 12.98
for $C_{29}H_{31}N_5O_5$	calc.:	C% 65.77	H% 5.90	N% 13.22

^{1}H-NMR ($CDCl_3$) δ: 8.39 (d, 1H, J = 6.4, arom); 8.03-7.87 (m, 2H, arom); 7.78-7-23 (m, 5H, arom); 4.99 (t, 2H, J = 5.6); 4.64 (t, 2H, J = 5.6); 4.38-4.17 (m, 1H); 3.93 (s, 3H); 3.90 (s, 3H); 3.62 (s, 3H); 2.63-2.15 (m, 3H); 2.09-1.82 (m, 4H).

N-[(7S)-1,2,3-Trimethoxy-9-oxo-10-(5-chloro-2*H*-benzotriazol-2-ethylamino)-5,6,7,9tetrahydrobenzo[a]heptalen-7-yl]acetamide (91)

To a solution of colchicine (0.14 g, 0.34 mmol) in MeOH (3 mL) were added 2-(2-Aminoethyl)-5-chloro-2*H*-benzotriazole (**I3**) (0.20 g, 1.02 mmol). The reaction mixture was stirred at r.t. for 72 h, and then the solvent is evaporated. The residue is purified by flash chromatography (SiO_2, $CHCl_3$:MeOH 9:1), obtaining 139 mg of amorphous solid. Yield = 72.4%

The elemental analysis provided the following results:

	found:	C% 62.08	H% 5.67	N% 12.25
for $C_{29}H_{30}ClN_5O_5$	calc.:	C% 61.75	H% 5.36	N% 12.42

^{1}H-NMR ($CDCl_3$) δ: 8.19-8.07 (m, 1H, arom); 7.96-7.80 (m, 2H, arom); 7.75-7-21 (m, 4H, arom); 5.11 (t, 2H, J = 5.2); 4.75 (t, 2H, J = 5.2); 4.31-4.14 (m, 1H); 3.93 (s, 3H); 3.90 (s, 3H); 3.66 (s, 3H); 2.72-1.88 (m, 7H).

N-[(7S)-1,2,3-Trimethoxy-9-oxo-10-(5-chloro-1*H*-benzotriazol-2-ethylamino)-5,6,7,9tetrahydrobenzo[a]heptalen-7-yl]acetamide (92)

To a solution of colchicine (0.14 g, 0.36 mmol) in MeOH (3 mL) were added 1-(2-Aminoethyl)-5-chloro-1*H*-benzotriazole (**I4**) (0.21 g, 1.07 mmol). The reaction mixture was stirred at r.t. for 72 h, and then the solvent is evaporated. The residue is purified by flash chromatography (SiO_2, $CHCl_3$:MeOH 9:1), obtaining 143 mg of amorphous solid. Yield = 70.4%

The elemental analysis provided the following results:

	found:	C% 61.80	H% 5.57	N% 12.39
for $C_{29}H_{30}ClN_5O_5$	calc.:	C% 61.75	H% 5.36	N% 12.42

^{1}H-NMR ($CDCl_3$) δ: 8.47 (d, 1H, J = 6.6, arom); 7.90 (d, 1H, J = 8.8, arom); 7.78-7.60 (m, 2H, arom); 7.55 (s, 1H, arom); 7.42-7.19 (m, 2H, arom); 4.99 (t, 2H, J = 5.2); 4.66 (t, 2H, J = 5.2); 4.20-3.99 (m, 1H); 3.93 (s, 3H); 3.90 (s, 3H); 3.60 (s, 3H); 2.59-2.14 (m, 3H); 2.09-1.79 (m, 4H).

N-[(7S)-1,2,3-Trimethoxy-9-oxo-10-(6-chloro-1*H*-benzotriazol-2-ethylamino)-5,6,7,9tetrahydrobenzo[a]heptalen-7-yl]acetamide (93)

To a solution of colchicine (0.12 g, 0.31 mmol) in MeOH (3 mL) were added 1-(2-Aminoethyl)-6-chloro-1*H*-benzotriazole (**I5**) (0.18 g, 0.92 mmol). The reaction mixture was stirred at r.t. for 72 h, and then the solvent is evaporated. The residue is purified by flash chromatography (SiO_2, $CHCl_3$:MeOH 9:1), obtaining 122 mg of amorphous solid. Yield = 69.7%

The elemental analysis provided the following results:

	found:	C% 61.80	H% 5.57	N% 12.39
for $C_{29}H_{30}ClN_5O_5$	calc.:	C% 61.75	H% 5.36	N% 12.42

^{1}H-NMR ($CDCl_3$) δ: 8.38-8.21 (m, 1H, arom); 8.06-7.81 (m, 2H, arom); 7.78-7.23 (m, 4H, arom); 5.06 (t, 2H, J = 5.6); 4.71 (t, 2H, J = 5.4); 4.22-4.02 (m, 1H); 3.96 (s, 3H); 3.92 (s, 3H); 3.62 (s, 3H); 2.62-1.78 (m, 7H).

N-[(7S)-1,2,3-Trimethoxy-9-oxo-10-(5-trifluoromethyl-2*H*-benzotriazol-2-ethylamino)-5,6,7,9tetrahydrobenzo[a]heptalen-7-yl]acetamide (94)

To a solution of colchicine (0.11 g, 0.28 mmol) in MeOH (3 mL) were added 2-(2-Aminoethyl)-5-trifluoromethyl-2*H*-benzotriazole (**I6**) (0.19 g, 0.83 mmol). The reaction mixture was stirred at r.t. for 72 h, and then the solvent is evaporated. The residue is purified by flash chromatography (SiO_2, $CHCl_3$:MeOH 9:1), obtaining 98 mg of amorphous solid. Yield = 58.7%

The elemental analysis provided the following results:

	found:	C% 60.14	H% 5.37	N% 11.66
for $C_{30}H_{30}F_3N_5O_5$	calc.:	C% 60.30	H% 5.06	N% 11.72

^{1}H-NMR ($CDCl_3$) δ: 8.27-8.12 (m, 1H, arom); 7.95-7.78 (m, 2H, arom); 7.76-7-29 (m, 4H, arom); 5.09 (t, 2H, J = 5.6); 4.68 (t, 2H, J = 5.6); 4.19-4.02 (m, 1H); 3.96 (s, 3H); 3.91 (s, 3H); 3.62 (s, 3H); 2.66-1.91 (m, 7H).

N-[(7S)-1,2,3-Trimethoxy-9-oxo-10-(5-trifluoromethyl-1*H*-benzotriazol-2-ethylamino)-5,6,7,9tetrahydrobenzo[a]heptalen-7-yl]acetamide (95)

To a solution of colchicine (0.12 g, 0.29 mmol) in MeOH (3 mL) were added 1-(2-Aminoethyl)-5-trifluoromethyl-1*H*-benzotriazole (**I7**) (0.20 g, 0.87 mmol). The reaction mixture was stirred at r.t. for 72 h, and then the solvent is evaporated. The residue is purified by flash chromatography (SiO_2, $CHCl_3$:MeOH 9:1), obtaining 106 mg of amorphous solid. Yield = 61.3%

The elemental analysis provided the following results:

	found:	C% 60.55	H% 5.29	N% 11.48
for $C_{30}H_{30}F_3N_5O_5$	calc.:	C% 60.30	H% 5.06	N% 11.72

^{1}H-NMR ($CDCl_3$) δ: 8.44 (d, 1H, J = 6.4, arom); 7.92 (d, 1H, J = 9, arom); 7.83-7.67 (m, 2H, arom); 7.56 (s, 1H, arom); 7.42-7.22 (m, 2H, arom); 5.03 (t, 2H, J = 5.2); 4.60 (t, 2H, J = 5.4); 4.18-3.98 (m, 1H); 3.93 (s, 3H); 3.90 (s, 3H); 3.66 (s, 3H); 2.64-2.17 (m, 3H); 2.11-1.80 (m, 4H).

