Synthesis and biological evaluation of novel thiazole- VX-809 hybrid derivatives as F508del correctors by QSAR-based filtering tools

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Abstract

The most common CF mutation, F508del, impairs the processing and gating of CFTR protein. This deletion results in the improper folding of the protein and its degradation before it reaches the plasma membrane of epithelial cells. Present correctors, like VX809 only induce a partial rescue of the mutant protein. Our previous studies reported a class of compounds, called aminoarylthiazoles (AATs), featuring an interesting activity as correctors. Some of them show additive effect with VX809 indicating a different mechanism of action. In an attempt to construct more interesting molecules, it was thought to generate chemically hybrid compounds, blending a portion of VX809 merged to the thiazole scaffold. This approach was guided by the development of QSAR analyses, which were performed based on the F508del correctors so far disclosed in the literature. This strategy was aimed at exploring the key requirements turning in the corrector ability of the collected derivatives and allowed us to derive a predictive model guiding for the synthesis of novel hybrids as promising correctors. The new molecules were tested in functional and biochemical assays on bronchial CFBE41o- cells expressing F508del-CFTR showing a promising corrector activity.

Abbreviations:

CF, cystic fibrosis; CFTR, CF transmembrane conductance regulator; AATs, aminoarylthiazoles; HS-YFP, halide-sensitive yellow fluorescent protein; QSAR, quantitative-structure activity relationship; HBTU, O-(Benzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium hexafluorophosphate; DIPEA, N,N-Diisopropylethylamine.

Keywords:

Aminoarylthiazole, corrector, QSAR, cystic fibrosis, CFTR, VX809.

1. Introduction

The genetic disease cystic fibrosis (CF) is caused by mutations in the cystic fibrosis transmembrane conductance regulator gene (CFTR), which encodes a cAMP-regulated chloride channel [1–4].

CFTR protein contain five domains: two nucleotide binding domains (NBD1 and NBD2), two transmembrane domains (MSD1 and MSD2), and one regulatory domain (R) [5]. So far, a large number of CF-causing mutations have been identified, which can be classified in six classes according to the mechanism leading to CFTR loss-of-function [6], such as reduction of protein expression, function, stability or a combination of these[7-9].

The most common genetic defect is the deletion of phenylalanine 508 (F508del), located in NBD1. F508del-CFTR is misfolded retained at the endoplasmic reticulum (ER), and rapidly degraded [10-12]. This kind of mutation leads to distinct defects in channel gating and cellular processing. Cystic fibrosis results in chronic lung infection, deterioration of lung function, and death.

The primary defects caused by CFTR mutations can be treated with drug-like small molecules, known as 'CFTR modulators', targeting specific defects caused by mutations in the CFTR gene. They are classified into five main groups including read-through agents, correctors, potentiators, stabilizers and amplifiers [13]. Even if the development of monotherapies could allow addressing specific molecular defect related to some CFTR mutations, a combination of treatments could rectify several multi-defects at the same time, achieving beneficial levels in the patients. Therefore, small-molecule therapy will likely require compounds that correct at least the two major underlying problems in CF: (a) CFTR misfolding and ER retention, and (b) defective channel gating [14-15].

In particular, the folding and the channel activity defect of F508del can be managed with the aforementioned modulators named correctors and potentiators, respectively.

Correctors improve the trafficking of mutant CFTR to the plasma membrane, instead, potentiators are believed to bind to F508del-CFTR at the cell surface and increase chloride channel gating.

While potentiators probably act by directly interacting with CFTR protein, the mechanism of action of correctors is unknown. They could directly bind to CFTR improving its folding and stability or regulate the expression/function of other proteins involved in CFTR processing and trafficking [16-17].

Because both a potentiator and corrector are likely required to treat cystic fibrosis [12] caused by the F508del mutation, CFTR is an attractive target for the development of a multiligand-containing drug, which represents an emerging paradigm in drug discovery [18].

During the last years, several efforts have been performed to identify new promising chemical entities able to behave as F508del -CFTR corrector and/or potentiator, relying on virtual screening protocols (VS) and deepening high-throughput screening strategies (HTS) [18-24].

The most promising data came from HTS approaches, conducted by several research groups and by Vertex Pharmaceuticals. In particular, Verkman and colleagues paved the way in the discovery of thiazole-containing correctors through HTS assays, disclosing corrector 4a that exhibited micromolar potency (EC_{50} < 3μM) [21]. This hit compound was further optimized, towards the design of novel series of bioisosteres experienced as CFTR correctors. These compounds included conformationally-locked bithiazoles, pyrazolylthiazoles and triazolo-bithiazoles, characterized by improved hydrophilicity and potency values (Figure 1) [18, 21, 23, 24].

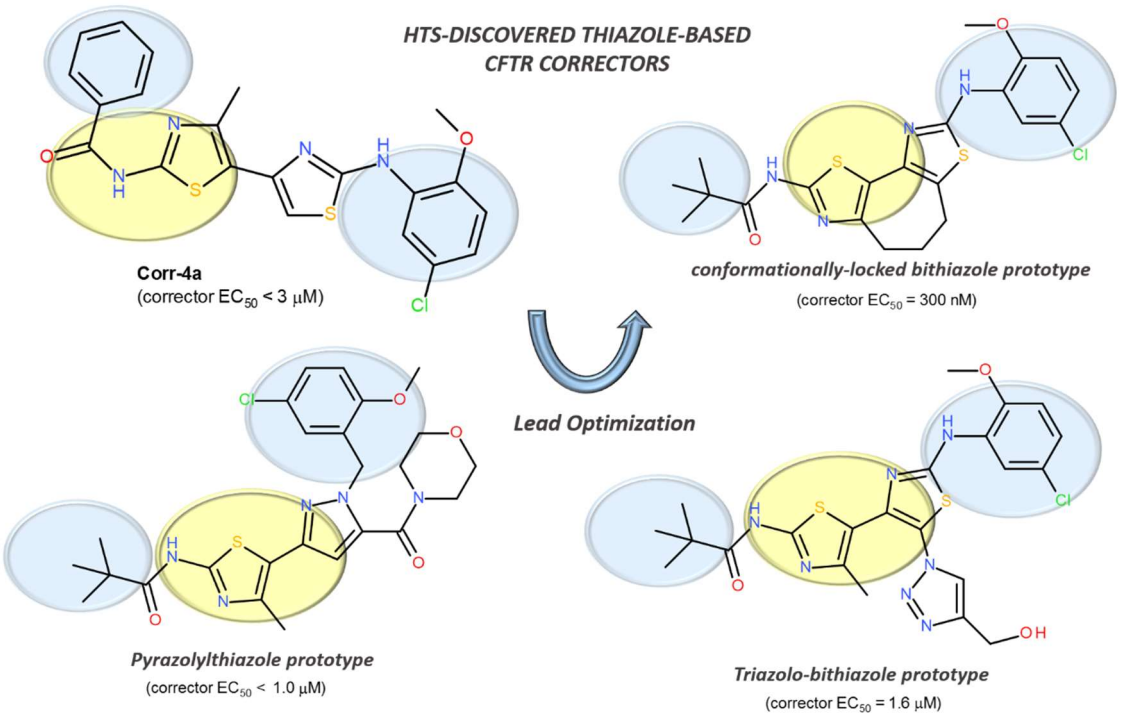

Figure 1. Chemical structure of the HTS-discovered thiazole-containing series of CFTR correctors. Recurrent hydrophobic and H-bonding features are highlighted in cyan and yellow, respectively.

Then, the same Authors also investigated further chemo-types, bearing a cyanoquinoline core linked to an aryl amide moiety by a variable tether, which displayed valuable corrector activities [25]. More recently, a screening campaign reported by other research groups enlightened a number of tetrahydropyridopyrimidines, properly decorated with aromatic substituents and basic moieties, as novel promising CFTR correctors [26]. Notably, both the two series display recurrent hydrophobic and H-bonding features if compared with the thiazole-based correctors, thanks to the bicyclic core and to the related aromatic substituents, maintaining adequate corrector ability (Figure 2).

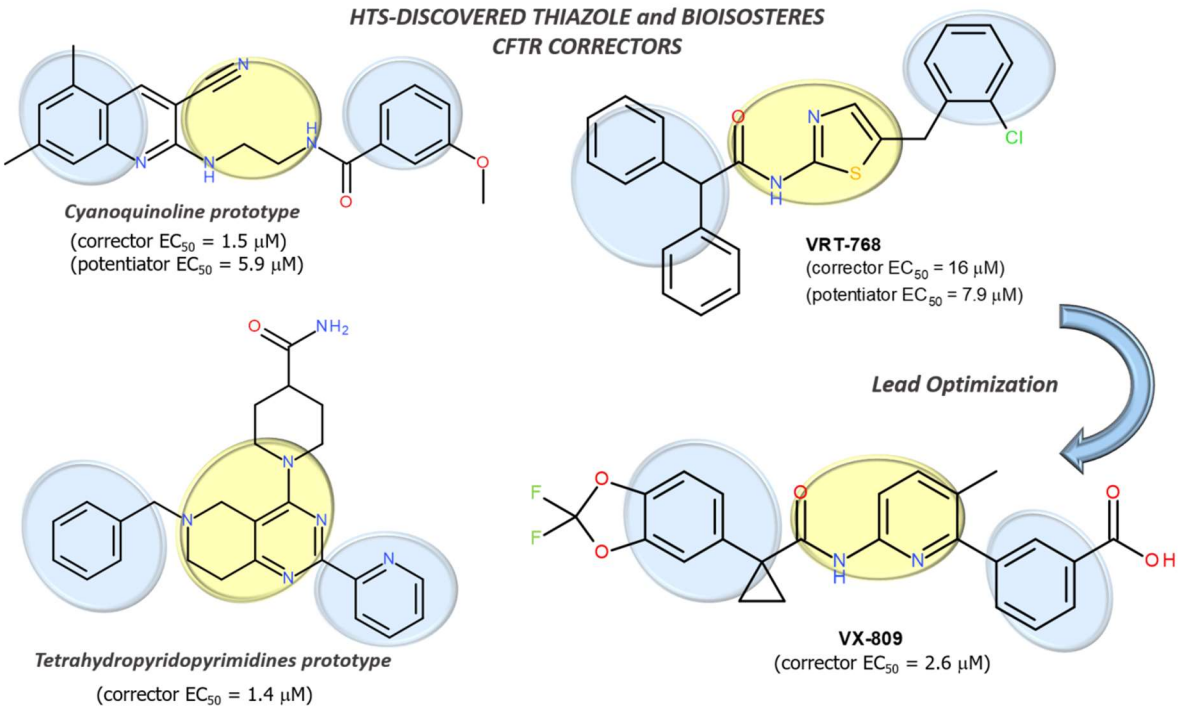

Figure 2. Chemical structure of the HTS-discovered thiazole and bioisosteres acting as CFTR correctors. Recurrent hydrophobic and H-bonding features are highlighted in cyan and yellow, respectively.

Starting from HTS, Vertex Pharmaceuticals discovered cycloalkyl carboxamide F508del-CFTR correctors, firstly identifying a thiazole-containing compound (VRT-768) as prototype (Figure 2). This compound was further optimized towards the well-known corrector VX-809 (Lumacaftor), bearing a pyrimidine ring as bioisosteres of the previous thiazole nucleus (Figure 2) [27].

In the search of new drugs for CF treatment, in our previous works [28, 29] we have shown how some aminoarylthiazoles (AATs) exhibit F508del modulator ability, being especially correctors and/or sometimes potentiators of the channel. Moreover, combined treatment of some of these correctors and VX809 resulted in additive effect. Indeed, incubation of CFBE41o- cells with some AATs together with VX-809 generated a five-fold increase in activity relative to vehicle-treated cells, suggesting for different mechanisms of action by the two chemo-types [29].

Concerning this issue, a number of in silico docking studies devised VX-809 directly targeting the full-length F508del-CFTR protein, with a possible binding site at the NBD1-ICL4 interface [30].

In this regard, recent literature from the Forman-Kay group highlights an allosteric coupling between the binding site of VX809 and the NBD1:ICL4 interface [31].

On the contrary, other hypotheses assert that VX-809 specifically interacts with TMD1 of CFTR [32-34], but does not increase the stability of F508del [32].

Nevertheless, the precise mechanism of action supporting the corrector ability featured by VX809 as well as that of AATs is still experimentally unclear, making the drug discovery and lead optimization process a challenging task.

On the other hand, a perspective of the whole chemical entities so far discovered allows deciphering common and recurrent pharmacophore features for CFTR correctors, including two aromatic-lipophilic moieties connected by at least one H-bond acceptor function (see Figures 1-2). Moreover, this information is in accordance with other studies proposed in the literature and focused on the development of pharmacological chaperone correctors in CF therapy, displaying aromatic centers and one H-bond acceptor moiety [35, 36].

On this basis, we deemed interesting to proceed our work about CFTR modulators applying quantitative-structure activity relationship (QSAR) on the aforementioned different chemical classes of correctors, in order to point out a limited number of chemical descriptors probably involved in their variable corrector ability trend. This information allowed providing useful guidelines to co-orchestrate the rational design of the newly synthesised CFTR correctors here proposed.

Indeed, the derived pool of descriptors was used as filtering tools guiding for the optimization of our AAT series. Based on these considerations and with the aim to improve the activity profile of the AATs, we have now designed and synthesised a library of novel derivatives to investigate their effect on mutant CFTR.

In particular, we continued our synthetic efforts exploring the effectiveness of novel derivatives, namely hybrid compounds, obtained by merging the benzodioxole moiety of VX809 with the thiazole

chemo-type of AATs. This represents the first step in the search of novel correctors rationally designed by fragment-based criteria, which is highly motivated by the well-known potency of VX809 so as by the interesting CFTR modulator ability experienced by the thiazole scaffold (Figure 3).

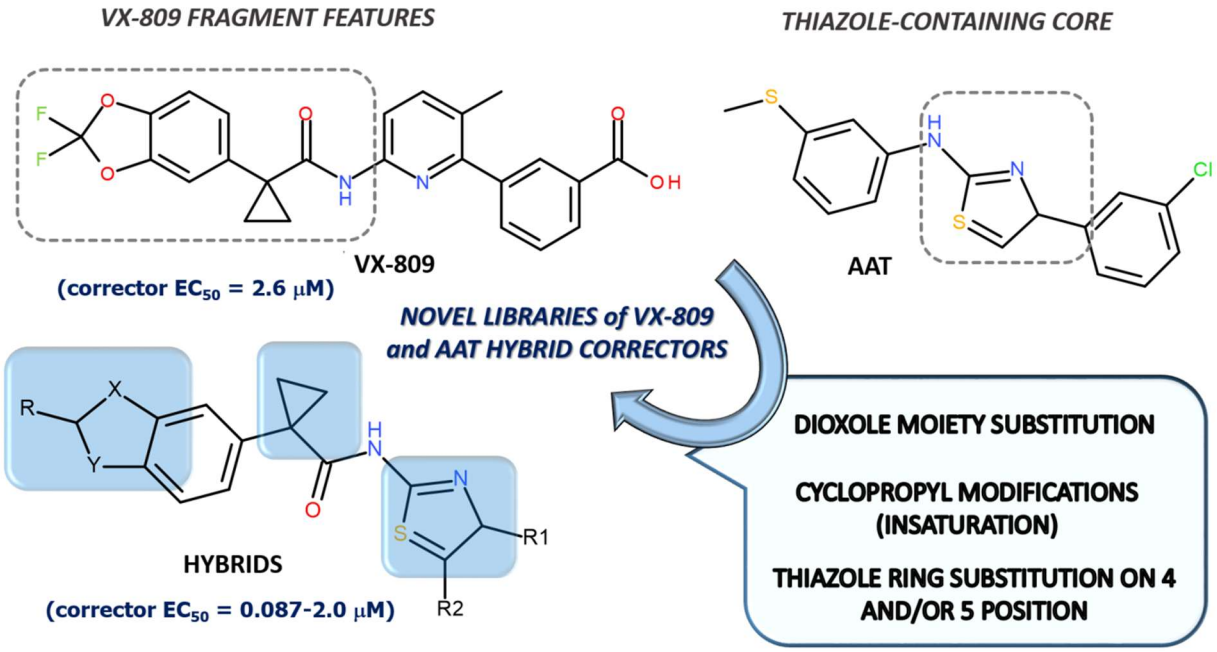

Figure 3. Chemical structure of the newly synthesised VX-809 and thiazole-containing hybrids. Merged moieties are shown by grey dot lines. Explored substituents of the hybrid-scaffold are highlighted in cyan.

The main issue to be addressed was to clarify the role played by the fluorinate benzodioxole portion when it is coupled with the thiazole-containing motif in maintaining and/or improving the corrector ability of the new compounds. In addition, the introduction of the carboxamide function linked at the thiazole core opens the possibility to better fulfil the specific pharmacophore requirements experienced by the previously mentioned correctors, discussed in the literature.

The set of compounds drawn and synthesised has been defined “hybrid compounds”, since their structure is really a hybrid between the AATs and the VX809 scaffolds. The design and synthesis of new libraries was planned in order to maximize the possibility of identify novel compounds endowed

with improved F508del corrector ability, filtering the most promising compounds based on the information obtained but the aforementioned QSAR analyses.

The ligand-based guided approach proved to deepen the discovery of VX-809-like hybrid structures bearing the thiazole core, endowed with a promising corrector activity. Further structural modifications merging other key moieties of VX809 and of AATs (following series of hybrids) will represent a rare opportunity to clarify whenever the thiazole scaffold could be endowed with improved CFTR modulator ability and will be discussed in due course.

2. Results and discussion

2.1. Chemistry

The lead structure of our derivatives can be split into three different parts: the benzodioxole substituted portion, the central cyclopropane moiety and the aminoarylthiazole substituted portion. To prepare the benzo [1, 3] dioxol-5-yl-cyclopropanecarboxylic acid **(1a)** we explored the reaction of arylacetonitriles with ethylene carbonate in K_2CO_3. The treatment of arylacetonitriles with ethylene carbonate in the presence of potassium carbonate at T=140°C should generate the cyclopropane ring on the structure but without a satisfactory yield [37].

The cyclopropanation of active methylene compounds is carried out using either ethylene dichloride or ethylene dibromide as the alkylating agent in the presence of a strong base like 50% aqueous sodium hydroxide in the presence of a phase transfer catalyst [38].

Initially, we performed the cyclopropanation using 1, 2-dibromoethane and K_2CO_3 in the presence of a catalytic amount of BTEAC as a phase transfer catalyst to furnish 1-(benzo[d] [1, 3] dioxol-5-yl) cyclopropanecarboxylic acid in very low yield via an intramolecular dialkylation reaction.

This reaction was repeated with bromochloroethane, and a strong base such as 50 % NaOH in aqueous media to furnish the product **1a** in 65% yield adapting to published procedures [39].

A similar protocol was used to synthesised the 1-(2, 2-difluorobenzo[d] [1, 3] dioxol-5-yl) cyclopropanecarboxylic acid **1b** starting from 2, 2-difluorobenzo[d] [1, 3] dioxole-5-carbaldehyde in four step as previously published with minor modifications [40].

The substituted aminoarylthiazole were synthesised in a range of variable yields via the classic Hantzsch thiazole synthesis refluxing the appropriate haloketone with an equimolar amount of the thiourea in absolute ethanol (Scheme 1) [41, 42].

In the search for a simple and effective means of obtaining aminothiazole derivatives, we used the selective monobromination of ketones. All the bromoketones that were not commercially available were readily prepared from the corresponding ketones utilizing *N*-bromosuccinimide (NBS), catalyzed by trimethylsilyl trifluoromethanesulfonate (TMSOTf) in acetonitrile [43]. This method is useful for the side-chain bromination of heteroaromatic carbonyl compounds without the ring brominations. (Scheme 1).

The aminothiazole of the analogue **5c** was synthesised, in a first moment, through the simple procedure from the precursor 1-(3-hydroxyphenyl) propan-1-one, its bromination and the final treatment with the thiourea but without success. The very low yield of the procedure the yield is due to the difficulty of selectively monobromination the ketone and of isolating the respective intermediate 2-bromo-(4-hydroxyphenyl) propan-1-one. The same synthetic troubles have been encountered in obtaining the derivative **5d** from the precursor 1-(4-hydroxyphenyl) propan-1-one.

To overcome such drawbacks we adopted a different strategy. There was a significant increase in yield of the condensation of the hydroxyl substituted propiophenone with thiourea and iodine to obtain 4-(2-amino-5-methylthiazol-4-yl)phenol or 3-(2-amino-5-methylthiazol-4-yl)phenol using microwave heating under solvent-free conditions as reported in the literature[44] (Scheme 2) .

The synthesis of amide derivatives of 2-aminothiazoles were achieved by condensation reaction of appropriate 2-amino-thiazole with the carboxylic group of cyclopropanecarboxylic acid derivatives with uronium salt HBTU /DIPEA activation in anhydrous DMF[29] (Scheme1).

A different synthetic route was used to synthesise **5f.** In fact the appropriate thiazole ethyl 4-(2-amino-5-methylthiazol-4-yl) benzoate was prepared starting from the corresponding derivative ethyl 4-propionylbenzoate.

Unfortunately we found, in pilot experiments, that the published routes to the carboxylic acid precursor, 4-carboxypropiophenone, from p-methylpropiophenone gave low and/or irreproducible yields. The oxidation of the methyl group with $KMnO_4$ [45] affords mainly a lot of sub-products rather than 4-carboxypropiophenone. To overcome these drawbacks we developed a more reliable synthesis from the commercially available starting material 4-bromopropiophenone according with the method of Rosowsky [46]. As shown in Scheme 3, treatment of 4-bromopropiophenone with CuCN in DMF afforded the nitrile, which on hydrolysis with KOH in acetonitrile was converted to carboxylic acid. The next acid esterification with ethanol completed the synthesis furnishing the ethyl 4-propionylbenzoate in good yield. The aminothiazole was thus built through the traditional procedure from this precursor, its bromination and the final treatment with the thiourea. The appropriate condensation reaction of ethyl 4-(2-aminothiazol-4-yl) benzoate with the carboxylic group of cyclopropanecarboxylic acid derivative promotes the formation of the esterified precursor which gives rise to the final derivative through hydrolysis.

The compound **5e** was indeed built starting from ethyl 4-acetylbenzoate. The ketone was then brominated with NBS as previously described and conjugated with thiourea in refluxing ethanol. The ethyl 4-(2-aminothiazol-4-yl) benzoate was then conjugated with **1a** affording an esterified derivative that was subjected to alkaline hydrolysis to obtain the final compound with the carboxyl group (Scheme 4).

The compound **7a** and **7b** ,that not containing the thiazole scaffold, were obtained by condensation of the carboxylic group of benzo [1, 3] dioxol-5-yl-cyclopropanecarboxylic acid **(1a)** with the amine derivatives 3-(methylthio) aniline and 3-aminoquinoline respectively (Scheme 5) .

2.2. QSAR analyses of F508del CFTR correctors

In the search of novel F508del correctors, we deemed interesting to proceed our work with the development of quantitative-structure activity relationship (QSAR) analyses, aimed at disclosing some relevant structural features exhibited by a number of known correctors, as described in the literature. Indeed, we built in silico a dataset including sixty-three compounds (**1-63**; see Supporting information S1-S3), being compounds **1** (pEC_{50} = 5.59) and **2** (pEC_{50} = 5.96) the well-known correctors VX-809 and corrector-4a, respectively. Most of the other derivatives were characterized by a number of the aforementioned tetrahydropyridopyrimidines (**4-30**), cyanoquinolines (**31-54**), and thiazole-containing molecules (**55-63**), as shown in Figure 4.

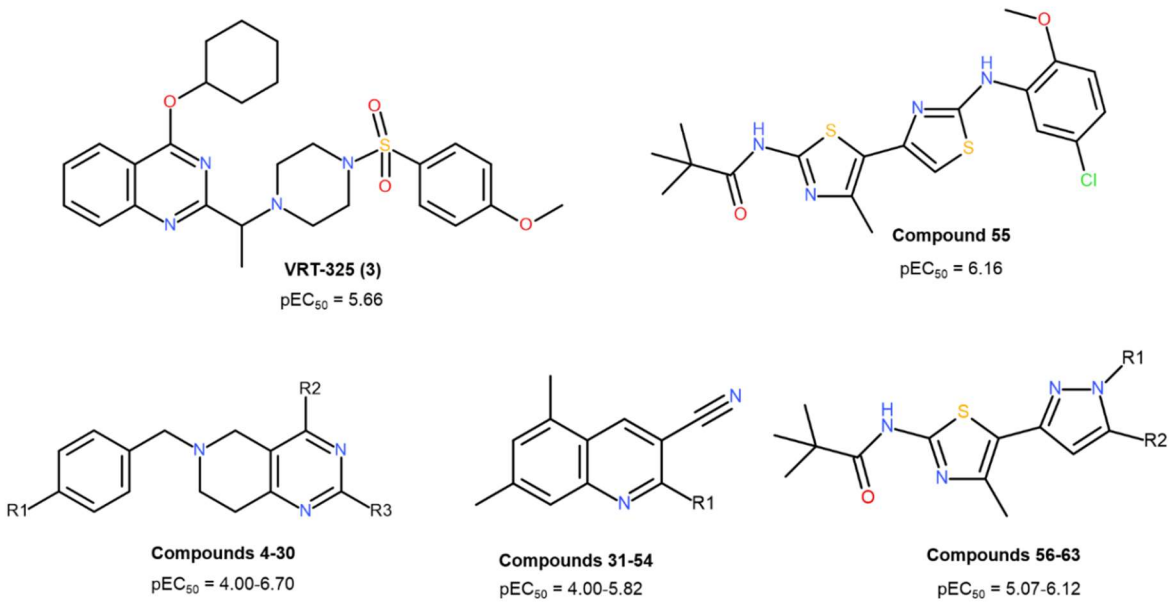

Figure 4. Chemical structure and biological activity range of the investigated F508del CFTR correctors

The predictive mathematical model was calculated by dividing compounds **1-63** into a training set including fifty molecules (**2-9**, **12-19**, **21**, **23**, **27-31**, **33**, **35**, **36**, **38-48**, **50-56**, **58-63**) for model generation, and into a test set with the others derivatives (**1**, **10**, **11**, **20**, **22**, **24-26**, **32**, **34**, **37**, **49**, **57**)

to evaluate the reliability of the mathematical relationship. In particular, the obtained statistical tool was generated by employing non-cross-validated PLS analysis to give a cross validated r^2 (r^2_{cv}) = 0.80 a non-cross validated r^2 (r^2_{ncv}) = 0.91, root mean square error (RMSE) = 0.220, a test set r^2 (r^2_{pred}) = 0.82. The predicted and experimental corrector ability values of all the compounds, together with the collected descriptors values, were reported as tables in Supporting information (S4).

In order to develop QSAR analyses, three hundred molecular descriptors (including 2D- and 3D-parameters) were calculated, by means of MOE software. 2D molecular descriptors are classified in seven clusters, which are related to physical properties (2D-I), subdivided surface areas (2D-II), atom and bond counts (2D-III), connectivity-based descriptors (2D-IV), partial charges descriptors (2D-V), pharmacophore features descriptors (2D-VI) and the so-called Adjacency and Distance Matrix Descriptors (2D-VII). 3D-descriptors consist of five groups, such as potential energy descriptors (3D-I), MOPAC descriptors (3D-II), Surface Area (3D-III), Volume and Shape Descriptors (3D-IV) and Conformation-Dependent Charge Descriptors (3D-V).

In this work, a limited cluster of descriptors was selected using the QSAR-Contingency module implemented in MOE. The top-fifty best ranked parameters, based on the contingency scoring function, were retained and further filtered by re-iterative partial least square (PLS) analyses, taking into account only those parameters exhibiting the highest relative importance (RI) values.

Following this method, we selected eight descriptors explaining for the corrector ability range of the collected library, being the most of them 2D descriptors (see Table 1). Indeed, three descriptors fall in the 2D-III cluster, while two other ones in the 2D-IV and 2D-VII, respectively. On the other hand, three 3D-descriptors were chosen, being two of them included in the 3D-III subtype, while the final one in 3D-I.

Both the two Vsurf descriptors underline a role played by adequate balance in hydrophilic exposed features and by an overall surface extent. This information is also supported by chi1 and weinerpol parameters, which suggest for a proper connectivity table and overall dimension of the ligand, turning in preferred branched and flexible substituents, over rigid, planar and much more extended groups.

As consequence, the presence of single bonds is suggested, being on the other hand limited the number of hydrogen atoms. This is in harmony with the overall need for a prominent polarity profile, to be unraveled along a branched chemical structure.

Quantitatively, the corrector ability of the compounds here studied is explained by the descriptors shown in Table 1 and by the following equation:

Eq. (1): pEC_{50} = -0.17522 + 1.54795 * chi1 -0.19794*weinerpol -0.03874*E_nb -0.12813*a_IC -0.17006*a_nH +0.24667*b_single +0.10392*vsurf_W8 +0.15617*vsurf_DD12

Based on these data, increased values of chi1, b_single, and vsurf descriptors should improve the potency of the compounds, being the two 2D descriptors the most effective in tuning potency (the calculated descriptors within the whole dataset are listed in Supporting information S4). In particular, most of the more potent derivatives (such as **27**, **28**; $pEC_{50} > 6.00$) display b_single and chi1 values spanning from 50 to 70, and from 15 to 18, while the less effective ones (such as **33**-**42**; $4.00 < pEC_{50} < 5.00$) fall between 30 to 60 and 12 and 17, respectively. This is in agreement with the lower potency trend experienced by the cyanoquinoline series (**31**-**54**; pEC_{50} = 4.00-5.82) with respect to the other ones, displaying b_single and chi1 values spanning from 28 to 37, and from 12 to 14, respectively. Interestingly, this turns in more planar and rigid structures and in poor levels of corrector ability. Conversely, tetrahydropyridopyrimidines (**3**-**30**; pEC_{50} = 4.00-6.70) and pyrazolylthiazoles such as **56**-**63** (pEC_{50} = 5.07-6.12) appeared to be well-suited, exhibiting mean values of b_single and chi1 descriptors of 54 and 17; and 44 and 16, respectively.

According to our model, too high values in weinerpol, E_nb, a_IC and a_nH descriptors could impair the potency of these correctors. In particular, lowering of weinerpol descriptor is accompanied by the introduction of flexible polar chains onto the central core of the molecules, suggesting for a folded conformation of the derivative, while higher values of this descriptor occurs when more extended features are chosen. Indeed, the tetrahydropyridopyrimidines **24**, **25** (pEC_{50} = 6.70) bearing a flexible basic chain in R2 (see Figure 4) display adequate weinerpol values (weinerpol = 46-47) if compared with the analogue **16** (pEC_{50} = 4.00; weinerpol = 55), featuring a piperazine-containing substituent.

Accordingly, at the cyanoquinoline series, the piperazine-based compounds **44**, **45** (pEC_{50} = 4.00) exhibit higher weinerpol values (weinerpol = 52) than the related linear aliphatic chain-containing analogues **50**, **51** (pEC_{50} = 5.34-5.37; weinerpol = 45), with expense in corrector potency. As consequence, it could be hypothesized that the proper weinerpol value moves around 50.

Concerning potential energy, E_nb values decrease in presence of proper chemical fragments linked to the central scaffold of the corrector, leading to effective intra-molecular contacts, such as intra-hydrogen bonds and hydrophobic interactions. This kind of conformation often turns in the aforementioned branched arrangement, improving the corrector ability of the compound. Indeed, tetrahydropyridopyrimidines bearing flexible chains in R2 together with H-bonding functions (such as **19**; pEC_{50} =6.70) could be involved in intramolecular H bonds with the protonated nitrogen atom of the tetrahydropyridine ring, displaying low levels in E_nb (**19**, E_nb = 87). On the contrary, the more rigid compound **18** (pEC_{50} = 5.46) exhibits higher E_nb (**18**, E_nb = 90). Accordingly, cyanoquinolines featuring basic and flexible ethylene diamine (**31**-**42**; pEC_{50} = 4.00-5.82), or propylene diamine (**49**-**54**; pEC_{50} = 4.00-5.52) chains in R1, exhibit E_nb mean values around 55, being able to be protonated and H-bonding the quinolone nitrogen atom. Conversely, the related rigid analogues with a piperazine ring in R1 (**43**-**48**; pEC_{50} = 4.00) are characterized by higher E_nb values, spanning from 65 to 71. Within the pyrazolylthiazole series, compound **63**, bearing an ethoxy-amine chain in R2, is the one endowed with the lower E_nb parameter (pEC_{50} = 5.52, E_nb =45). Finally, lowering in a_IC and a_nH parameters proved to be beneficial for the corrector ability of the derivatives here investigated, promoting the design of poorly planar scaffolds enriched with polar moieties, within overall limited and bulky conformations.

2.3. Rational design of AAT-VX809 hybrids

Based on one our previous study [29], in which we modified the AAT structure at different positions, for the identification of derivatives with improved potency and efficacy, we chose to initially build a series of substituted analogues. The modifications were made in one region at a time, to determine the contribution of each particular structural portion to bioactivity. Such modifications led to a variable range of activities that helped us in the generation of a plausible SAR, as will be described below.