N-[(7S)-1,2,3-Trimethoxy-9-oxo-10-(6-trifluoromethyl-1*H*-benzotriazol-2-ethylamino)-5,6,7,9tetrahydrobenzo[a]heptalen-7-yl]acetamide (96)

To a solution of colchicine (0.13 g, 0.32 mmol) in MeOH (3 mL) were added 1-(2-Aminoethyl)-6-trifluoromethyl-1*H*-benzotriazole (**I8**) (0.22 g, 0.96 mmol). The reaction mixture was stirred at r.t. for 72 h, and then the solvent is evaporated. The residue is purified by flash chromatography (SiO_2, $CHCl_3$:MeOH 9:1), obtaining 120 mg of amorphous solid. Yield = 62.8%

The elemental analysis provided the following results:

	found:	C% 60.51	H% 5.31	N% 11.67
for $C_{30}H_{30}F_3N_5O_5$	calc.:	C% 60.30	H% 5.06	N% 11.72

^{1}H-NMR ($CDCl_3$) δ: 8.41-8.24 (m, 1H, arom); 8.08-7.84 (m, 2H, arom); 7.77-7.25 (m, 4H, arom); 5.07 (t, 2H, J = 5.6); 4.68 (t, 2H, J = 5.6); 4.21-3.99 (m, 1H); 3.95 (s, 3H); 3.91 (s, 3H); 3.66 (s, 3H); 2.65-1.76 (m, 7H).

Computational Chemistry

In order to rationalize SARs, docking studies were carried out on benzotriazole and benzodiazole derivatives with tubulin dimer. The X-ray structure of colchicine-tubulin complex (PDB code 4O2B) was used as starting structure and modified as follows: i) only chains A and B were considered; ii) colchicine as well as all the water molecules were removed from the complex; iii) GTP molecule was included in the calculations considering its proximity to colchicine binding site. The ligand molecules were built by MOE (Molecular Operating Environment) Builder module,

parameterized according to the MMFF94x forcefield and energy minimized. Docking simulations were carried out using Autodock 4.2 program. Hydrogens were added to the modified protein coordinates and partial charges were assigned according to Gasteiger-Huckel method. The ligand “root” was defined automatically and all bonds were allowed to freely rotate. A 50 x 60 x 50 grid (grid spacing 0.375 Å) was centred on the colchicine binding site and electrostatic and affinity maps for each atom type of the ligand were calculated. The docking search was performed over 100 conformers using the Genetic Algorithm Local Search protocol as implemented in AutoDock (Population size: 150; Rate of gene mutation: 0.02; Rate of Crossover: 0.8). The docking poses were clustered (rmsd 2.0 Å) and the best conformation of the low energy, highest populated cluster was select as the binding conformation. All the calculations were carried out on ***PC (Windows 8.1 o.s). Model analyses were performed using the CCP4 program suite [Collaborative Computational Project, Number 4 *Acta Crystallogr.* **1994**, *D50*, 760]. The program MOE was used to draw the figures.

Chapter 5 Conclusions

This dissertation work describes two research lines: the first one regarded the synthesis, characterization and biological evaluation of two different pharmacological classes of quinolizidine derivatives (anti-leishmaniasis and anti-Alzheimer's Disease); the second part developed the investigation of benzotriazole derivatives with potential antiproliferative activity. in second part, in particular, I described the synthesis, characterization and evaluation of a class of benzotriazole trimethoxybenzoic esters and a class of colchicine-benzotriazole hybrids; both the class may exert an anti-tubulin activity. Regarding the class of the colchicine-benzotriazole hybrids I also carried out docking studies in order to evaluate the compound's interactions within the tubulin binding site comparing to those of colchicine.

Section 1: Quinolizidine derivatives

Alkaloids are a very heterogenous class of natural compounds, some of which are utilized in therapy, whereas others, despite exerting some biological activity, are excluded from clinical practice because of their low safety profile and high toxicity. However, the modifications of the alkaloids structure in order to make them suitable for clinical use is a widely utilized synthetic process to obtain semi-synthetic compounds with higher therapeutic index and less toxicity. The research group where I attended my PhD has focused over the years on the class of quinolizidine alkaloids, molecules characterized by a peculiar bulky and basic nucleus in which often dialkylaminoalkyl or cycloalkylaminocyclic fragments inserted into the structure can be observed. Quinolizidine alkaloids, therefore, have been used as starting compounds to synthesize novel molecules in which the basic nucleus is linked to opportune chemical moieties or suitable modified. In particular, based on the positive results in the literature and achieved by the research group, I focused my research on cytisine and lupinine derivatives. On the basis of SAR studies that indicated some essential characteristics for the antileishmaniasis activity (a lipophilic chain, a bulky cationic head and a specific chain length) I first synthesized the palmitic ester of lupinine and a small series of its quaternary ammonium salts with a variable cationic head; these derivatives possess two structural

characteristics in common with miltefosine : a chain of 16 carbon atoms and a cationic head. I also synthesized some cytisine derivatives in which cytisine is substituted with alkyl chains and alkylaryl portions and their corresponding quaternary N-methyl ammonium salts. Some of these compounds have been tested and it emerged that in the series of the lupinine derivatives the benzyl cationic head improves the activity; the importance of the benzyl cationic head and the possible influence of substituents on the phenyl ring may thus be hypothesized. In the series of the cytisine derivatives the optimal chain length appears to be 14 carbon atoms., however the synthesis and the essays on compounds with shorter alkyl chains (C6-C10) could be useful to continue the investigation; moreover, the insertion of halide substituents on the aromatic ring of the compounds could be performed in order to verify a greater activity, as the literature suggests. Finally, it is noteworthy that all the compounds exert a low cytotoxicity (IC50 > 100 μM) on the HMEC-1 cell line. Based on the few results available, a general low antileishmaniasis activity of these compounds can be confirmed. However some of these novel molecules could be tested on *Plasmodium falciparum*, since other compounds previously synthesized have shown a higher activity against this kind of protozoa. Eventually, the first series of compounds may act as antitubercular agents, as in the literature lupinyl palmitate is reported to exert an antitubercular effect against *M. tuberculosis* R37RV, causing a bacterial inhibition of 98% at the concentration of 6.25 μg/mL.

I also carried out a research line on potential anti-Alzheimer compounds. Recent studies on this disease demonstrated the higher effectiveness of multi-target drugs instead of mono-therapy, owing to the multifactorial pathogenesis of the syndrome, the higher patients' compliance and less onset of side effects. The research lab where I carried out my PhD focused the research on potential dual AChE/BChE inhibitors derived from naphto- and anthraquinones linked to the quinolizidine (lupinine) nucleus, in order to obtain more effective ChEs inhibitors that, at the same time, would be able to inhibit Aß aggregation both acting on the PAS-induced aggregation and through a direct mechanism. Some compounds of remarkable interest have emerged especially as acetylcholinesterase inhibitors, with micromolar and, in some cases, submicromolar IC_{50}. On the basis of the interesting results already obtained, I synthesized 8 novel anthraquinone and 8 naphtoquinone derivatives; the basic nucleus has been varied, utilizing cytisine, another quinolizidine alkaloid, as the starting molecule. These novel derivatives permitted to investigate structural variations concerning the linker lenght and the

substitution in position 3 of the cytisine nucleus. In comparison with the previous naphtoquinone analogues presenting the lupinine-derived basic head, these compounds exert a marked decrease of the inhibitor activity toward AChE but an improvement of the activity toward BChE. However, the importance of the presence of the chlorine atom in position 3 of the naphtoquinone nucleus has been confirmed for the naphtoquinone derivatives. The linker elongation doesn't appear to influence the biological activity, although the importance of the linker length has been already highlighted in other derivatives. Nevertheless, the compound with n = 3 e R = Cl appears to be endowed with the optimal distance for the interaction with the enzyme. No significant selectivity toward one of the two enzymes has been observed, except for one compound which has also been tested to evaluate its ability in the inhibition of Aβ aggregation, showing a low/moderate effect

Finally, the cytisine derivatives, unlike the lupinyl derivatives, appear to benefit more from the conjugation with the anthraquinone tryciclic system than with the naphtoquinone byciclic nucleus.