To begin the investigation, information around compound was generated by using the central amide bond to synthesise analogs, and in this first phase to probe independently the role of both the benzodioxole and the thiazoles moieties. A range of distinct analogs was designed to examine the requirements for the action (Table 2).

Despite of the limited number of the (available) collected F508del CFTR correctors, the QSAR analyses allowed to derive some preliminary information and useful filtering tools to continue our work in the design and development of novel AAT-containing derivatives, even merging key moieties featured by VX-809. Among the proposed substitutions, some of them proved to be better ranked and filtered taking into account the previously mentioned adequate ranges of the b_single, chi1 and weinerpol descriptors (see Supporting Information S5). Indeed, those derivatives bearing an ester moiety at the thiazole position 5 (**2b-2d**) exhibited comparable weinerpol and chi1 mean values (around 52 and 15, respectively) with respect to the suggested thresholds (weinerpol around 50, chi1 spanning from 15 to 18), while the b_single values was of 36. Accordingly, we considered them as the most potent within the whole series. In addition, this information has drawn some guidelines for further optimization, suggesting for more substitutions with non-aromatic rings and/or with more flexible substituents, in order to increase this parameter to the aforementioned favorable trend (b_single spanning from 50 to 70).

Notably, **2a** is the only one compounds of the newly synthesised derivative to exhibit high chi1 values (around 16), revolving around a bulky branched chemical structure. This data seems to confirm the information coming from the QSAR study.

According to our model, removing the fluorine-containing benzodioxole moiety of VX-809 such as in **1-5** series, as well as maintaining the fluorinate motif (compounds **6** series), allowed to derive a number of congeners sometimes endowed with quite comparable potency trend (see Supporting Information S5). Indeed, the predicted pEC_{50} values of **5a** (pred. pEC_{50} = 6.59), **5b** (pred. pEC_{50} = 6.56), and **3c** (pred. pEC_{50} = 6.17) were comparable with those of **6d** (pred. pEC_{50} = 6.15), **6c** (pred. pEC_{50} = 6.29) and **6b** (pred. pEC_{50} = 5.79). Notably, these results were in good agreement with the biological assays discussed in the following sections concerning **5a**, **5b**, **3c** (Exp. pEC_{50} = 5.64-5.74) with respect to **6d**, **6c**, **6b** (Exp. pEC_{50} = 5.27-5.89).

Removing bulky groups by the thiazole position 5 was predicted to be allowed to design compounds endowed with promising potency values, as well as bearing a methyl group at the same position, especially in presence of when accompanied by a *meta* or *para*-substituted phenyl ring linked at the thiazole position 4. Accordingly, the methyl-substituted AATs **3a**, **3b**, **5c**, **5d** (Pred. pEC_{50} = 5.95-6.47) were predicted to be quite effective as the unsubstituted analogues **4a**, **4b**, **5b**, **5a** (Pred. pEC_{50} = 6.08-6.36).

Removing the thiazole core could in any case be well-tolerated, as shown by the calculated potency trend for **7a** and **7b** (Pred. pEC_{50} = 4.39-5.40). Conceivably, this kind of scaffold was able to fulfill the pharmacophore requirement we previously mentioned for the collected correctors, which exhibited two aromatic cores linked by one H-bonding function. As consequence, also compounds **7a** and **7b** featured modest corrector ability.

2.4. Effect of compounds as correctors of F508del-CFTR trafficking defect

To corroborate our model all designed compounds were then synthesised and tested to investigate the structure-activity relationships as correctors of F508del-CFTR (Table 2).

By using the YFP functional assay on F508del-CFTR CFBE41o- cells, we tested the compounds after 24 hours incubation at different concentrations to extrapolate the EC50 values of the compounds as correctors of mutant CFTR (Table 2). Activity, after incubation with these hybrids, of F508del-CFTR in the plasma membrane was determined by measuring the rate of HSYFP quenching caused by iodide influx as previously published [29]. Activity, after incubation with these hybrids, was compared to that of cells treated with vehicle alone (DMSO) or with the known corrector VX-809 (1 μM).

All the compounds show activity as correctors except for the compound **7c** with a not detectable EC_{50} value. The most active compounds of the series, in according with the predicted pEC_{50} (Pred. pEC_{50} = 6.97-7.21), were **2a** (EC_{50}=0.09μM; Exp. pEC_{50} = 7.06), **2b** (EC_{50} = 0.3μM; Exp. pEC_{50} = 6.52), **2c** (EC_{50} = 0.55μM; Exp. pEC_{50} = 6.26) and **2d** (EC_{50} = 0.90 μM; Exp. pEC_{50} = 6.05). All the compounds were characterized by a substitution on position 5 of the thiazole ring with two different portions like benzoyl group for **2a** and ethyl ester for the others derivatives. The presence of halogens in *meta* or *para*-substituted phenyl ring linked at the thiazole position 4 was well-tolerated and did not change much the activity.

We investigate whether the substitution at the C-5 of the 2-aminothiazole was fundamental for the activity and, if so, which characteristics these substituents should possess. In fact, the presence of a bulky substituent (benzoyl or ethyl acetate) at the C-5 might be useful in terms of improving some pharmacokinetic characteristics. For example, a small group such as the methyl did not improve the activity compared to the counterpart (see **3a** vs **2d** or **3b** vs **2b**, **3c** vs **2c** and **3d** vs **2a**), where the EC_{50} were higher compared to the precursor, even if is well-tolerated. Nevertheless, the methyl group allowed an increase in activity in the case of compound **3e** (EC_{50}= 0.90 μM; Exp. pEC_{50} = 6.05), where a different halogen (F) in the *para* position of the ring was substituted to the chloride in the most active of this series (**3d** with EC_{50} of 1.60 μM; Exp. pEC_{50} = 5.80). It is reasonable to assume that the introduction of a fluorine atom in the molecule led to an increase in hydrophobicity with a small bulkiness, a characteristic likely to be important for activity.

Activity was significantly decreased when maintaining the same substituents on the 4-position, the thiazole ring was unsubstituted at the position 5 (see compounds **4a-c** and their EC_{50}). The same results were obtained with compound without benzoyl group (data not shown).

We might speculate that the phenyl ring, by virtue of its low flexibility, can accommodate in a favorable manner if the position 5 is suitably substituted. These data suggest how the thiazole ring plays a pivotal role in the position of the entire construct. In particular, the substitutions at the C-5 position of the thiazole were optimal when accompanied by a carbonyl group like in the molecule **2a.**

A second series of derivatives comprised different substitutions on the position 4 of the thiazole by changing the substitution pattern of the phenyl ring.

We also investigated some compounds with a common feature like the presence of halogen groups in the ring. In particular we evaluated two derivatives with chlorine and bromine in *para* (**4a** and **4b;** Exp. pEC_{50} = 5.54 and 5.31), exhibiting weak corrector ability (EC_{50} =2.9 μM and EC_{50}=4.9μM respectively). As the most active contained chlorine in *para* (**4a** EC_{50}=2.9μM), we decided to insert in the same position iodine (**4d**) leading to a significant increase in activity (EC_{50}=1.2 μM; Exp. pEC_{50} = 5.92). Actually, the electronic effects of the halogens were ascribed to their inductive electron attractive properties and such properties are maximal for chlorine and bromine, less marked for iodine. Importantly, moving the chlorine group from the *para* (**4a**) to the *meta* position (**4c**) impaired the corrector ability (EC_{50} = 8.2 μM; Exp. pEC_{50} = 5.09).

Surprisingly, the replacement of chlorine with fluorine in the *meta* position of the phenyl ring (**4e** EC_{50} = 2.1 μM; Exp. pEC_{50} = 5.68) led to a high decrease in activity compared to the aforementioned *meta* chlorine analog **4c**. These results were consistent with the notion that at this position the activity is somehow dependent on the size of the substituent (chlorine is bigger than fluorine). Conceivably, the introduction of an additional fluorine atom in the molecule led to an increase in hydrophobicity a characteristic convenient for corrector activity.

We also speculated whenever F508del-CFTR correction activity could be achieved with the presence polar groups on *meta* or *para*-substituted phenyl ring, linked at the thiazole position 4. The

introduction of this moiety was hypothesized in relationship to the good predicted pEC_{50} values of some of these. First, we investigated the effect of *para*-substitution with an electron-donating such a hydroxyl in the compound **5a**, where the effect of correction was good (EC_{50} =1.8 μM; Exp. pEC_{50} = 5.74). Conversely, the presence in **5b** of the same substituent in *meta* caused a slight decrease in activity (EC_{50} =2.3 μM; Exp. pEC_{50} = 5.64).

Interestingly, a small group such as the methyl in position 5 improved the activity, if compared to the counterpart, only when accompanied by a hydroxyl group in *meta* (**5c** vs **5b**), where the EC_{50} value increase from 1.2 μM (Exp. pEC_{50} = 5.92) to 2.3 μM (Exp. pEC_{50} = 5.64).On the other hand, the same substitution was detrimental when involved the *para* position, as shown by **5d** (EC_{50} = 3.9 μM; Exp. pEC_{50} = 5.41) if compared with the previously mentioned **5a**.

In agreement with the previous observations, we synthesised two different analogs **5e** and **5f** containing electron-withdrawing group like the carboxyl feature in the *para* position. Only the compound with a methyl group in position 5, called **5f** maintained adequate corrector ability (EC_{50} = 2.9 μM; Exp. pEC_{50} = 5.54) while the molecule **5e**, where the substitution in position 5 was absent, was less potent (EC_{50} >5 μM; Exp. pEC_{50} < 5.30).

The precise positioning of the hydrogen-bond acceptor on the phenyl ring resulted therefore to be important in enhancing activity.

Changing the polar moiety with an acetamide one caused a decrease in potency (see **5g**; EC_{50} >5 μM).

According to our model, we inserted fluorine atoms in some derivatives, as the typical benzodioxole moiety of VX-809, to compare our hybrids with respect to the fluorinate analogues. Five different derivatives were synthesised. Two of these (**6a** and **6b;** Exp. pEC_{50} = 5.42 and 5.27), exhibiting the methyl in position 5 and one halogen on the aromatic ring in position 4, have a significantly worse activity than the non-fluorinated, (**6a** EC_{50}= 3.8 μM against **3b,** EC_{50} =1.9 μM and **6b** EC_{50} =5.4 μM against **3c** EC_{50} = 2.3 μM) .

In the other analogs where the methyl group was not present, like **6c, 6d** and **6e (**Exp. pEC_{50} = 5.89,5.77 and 5.40), the introduction of two fluorine led to comparable or more effective compounds

with respect to the not fluorinate analogues **4c**, **5a**, **5b** (Exp. pEC_{50} = 5.09, 5.74 and 5.64). Perhaps, the presence of two additional fluorine atoms in the backbone of the hybrids could increase the hydrophobicity making it similar to the one with the methyl substituents.

To complete the studies, we synthesised a panel of compounds with particular features as the removal of the thiazole ring. The replacement of the aminothiazole core proved to be tolerated when the original scaffold was substituted with 3-methylthioaniline portion (**7a** with EC_{50} = 8 μM; Exp. pEC_{50} = 5.10), or overall with quinolin-8-amine portion (**7b** with EC_{50} = 2.2 μM; Exp. pEC_{50} = 5.66). This modification, in fact fulfilled the pharmacophore requirements experienced by the so far known correctors, such as two aromatic cores linked by one H-bonding function.

On the other hand, this hypothesis was also reinforced by non-activity detected in **7c** derivative that does not contain aromatic portions linked to the thiazole ring but a methyl in position 4 and an acetyl group in 5.

We then selected ten best potent and effective compounds in terms of CFTR rescue to determine the dose-response relationships (Figure 5). We treated F508del-CFTR expressing CFBE41o- cells for 24 h with selected compounds and we then determined mutant CFTR activity by using the YFP functional assay (see Experimental section for details).

Such data were in harmony with the previous information regarding possible substituents in scaffold obtained by the QSAR analyses. The extrapolated biological activities of all these derivatives ultimately are not much different from those parameters analyzed in Supporting Information S5.

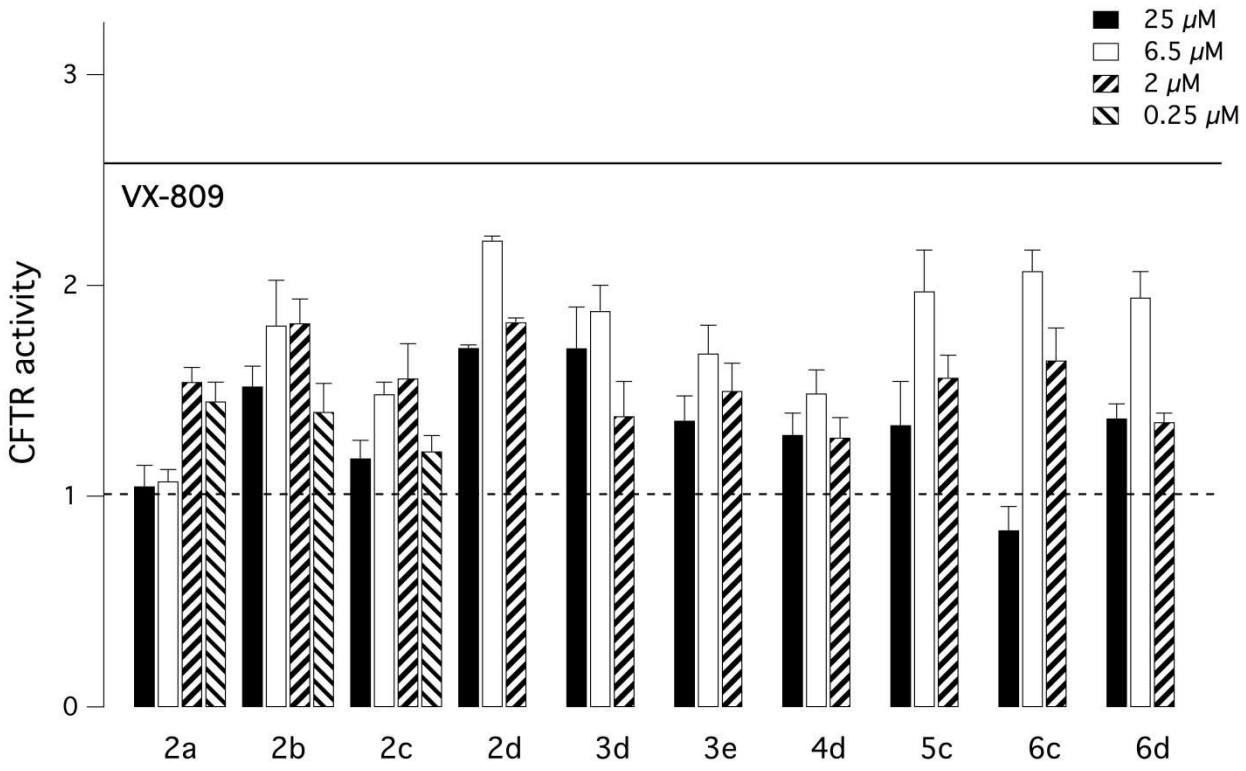

Figure 5. Effects of most active compounds on F508del-CFTR activity. The bar graph reports normalized F508del-CFTR activity in CFBE41o-cells after 24 h treatment with the indicated compounds at different concentrations. Activity was measured with the HS-YFP assay. The line indicates the level of activity in cells treated with corrector VX-809 (1 μM), while the dashed line indicates activity in cells treated with vehicle alone.

Considering that some of the AATs previously described [29] showed additivity with VX-809, we evaluated whether the new derivatives displayed similar effect. Therefore, we treated F508del-CFTR expressing CFBE41o- cells for 24 h with selected compounds (at 2, 10 or 25 μM) in combination with VX-809 (1 μM) and we then evaluated rescue of mutant CFTR activity by using the YFP functional assay. The results demonstrated that the new hybrid show no additive effect with VX-809 (data not shown).

We then considered rescue of processing defect biochemically by observing the electrophoretic mobility of CFTR protein. In Western blots, CFTR protein is detected as two bands, named B and C, of approximately 150 and 170 kDa, respectively. Band B corresponds to partially glycosylated CFTR

residing in the ER. Band C is instead the mature fully processed CFTR that has passed through the Golgi. The prevalent form in cells expressing wild-type CFTR is band C. Lysates of cells expressing F508del-CFTR show primarily band B, consistent with the severe trafficking defect caused by the mutation (Fig. 6A). To evaluate the effect of hybrid compounds on CFTR electrophoretic mobility, we treated F508del-CFTR/HS-YFP expressing CFBE41o- cells with DMSO (vehicle alone) or test compounds or VX-809 (1 μM, as positive control). The following day, cells were lysed and lysates were subjected to SDS-PAGE followed by western blotting. Western blot images were analyzed with ImageJ software. For each lane, CFTR bands, analyzed as ROI, were quantified after normalization for GAPDH to account for total protein loading. Treatment of F508del-CFTR cells with corrector VX-809 significantly enhanced expression of mature CFTR (band C), resulting in a change of the C band / B band ratio. Similarly, treatment with some of the compounds resulted in a significant increase of the C band / B band ratio, although less marked as compared to VX-809 (Fig. 6B).