Section 2: Benzotriazole derivatives and colchicine-benzotriazole hybrids

The second part of my research work regarded synthesis, characterization and biological tests on benzotriazole derivatives and colchicine-benzotriazole hybrids. On these latter compounds I also carried out docking studies in order to investigate their interaction with the tubulin dimer. Benzotriazoles are a class of heterocyclic compounds endowed with peculiar chemical characteristics and pharmacological activity. In particular they are widely studied as antitumor compounds; benzotriazoles derivatives are classified based on their antitumor mechanism of action. I focused, in particular, on the synthesis of benzotriazoles that potentially inhibit tubulin polymerization. This mechanism of action is also exerted by colchicine, a well known anti-proliferative agent, whose clinical utilization is limited by its low therapeutic index. On the basis of data found in literature and previous research carried out by the research group where I accomplished my PhD, I synthesized a series of 8 benzoyl benzotriazoles and a series of 32 benzotriazole trimethoxybenzoic esters. The evaluation of the cytotoxicity of the compounds synthesized has been carried out by Dr. Maurizio Viale and Dr. Marco Ponassi– IRCCS San Martino-on different tumor cell lines. Among the first series two compounds exerted a percentage of cell proliferation inhibition up to 70% and for them IC_{50} value was evaluated. In the second series IC_{50} was evaluated only for

the most active compound. Based on the results obtained it can be confirmed that the linker of two carbon atoms between the benzotriazolic nucleus and the benzoic residue leads to a higher antiproliferative activity. The limited number of active compounds may be due to a very specific action of the molecules and therefore a research should be carried out to identify the specific structural characteristics necessary to interact with the target.

Then, in order to ameliorate anti-proliferative activity and decrease colchicine toxicity, I devised and synthesized novel 8 colchicine-benzotriazole hybrids. I performed docking studies to compare colchicine-benzimidazole and colchicine-benzotriazole hybrids to determine which scaffold gave the best interaction within the binding site. The results indicated the colchicine-benzotriazole conjugates as a promising scaffold to develop novel and potent tubulin inhibitors. Then, a screening through Autodock has been carried out on benzotriazole-colchicine hybrids I synthesized, bearing Cl (electron-withdrawing group) or OCH_3 (electron-donating group) at position 5 or 6 of the benzotriazole ring. For all the tested compounds nanomolar K_i value were calculated and the 6-Cl derivative emerged to be the most active compounds of the series with a predicted K_i value of 0.41 nM. These data suggest that the presence of an electron-withdrawing group ameliorate the interaction of the complex within the tubulin binding site. This finding could be taken into consideration for structural modifications of these colchicine-benzotriazole derivatives.

List of References

Ahmed K., Gerber DA., Cochet C., Joining the cell survival squad: an emerging role for protein kinase CK2, Trends Cell. Biol. 12 (2002) 226-230.

Alvar J., Velez ID., Bern C., Herrero M., Desjeux P., Cano J., Jannin J., den Boer M.; WHO Leishmaniasis Control Team (2012) Leishmaniasis worldwide and global estimates of its incidence. PLoS ONE 7: e35671.

Alvarez A., Bronfman F., Pérez C.A., Vicente M., Garrido J., Inestrosa N.C., Acetylcholinesterase, a senile plaque component, affects the fibrillogenesis of amyloid-beta-peptides. Neurosci. Lett. 1995, 201, 49-52

Anand R., Gill, K.D., Mahdi A.A., Therapeutics of Alzheimer's disease: past, present and future. Neuropharm. 2014, 76 (A), 27-50.

Ancelin M. L., Vial H. J., Regulation of phosphatidylcholine biosynthesis in Plasmodium-infected erythrocytes. Biochim. Biophys. Acta, 1989, 1001, 82-89.

Anderson J.P., Chen Y.,Kim K.S., Robakis, N.K., An alternative secretase cleavage produces soluble Alzheimer amyloid precursor protein containing a potentially amyloidogenic sequence. J. Neurochem. 1992, 59, 2328-2331.

Auffinger P., Hays F.A., Westhof E., Ho P.S. Halogen bonds in biological molecules. PNAS 2004, 11, 16789-16794.

Baiocco P., Ilari A., Ceci P., et al. Inhibitori effect of silver nanoparticles on trypanothione reductase activity and leishmania infantum proliferation. ACS Med Chem Lett 2010, 2, 203-233.

Balana-Fouce R., Requera RM., Cubr´ıa JC., and Ord on ez, Gen D., Pharmacol., 1998, 30, 435–443.

Bandiera T., Lansen J., Post C., Varasi M., Inhibitors of Aβ peptide aggregation as potential anti-Alzheimer agents. Curr. Med. Chem. 1997, 4, 159-170.

Bartus, R.T., Dean 3rd R.L., Beer B., Lippa A.S., The cholinergic hypothesis of geriatric memory dysfunction. Science 1982, 217, 408-414.

Battistutta R., De Moliner E., Sarno S., Zanotti G., Pinna LA., Structural features underlying selective inhibition of protein kinase CK2 by ATP sitedirected tetrabromo-2-benzotriazole, Protein Sci. 10 (2001) 2200-2206.

Beckett C.,Nalivaeva N.N., Belyaev N.D., Turner A.J., Nuclear signalling by membrane protein intracellular domains: the AICD enigma, Cell Signal 24 (2012) 402e409.

Berman JD., Badaro R., Thakur CP., Wasunna KM., Behbehani K., Davidson R., Kuzoe F., Pang L., Weerasuriya K. and Bryceson ADM., Bull W.H.O., 1998, 76, 25–32.

Bern C., Adler-Moore J., Berenguer J., Boelaert M., den M., Boer M., Davidson RN., Figueras C., Gradoni L., Kafetzis DA., Ritmeijer K., Rosenthal E., Royce C., Russo R., Sundar S., and Alvar J., Clin. Infect. Dis., 2006, 43, 917–924.

Bhardwaj Bharti., Lata and Kushwaha, Khushbu., Benzotriazole: A Medicinally important Heterocyclic Moiety. Asian Journal of Biochemical and Pharmaceutical Research. (2013), 1, 2231-2560, Volume 4.

Bian Y., Ye M., Wang Ch., Cheng K., Song Ch., Dong M., Pan Y., Qin H., Zou H., Global Screening of CK2 Kinase Substrates by an integrated phosphoproteomics workflow, Sci. Rep. 3 (2013) 3460.

Bibby AC., Litchfield DW., The multiple personalities of the regulatory subunit of protein kinase CK2: CK2 dependent and CK2 independent roles reveal a secret identity for CK2beta, Int. J. Biol. Sci. 1 (2005) 67-79.

Bodhe PV., Kotwani RN., Kirodian BG., Pathare AV., Pandey AK., Thakur CP., and Kshirsagar NA., Trans. R. Soc. Trop. Med. Hyg., 1999, 93, 314–318.

Bohlmann F., Schumann, D. The Alkaloids, 9, (1967) p 175-221.

Boido V., Ercoli M., Sparatore F., XVII Congr. Naz. Soc. Chim. Ital. Janua Chem 92, Genova 1992; Atti p. 100. XI Conv. Naz. Div. Chim. Farmac. Bari 1994, Atti p. 99.

Bovet O., Bovet-Nitti F., Medicaments du Systeme nerveux végétatif, Karper S. Basilea 1948, p. 604.