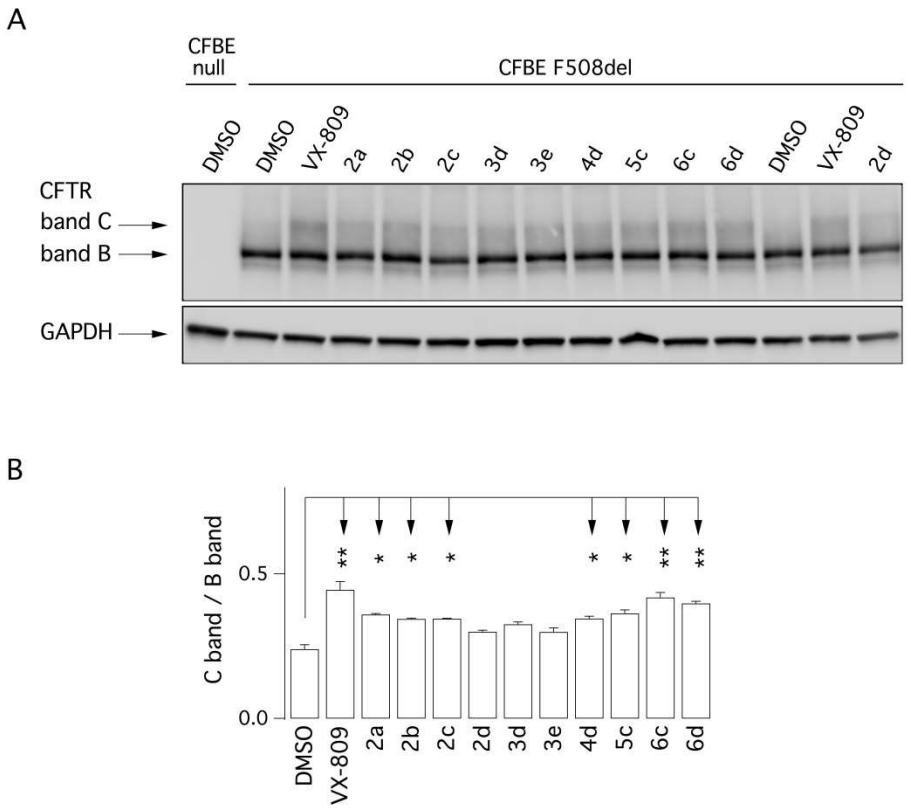

Figure 6. Biochemical analysis of the F508del-CFTR expression pattern. A. The figures show the electrophoretic mobility of F508del-CFTR in CFBE41o- cells after 24 h treatment with indicated compounds (2a: 2 μM; others: 5 μM) or vehicle alone (DMSO) or VX-809 (1 μM). Arrows indicate complex-glycosylated (band C) and core-glycosylated (band B) forms of CFTR protein. B. Quantification of CFTR bands. Data are expressed as C band / B band ratio normalized for the value observed in cells treated with DMSO. Data are expressed as means ± SEM, n = 3 independent experiments. Statistical significance was tested by parametric ANOVA followed by the Dunnet multiple comparisons test (all groups against the control group). Symbols indicate statistical significance versus DMSO: ** $P < 0.01$, * $P < 0.05$.

2.5. Analysis of biological activity of compounds as potentiators of F508del-CFTR gating defect

In the evaluation of the all molecules we investigated the possibility that some hybrids compounds could show dual activities of corrector and potentiator as some of thiazolic derivatives demonstrated in our previous works [28-29].

Therefore, all compounds were tested as potentiators after a 30 min stimulation in the presence of forskolin (20 μM), on F508del-CFTR / HS-YFP expressing CFBE41o- cells, following rescue of the mutant channel by low temperature incubation for 24 h. We found that all of these were ineffective in the potentiator assay, with the exception of **5b, 5c** and **7b.** However, the efficacy of these molecules was partial (nearly 50% of the maximal effect elicited by genistein of VX-770) and their potency low, as the potentiator activity was observed only at high concentration (25μM) while no activity was observed at the concentration of use of VX-770 (1 μM) (Fig. 7).

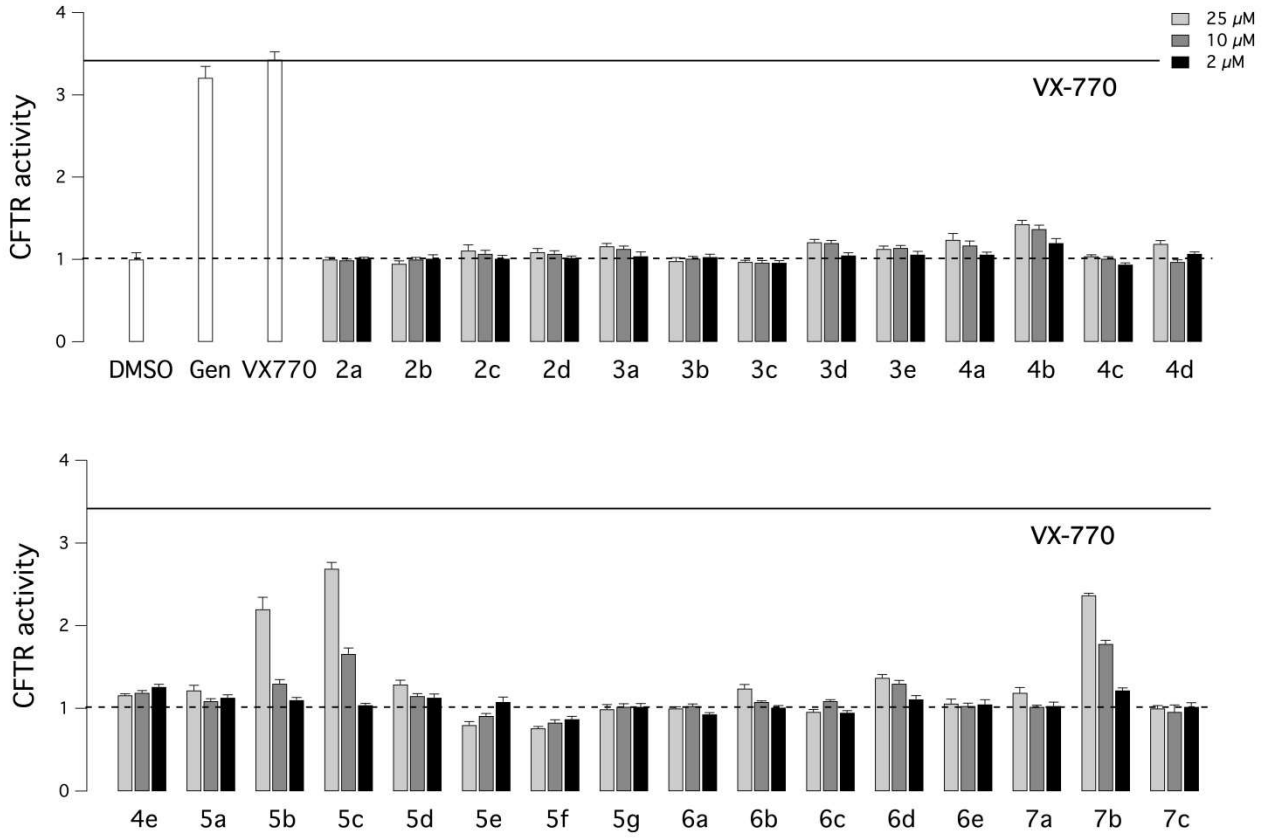

Figure 7. Activity of all compounds as potentiators. The bar graphs report F508del-CFTR activity in CFBE41o- cells after acute treatment with the indicated compounds (2, 10 and 25 μM) in the presence of forskolin (20 μM). Activity was measured with the HS-YFP assay. For each compound, the activity was normalized for the activity detected in negative control (cells stimulated with forskolin alone).The line indicates the level of activity in cells treated with potentiator VX-770 (1 μM) and Genistein (25 μM) while the dashed line indicates activity in cells treated with forskolin alone.

3. Conclusion

Herein we reported a ligand-based approach including quantitative-structure activity relationship (QSAR), developed in order to decipher a limited number of chemical descriptors turning in the rational design and optimization of compounds called VX-809 and thiazole-containing hybrids. Thus, this work showed for the first time, the effectiveness of the ligand based studies on finding new CFTR

modulators, performing mathematical predictive models on the corrector chemo-types so far available. The derived model allowed us to guide and prioritize the most promising hybrids to be synthesised in the following synthetic routes**.**

The ligand-based hybrid-driven approach demonstrated the effectiveness of VX-809-like chemo-types, bearing the thiazole nucleus, as endowed with corrector activities. Indeed, we have identified a number of compounds featuring comparable potency with respect to VX809, revealing those feature of the thiazole core which could be further optimized for an improvement in the activity. Among them, we identified compounds **2a**-**2d** (Exp. pEC_{50} = 7.06, 6.52, 6.26 and 6.05) which experienced a higher potency than the prototype VX809 (Exp. pEC_{50} = 5.59).

Unfortunately, no compounds showed additive effect with VX809. We suppose that the common scaffold of all hybrids and VX-809 i.e. benzo [1, 3] dioxol-5-yl-cyclopropanecarboxylic moiety can aim molecules over the same target and in that case prevent the combined action of the two substances.

Notably, the QSAR model efficiently predicted the biological trend of these newly synthesised derivatives (Pred. pEC_{50} = 7.21, 7.17, 7.14 and 6.97), being therefore worthy of a further development and optimization process towards the design of novel correctors.

4. Experimental Protocols

4.1. Chemistry

Reagents and solvents were purchased from Sigma Aldrich, Alfa Aesar and VWR and used as received unless otherwise indicated. Solvent removal was accomplished with a rotary evaporator at ca.10-50 Torr. The analytical instrument used was an Agilent 1100 high performance liquid chromatograph (HPLC). The analytical HPLC column was a Waters µBondapack C18, 3.9 id x 300 mm length.

The preparative HPLC Agilent 1260 Infinity preparative HPLC and the column used for preparative chromatography was a Phenomenex C18 Luna 21.2 x 250 mm length. The analysis of the intermediates and the raw products was performed by liquid chromatography-electrospray mass spectrometry (HPLC–ESI–MS) using an Agilent 1100 series LC/MSD ion trap instrument.

HRMS experiment were performed using Q Exactive Orbitrap instrument by Thermo Scientific.

The nuclear magnetic resonance (NMR) spectrometer was a Varian Gemini 200 MHz

The proton spectra were acquired at 200 MHz while carbon spectra were acquired at 50 MHz, at room temperature. Chemical shifts are reported in δ units (ppm) relative to TMS as an internal standard. Coupling constants (J) are reported in Hertz (Hz).

All the raw powders obtained were purified with preparative HPLC using the following gradient: from 0 to 5 min at 20% eluent B, then from 5 min to 40 min to 100% eluent B, from 40 to 45 min at 100% eluent B. Eluent A was water + 0.1% formic acid (FOA) and eluent B was acetonitrile + 0.1% FOA. All analogues submitted for testing were judged to be of 95% or higher purity based on analytical HPLC/MS analysis.

Compound purity was determined by integrating peak areas of the liquid chromatogram, monitored at 254 nm.

4.1.1. Synthesis of benzo [1, 3] dioxol-5-yl-cyclopropanecarboxylic acid (1a)

A mixture of benzo [1, 3] dioxole-5- carbonitrile (800 mg, 5 mmol), 1-bromo-2-chloro-ethane (1.4 ml, 17 mmol), and benzyltriethylammonium chloride (23 mg, 0.1 mmol) was heated to 75 °C and then 50 percent (wt. /wt.) aqueous sodium hydroxide (5 mL) was slowly added. The reaction was stirred at T= 75 °C for 24 hours. After this time 1-bromo-2-chloro-ethane (700 µl, 8.5 mmol) and 50 percent (wt. /wt.) aqueous sodium hydroxide (1 mL) were added to insure complete formation of the cyclopropyl moiety (about 12 hours). The reaction was then heated to T=150 °C for about 48 hours to insure complete conversion from the nitrile to the carboxylic acid. When the hydrolysis was

complete (controlled with HPLC-MS) the dark brown mixture was diluted with water (10 ml) and extracted three times with equal volumes of dichloromethane to remove all the sub-products. The basic aqueous solution was acidified with concentrated hydrochloric acid to pH=2. The reaction mixture was centrifuged at 4000g for 5 minutes and the pellet was washed with 1 M hydrochloric acid (3 x 1 ml). The solid material was dissolved in dichloromethane (5 ml) and extracted twice with equal volumes of 1 M hydrochloric acid. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The product was purified by preparative HPLC to afford the title compound (670 mg, 65%).

^{1}H NMR (200 MHz, $CDCl_3$) 6.94-6.81 (m, 3H), 5.97 (s, 2H), 1.73-1.61 (m, 2H), 1.38-1.19 (m, 2H).

^{13}C NMR (50 MHz, $CDCl_3$) δ, 179.9, 146.2, 145.8, 131.4, 122.5, 110.1, 106.9, 100.0, 27.5, 16.6

HRMS (ESI) calculated for $C_{11}H_{11}O_4$: 207.0657 $[M + H]^+$ found 207.0651

4.1.2. Synthesis of 1-(2, 2-difluorobenzo [1, 3] dioxol-5-yl) cyclopropanecarboxylic acid (1b)

To a solution of 2, 2-difluorobenzo [1, 3] dioxol-5-yl-methanol (930 mg, 5 mmol) in anhydrous THF (5 ml) was added $NaBH_4$ (208 mg, 5.5 mmol) in portions at T=0°C in ten minutes. The mixture was then stirred at the same temperature for about 2h (controlled with HPLC). H_2O (1.5 ml) was added and the mixture was stirred for about 15 min. The mixture was then extracted with diethyl ether (3 x 3 ml) and washed with water (2 x 1 ml). The organic phase was completely dried and lyophilized to obtain 2,2-difluorobenzo [1, 3] dioxol-5-yl-methanol as colorless oil and used without further purification (865 mg, 85%).

A solution of 2, 2-difluorobenzo [1, 3] dioxol-5-yl-methanol (865 mg, 4.6 mmol) in thionyl chloride (2 ml) was stirred at room temperature upon completeness. When the reaction was complete (about 1 h), the solution was concentrated under vacuum to remove the excess of thionyl chloride. Then, the residue was resuspended in a solution of dichloromethane and saturated $NaHCO_3$ 1:1 (2 ml). The aqueous layer was then back-extracted with dichloromethane (2 x 1 ml) and the combined organic

layers were dried over Na_2SO_4 and finally concentrated to vacuum to give 5 (chloromethyl)-2,2-difluorobenzo [1, 3] dioxole (823 mg, 87%) which was used directly in the next step.

A mixture of 5(chloromethyl)-2,2-difluorobenzo [1, 3] dioxole (823 mg, 3.99 mmol) and KCN (519 mg, 7.98 mmol) was stirred in dimethyl sulfoxide (DMSO) (3 ml) for 3 h at room temperature. H_2O (1.5 ml) was added and the mixture extracted with ethyl acetate (EtOAc) (3 x 3 ml) and the organic phases were dried over Na_2SO_4 and concentrated under reduced pressure. The product was purified by preparative HPLC to afford 2(2, 2-difluorbenzo [1, 3] dioxol-5-yl)-acetonitrile (432 mg, 55%).

A mixture of 2(2, 2-difluorbenzo [1, 3] dioxol-5-yl)-acetonitrile (432 mg, 2.2 mmol), 1-bromo-2-chloro-ethane (615 µl, 7.5 mmol) and benzyltriethylammonium chloride (5 mg, 0.02 mmol) was heated to T= 75 °C and then 50 percent (wt. /wt.) aqueous sodium hydroxide (5 ml) was slowly added. The reaction was stirred at T= 75 °C for 24 hours. After this time 1-bromo-2-chloro-ethane (310 microlitres, 3.8 mmol) and 50 percent (wt. /wt.) aqueous sodium hydroxide (1 mL) were added to insure the complete formation of the cyclopropyl moiety (about 12-14 hours). The reaction was then heated to T= 150 °C for about 60 hours to insure complete conversion from the nitrile to the carboxylic acid. When the hydrolysis was complete (controlled with HPLC-MS) the dark brown mixture was diluted with water (10 mL) and extracted three times with equal volumes of dichloromethane to remove all the sub-products. The basic aqueous solution was acidified with concentrated hydrochloric acid to pH = 1.The reaction mixture was centrifuged at 4000g for 5 minutes and the pellet was washed with 1 M hydrochloric acid (3 x 1 ml). The solid material was dissolved in dichloromethane (5 mL) and extracted twice with equal volumes of 1 M hydrochloric acid. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The product was purified by preparative HPLC to afford the title compound (276 mg, 52%).