Bretner M., Najda-Bernatowicz A., Lebska M., Muszynska G., Kilanowicz A., Sapota A., New inhibitors of protein kinase CK2, analogues of benzimidazole and benzotriazole, Mol. Cell. Biochem.. 316 (2008) 87-89.

Briguglio I., Piras S., Corona P., Gavini E., Nieddu M., Boatto G., Carta A., Benzotriazole: An overview on its versatile biological behavior. European Journal Of Medicinal Chemistry. (2015) 97, 612-648.

Calas M., Cordina G., Bompart J., Ben Bari M., Jei T., Ancelin M. L., Vial H. J., Antimalarial activity of molecules interfering with Plasmodium falciparum phospholipid metabolism. Structure-activity relationship analysis. J. Med. Chem., 1997, 40, 3557-3566.

Calas M., Ancelin M. L., Cordina G., Portefaix P., Piquet G., Antimalarial activity of compounds interfering with Plasmodium falciparum phospholipid metabolism: comparison between mono- and bisquaternary ammonium salts. J. Med. Chem., 2000, 43, 505-516.

Canu C., Boido V., Sparatore F., Farmaco, 54 (1999) 438.

Carta A., Briguglio I., Piras S., Boatto G., La Colla P., et al. (2011) 3-Aryl-2-[1Hbenzotriazol-1-yl]acrylonitriles: a novel class of potent tubulin inhibitors. Eur J Med Chem 46: 4151-4167.

Carta A., Sanna P., Palomba M., Vargiu L., La Colla M., and Loddo R., European Journal of Medicinal Chemistry., 2002, 37, 891.

Castellani R.J., Lee H.G., Zhu X., Nunomura A., Perry G., Smith M.A., Neuropathology of Alzheimer disease: pathognomonic but not pathogenic. Acta Neuropathol. 2006, 111, 503-509.

Cecilio P., Perez-Cabezas B., Santarem N., Maciel J., Rodrigues V., Cordeiro A., Silva da., (2014) Deception and manipulation: the arms of Leishmania, a successful parasite. Front Immunol 5: 480.

Chojnacki Konrad., Winska Patrycja., Skierka Katarzyna., Wielechowska Monika., Bretner Maria., Synthesis, in vitro antiproliferative activity and kinase profile of new benzimidazole and benzotriazole derivatives. Bioorganic Chemistry. (2017) 72, 1-10.

Colombo R., Carotti A., Catto M., Racchi M., Lanni C.,Verga L., Caccialanza G., De Lorenzi E., CE can identify small molecules that selectively target soluble oligomers of amyloid ß protein and display antifibrillogenic activity. Electrophoresis 2009, 30, 1418-1429.

Cro SL., Seifert K., and Duchene M., Mol. Biochem. Parasitol., 2003, 126, 165–172.

Croft SL. and Coombs GH., Trends Parasitol., 2003, 19, 502–508; Fernandes FR., Ferreira WA., Campos MA., Ramos GS., Kato KC., Almeida GG., Junior JDC., Melo MN., Demicheli C., and Frezarda F., F.Antimicrob. Agents Chemother., 2013, 57, 4229–4236.

Daqllemagne MJ., Hejmans C., The Alkaloids, 5, (1955) 121.

Dartigues, J.F. Alzheimer's disease: a global challenge for the 21st century. Lancet Neurol. 2009, 8, 1082-1083.

Davie JR., Covalent modifications of histones: expression from chromatin templates, Curr. Opin. Genet. Dev. 8 (1998) 173-178.

Davies P., Maloney A.J., Selective loss of central cholinergic neurons in Alzheimer' disease. Lancet 1976, 2, 1403.

Davis D., Benzotriazole, a plant-growth regulator, Science 120 (1954) 989.

De Ferrari G.V., Canales M.A., Shin I., Weiner L.M., Silman I., Inestrosa N.C., A structural motif of acetylcholinesterase that promotes amyloid beta-peptide fibril formation. Biochemistry 2001, 40, 10447-10457.

De Vries HJ., Reedijk SH., Schallig HD., (2015) Cutaneous leishmaniasis: recent developments in diagnosis and management. Am J Clin Dermatol 16: 99-109.

Diniz SA., Silva FL., Carvalho AV., Neta Bueno R., Guerra RM., Abreu-Silva AL.,

Santos RL., (2008) Animal reservoirs for visceral leishmaniasis in densely populated urban areas. J Infect Dev Ctries 2: 24-33. doi:10.3855/jidc.318.

Drummond DC., Noble CO., Kirpotin DB., Guo Z., Scott GK., Benz CC., Clinical development of histone deacetylase inhibitors as anticancer agents, Annu. Rev. Pharmacol. Toxicol. 45 (2005) 495-528.

Ellman G.L., Courtney D., Andres V., Featherstone R.M.. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 1961, 7, 88-95.

El Rashedy AA., Aboul-Enein HY., Benzimidazole derivatives as potential anticancer agents, Mini Rev. Med. Chem. 13 (2013) 399-407.

El-Asmy HA., Butler IS., Mouhri ZS., Jean-Claude BJ., Emmam MS., et al. (2014) Zinc(II), ruthenium(II), rhodium(III), palladium(II), silver(I), platinum(II) and MoO22+complexes of 2-(2'-hydroxy-5'-methylphenyl)-benzotriazole as simple or primary ligand and 2,2'-bipyridyl, 9,10-phenanthroline or triphenylphosphine as secondary ligands: structure and anticancer activity. J Mol Struct 1059: 193201.

Faust RA., Gapany M., Tristani P., Davis A., Adams GL., Ahmed K., Elevated protein kinase CK2 activity in chromatin of head and neck tumors: association with malignant transformation, Cancer Lett. 101 (1996) 31-35.

Feng Y.,Wang X., Antioxidant terapie for Alzheimer's disease. Oxid. Med. Cell. Longev. 2012. Doi 10.1155/2012/472932

Fernandes W.A., Ferreira M.A., Campos G.S., Ramos K.C., Kato G.G., Almeida J.D.C., Junior M.N., Melo C.,Demicheli and Frezarda F., Antimicrob. Agents Chemother., 2013, 57, 4229–4236.

Field V., Gautret P., Schlagenhauf P., Burchard GD., Caumes E., Jensenius M., Castelli F., Gkrania-Klotsas E., Weld L., Lopez-Velez R., de Vries P., von Sonnenburg F., Loutan L., Parola P.; EuroTravNet network (2010) Travel and migration associated infectious diseases morbidity in Europe, 2008. BMC Infect Dis 10: 330.

Freitas-Junior LH., Chatelain E., Kim HA. and Siqueira-Neto JL., Int. J. Parasitol.:

Drugs Drug Resist., 2012, 2, 11–19.

Freund M., Friedmann A. Ber. 1901, 34, 605-619

Fries DS., and Fairlamb AH., in Burger's Medicinal Chemistry and Drug Discovery, ed. D. J. Abraham, John Wiley & Sons, 6th edn, 2003, vol. 5, pp. 1033–1087.

Fu J., Yang Y., Zhang XW., Mao WJ., Zhang ZM., Zhu, HL., Discovery of 1H-benzo[d][1,2,3]triazol-1-yl 3,4,5-trimethoxybenzoate as a potential antiproliferative agent by inhibiting histone deacetylase, Bioorg. Med. Chem. 18 (2010) 8457-8462.

Fukuda M., Kanou F., Shimada N., Sawabe M., Saito Y., Murayama S. et al. Elevated levels of 4-hydroxynonenal-histidine Michael adduct in the ippocampi of patients with Alzheimer's disease. Biomed. Res. 2009, 30, 229-233.

Gangneux JP., Sulahian A., Garin YJ. and Derouin F. Trans. R. Soc. Trop. Med. Hyg., 1996, 90, 574–577.