^{1}H NMR (200 MHz, $CDCl_3$) 7.17-6.96 (m, 3H), 1.82-1.65 (m, 2H), 1.39-1.22 (m, 2H).

^{13}C NMR (50 MHz, $CDCl_3$) δ, 179.4, 142.4, 141.9, 135.7, 133.7, 130.6, 124.6, 111.0, 107.9, 27.6 16.6

HRMS (ESI) calculated for $C_{11}H_9N_2O_4F_2$: 243.0469 $[M + H]^+$ found 243.0464

4.1.3. General procedure A: preparation of ketones brominated using *N*-bromosuccinimide

Ketone (1 eq) and *N*-bromosuccinimide (NBS) (2 eq) were solved in acetonitrile and trimethylsilyl trifluoromethanesulfonate (TMS-OTf) (1 eq) was added. The reactions were stirred at T=40°C until completeness, diluted with diethyl ether (2 ml), washed with H_2O (3 x 2 ml), dried over Na_2SO_4 and concentrated under reduced pressure. This procedure provided bromoketones intermediates in 70-90% overall yield, with purities generally>90% as determined by HPLC-MS. The compounds were used without further purification.

4.1.4. General procedure B: synthesis of aminoarylthiazole analogues

Haloketones (1 eq) were conjugated with thioureas (1 eq) in anhydrous ethanol (EtOH) (1 ml). Reaction was stirred at reflux from 2 to 24 hours until the reaction was judged complete (HPLC-MS). The mixture was then extracted with ethyl acetate (3 x 3 ml). The organic phases were concentrated in vacuum and lyophilized. Products were verified by HPLC and MS and purified with preparative HPLC. Relevant fractions were collected and concentrated to afford the desired product in 40-95% yields, with purity of >95% as determined by HPLC-MS.

4.1.5. General procedure C: conjugation of cyclopropanecarboxylic acid derivative with aminothiazole or amine.

The benzo [1, 3] dioxol-5-yl-cyclopropanecarboxylic acid (1 eq) was resuspended in anhydrous DMF (1 ml), HBTU (1 eq) and DIPEA (1 eq) were added. The reaction was vigorously stirred for 5 minutes and the appropriate thiazole or aminic derivative (1 eq) in anhydrous DMF (500 µl) was added, and the reaction was kept at T=40°C until completeness (from 14 h to 24 h) depending from reactants. The mixture was concentrated under vacuum and after extraction with organic solvent (3 x 3 ml), washed with H_2O (3 x 2 ml). The organic phases were dried over Na_2SO_4 and concentrated under

reduced pressure and concentrated under vacuum. The crude product was then purified by preparative HPLC; the peak of interest was concentrated in vacuum and finally lyophilized to obtain the final compound.

4.1.5.1. 1-(benzo[d] [1, 3] dioxol-5-yl)-N-(5-benzoyl-4-phenylthiazol-2-yl) cyclopropanecarboxamide (2a)

2-bromo-1, 3-diphenylpropano-1, 3-dione (30.3 mg, 0.1 mmol) was conjugated with thiourea (7, 6 mg, 0.1 mmol) in anhydrous EtOH (1 ml) using the general procedure B previously described. The intermediate compound obtained, 2-amino-(4-phenylthiazol-5-yl) phenylmethanone was verified by HPLC and MS and purified with preparative HPLC. The thiazole (12.5 mg, 0.06 mmol) was dissolved in anhydrous DMF (500 µL) and conjugated, as using the general procedure C, with 1-(benzo[d] [1, 3] dioxol-5-yl) cyclopropanecarboxylic acid (12.5 mg, 0.06 mmol). The reaction mixture was stirred at 50°C. After 20 h the reaction was complete. The mixture was concentrated under vacuum and after extraction with ethyl acetate (3 x 3 ml), washed with H_2O (3 x 2 ml). The organic phase was dried over Na_2SO_4, concentrated under reduced pressure and purified by preparative HPLC. The peak of interest was concentrated to obtain the title compound with purity of >95% as determined by HPLC-MS (14 mg, 30 %).

^{1}H NMR (200 MHz, $CDCl_3$): δ), 8.93 (s, broad, 1H), 7.96-7.39 (m, 10H), 7.08-6.83 (m, 3H), 6.07 (s, 2H), 2.02-1.63 (m, 2H), 1.45-1.19 (m, 2H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 193.6, 172.1, 164.2, 153.8, 148.0, 145.1, 138.2, 136.9, 133.5, 132.4, 129.5, 129.2, 128.6, 128.5, 127.0, 116.5, 112.5, 107.8, 100.3, 29.7, 16.5.

HRMS (ESI) calculated for $C_{27}H_{21}N_2O_4S$: 469.1222 $[M + H]^+$ found 469.1213

4.1.5.2. Ethyl 2-(1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-4-(4-bromophenyl)thiazole-5 carboxylate (2b)

Ethyl 2-amino-4-(4-bromophenyl) thiazole-5-carboxylate (32.7 mg, 0.1 mmol) was dissolved in anhydrous DMF (600 µL) and conjugated, using the general procedure C, with 1-(benzo[d] [1, 3] dioxol-5-yl) cyclopropanecarboxylic acid (20 mg, 0.1 mmol). The reaction mixture was stirred at T=50°C. After 24 h the reaction was complete. The mixture was concentrated under vacuum and after extraction with dichloromethane (3 x 3 ml), washed with H_2O (3 x 2 ml). The organic phase was dried over Na_2SO_4, concentrated under reduced pressure and purified by preparative HPLC. The peak of interest was concentrated to obtain the title compound with purity of >95% as determined by HPLC-MS (15 mg, 29%).

^{1}H NMR (200 MHz, $CDCl_3$): δ 8.81 (s, broad, 1H), 7.61-7.49 (m, 4H), 7.02-6.81 (m, 3H), 6.06 (s, 2H), 4.28 (q, J = 7.2 Hz, 2H), 1.98-1.62 (m, 2H), 1.44-1.22 (m, 5H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 171.6, 160.6, 158.2, 153.9, 147.5, 147.2, 131.8, 130.1, 129.9, 129.4, 123.6, 122.4, 115.7, 110.1, 100.5, 60.2, 29.3, 17.4, 13.1.

HRMS (ESI) calculated for $C_{23}H_{20}B_rN_2O_5S$: 515.0276 $[M + H]^+$ found 515.02675

4.1.5.3. Ethyl 2-(1-(benzo[d] [1, 3] dioxol-5-yl) cyclopropanecarboxamido)-4-(3-chlorophenyl) thiazole-5-carboxylate (2c)

Ethyl (3-chlorobenzoyl) acetate (18.6 µl, 0.1 mmol) was brominated with *N*-bromosuccinimide (NBS) using the general procedure A previously described, providing ethyl 2-bromo-3-(3-chlorophenyl)-3-oxopropanoate intermediate. Purity was verified by HPLC-MS. Intermediate compound (18.3 mg, 0.06 mmol) was then conjugated with thiourea (4, 5 mg, 0.06 mmol) in anhydrous EtOH (1 ml) using the general procedure B. The intermediate compound obtained, ethyl 2-amino-4-(3-chlorobenzoyl) thiazole-5-carboxylate was verified by HPLC and MS and purified with preparative HPLC. Then the thiazole (14 mg, 0.05 mmol) was conjugated with 1-(benzo[d] [1, 3] dioxol-5-yl) cyclopropanecarboxylic acid (10 mg, 0.05 mmol) using the general procedure C

previously described. The reaction mixture was stirred at T=50°C. After 20 h the reaction was complete. The mixture was concentrated under vacuum and after extraction with ethyl acetate (3 x 3 ml), washed with H_2O (3 x 2 ml). The organic phase was dried over Na_2SO_4, concentrated under reduced pressure and purified by preparative HPLC. The peak of interest was concentrated to obtain the title compound with purity of >95% as determined by HPLC-MS (18.8 mg, 40 %).

^{1}H NMR (200 MHz, $CDCl_3$): δ 8.64 (s, broad, 1H), 7.63-7.48 (m, 2H), 7.42-7.24 (m, 2H), 7.04-6.78 (m, 3H), 6.04 (s, 2H), 4.33 (q, J = 7 Hz, 2H), 2.01-1.67 (m, 2H), 1.51-1.07 (m, 5H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 174.4, 167.0, 164.2, 153.8, 148.1, 145.0, 136.9, 134.9, 134.3, 132.4, 130.4, 129.4, 128.9, 125.1, 116.5, 112.5, 107.8, 100.3, 60.1, 29.7, 16.6, 13.6.

HRMS (ESI) calculated for $C_{23}H_{20}ClN_2O_5S$: 471.0781 $[M + H]^+$ found 471.0774.

4.1.5.4. Ethyl 2-(1-(benzo[d] [1, 3] dioxol-5-yl) cyclopropanecarboxamido)-4-(4-chlorophenyl) thiazole-5-carboxylate (2d)

Ethyl 2-amino-4-(4-chlorophenyl)-1, 3-thiazole-5-carboxylate (28.3 mg, 0.1 mmol) was dissolved in anhydrous DMF (500 µL) and conjugated, using the general procedure C, with 1-(benzo[d] [1, 3] dioxol-5-yl) cyclopropanecarboxylic acid (20 mg, 0.1 mmol). The reaction mixture was stirred at T = 50°C. After 24 h the reaction was complete. The mixture was concentrated under vacuum and after extraction with ethyl acetate (3 x 3 ml), washed with H_2O (3 x 2 ml). The organic phase was dried over Na_2SO_4, concentrated under reduced pressure and purified by preparative HPLC. The peak of interest was concentrated to obtain the title compound with purity of >95% as determined by HPLC-MS (17.8 mg, 38 %).

^{1}H NMR (200 MHz, $CDCl_3$): δ 7.65-7.48 (m, 2H), 7.42-7.27 (m, 2H), 7.02-6.82 (m, 3H), 6.04 (s, 2H), 4.31 (q, J = 7.2 Hz, 2H), 1.98-1.62 (m, 2H), 1.52-1.03 (m, 5H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 173.8, 167.2, 164.2, 153.8, 148.0, 145.1, 136.9, 133.8, 132.4, 131.6, 129.4, 129.4, 128.4, 116.5, 112.5, 107.8, 100.3, 60.1, 29.7, 15.9, 13.6.

HRMS (ESI) calculated for $C_{23}H_{20}ClN_2O_5S$: 471.0781 $[M + H]^+$ found 471.0776.

4.1.5.5. 1-(benzo[d] [1, 3] dioxol-5-yl)-N-(4-(4-chlorophenyl)-5-methylthiazol-2 yl) cyclopropanecarboxamide (3a)

1-(4-chlorophenyl) propan-1-one (34 mg, 0.15 mmol) was brominated with *N*-bromosuccinimide (NBS) using the general procedure A, providing ethyl 2-bromo-1-(4-chlorophenyl) propan-1-one intermediated. The purity was verified by HPLC-MS without further purification. Intermediate compound (29.7 mg, 0.12 mmol) was then conjugated with thiourea (9 mg, 0.12 mmol) in anhydrous EtOH (1 mL) using the general procedure B. The intermediate compound obtained, 4-(4-chlorophenyl)-5-methylthiazol-2-amine, was verified by HPLC and MS and purified with preparative HPLC. The thiazole (25 mg, 0.1 mmol) was conjugated with 1-(benzo[d] [1, 3] dioxol-5-yl) cyclopropanecarboxylic acid (20 mg, 0.1 mmol) using the general procedure C. The reaction mixture was stirred at T= 50°C. After 18 h the reaction was complete. The mixture was concentrated under vacuum and after extraction with ethyl acetate (3 x 3 ml), washed with H_2O (3 x 2 ml). The organic phase was dried over Na_2SO_4, concentrated under reduced pressure and purified by preparative HPLC. The peak of interest was concentrated to obtain the title compound with purity of >95% as determined by HPLC-MS (26.6 mg, 43 %).

^{1}H NMR (200 MHz, $CDCl_3$): δ 9.11 (s, broad, 1H), 7.73-7.51 (m, 2H), 7.46-7.19 (m, 2H), 7.01-6.73 (m, 3H), 6.05 (s, 2H), 2.48 (s, 3H), 1.87-1.64 (m, 2H), 1.43-1.16 (m, 2H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 172.6, 163.3, 148.8, 148.2, 145.1, 136.9, 133.8, 131.6, 129.4, 128.4, 118.1, 116.5, 112.5, 107.8, 100.3, 29.7, 16.7, 13.6.

HRMS (ESI) calculated for $C_{21}H_{18}ClN_2O_3S$: 413.0727 $[M + H]^+$ found 413.0415.

4.1.5.6. 1-(benzo[d][1,3]dioxol-5-yl)-N-(4-(4-bromophenyl)-5-methylthiazol 2yl)cyclopropanecarboxamide (3b)

2-bromo-1-(4-bromophenyl)propan-1-one (60 mg, 0.2 mmol) was conjugated with thiourea (15 mg, 0.2 mmol) in anhydrous EtOH (1 mL) using the general procedure B. The intermediate compound obtained, 4-(4-bromophenyl)-5-methylthiazol-2-amine was verified by HPLC and MS and purified with preparative HPLC. Then the thiazole (48 mg, 0.18 mmol) was conjugated, using the general procedure C, with 1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid (37 mg, 0.18 mmol). The reaction mixture was stirred at T=50°C. After 22 h the reaction was complete. The mixture was concentrated under vacuum and after extraction with ethyl acetate (3 x 3 ml), washed with H_2O (3 x 2 ml). The organic phase was dried over Na_2SO_4, concentrated under reduced pressure and purified by preparative HPLC. The peak of interest was concentrated to obtain the title compound with purity of >95% as determined by HPLC-MS (38.5 mg, 42 %).

^{1}H NMR (200 MHz, $CDCl_3$): δ 8.74 (s, broad, 1H), 7.69-7.49 (m, 2H), 7.44-7.23 (m, 2H), 7.03-6.79 (m, 3H); 6.04 (s, 2H), 2.51 (s, 3H), 1.97-1.64 (m, 2H), 1.47-1.23 (m, 2H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 175.3, 162.8, 148.0, 145.1, 136.9, 116.5, 112.5, 107.8, 100.3, 56.9, 32.3, 27.3, 25.7, 23.4, 18.8, 16.3, 14.8, 11.6, 6.8.

HRMS (ESI) calculated for $C_{21}H_{18}BrN_2O_3S$: 457.0221 $[M + H]^+$ found 457.0215.

4.1.5.7. 1-(benzo[d][1,3]dioxol-5-yl)-N-(4-(3-chlorophenyl)-5-methylthiazol-2yl)cyclopropanecarboxamide (3c)

2-bromo-1-(3-chlorophenyl)propan-1-one (25 mg, 0.1 mmol) was conjugated with thiourea (7.6 mg, 0.1 mmol) in anhydrous EtOH (1 mL) using the general procedure B. The intermediate compound obtained, 4-(3-chlorophenyl)-5-methylthiazol-2-amine was verified by HPLC and MS and purified with preparative HPLC. The intermediate (20 mg, 0.09 mmol) was conjugated, using the general procedure C, with 1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid (18.5 mg, 0.09 mmol). The reaction mixture was stirred at T=50°C. After 18 h the reaction was complete. The mixture was concentrated under vacuum and after extraction with ethyl acetate (3 x 3 ml), washed with H_2O (3 x

2 ml). The organic phase was dried over Na_2SO_4, concentrated under reduced pressure and purified by preparative HPLC. The peak of interest was concentrated to obtain the title compound with purity of >95% as determined by HPLC-MS (21.5 mg, 52 %).

^{1}H NMR (200 MHz, $CDCl_3$): δ 8.79 (s, broad, 1H), 7.67-7.38 (m, 4H), 7.02-6.83 (m, 3H), 6.05 (s, 2H), 2.58 (s, 3H), 1.89-1.65 (m, 2H), 1.47-1.21 (m, 2H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 174.2, 163.3, 148.8, 148.1, 145.1, 136.9, 134.9, 134.3, 130.4, 129.4, 128.9, 125.1, 118.1, 116.5, 112.5, 107.8, 100.3, 29.7, 16.7, 11.1.