Ghavidel A., Schultz MC., TATA binding protein-associated CK2 transduces DNA damage signals to the RNA polymerase III transcriptional machinery, Cell 106 (2001) 575-584.

Giacobini E., Selective Inhibitors of Butyrylcholinesterase. Drugs Aging 2001, 18, 891-898.

Globocan, Cancer Fact Sheets, 2012. http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx.

Gradoni L., Davidson RN., Orsini S., Betto P., and Giambenedetti M., J. Drug Targeting, 1993, 1, 311–316.

Greig N.H., Utsuki T., Yu Q.S., Zhu X., Holloway H.W., Perry T.A., Lee B., Ingram D.H., Lahiri D.K., A new therapeutic target in AD treatment: attention to butyrylcholinesterase. Curr. Med. Res. Opin. 2001, 17, 159-165

Grimaldi Jr G., Tesh, RB., and McMahon-Pratt D., Am. J. Trop. Med. Hyg., 1989, 41, 687–725.

Grunstein M., Histone acetylation in chromatin structure and transcription, Nature 389 (1997) 349-352.

Guillozet A.L.,Smiley J.F., Mash D.C., Mesulam M.M., Butyrylcholinesterase in the life cycle of amyloid plaques. Ann Neurol. 1997, 42, 909-918.

Haass C., Koo E.H., Mellon A., Hung A.Y., Selkoe, D.J., Targeting of cell surface beta-amyloid precursor protein to lysosomes: alternative processing into amyloid-bearing fragments. Nature 1992, 357, 500-503.

Hamilton JG., Parasite, 2008, 15, 252–256.

Handley MS., King FD., McRitchie B., Turner DH., Watts EA., J. Med. Chem. 28 (1985) 1843.

Hazard R., Bases pharmacodynamiques de la thérapie du sisteme nerveux autonome, Doin G. Paris, 1956, p. 108.

Henry TA., “The Plant alkaloids”, 4th Edition Churchill Ltd, London 1949 Pelletier SW., “Chemistry of the Alkaloids”, Van Nostrand, New York 1970.

Hepburn NC., Postgrad, J., Med., 2003, 49, 50–54.

Hessenauer A., Schneider CC., Goetz C., and Montenarh M., Cell. Signalling, 2011, 23, 145–151.

Howell LA., Howman A., O'Connell MA., Mueller A., Searcey M., (2009) Synthesis and evaluation of 9-aminoacridines derived from benzyne click chemistry. PubMed Bioorg Med Chem Lett 19: 5880-5883.

http://www.who.int/mediacentre/factsheets/fs297/en/

Husain A., Rashid M., Mishra R., Parveen S., Shin D.S., Kumar D., Benzimidazole bearing oxadiazole and triazolo-thiadiazoles nucleus: design and synthesis as anticancer agents, Bioorg. Med. Chem. Lett. 22 (2012) 5438-5444.

Hussain H., Al-Hurrasi A., Al-Rawahi A., Green IR. and Gibbons S., Chem. Rev., 2014, 114, 10369–10428; Nagle AS., Khare S., Kumar AB., Supek F., Buchynskyy A., Mathison CJN., Chennamaneni, NK., Pendem N., Buckner FS., Gelb MH., and Molteni V., Chem. Rev., 2014, 114, 11305–11347; Alvar

J., Cro S., and Olliaro P., Adv. Parasitol., 2006, 61, 224–261; B. L. Herwaldt, Lancet, 1999,354, 1191–1199.

I numeri del cancro in Italia 2016. Associazione Italiana Di Oncologia Medica.

Inestrosa N.C., Alvarez A., Pérez C.A., Moreno R.D., Vicente M., Linker C., Casanueva O.I., Soto C., Garrido J., Acetylcholinesterase accelerates assembly of amyloid-beta-peptides into Alzheimer's fibrils: possible role of the peripheral site of the enzyme. Neuron 1996, 16, 881-891.

Ing HR., Patel RD., J. Chem. Soc. 1936, 1774; J. Pharmacol 59 (1937) 301.

Ishida M., Shinozaki H., Brit. J. Pharmacol. 82 (1984) 523-531.

Jemal A., Bray F., Center MM., Ferlay J., Ward E., Forman D., Global cancer statistics, Cancer J. Clin. 61 (2011) 69-90.

Jhingran A., Chawla B., Saxena S., Barrett MP., and Madhubala R., Mol. Biochem. Parasitol., 2009, 164, 111–117.

Johnson RH. and Einstein HE., Ann. N. Y. Acad. Sci., 2007, 1111, 434–441.

Johnstone RW., Histone-deacetylase inhibitors: novel drugs for the treatment of cancer, Nat. Rev. Drug. Discov. 1 (2002) 287-299.

Kayed R., Thompson J.L., McIntire T.M., Milton S.C., Cotman C.W., Glabe C.G., Common structure of soluble amyloiod oligomers implies common mechanism of phatogenesis. Science 2003, 300, 486-489.

Kale R., Prasad V., Mohapatra P., Tiwari V., Recent developments in benzotriazole methodology for construction of pharmacologically important heterocyclic skeletons, Monatsh. für Chem. - Chem. Mon. 141 (2010) 1159-1182.

Katritzky AR., Rogovoy BV., Benzotriazole: an ideal synthetic auxiliary, Chemistry 9 (2003) 4586-4593.

Katritzky AR., Rogovoy BV., Chassaing C., Vvedensky V., Di(benzotriazol-1-yl)methanimine: a new reagent for the synthesis of tri- and tetrasubstituted guanidines, J. Org. Chem. 65 (2000) 8080-8082.

Katritzky AR., Xie L., Toader D., Serdyuk L., General and efficient carbon insertion route to one-carbon-homologated alpha-aryl, alpha-Alkenyl, alpha-alkoxy, and alpha-phenylthio alkyl ketones, J. Am. Chem. Soc. 117 (1995) 12015-12016.

Katznel'son MM., Kabachnik MJ., Compt Rend Acad Sci. URSS 1 (1935) 125; Chem. Abstr. 29 (1935) 3681.

Kawas, C.H., Clinical practice. Early Alzheimer's disease. N. Engl. J. Med. 2003, 349, 1056-1063.

Khalafi A., Zare A., Parhami A., Soltani Rad MN., and Nejabat, G.R., J. Iranian Chem. Soc. September, 2007; 4(3): 271-278.

Khan W., and Kumar N., J. Drug Targeting, 2011, 19, 239–250.

Kheirandish F., Shara AC., Kazemi B., Mohebali M., Sarlak A., Tarahi MJ., Holakouee K., and Hajaran H., Iran. J. Parasitol., 2013, 8, 382–388.

Kirkitadze M.D., Kowalska A. Molecular mechanisms initiating amyloid beta-fibril formation in Alzheimer's disease. Acta Biochim. Pol. 2005, 52, 417-423.

Knunyants IL., Benevolenskaya ZV., Gen, J., Chem. (USSR) 7 (1937) 2930; Chem. Abstr. 32 (1938) 5404.

Kulshreshtha A., Piplani P., Current pharmacotherapy and putative diesease-modifying therapy for Alzheimner's disease, Neurol Sci, 2016, DOI 10.1007/s10072-016-2625-7

Leonard NI., The Alkaloids, 3, (1953) p 120-157; 7, (1960) p 253-317.

Litchfield DW., Protein kinase CK2: structure, regulation and role in cellular decisions of life and death, Biochem.. J. 369 (2003) 1-15.

Lopez-Vallejo F., Castillo R., Yepez-Mulia L., Medina-Franco JL., Benzotriazoles and indazoles are scaffolds with biological activity against Entamoeba histolytica, J. Biomol. Screen. 16 (2011) 862e868.

Lotfi-Jam K., Carey M., Jefford M., Schofield P., Charleson C., Aranda S., Nonpharmacologic strategies for managing common chemotherapy adverse effects: a Systematic review, J. Clin. Oncol. 26 (2008) 5618-5629.

Maarouf M., Adeline MT., Solignac M., Vautrin D., and Robert-Gero M., Parasite, 1998, 5, 167–173.

Maarouf M., Lawrence F., Cro SL. and Robert-Gero M., Parasitol. Res., 1995, 81, 421-425.

Mai A., Massa S., Ragno R., Cerbara I., Jesacher F., Loidl P., Brosch G., 3-(4-Aroyl-1-methyl-1H-2-pyrrolyl)-N-hydroxy-2-alkylamides as a new class of synthetic histone deacetylase inhibitors. 1. Design, synthesis, biological evaluation, and binding mode studies performed through three different docking procedures, J. Med. Chem. 46 (2003) 512-524.

Makowska M., Lukowska-Chojnacka E., Winska P., Kus A., Bilinska-Chomik A., Bretner M., Design and synthesis of CK2 inhibitors, Mol. Cell. Biochem. 356 (2011) 91-96.

Mattson. P.M.,Pathways towards and away from Alzheimer’s disease. Nature 2004, 430, 631-639

McKhann G., Drachman D., Folstein, M., Katzman, R., Price D., Stadlan E.M., Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984, 34, 939-944.

Mega, M.S., Cummings, J.L., Fiorello, T., Gornbein, J., The spectrum of behavioral changes in Alzheimer's disease. Neurology 1996, 46, 130-135.

Meggio F., Shugar D., Pinna LA., Ribofuranosyl-benzimidazole derivatives as inhibitors of casein kinase-2 and casein kinase-1, Eur. J. Biochem.. 187 (1990) 89-94.

Meheus F., Balasegaram M., Olliaro P., Sundar S., Rijal S., Faiz MdA., and Boelaert M., PLoS Neglected Trop. Dis., 2010, 4, e818.

Meyerhoff A., Clin. Infect. Dis., 1999, 28, 42–48.

Milosevic NP., Dimova VB., Perisic-Janjic NU RP TLC data in correlation studies with in silico pharmacokinetic properties of benzimidazole and benztriazole derivatives. Eur J Pharm Sci, 2013; 49: 10-17.

Mishra J., Saxena A., and Singh S., Curr. Med. Chem, 2007, 14, 1153–1169.

Monge-Maillo B., Norman F., Cruz I., Alvar J., Lopez-Velez R., (2014) Visceral leishmaniasis and HIV coinfection in the Mediterranean region. PLoS Negl Trop Dis 8, e3021.

Moreira, P.I., Nunomura A., Nakamura M., Takeda A., Shenk J.C., Aliev G. et al. Nucleic acid oxidation in Alzheimer disease. Free Rad. Biol. Med. 2008, 44, 1493-1505.

Murray BW., Miller N., Durability of kinase-directed therapies-a network perspective on response and resistance, Mol. Cancer Ther. 14 (9) (2015) 1975–1984.

Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods, 1983, 65, 55-63.

Muthuraju, S., Maiti, P. Solanki, P., Sharma A.K., Amitabh, Singh S.B., Prasad D., Ilavazhagan G., Acetylcholinesterase inhibitors enhance cognitive functions in rats following hypobaric hypoxia. Behav. Brain Res. 2009, 203, 1-14.

National Institute of Health. MedlinePlus: Alzheimer's disease. http://www.nlm.nih.gov/medlineplus/alzheimersdisease.html. Accessed 10 Nov 2015.

Necula M., Kayed R., Milton S., Glabe C.G., Small molecule inhibitors of aggregation indicate that amyloid b-oligomeration and fibrillization pathways are independent and distinct. J. Biol. Chem. 2007, 282, 10311-10324.

New RRC., Chance ML., and Heath, S., J. Antimicrob. Chemother., 1981, 8, 371–381.

Novelli F., Tesi di dottorato di Ricerca, Genova, 1987

Novelli F., Sparatore F., Farmaco, 48 (1993) 1021

Nyland R.L., Luo M., Kelly M.R., Borch R.F. Design and synthesis of novel quinone inhibitors targeted to the redox function of apurinic/apyrimidinic endonuclease 1/redox enhancing factor-1 (Ape1/Ref-1). J. Med. Chem. 2010, 53, 1200-1210.

Okwor I., Uzonna JE. (2013) The immunology of leishmania/HIV coinfection. Immunol Res 56: 163-171.

Pabreza LA., Dhawan S., Kellar KJ., Molec. Pharmacol. 39 (1991) 9.

Pace D. (2014) Leishmaniasis. J Infect 69 S10-18.

Pagano MA., Bain J., Kazimierczuk Z., Sarno S., Ruzzane M., Di Maira G., Elliott M., Orzeskjj A., Cozza G., Meggio F., and Pinna LA., Biochem. J., 2008, 415, 353.

Paglietti G., Sanna P., Carta A., Sparatore F., Vazzana I., Peana A., Satta M., Choleretic activity of 3-[ring substituted benzotriazol-1(2)-yl]alkanoic and alkenoic acids, Farm. Sci. 40 (1994) 693e702.

Pakudina ZP., Yunusov SY., Izvest. Akad. Nauk. Uzbek.S.S.R. Ser. Khim. Nauk. (1957) 69; Chem. Abstr. 54 (1960) 6783 Luputiu, G., Moll, F., Arch. Pharm. 304 (1971) 151.

Pardossi-Piquard R., Checler F., The physiology of the b-amyloid precursor protein intracellular domain AICD, J. Neurochem. 120 (Suppl 1) (2012) 109e124.

Patel TA., and Lockwood, D. N. Trop. Med. Int. Health, 2009, 14, 1064–1070.

Perez-Victoria FJ., Gamarro F., Ouellette M. and Castanys SJ., Biol. Chem., 2003, 278, 49965–49971.

Peter W., Indian J. Med. Res., 1981, 73, 1–18.

Piccionello AP., Guarcello A., (2010) Bioactive compounds containing benzoxadiazole, benzothiadiazole, benzotriazole. Curr Bioact Compd 6: 266283.

Pike C.J., Burdick D., Walencewicz A.J.,Glabe C.G., Cotman C.W., Neurodegeneration induced by beta-amyloid peptides in vitro: the role of peptide assembly state. J. Neurosci. 1993, 13, 1676-1687.

Pinna LA., Meggio F., Protein kinase CK2 (“casein kinase-2”) and its implication in cell division and proliferation, Prog. Cell. Cycle Res. 3 (1997) 77-97.

Pyerin W., Ackermann K., The genes encoding human protein kinase CK2 and their functional links, Prog. Nucleic Acid. Res. Mol. Biol. 74 (2003) 239-273.

Pyerin W., Ackermann K., Transcriptional coordination of the genes encoding catalytic (CK2alpha) and regulatory (CK2beta) subunits of human protein kinase CK2, Mol. Cell. Biochem. 227 (2001) 45-57.

Rakhimov S. B., Islamova Z. I., Vinogradova V. I., Khushbaktova, Z. A., Osipova, S. O. Syrova, V. N. Synthesis and antiparasitic activity of N-benzylcytisine derivatives. Pharm. Chem. J. 2013, 47, 209-212

Ramos H., Valdivieso E., Gamargo M., Dagger F. and Cohen BE., Membr J., Biol., 1996, 152, 65–75.

Raschack M., Arzneim. Forsch. 24 (1974) 753.

Reithinger R, Dujardin JC, Louzir H, Pirmez C, Alexander B, Brooker S. Cutaneous leishmaniasis. Lancet Infect Dis. 2007; 7: 581-596.

Ren Yu., Zhang Ling., Zhou Cheng-He., and Geng Rong-Xia., Recent development of Benzotriazole-based Medicinal drugs. Medicinal Chemistry (2014) 640-662, Volume 4(9).