HRMS (ESI) calculated for $C_{21}H_{18}ClN_2O_3S$: 413.0727 $[M + H]^+$ found 413.0715.

4.1.5.8. 1-(benzo[d][1,3]dioxol-5-yl)-N-(5-methyl-4-phenylthiazol-2-yl)cyclopropanecarboxamide(3d)

2-bromo-1-phenylpropan-1-one (21.2 mg, 0.1 mmol) was conjugated with thiourea (7.6 mg, 0.1mmol) in anhydrous EtOH (1 mL) using the general procedure B. The intermediate compound obtained, 5-methyl-4-phenylthiazol-2-amine, was verified by HPLC and MS and purified with preparative HPLC. Then the compound (17 mg ,0.09 mmol) was conjugated, using the general procedure C, with 1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid (18.5 mg, 0.09 mmol). The reaction mixture was stirred at T=50°C. After 19 h the reaction was complete. The mixture was concentrated under vacuum and after extraction with dichloromethane (3 x 3 ml), washed with H_2O (3 x 2 ml). The organic phase was dried over Na_2SO_4, concentrated under reduced pressure and purified by preparative HPLC. The peak of interest was concentrated to obtain the title compound with purity of >95% as determined by HPLC-MS (19 mg, 51%).

^{1}H NMR (200 MHz, $CDCl_3$): δ 8.59 (s, broad, 1H), 7.53-7.28 (m, 5H), 7.03-6.89 (m, 3H), 6.06 (s, 2H), 2.40 (s, 3H), 1.94-1.65 (m, 2H), 1.39-1.17 (m, 2H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 173.7, 163.3, 148.8, 148.1, 145.2, 136.9, 133.5, 129, 128.5, 127.1, 118.1, 116.5, 112.5, 107.8, 100.3, 29.7, 16.6, 11.1.

HRMS (ESI) calculated for $C_{21}H_{19}N_2O_3S$: 379.1116 $[M + H]^+$ found 379.1105.

4.1.5.9. 1-(benzo[d][1,3]dioxol-5-yl)-N-(4-(4-fluorophenyl)-5-methylthiazol 2yl)cyclopropanecarboxamide (3e)

1-(4-fluorophenyl)propan-1-one (15.5 mg, 0.1 mmol) was brominated with *N*-bromosuccinimide (NBS) using the general procedure A, providing 2-bromo-1-(4-fluorophenyl)propan-1-one intermediate. Purity was verified by HPLC-MS. The intermediate compound without further purification (20.5 mg, 0.09 mmol) was then conjugated with thiourea (6.9 mg, 0.09 mmol) in anhydrous EtOH (1 mL) using the general procedure B. The intermediate obtained, 4-(4-fluorophenyl)-5-methylthiazol-2-amine was verified by HPLC and MS and purified with preparative HPLC. Then the thiazole (14.5 mg, 0.07 mmol) was conjugated with 1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid (14.5 mg, 0.07 mmol) using the general procedure C. The reaction mixture was stirred at T=50°C. After 16 h the reaction was complete. The mixture was concentrated under vacuum and after extraction with ethyl acetate (3 x 3 ml), washed with H_2O (3 x 2 ml). The organic phase was dried over Na_2SO_4, concentrated under reduced pressure and purified by preparative HPLC. The peak of interest was concentrated to obtain the title compound with purity of >95% as determined by HPLC-MS (15.8 mg, 40 %).

^{1}H NMR (200 MHz, $CDCl_3$): 8.93 (s, broad, 1H), 7.73-7.51 (m, 2H), 7.45-7.27 (m, 2H), 7.02-6.81 (m, 3H), 6.05 (s, 2H), 2.48 (s, 3H), 1.95-1.66 (m, 2H), 1.42-1.18 (m, 2H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 172.7, 164.2, 162.1, 150.8, 148.0, 145.2, 136.9, 130.6, 129.1, 116.5, 116.1, 112.5, 107.8, 104.4, 100.3, 28.7, 16.5.

HRMS (ESI) calculated for $C_{21}H_{18}FN_2O_3S$: 397.1022 $[M + H]^+$ found 397.1012.

4.1.5.10. 1-(benzo[d][1,3]dioxol-5-yl)-N-(4-(4-chlorophenyl)thiazol-2-yl)cyclopropanecarboxamide (4a)

2-Amino-4-(4-chlorophenyl)thiazole (21 mg, 0.1 mmol) was dissolved in anhydrous DMF (500 µL) and conjugated, using the general procedure C, with 1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid (20.5 mg, 0.1 mmol). The reaction mixture was stirred at T=50°C. After 17 h the reaction was complete. The mixture was concentrated under vacuum and after extraction with dichloromethane (3 x 3 ml), washed with H_2O (3 x 2 ml). The organic phase was dried over Na_2SO_4, concentrated under reduced pressure and purified by preparative HPLC. The peak of interest was concentrated to obtain the title compound with purity of >95% as determined by HPLC-MS (18.4 mg, 46 %).

^{1}H NMR (200 MHz, $CDCl_3$): δ 8.56 (s, broad, 1H), 7.82 (s, 1H), 7.69-7.52 (m, 2H), 7.48-7.24 (m, 2H), 7.02-6.77 (m, 3H), 6.05 (s, 2H), 1.91-1.69 (m, 2H), 1.49-1.25 (m, 2H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 173.3, 164.2, 150.8, 148.1, 145.0, 133.8, 131.6, 129.4, 128.4, 116.5, 112.5, 107.8, 104.4, 100.3, 29.7, 16.1.

HRMS (ESI) calculated for $C_{20}H_{16}ClN_2O_3S$: 399.0570 $[M + H]^+$ found 399.0557.

4.1.5.11. 1-(benzo[d][1,3]dioxol-5-yl)-N-(4-(4-bromophenyl)thiazol-2-yl)cyclopropanecarboxamide (4b)

2-bromo-1-(4-bromophenyl)ethanone (41.6 mg, 0.15 mmol) was conjugated with thiourea (11.4 mg, 0.15 mmol) in anhydrous EtOH (1 ml) using the general procedure B. The intermediate compound obtained, 4-(4-bromophenyl)thiazol-2-amine was verified by HPLC and MS and purified with preparative HPLC. Then the thiazole (30.5 mg, 0.12 mmol) was dissolved in anhydrous DMF (700 µL) and conjugated, using the general procedure C, with 1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid (25 mg, 0.12 mmol). The reaction mixture was stirred at T=50°C. After 22 h the reaction was complete. The mixture was concentrated under vacuum and after

extraction with ethyl acetate (3 x 3 ml), washed with H_2O (3 x 2 ml). The organic phase was dried over Na_2SO_4, concentrated under reduced pressure and purified by preparative HPLC. The peak of interest was concentrated to obtain the title compound with purity of >95% as determined by HPLC-MS (23 mg, 35 %).

^{1}H NMR (200 MHz, $CDCl_3$): δ 8.93 (s, broad, 1H), 7.94 (s, 1H), 7.66-7.43 (m, 2H), 7.41-7.27 (m, 2H), 7.02-6.81 (m, 3H), 6.05 (s, 2H), 2.03-1.69 (m, 2H), 1.48-1.25 (m, 2H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 177.6, 164.2, 150.8, 148.0, 145.1, 136.9, 132.5, 132.3, 129.2, 123.1, 116.5, 112.5, 107.8, 104.4, 100.3, 29.7, 16.4.

HRMS (ESI) calculated for $C_{20}H_{16}BrN_2O_3S$: 443.0065 $[M + H]^+$ found 443.0057.

4.1.5.12. 1-(benzo[d][1,3]dioxol-5-yl)-N-(4-(3-chlorophenyl)thiazol-2-yl)cyclopropanecarboxamide (4c)

2-bromo-1-(3-chlorophenyl)ethanone (25 mg, 0.1 mmol) was conjugated with thiourea (7.6 mg, 0.1 mmol) in anhydrous EtOH (1 ml) using the general procedure B. The intermediate compound obtained, 4-(3-chlorophenyl)thiazol-2-amine, was verified by HPLC and MS and purified with preparative HPLC.

The thiazole (17 mg, 0.08 mmol) was then dissolved in anhydrous DMF (500 µL) and conjugated, using the general procedure C, with 1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid (16.mg, 0.08 mmol). The reaction mixture was stirred at T= 50°C. After 20 h the reaction was complete. The mixture was concentrated under vacuum and after extraction with dichloromethane (3 x 3 ml), washed with H_2O (3 x 2 ml). The organic phase was dried over Na_2SO_4 , concentrated under reduced pressure, purified by preparative HPLC and the peak of interest was concentrated to obtain the title compound, with purity of >95% as determined by HPLC-MS (14 mg, 35%).

^{1}H NMR (200 MHz, $CDCl_3$): δ 9.03 (s, broad, 1H), 7.88 (s, 1H), 7.71-7.35 (m, 4H), 7.06-6.84 (m, 3H), 6.06 (s, 2H), 1.83-1.57 (m, 2H), 1.35-1.08 (m, 2H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 172.9, 164.2, 150.8, 148.0, 145.1, 136.9, 134.9, 134.3, 130.4, 129.4, 128.9, 125.1, 116.5, 112.5, 107.8, 104.4, 100.3, 29.7, 16.5.

HRMS (ESI) calculated for $C_{20}H_{16}ClN_2O_3S$: 399.0570 $[M + H]^+$ found 399.0560.

4.1.5.13. 1-(benzo[d][1,3]dioxol-5-yl)-N-(4-(4-iodophenyl)thiazol-2-yl)cyclopropanecarboxamide (4d)

4′-Iodoacetophenone (25 mg, 0.1 mmol) was brominated with *N*-bromosuccinimide (NBS) using the general procedure A, providing 2-bromo-1-(4-iodophenyl)ethanone intermediate. Purity was verified with HPLC-MS and used without further purification. Intermediate compound (19.5 mg, 0.06 mmol) was then conjugated with thiourea (4.5 mg, 0.06 mmol) in anhydrous EtOH (1 mL) using the general procedure B. The intermediate compound obtained, 4-(4-iodophenyl)thiazol-2-amine was verified by HPLC and MS and purified with preparative HPLC. The thiazole (15 mg, 0.05 mmol) was conjugated with 1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid (5.5 mg 0.05 mml) using the general procedure C. The reaction mixture was stirred at T=50°C. After 24 h the reaction was complete. The mixture was concentrated under vacuum and after extraction with dichloromethane (3 x 3 ml), washed with H_2O (3 x 2 ml). The organic phase was dried over Na_2SO_4, concentrated under reduced pressure and purified by preparative HPLC. The peak of interest was concentrated to obtain the title compound with purity of >95% as determined by HPLC-MS (15.6 mg, 32 %).

^{1}H NMR (200 MHz, $CDCl_3$): δ 9.17 (s, broad, 1H), 7.89 (s, 1H), 7.62-7.44 (m, 2H), 7.37-7.15 (m, 2H), 7.01-6.75 (m, 3H), 5.98 (s, 2H), 2.03-1.83 (m, 2H), 1.45-1.21 (m, 2H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 174.1, 164.2, 150.8, 148.0, 145.1, 137.9, 136.9, 132.4, 128.6, 116.5, 112.5, 107.8, 104.4, 100.3, 95.3, 29.7, 16.6.

HRMS (ESI) calculated for $C_{20}H_{16}IN_2O_3S$: 490.9926 $[M + H]^+$ found 490.9915.

4.1.5.14. 1-(benzo[d][1,3]dioxol-5-yl)-N-(4-(3-fluorophenyl)thiazol-2-yl)cyclopropanecarboxamide(4e)

2'-bromo-1-(3-fluorophenyl)ethanone (43.4 mg, 0.2 mmol) was conjugated with thiourea (15 mg, 0.2 mmol) in anhydrous EtOH (1 ml) using the general procedure B. The intermediate compound obtained, 4-(3-fluorophenyl)thiazol-2-amine was verified by HPLC and MS and purified with preparative HPLC. Then the intermediate thiazole (33 mg, 0.17 mmol) was conjugated, using the general procedure C, with 1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid (35 mg, 0.17 mmol). The reaction mixture was stirred at T=50°C. After 28 h the reaction was complete. The mixture was concentrated under vacuum and after extraction with dichloromethane (3 x 3 ml), washed with H_2O (3 x 2 ml). The organic phase was dried over Na_2SO_4, concentrated under reduced pressure and purified by preparative HPLC. The peak of interest was concentrated to obtain the title compound with purity of >95% as determined by HPLC-MS (23 mg, 30%).

^{1}H NMR (200 MHz, $CDCl_3$): δ 8.93 (s, broad, 1H), 7.91 (s, 1H), 7.68-7.33 (m, 4H), 7.04-6.85 (m, 3H), 6.05 (s, 2H), 1.87-1.61 (m, 2H), 1.39-1.14 (m, 2H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 175.8, 164.2, 162.6, 150.8, 148.1, 145.0, 136.9, 135.1, 127.6, 122.6, 116.7, 115.5, 115.2, 112.5, 107.8, 104.5, 100.3, 29.7, 16.8.

HRMS (ESI) calculated for $C_{20}H_{16}FN_2O_3S$: 383.0866 $[M + H]^+$ found 383.0856.

4.1.5.15. 1-(benzo[d][1,3]dioxol-5-yl)-N-(4-(4-hydroxyphenyl)thiazol-2yl)cyclopropanecarboxamide(5a)

4-(2-Amino-1,3-thiazol-4-yl)phenol (19 mg, 0.1 mmol) was solved in anhydrous DMF (400 μL) and conjugated, using the general procedure C, with 1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid (20 mg, 0.1 mmol). The reaction mixture was stirred at T=50°C. After 16 h the reaction was complete. The mixture was concentrated under vacuum and after extraction with dichloromethane (3 x 3 ml), washed with H_2O (3 x 2 ml). The organic phase was dried over Na_2SO_4, concentrated under

reduced pressure and purified by preparative HPLC. The peak of interest was concentrated to obtain the title compound with purity of >95% as determined by HPLC-MS (15.9 mg, 42%).

^{1}H NMR (200 MHz, $CDCl_3$): δ 9.54 (s, broad, 1H), 7.88 (s, 1H), 7.69-7.48 (m, 2H), 7.45-7.28 (m, 2H), 7.07-6.79 (m, 3H), 6.00 (s, 2H), 5.05 (s, broad, 1H), 1.83-1.57 (m, 2H), 1.41-1,18 (m, 2H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 171.7, 164.2, 158.3, 150.8, 148.0, 145.1, 136.9, 128.4, 126.1, 116.5, 116.2, 112.5, 107.8, 104.4, 98.3, 29.4, 16.3.

HRMS (ESI) calculated for $C_{20}H_{17}N_2O_4S$: 381.0909 $[M + H]^+$ found 381.0899.

4.1.5.16. 1-(benzo[d][1,3]dioxol-5-yl)-N-(4-(3-hydroxyphenyl)thiazol-2yl)cyclopropanecarboxamide(5b)

2-bromo-1-(3-hydroxyphenyl)ethanone (43 mg, 0.2 mmol) was conjugated with thiourea (15 mg, 0.2 mmol) in anhydrous EtOH (1 ml) using the general procedure B. The intermediate compound obtained, 3-(2-aminothiazol-4-yl)phenol, was verified by HPLC and MS and purified with preparative HPLC. Then the intermediate thiazole (28.8 mg, 0.15 mmol) was conjugated, using the general procedure C ,with 1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid (31 mg, 0.15 mmol). The reaction mixture was stirred at T= 50°C. After about 16 h the reaction was complete. The mixture was concentrated under vacuum and after extraction with dichloromethane (3 x 3 ml) washed with H_2O (3 x 2 ml). The organic phase was dried over Na_2SO_4, concentrated under reduced pressure and purified by preparative HPLC. The peak of interest was concentrated to obtain the title compound with purity of >95% as determined by HPLC-MS (24.3 mg, 32 %).

^{1}H NMR (300 MHz, CDCl3): δ 8.78 (s, broad, 1H), 7.92 (s, 1H), 7.65-7.32 (m, 4H), 7.02-6.78 (m, 3H), 6.05 (s, 2H), 4.83 (s, broad, 1H), 1.83-1.59 (m, 2H), 1.36-1.09 (m, 2H).

^{13}C NMR (75 MHz, CDCl3): δ 172.9, 164.2, 157.5, 150.2, 148.2, 145.2, 137, 134.4, 130.6, 120.1, 116.0, 115.9, 111.8, 107.2, 105.0, 101.2, 29.7, 15.9.

HRMS (ESI) calculated for $C_{20}H_{17}N_2O_4S$: 381.0909 $[M + H]^+$found 381.0899.