Reynolds AK., The Alkaloids, 5, (1955) 179-183.

Rink M., Grabowski K., Arch. Pharm. 289 (1956) 695 Engelmann K., Raake W., Petter A., Arzneim. Forsch. 24 (1974) 759 Schoen U., Kehrbach W., Hachmeister B., Buschmann G., Ger. Offen.DE 3,522,475 e DE 3,643,402; Chem. Abstr. 106 (1987) 156761s e 110 (1989) 63753h.

Robert A., Smiley “Phenylene- and Toluenediamines” in Ullmann's Encyclopedia of Industrial Chemistry, 2002, Wiley-VCH, Weinheim. doi:10.1002/14356007.a19_405.

Roher A.E., Baudry J., Chaney M.O., Kuo Y.M., Stine W.B., Emmerling M.R., Oligomerization and fibril assembly of the amyloid-beta protein. Biochim. Biophys. Acta 2000, 1502, 31-43.

Roses A.D., A model for susceptibility polymorphisms for complex diseases: apolipoprotein E and Alzheimer disease, Neurogenetics 1997, 1, 3-11.

Rosini M Simoni E., Milelli A., Minarini A, Melchiorre C.. Oxidative stress in Alzheimer's disease: are we connecting the dots? J. Med. Chem. 2014, 57, 2821-2831.

Rosini M., Simoni E., Caporaso R., Minarini A., Future Med. Chem (2016) 8(6),697-711.

Roychoudhury J., and Ali N., Indian J. Biochem. Biophys., 2008, 45, 16–22; Ferreira CS., Martins PS., Demicheli C., Brochu C., Ouellette M., and Frezard F., Biometals, 2003, 16, 441–446.

Rubiano LC., Miranda MC., Muvdi-Arenas S., Montero LM., Rodrìguez-Barraquer I., Garcerant D., Prager M., Osorio L., Rojas MX., P´erez M., Nicholls RS., and Gore-Saravia N., J. Infect. Dis., 2012, 205, 684–692.

Saha AK., Mukherjee T., and Bhaduri A., Mol. Biochem. Parasitol., 1986, 19, 195–200.

Saito K., et al: risultati inediti; citati da: Ohmiya S., Saito K., Murakoshi I., The Alkaloids, 47, (1995) 100.

Sangshetti JN., Kalam Khan FA., Kulkarni AA., Arote R., and Patil RH., Antileishmanial drug discovery: comprehensive review of the last 10 years, RSC Adv, 2015, 5, 32376–32415.

Santini F.,Tesi di laurea: Derivati della tiolupinina con potenziale attività antimalarica, Genova, 2008.

Sarno S., Reddy H., Meggio F., Ruzzene M., Davies SP., Donella-Deana A., Shugar D., Pinna LA., Selectivity of 4,5,6,7-tetrabromobenzotriazole, an ATP site-directed inhibitor of protein kinase CK2 ('casein kinase-2'), FEBS Lett. 496 (2001) 44-48.

Savelli F., Sparatore F., XI Congr. Naz. Soc. Chim. Ital., Perugia 1972, Atti A 15; Studi Sassaresi 53 (1975) 409.

Sease Catherine., "Benzotriazole: A Review for Conservators". Studies in Conservation 2, 1978; 23(2): 76–85. doi:10.2307/1505798. JSTOR 1505798.

Seifert K., and Cro, Antimicrob, S. L., Agents Chemother., 2006, 50, 73–79.

Seilheimer B., Bohrmann B., Bondolfi L., Muller F., Stuber D., Dobeli H., The toxicity of the Alzheimer's beta-amyloid peptide correlates with a distinct fiber morphology. J. Struct. Biol. 1997, 119, 59-71.

Seldin DC., Leder P., Casein kinase II alpha transgene-induced murine lymphoma: relation to theileriosis in cattle, Science 267 (1995) 894-897.

Seubert P., Oltersdorf T., Lee M.G., Barbour R., Blomquist C., Davis D.L., Bryant K., Fritz L.C., Galasko D., Thal L.J., Liberburg I., Shenk, D.B., Secretion of beta-amyloid precursor protein cleaved at the amino terminus of the betaamyloid peptide. Nature 1993, 361, 260-263.

Shaughnessy LW, Locascio JJ, Growdon JH (2008) Long-term course and effectiveness of combination therapy in Alzheimer disease. Alzheimer Dis Assoc Disord 22:209–221

Scherzer R., Gazit E., Segal D., Naphthoquinone derivatives useful for prevention of amyloid deposits and treatment of diseases involving amyloidogenesis. Pat. WO 2010026592A1 (2010).

Shibata S., Mishikana Y., Yakugaku Zasshi 81 (1961) 1635 [Drug Fut. 9 (1984) 29].

Singh G., Kumar R., Swett J., Zajc B. (2013) Modular synthesis of N-vinyl benzotriazoles. Org Lett 15: 4086-4089.

Singh N., Kumar M. and Singh RK., Asian Pac. J. Trop. Med., 2012, 5, 485–497.

Singh N., Mishra BB., Bajpai S., Singh RK. and Tiwari VK., Bioorg. Med. Chem., 2014, 22, 18-45.

Singh S., (2014) Changing trends in the epidemiology, clinical presentation, and diagnosis of Leishmania-HIV co-infection in India. Int J Infect Dis 29:103-112.

Singh S., and Sivakumar R., J. Infect. Chemother., 2004, 10, 307–315.

Snegur LV., Nekrasov YS., Sergeeva NS., Zhilina ZV., Gumenyuk VV. et al. (2008) Ferrocenylalkyl azoles: bioactivity, synthesis, structure. Appl Organometal Chem 22: 139-147.

Soto C., Protein misfolding and disease; protein refolding and therapy. FEBS letters 2001, 498, 204-207.

Sparatore F., Ann. Chim. 52 (1962) 259.

Sparatore F., La Rotonda MI., Ramundo E., Silipo C., Vittoria A., Effect of benzotriazole derivatives on plant growth. II, Farm. Sci. 33 (1978) 924e944.

Stefani M., Protein misfolding and aggregation: new examples in medicine and biology of the dark side of the protein world. Biochim. Biophys. Acta 2004, 1739, 5-25.

Sultana R., Mecocci P., Mangialasche F., Cecchetti R., Baglioni M., Butterfield D.A., Increased protein and lipid oxidative damage in mitochondria isolated from lymphocytes from patients with Alzheimer's disease: insights into the role of oxidative stress in Alzheimer's disease and initial investigations into a potential biomarker for this dementing disorder. J. Alzheimer Dis. 2011, 24, 77-84.

Sundar S., Agrawal N., Arora R., Agarwal D., Rai M. and Chakravarty J., Clin. Infect. Dis., 2009, 49, 914–918.

Sundar S., and Chakravarty J., Expert Opin. Invest. Drugs, 2008, 17, 787–794.

Sundar S., and Chatterjee, M., Indian J. Med. Res., 2006, 123, 345–352.

Sundar S., Chakravarty J., Rai VK., Agrawal N., Singh SP., Chauhan V., and Murray H., Clin. Infect. Dis., 2007, 45, 556– 561.

Sundar S., Jha T. K., Sindermann H., Junge K., Bachmann P., and Berman J., Pediatr. Infect. Dis. J., 2003, 22, 434–438.

Sundar S., Jha TK., Thakur CP., Sinha PK. and Bhattacharya SK., Engl N., J. Med., 2007, 356, 2571–2581.

Sundar S., Mehta H., Suresh AV., Singh SP., Rai M., and Murray HW., Clin. Infect. Dis., 2004, 38, 377–383.