4.1.5.17. 1-(benzo[d][1,3]dioxol-5-yl)-N-(4-(3-hydroxyphenyl)-5-methylthiazol-2 yl)cyclopropanecarboxamide (5c)

1-(3-hydroxyphenyl)-1-propanone (15 mg, 0.1 mmol),thiourea (15 mg, 0.2 mmol) and iodine (25,4 mg, 0.1 mmol) were placed in an open vessel. The mixture was subjected to MW irradiation (100 Watt) in microwave for about 5 minutes. Then the reaction mixture was checked with HPLC-MS, diluted with ethyl acetate and washed with H_2O (3 x 2 ml), dried over Na_2SO_4 and concentrated under reduced pressure. The intermediate product, 3-(2-amino-5-methylthiazol-4-yl)phenol (19 mg, 0.09 mmol), was dissolved in anhydrous DMF (600 µL) and conjugated, using the general procedure C, with 1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid (19 mg, 0.09 mmol). The reaction mix was stirred at T=50°C. After 20 h the reaction was complete. The mixture was concentrated under vacuum and after extraction with ethyl acetate (3 x 3 ml), washed with H_2O (3 x 2 ml). The organic phase was dried over Na_2SO_4, concentrated under reduced pressure and purified by preparative HPLC. The peak of interest was concentrated to obtain the title compound with purity of >95% as determined by HPLC-MS (11.5 mg, 30 %).

^{1}H NMR (200 MHz, $CDCl_3$): δ 7.67-7.22 (m, 4H), 7.02-6.76 (m, 3H), 6.05 (s, 2H), 3.44 (s, broad, 1H), 2.50 (s, 3H), 1.84-1.69 (m, 2H), 1.45-1.21 (m, 2H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 171.6, 160.6, 158.2, 153.9, 147.5, 147.2, 131.8, 130.1, 129.9, 129.4, 123.6, 122.4, 115.7, 110.1, 108.1, 100.5, 60.2, 29.3, 17.4, 13.1.

HRMS (ESI) calculated for $C_{21}H_{19}N_2O_4S$: 395.1065 $[M + H]^+$ found 395.1055.

4.1.5.18. 1-(benzo[d][1,3]dioxol-5-yl)-N-(4-(4-hydroxyphenyl)-5-methylthiazol-2 yl)cyclopropanecarboxamide (5d)

1-(4-hydroxyphenyl)propan-1-one (15 mg, 0.1 mmol), thiourea (15 mg, 0.2 mmol) and iodine (25.4 mg, 0.1 mmol) were placed in an open vessel. The mixture was subjected to MW irradiation (100 Watt) in microwave for about 8-10 minutes. Then the reaction mixture was checked with HPLC-MS, diluted with ethyl acetate and washed with H_2O (3 x 2 ml), dried over Na_2SO_4 and concentrated under reduced pressure. The intermediate product, 4-(2-amino-5-methylthiazol-4-yl)phenol (19 mg, 0.09 mmol), was solved in anhydrous DMF (500 µL) and conjugated, using the general procedure C, with 1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid (19 mg, 0.09 mmol). The reaction mixture was stirred at T=50°C. After 20 h the reaction was complete. The mixture was concentrated under vacuum and after extraction with ethyl acetate (3 x 3 ml), washed with H_2O (3 x 2 ml). The organic phase was dried over Na_2SO_4, concentrated under reduced pressure and purified by preparative HPLC. The peak of interest was concentrated to obtain the title compound with purity of >95% as determined by HPLC-MS (13.5 mg, 35 %).

^{1}H NMR (200 MHz, $CDCl_3$): δ 8.75 (s, broad, 1H), 7.72-7.56 (m, 2H), 7.49-7.23 (m, 2H), 7.08-6.85 (m, 3H), 5.98 (s, 2H), 4.79 (s, broad, 1H), 2.46 (s, 3H), 1.87-1.62 (m, 2H), 1.37-1.13 (m, 2H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 172.4, 163.3, 158.3, 149.8, 148.1, 145.4, 136.9, 128.4, 126.1, 118.2, 116.5, 116.2, 112.5, 107.8, 100.3, 29.7, 16.3, 11.1.

HRMS (ESI) calculated for $C_{21}H_{19}N_2O_4S$: 395.1065 $[M + H]^+$ found 395.1055.

4.1.5.19. 4-(2-(1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)thiazol-4-yl)benzoic acid (5e)

Ethyl 4 -acetylbenzoate (19 mg, 0.1 mmol) was brominated with *N*-bromosuccinimide (NBS) using the general procedure A, providing ethyl 4-(2-bromoacetyl)benzoate intermediate. Purity was verified by HPLC-MS. The intermediate compound without further purification (24 mg, 0.09 mmol) was then conjugated with thiourea (6.9 mg, 0.09 mmol) in anhydrous EtOH (1 mL) using the general procedure

B. The intermediate compound obtained, ethyl 4-(2-aminothiazol-4-yl)benzoate was verified by HPLC and MS and purified with preparative HPLC.

Then, the intermediate thiazole (17.5 mg ,0.07 mmol) was solved in anhydrous DMF (600 μL) and conjugated, using the general procedure C, with 1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid (14.5 mg, 0.07 mmol). The reaction mixture was stirred at T=50°C. After 24 h the reaction was complete. The mixture was concentrated under vacuum and after extraction with ethyl acetate (3 x 3 ml), washed with H_2O (3 x 2 ml). The organic phase was dried over Na_2SO_4, concentrated under reduced pressure and purified by preparative HPLC. The peak of interest was concentrated to obtain ethyl 4-(2-(1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)thiazol-4-yl)benzoate. The compound was then resuspended in acetonitrile and NaOH 1N 1:1 (2 ml). The hydrolysis was complete after 16 h at room temperature. After extraction with EtOAc (3 x 2 ml), the organic phase was concentrated under vacuum to obtain 4-(2-(1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)thiazol-4-yl)benzoic acid with purity of >95% as determined by HPLC-MS (16 mg, 39 %).

^{1}H NMR (200 MHz, $CDCl_3$): δ 10.94 (s, broad, 1H), 8.73 (s, broad, 1H), 8.02 (s, 1H), 7.74-7.51 (m, 2H), 7.40-7.16 (m, 2H), 7.04-6.85 (m, 3H), 6.01 (s, 2H), 1.96-1.73 (m, 2H), 1.47-1.19 (m, 2H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 171.7, 167.2, 158.3, 150.8, 148.1, 145.3, 136.9, 128.4, 126.1, 116.5, 116.2, 112.5, 107.8, 102.4, 100.3, 28.7, 15.6.

HRMS (ESI) calculated for $C_{21}H_{17}N_2O_5S$: 409.0858 $[M + H]^+$found 409.0849.

4.1.5.20. 4-(2-(1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-5-methylthiazol-4-yl)benzoic acid (5f)

A mixture of 4-bromopropiophenone (105 mg, 0.5 mmol) and CuCN (59 mg, 0.65 mmol) in dry DMF (0.5 mL) was refluxed for 10 h, then poured into H_2O (1,5 mL) containing $FeCl_3$ (162 mg,1 mmol) and 12 N HCl (230 μl). The mixture was kept in a water bath at 70 °C for 20 min and left at room

temperature overnight. The solid precipitated was filtered and dissolved in diethyl ether (2 ml) and the solution was washed with H_2O (2 x 2 ml).

The organic layer was dried ($MgSO_4$) and evaporated to obtain 4-propionylbenzonitrile (59 mg 74%) with high purity.

4-Propionyl-benzonitrile (46 mg, 0.29 mmol) was refluxed in EtOH (1 ml) and KOH (37 mg, 0.64 mmol) for 2 h to permit the complete hydrolysis of the nitrile. The ethanol solution was evaporated and the residue suspended in water (1 ml). The reaction mixture was cooled in ice and adjusted to pH =2 with 12 N HCl. The slurry was extracted with ethyl acetate (3 x 1 ml). The combined organic phases were washed with water and saturated sodium chloride, dried over sodium sulfate and evaporated. After lyophilization we obtain 4-propionyl-benzoic acid (44 mg, 86%).

The foregoing acid (37 mg, 0.2 mmol) was dissolved in ethanol (1 ml) and treated with 97% sulfuric acid (0.1 ml). The mixture was stirred at room temperature for 4 h, then at T=70^0C for 2 h and lastly T= 40^0C for 16 h. After evaporation of ethanol, the residue was suspended in water (1 ml) and extracted with ethyl acetate (3 x 1 ml). The combined organic phases were washed with water (2 x 1 ml), saturated sodium chloride, dried over sodium sulfate and evaporated. The residue ethyl 4-propionylbenzoate was isolated as colorless oil (34.5 mg, 83%).

Ethyl 4-propionylbenzoate (27 mg, 0.13 mmol) was brominated with *N*-bromosuccinimide (NBS) using the general procedure A, providing ethyl 4-(2-bromopropanoyl)benzoate intermediate. Purity was verified by HPLC-MS. The intermediate compound without further purification (25 mg, 0.09 mmol) was then conjugated with thiourea (6.9 mg, 0.09 mmol) in anhydrous EtOH (1 ml) using the general procedure B. The compound obtained, ethyl 4-(2-aminothiazol-4-yl)benzoate, was verified by HPLC and MS and purified with preparative HPLC.

Then, the intermediate thiazole (19.8 mg, 0.08 mmol) was dissolved in anhydrous DMF (600 µL) and conjugated, using the general procedure C, with 1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid (16.5 mg, 0.08 mmol). The reaction mixture was stirred at T=50°C. After 24 h the reaction was complete. The mixture was concentrated under vacuum and after extraction with ethyl acetate (3 x 3

ml), washed with H_2O (3 x 2 ml). The organic phase was dried over Na_2SO_4, concentrated under reduced pressure and purified by preparative HPLC. The peak of interest was concentrated to obtain ethyl 4-(2-(1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-5-methylthiazol-4-yl)benzoate. The compound was then resuspended in acetonitrile and NaOH 1N 1:1 (2 ml). The hydrolysis was complete after 24 h at room temperature. After extraction with EtOAc (3 x 2 ml), the organic phase was concentrated under vacuum to obtain 4-(2-(1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-5-methylthiazol-4-yl)benzoic acid with purity of >95% as determined by HPLC-MS (15 mg, 45 %).

^{1}H NMR (200 MHz, CDCl3): δ 11.43 (s, broad, 1H), 8.90 (s, broad, 1H), 7.78-7.53 (m, 2H), 7.42-7.23 (m, 2H), 7.02-6.78 (m, 3H), 6.06 (s, 2H), 2.53 (s, 3H), 1.85-1.59 (m, 2H), 1.32-1.08 (m, 2H).

^{13}C NMR (50 MHz, CDCl3): δ 174.7, 167.6, 158.3, 150.8, 148.0, 145.0, 136.9, 128.4, 126.1, 116.5, 116.2, 114.5, 107.8, 102.4, 100.4, 28.7, 15.6 ,11.7.

HRMS (ESI) calculated for $C_{22}H_{19}N_2O_5S$: 423.1015 $[M + H]^+$ found 423.1023.

4.1.5.21. N-(4-(4-acetamidophenyl)thiazol-2-yl)-1-(benzo[d][1,3]dioxol-5 yl)cyclopropanecarboxamide (5g)

N-(4-(2-aminothiazol-4-yl)phenyl)acetamide (35 mg, 0.15 mmol) was solved in anhydrous DMF (600 µL) and conjugated, using the general procedure C, with 1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid (31 mg, 0.15 mmol). The reaction mixture was stirred at T=50°C. After 18 h the reaction was complete. The mixture was concentrated under vacuum and after extraction with ethyl acetate (3 x 3 ml), washed with H_2O (3 x 2 ml). The organic phase was dried over Na_2SO_4, concentrated under reduced pressure and purified by preparative HPLC. The peak of interest was concentrated to obtain the title compound with purity of >95% as determined by HPLC-MS. (26 mg, 47 %).

^{1}H NMR (200 MHz, DMSO-d6) δ 8.55 (d, J = 8.0 Hz, 1H), 7.79 (d, J = 8.4 Hz, 2H), 7.61 (d, J = 8.4 Hz, 2H), 6.97-6.62 (m, 3H), 5.98 (s, 2H), 2.89 (s, 3H), 1.42-1.35 (m, 2H), 1.18-0.98 (m, 2H).

^{13}C NMR (50 MHz, DMSO-d6) δ, 175.0, 150.7, 146.3, 145.5, 139.2, 134.2, 133.4, 128.6, 128.4, 125.6, 122.8, 120.3, 110.5, 107.1, 100.4, 27.8, 15.6.

HRMS (ESI) calculated for $C_{22}H_{20}N_3O_4S$: 422.1174 $[M + H]^+$ found 422.1163.

4.1.5.22. N-(4-(4-bromophenyl)-5-methylthiazol-2-yl)-1-(2,2-difluorobenzo[d][1,3]dioxol-5 yl)cyclopropanecarboxamide (6a)

4′-Bromopropiophenone (42,5 mg, 0.2 mmol) was brominated with *N*-bromosuccinimide (NBS) using the general procedure A, providing brominated intermediate compound, 2-bromo-1-(4-bromophenyl)propan-1-one. The purity was verified by HPLC-MS. Intermediate compound without further purification (35 mg, 0.12 mmol) was then conjugated with thiourea (9.5 mg, 0.12 mmol) in anhydrous EtOH (1 ml) using the general procedure B. The compound obtained, 4-(4-bromophenyl)-5-methylthiazol-2-amine, was verified by HPLC and MS and purified with preparative HPLC. Then the thiazole (16 mg, 0.06 mmol) was dissolved anhydrous DMF (400 µl) and conjugated, using the general procedure C, with 1-(2,2-difluorobenzo[1,3]dioxol-5-yl)cyclopropanecarboxylic acid (14.5 mg, 0.06 mmol). The reaction mixture was stirred at T=50°C. After 18 h the reaction was complete. The mixture was concentrated under vacuum and after extraction with ethyl acetate (3 x 3 ml), washed with H_2O (3 x 2 ml). The organic phase was dried over Na_2SO_4, concentrated under vacuum and the final product was purified by preparative HPLC. The peak of interest was concentrated to obtain the title compound with purity of >95% as determined by HPLC-MS (19.7 mg, 21%).

^{1}H NMR (200 MHz, $CDCl_3$): δ 7.73-7.54 (m, 2H), 7.38-7.20 (m, 2H), 7.01-6.76 (m, 3H), 2.43 (s, 3H), 2.03-1.79 (m, 2H), 1.49-1.22 (m, 2H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 204.2, 173.2, 163.3, 148.8, 145, 136.9, 135.5, 132.3, 129.2, 123.1, 118.1, 116.5, 112.5, 107.8, 29.7, 16.6, 11.1.

HRMS (ESI) calculated for $C_{21}H_{16}BrF_2N_2O_3S$: 493.0033 $[M + H]^+$ found 493.0022.

4.1.5.23. N-(4-(3-chlorophenyl)-5-methylthiazol-2-yl)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamide (6b)

2-bromo-1-(3-chlorophenyl)propan-1-one (50 mg, 0.2 mmol) was conjugated with thiourea (15 mg, 0.2 mmol) in anhydrous EtOH (1 mL) using the general procedure B. The intermediate compound obtained, 4-(3-chlorophenyl)-5-methylthiazol-2-amine was verified by HPLC and MS and purified with preparative HPLC.

4-(3-chlorophenyl)-5-methylthiazol-2-amine (34 mg, 0.15 mmol) was dissolved in anhydrous DMF (500 µl) and conjugated, using the general procedure C, with 1-(2,2-difluorobenzo[1,3]dioxol-5-yl)cyclopropanecarboxylic acid (36 mg, 0.15 mmol). The reaction mixture was stirred at T=50°C. After 18 h the reaction was complete. The mixture was concentrated under vacuum and after extraction with ethyl acetate (3 x 3 ml), washed with H_2O (3 x 2 ml). The organic phase was dried over Na_2SO_4, concentrated under reduced pressure and purified by preparative HPLC. The peak of interest was concentrated to obtain the title compound with purity of >95% as determined by HPLC-MS (43 mg, 49 %).

^{1}H NMR (200 MHz, $CDCl_3$): δ 9.07 (s, broad, 1H), 7.70-7.43 (m, 4H), 7.04-6.80 (m, 3H), 2.51 (s, 3H), 1.84-1.59 (m, 2H), 1.41-1.20 (m, 2H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 205.1, 171.9, 163.3 148.8, 148.1, 145.0, 136.9, 134.9, 134.3, 130.4, 129.4, 128.4, 125.1, 118.1, 116.5, 112.5, 107.8, 29.7, 15.9, 11.1.

HRMS (ESI) calculated for $C_{21}H_{16}ClF_2N_2O_3S$: 449.0538 $[M + H]^+$ found 449.0530.