Swider R., Maslyk M., Zapico JM., Coderch C., Panchuk R., Skorokhyd N., Schnitzler A., Niefind K., de Pascual-Teresa B., and Ramos A., Synthesis, Biological activity and structural study of new benzotriazole-based protein kinase CK2 inhibitors. Royal Society of Chemistry. (2015) 5, 72482-72494.

Szyszka R., Grankowski N., Felczak K., and Shugar D., Biochem. Biophys. Res. Commun., 1995, 208, 418.

Szyszka, R., Grankowski N., Felczak K., Shugar D., Halogenated benzimidazoles and benzotriazoles as selective inhibitors of protein kinases CK I and CK II from Saccharomyces cerevisiae and other sources, Biochem.. Biophys. Res. Commun. 208 (1995) 418-424.

Tang YD., Zhang JQ., Zhang SL., Geng RX., Zhou CH., Synthesis and characterization of thiophene-derived amido bis-nitrogen mustard and its antimicrobial and anticancer activities. Chin J Chem, 2012; 30: 1831-1840.

Tariot PN, Farlow MR, Grossberg GT, Graham SM, McDonald S, Gergel I, Memantine study group (2004) Memantine treatment in patients with moderate to severe Alzheimer disease already receiving donepezil: a randomized controlled trial. JAMA 291:317–324

Tasso, B.; Catto, M.; Nicolotti, O.; Novelli, F.; Tonelli, M.; Giangreco, I.; Pisani, L.; Sparatore, A.; Boido, V.; Carotti, A.; Sparatore, F. Quinolizidinyl derivatives of bi- and tricyclic systems as potent inhibitors of acetyl- and butyrylcholinesterase with potential in Alzheimer's disease. Eur. J. Med. Chem., 2011, 46, 2170-2184.

Thakur CP., Singh RK., Hassan SM., Kumar R., Narain S., and Kumar A., Trans. R. Soc. Trop. Med. Hyg., 1999, 93, 319– 323.

Tlegenov R. T., Synthesis and antituberculosis activity of lupinine palmitate, Pharm. Chem. J., 2011, 42, 607-608.

Tonelli M., Tasso B., Mina L., Paglietti G., Boido V., Sparatore F.. Primary anti-proliferative activity evaluation of 1-(quinolizidin-1'-yl)methyl and 1-(ω-tert-

amino)alkyl-substituted 2-phenyl-, 2-benzyl- and 2-[(benzotriazol-1/2-yl)methyl]benzimidazoles on human cancer cell lines. Mol Divers 17, (2013), 409-419.

Tonelli, M.; Catto, M.; Tasso, B.; Novelli, F.; Canu, C.; Iusco, G.; Pisani, L.; De Stradis, A.; Denora, N.; Sparatore, A.; Boido, V.; Carotti, A.; Sparatore, F. Multitarget Therapeutic Leads for Alzheimer's Disease: Quinolizidinyl Derivatives of Bi- and Tricyclic Systems as Dual Inhibitors of Cholinesterases and β-Amyloid (Aβ) Aggregation. ChemMedChem, 2015, 10, 1040-1053.

Turabekova, M. A.; Vinogradova, V. I.; Werbovetz, K. A.; Capers, J.; Rasulev, B. F.; Levkovich, M. G.; Rakhimov, S. B.; Abdullaev, N. D. Structure-activity relationship investigations of Leishmanicidal N-benzylcytisine derivatives. Chem. Biol. Drug Des., 2011, 78, 183-189.

Turchetti AP., Souza TD., Paixao TA., Santos RL., (2014) Sexual and vertical transmission of visceral leishmaniasis. J Infect Dev Ctries 8: 403-407. doi:10.3855/jidc.4108.

Unger C., Peukert M., Sindermann H., Hilgard P., Nagel G., and Eibl H., Cancer Treat. Rev., 1990, 17, 243–246.

Vial H. J., Ancelin M. L., Malarial lipids. In Subcellular biochemistry; J. L. Avila, J. R. Harris, Eds. Plenum Press: New York, 1992, 259-306.

Vial H. J., Eldin P., Tielens A. G., Van Hellemond J. J., Phospholipids in parasitic protozoa. Mol. Biochem. Parasitol., 2003, 126, 143-154.

Vial H. J., Mamoun C., Plasmodium lipids: metabolism and function. In Molecular approach to malaria; I. Sherman, Ed. ASM Press: Washington DC, 2005, 327-352.

Volodina Iu L., Shtil AA., [Casein kinase 2, the versatile regulator of cell survival], Mol. Biol. Mosk. 46 (2012) 423-433.

Wadhone P., Maiti M., Agarwal R., Kamat V., Martin S., and Saha B., J. Immunol., 2009, 182, 7146–7154.

Wasik R., Winska P., Poznanski J., Shugar D., Synthesis and physico-chemical properties in aqueous medium of all possible isomeric bromo analogues of benzo-1H-triazole, potential inhibitors of protein kinases, J. Phys. Chem. B 116 (2012) 7259-7268.

Weidemann A., König G., Bunke D., Fischer P., Salbaum J.M., Masters C.L., Beyreuther K., Identification, biogenesis, and localization of precursors of Alzheimer's disease A4 amyloid protein. Cell 1989, 57, 115-126.

Wimo, A., Jonsson, L., Bond, J., Prince, M., Winblad, B., Alzheimer Disease International. The worldwide economic impact of dementia 2010. Alzh. Dement 2013, 9, 1-11.

Winterfeld K., Hoffmann E., Arch. Pharm. 275 (1937) 5 e 526 Jack, W., Arch. Exptl. Pathol. Pharmakol. 200 (1942) 528.

World Health Organization (2014) Global Health Observatory Data – Leishmaniasis. Available at: http://www.who.int/gho/neglected_diseases/leishmaniasis/en/ Global Health Observatory. Accessed on September 21, 2016.

World Health Organization, Regional Office for Europe (2014) Strategic framework for-leishmaniasis control in the WHO European Region 2014-2010. Available at: www.euro.who.int/_data/assets/pdf_file/0017/245330/

Xie M., Zhou W., Zhang Y., Chin. Med. J., 96 (1983) 145; citato in Drugs Fut. 9 (1984) 29.

Yardley V., and Croft SL., Antimicrob. Agents Chemother., 1997, 41, 752–756.

Yavar R., Hadi K., Reza AM., Mohebali M., Hasan B., Ali OM., Sina R., Habib BH., Abodolrahim H. and Manuchehr G., Asian Pac. J. Trop. Biomed., 2013, 3, 825–829.

Yuan J., Zhong Y., Li SL., Zhao X., Luan GQ., et al. Triazole and benzotriazole derivatives as novel inhibitors for p90 ribosomal S6 protein kinase 2: synthesis, molecular docking and SAR analysis. Chin J Chem, 2013; 31: 1192-1198.

Zaghloul IY. and Al-Jasser M., Trop Ann., Med. Parasitol, 2004, 98, 793-800.

Zakusov VV.,Farmacologiia, Medgiz, Mosca 1960, p. 203.

Zerovnik E., Amyloid-fibril formation. Proposed mechanisms and relevance to conformational disease. E. J. Biochem. 2002, 269, 3362-3371.

Zhang S., Luo Y., Qiang He L., Jun Liu Z., Qin Jiang A., Hua Yang Y., and Liang Zhu H., Bioorganic & Medicinal Chemistry. 2013, 21, 3723.

Zhang S., Zhang H., Li-Hong Sun D., Ma C., Wang W., Wan J., Qu B., Eur. J. Pharm., 2008, 584, 144.

Zheng H., Koo E.K.,The amyloid precursor protein. beyond amyloid. Mol. Neurodegener. 2006, 3, 1-5.

Zhou CH., Zhang YY., Yan CY., Wan K., Gan LL., et al. (2010) Recent researches in metal supramolecular complexes as anticancer agents. Anti-Cancer Agents Med Chem 10: 371-395.