4.1.5.24. 1-(benzo[d][1,3]dioxol-5-yl)-N-(4-(3-hydroxyphenyl)thiazol-2-yl)cyclopropanecarboxamide (6c)

2-bromo-1-(3-hydroxyphenyl)ethanone (21.5 mg, 0.1 mmol) was conjugated with thiourea (7.6 mg, 0.1 mmol) in anhydrous EtOH (1 mL) using the general procedure B. The intermediate compound obtained, 3-(2-aminothiazol-4-yl)phenol, was verified by HPLC and MS and purified with preparative HPLC. Then the intermediate thiazole (15.4 mg, 0.08 mmol) was dissolved in anhydrous DMF (500 µl) and conjugated, using the general procedure C, with 1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid (19 mg, 0.08 mmol). The reaction mixture was stirred at T=50°C. After 22 h the reaction was complete. The mixture was concentrated under vacuum and after extraction with ethyl acetate (3 x 3 ml), washed with H_2O (3 x 2 ml). The organic phase was dried over Na_2SO_4, concentrated under reduced pressure and purified by preparative HPLC. The peak of interest was concentrated to obtain the title compound with purity of >95% as determined by HPLC-MS (25 mg, 60 %).

^{1}H NMR (200 MHz, $CDCl_3$): δ 8.90 (s, broad, 1H), 7.85 (s, 1H), 7.67-7.31 (m, 4H), 7.03-6.81 (m, 3H), 1.83-1.59 (m, 2H), 1.41-1.21 (m, 2H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 205.1, 175.6, 164.2, 157.8, 150.8, 148.0, 145.3, 136.9, 134.9, 130.4, 119.6, 116.5, 115.7, 115.2, 112.5, 107.8, 104.4, 29.7, 16.2.

HRMS (ESI) calculated for $C_{20}H_{15}F_2N_2O_4S$: 417.0721 $[M + H]^+$ found 417.0710.

4.1.5.25. 1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-N-(4-(4-hydroxyphenyl)thiazol yl)cyclopropanecarboxamide (6d)

4-(2-Amino-1,3-thiazol-4-yl)phenol (20 mg, 0.1 mmol) was solved in anhydrous DMF (500 µl) and conjugated, using the general procedure C, with 1-(2,2-difluorobenzo[1,3]dioxol-5-yl)cyclopropanecarboxylic acid (24 mg, 0.1 mmol). The reaction mixture was stirred at T= 50°C. After 24 h the reaction was complete. The mixture was concentrated under vacuum and after extraction with ethyl acetate (3 x 3 ml), washed with H_2O (3 x 2 ml). The organic phase was dried over Na_2SO_4, concentrated under reduced pressure and purified by preparative HPLC. The peak of

interest was concentrated to obtain the title compound with purity of >95% as determined by HPLC-MS. (28 mg, 69 %).

^{1}H NMR (200 MHz, $CDCl_3$): δ 8.97 (s, broad, 1H), 7.92 (s, 1H), 7.83-7.59 (m, 2H), 7.43-7.11 (m, 2H), 7.00-6.78 (m, 3H), 4.02 (s, broad, 1H), 1.87-1.58 (m, 2H), 1.44-1.22 (m, 2H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 204.8, 174.3, 164.2, 157.3, 150.2, 148.2, 145.1, 137.1, 128.9, 125.6, 116.3, 116.0, 111.8, 107.4, 105.3, 29.2, 16.6.

HRMS (ESI) calculated for $C_{20}H_{15}FN_2O_4S$: 417.0721$[M + H]^+$ found 417.0708.

4.1.5.26. N-(4-(3-chlorophenyl)thiazol-2-yl)-1-(2,2-difluorobenzo[d][1,3]dioxol 5 yl)cyclopropanecarboxamide (6e)

2-bromo-1-(3-chlorophenyl)ethanone (23.4 mg, 0.1 mmol) was conjugated with thiourea (7,6 mg,0.1mmol) in anhydrous EtOH (1 mL) using the general procedure B. The intermediate compound obtained, 4-(3-chlorophenyl) thiazol-2-amine was verified by HPLC and MS and purified with preparative HPLC. The thiazole (19 mg, 0.09 mmol) was dissolved in anhydrous DMF (500 µl) and conjugated, using the general procedure C, with 1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid (22 mg, 0.09 mmol). The reaction mixture was stirred at T =50°C. After 24 h the reaction was complete. The mixture was concentrated under vacuum and after extraction with ethyl acetate (3 x 3 ml), washed with H_2O (3 x 2 ml). The organic phase was dried over Na_2SO_4, concentrated under reduced pressure and purified by preparative HPLC. The peak of interest was concentrated to obtain the title compound with purity of >95% as determined by HPLC-MS (22 mg, 51 %).

^{1}H NMR (200 MHz, $CDCl_3$): δ 9.03 (s, broad, 1H), 7.98 (s, 1H), 7.72-7.43 (m, 4H), 7.05-6.88 (m, 3H), 1.79-1.51 (m. 2H), 1.36-1.14 (m, 2H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 211.8, 174.9, 164.2, 150.8, 148.3, 145.1, 136.9, 134.9, 134.3, 130.4, 129.4, 128.9, 125.1, 116.5, 112.5, 107.8, 104.4, 29.7, 16.6.

HRMS (ESI) calculated for $C_{20}H_{14}ClF_2N_2O_3S$: 435.0382 $[M + H]^+$ found 435.0372.

4.1.5.27. 1-(benzo[d][1,3]dioxol-5-yl)-N-(3-(methylthio)phenyl)cyclopropanecarboxamide (7a)

3-(methylthio)aniline (21 mg, 0.15 mmol) was dissolved in in anhydrous DMF (500 µL) and conjugated, using the general procedure C, with 1-(benzo[d][1,3]dioxol-5-yl) cyclopropanecarboxylic acid (31 mg, 0.15 mmol). The reaction mixture was stirred at T= 60°C. After 16 h the reaction was complete. The mixture was concentrated under vacuum and after extraction with ethyl acetate (3 x 3 ml), washed with H_2O (3 x 2 ml). The organic phase was dried over Na_2SO_4 , concentrated under reduced pressure , the final product was purified by preparative HPLC and the peak of interest was concentrated to obtain the title compound, with purity of >95% as determined by HPLC-MS (22.6 mg, 46%).

^{1}H NMR (200 MHz, $CDCl_3$): δ 7.42 (t, *J* = 0.9 Hz, 1H), 7.24-7.14 (m, 2H), 7.14-6.79 (m, 4H), 6.06 (s, 2H), 2.48 (s, 3H), 1.82-1.61 (m, 2H), 1.23-1.04 (m, 2H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 171.2, 147.1, 146.6, 138.3, 137.3, 131.6, 128.0, 123.5, 121.1, 116.3, 115.1, 110.2, 107.8, 100.4, 29.9, 15.6, 14.7.

HRMS (ESI) calculated for $C_{18}H_{18}NO_3S$: 328.1007 $[M + H]^+$ found 328.0910.

4.1.5.28. 1-(benzo[d][1,3]dioxol-5-yl)-N-(quinolin-8-yl)cyclopropanecarboxamide (7b)

3-Aminoquinoline (21.6 mg, 0.15 mmol) was dissolved in anhydrous DMF (500 µL) and conjugated, using the general procedure C, with 1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid (31 mg, 0.15 mmol). The reaction mixture was stirred at T =60°C. After 10 h the reaction was complete. The mixture was concentrated under vacuum and after extraction with ethyl acetate (3 x 3 ml), washed with H_2O (3 x 2 ml). The organic phase was dried over Na_2SO_4, concentrated under reduced pressure

and purified by preparative HPLC. The peak of interest was concentrated to obtain the title compound with purity of >95% as determined by HPLC-MS (27.4 mg, 55 %).

^{1}H NMR (200 MHz, $CDCl_3$): δ 9.23 (s, broad, 1H), 8.21-7.94 (m, 3H), 7.75-7.28 (m, 3H), 7.09-6.74 (m, 3H), 5.97 (s, 2H), 1.85-1.51 (m, 2H), 1.41-1.23 (m, 2H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 181.7, 148.7, 148.1, 145.0, 137.9, 136.9, 136, 134.2, 128.6, 126.6, 120.4, 116.5, 115.7, 114.3, 112.5, 107.8, 100.3, 29.5, 16.6.

HRMS (ESI) calculated for $C_{20}H_{17}N2O_3$: 333.1239$[M + H]^+$ found 333.1228.

4.1.5.29. N-(5-acetyl-4-methylthiazol-2-yl)-1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamide (7c)

1-(2-amino-4-methylthiazol-5-yl)ethanone (23 mg, 0.15 mmol) was solved in anhydrous DMF (600 µL) and conjugated, using the general procedure C, with 1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid (31 mg, 0.15 mmol). The reaction mixture was stirred at T=50°C. After 18 h the reaction was complete. The mixture was concentrated under vacuum and after extraction with ethyl acetate (3 x 3 ml), washed with H_2O (3 x 2 ml). The organic phase was dried over Na_2SO_4, concentrated under reduced pressure and purified by preparative HPLC. The peak of interest was concentrated to obtain the title compound with purity of >95% as determined by HPLC-MS (36 mg, 71 %).

^{1}H NMR (200 MHz, DMSO-d6) δ 8.54 (d, J = 8.4 Hz, 1H) 6.92-6.63 (m, 2H), 5.98 (s, 2H), 2.89 (s, 3H), 2.73 (s, 3H) 1.42-1.25 (m, 2H), 1.16-0.94 (m, 2H)

^{13}C NMR (50 MHz, DMSO-d6) δ, 175.0, 150.7, 146.3, 145.5, 133.5, 128.4, 122.8, 120.3, 110.6, 107.8, 107.1, 100.4, 35.3, 30.3, 27.8, 15.6.

HRMS (ESI) calculated for $C_{17}H_{17}N_2O_4S$: 345.0909 $[M + H]^+$ found 345.0898.

4.2. Molecular modeling.

The compounds **1-63** analyzed in this work by means of QSAR analysis as well as the newly synthesised VX809-AAT hybrids were built, parameterized (AM1 partial charges as calculation method) and energy minimized within MOE [MOE: Chemical Computing Group Inc. Montreal. H3A 2R7 Canada. http://www.chemcomp.com.] using MMFF94 force field.

For all the compounds the proper protonation state at physiological pH has been taken into account, by means of the molecular database wash tools implemented in MOE.

Most of the compounds **1-63** was discovered and biologically evaluated by the same research group, following comparable evaluation methods. This makes the derived potency values adequate to perform statistical analyses.

Any compound was explored in terms of geometry and conformation energy by means of the systematic conformational search tool implemented in MOE.

Systematic Conformational Search generates molecular conformations by systematically rotating bonds in a molecule by discrete increments. The purpose of the Systematic Conformational Search is to generate a collection of reasonable molecular conformations, which may or may not be at local minima. A generated conformation is rejected if it contains two atoms whose mutual van der Waals energy exceeds a threshold (by default, 10 kcal/mol). This ensures that the output conformations contain no conformations with heavily overlapped atoms. Conformations generated by this method may be strained due to bonded interactions as well as some non-bonded strain.

The selection of the proper conformers for the following QSAR analyses was devised also taking into account the best ranked conformation which better proved to overlap the corrector **5**. This compound was used as reference compound since its poor flexibility profile, combined with adequate corrector behavior.

QSAR studies were performed based on calculations of three hundred of -molecular descriptors, including 2D- and 3D parameters, by means of MOE software. 2D molecular descriptors are defined to be numerical properties that can be calculated from the connection table representation of a molecule (e.g., elements, formal charges and bonds, but not atomic coordinates). 2D descriptors are,

therefore, not dependent on the conformation of a molecule and are most suitable for large database studies. They include descriptors related to physical properties, subdivided surface areas, atom and bond counts, connectivity-based descriptors, partial charges descriptors, pharmacophore features descriptors and the so-called Adjacency and Distance Matrix Descriptors. The 3D-descriptors consist of potential energy descriptors, MOPAC descriptors, Surface Area, Volume and Shape Descriptors and Conformation Dependent Charge Descriptors.

The number of descriptors was filtered and further reduced to the selected ones, using the QSAR-Contingency module implemented in MOE and by, re-iterative partial least square (PLS) analyses, within the same software.

4.2.1. Statistical analysis pruning descriptors

All the analyses here reported were performed applying the cheminformatics and QSAR packages of MOE, including molecular descriptors calculation. Afterwards 302 molecular descriptors (2D and 3D) were computed by MOE and the resulting matrix was submitted to the statistical analyses and Quantitative Structure Activity Relationships (QSAR), objects of the present work. In addition, QuaSAR-Contingency was employed for pruning molecular descriptors, while the QSAR analysis module of MOE software was applied to generate the final mathematical models.

The statistical application QuaSAR-Contingency performed a bivariate analysis between the molecular descriptors calculated for the corrector EC_{50} values, expressed as pEC_{50}. By a correlation and contingency analysis, the applied statistical tool ranked all the descriptors based on four coefficients, in order to express their influence on the dependent variable (corrector pEC_{50}). All the calculated coefficients with the related acceptable value are listed in Table 3.

To discard the less significant descriptors at this level, a unique score (S) was computed by summing all the coefficient values for each descriptor and only those descriptors with $S > 1.2$ were saved.

Following this procedure, we limited the number of descriptors, based on the calculated S values, in order to perform the following PLS iterations and QSAR analyses.

4.2.2. Quantitative structure-activity relationship (QSAR)

In order to perform quantitative structure-activity relationship (QSAR) studies, fifty correctors were included into the training set, for model generation, and the other ones into the test set, for model validation. The compounds were divided into the training and the test set pools manually, based on representative criteria of the overall biological activity trend and structural variations.

QSAR was performed by the application of various iterations of partial least-squares (PLS) multivariate analysis, considering the molecular descriptors as independent variables and corrector pEC_{50} values as dependent variable. Data fitting was accomplished using this regression analysis, which is useful when there are a number of independent variables, descriptor pool, relative to the number of dependent variables, the pIC_{50}. At each iteration the relative importance of every descriptors in influencing corrector ability was calculated, therefore the less important ones were discarded in the following PLS analysis, until the generation of the final linear regression model. At each PLS, Leave One Out method was used to check the internal predictability of the derived models. The predictive ability of the derived model was evaluated for the test set compounds (expressed as $r2_{pred}$), by using the following equation:

$$r^2_{pred} = (SD - PRESS)/SD$$

SD is the sum of the squared deviations between the biological activities of the test set molecules and the mean activity of the training set compounds and PRESS is the sum of the squared deviation between the observed and the predicted activities of the test set compounds.

4.3. Biological evaluations

4.3.1. Cell culture.

The bronchial epithelial cell line CFBE41o- with stable co-expression of F508del-CFTR and the halide-sensitive yellow fluorescent protein (HS-YFP) was cultured with MEM medium supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin.

4.3.2. Fluorescence assay for CFTR activity.

CFBE41o- cells with expression of mutant CFTR and HS-YFP were plated on clear-bottom 96-well black microplates (Corning Life Sciences, Acton, MA) at a density of 50,000 cells/well and kept at 37 °C in 5% CO2 for 24 hours. For the corrector assay, CFBE41o- cells were treated for further 24 hours with compounds and/or VX-809. After treatment, the culture medium was removed and cells in each well were stimulated for 30 min at 37 °C with 60 µl PBS (containing 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 1 mM $CaCl_2$, and 0.5 mM $MgCl_2$) plus forskolin (20 µM) and genistein (50 µM).

For determination of potentiator activity on F508del-CFTR, CFBE41o-cells were incubated for 24 h at 27 °C to allow trafficking of the mutant protein to plasma membrane. Cells were then stimulated with for 30 min with PBS containing forskolin (20 mM) plus the compound to be tested at the desired concentration.

At the time of assay, microplates carrying CFBE41o- cells were transferred to a microplates reader (FluoStar Galaxy; BMG Labtech, Offenburg, Germany). The plate reader was equipped with high-quality excitation (HQ500/20X: 500 ± 10 nm) and emission (HQ535/30M: 535 ± 15 nm) filters for YFP (Chroma Technology, Brattleboro, VT). The assay consisted of a continuous 14 s fluorescence reading with 2 s before and 12 s after injection of an iodide-containing solution (165 µl of a modified PBS containing I^- instead of Cl^-; final I^- concentration in the well: 100 mM). Data were normalized to the initial background-subtracted fluorescence. To determine fluorescence quenching rate

associated with I^- influx, the final 10 s of data for each well were fitted with an exponential function to extrapolate initial slope (dF/dt).

Dose-response relationships from each experiment were fitted with the Hill equation using the Igor software (WaveMetrics) to calculate EC50, maximal effect, and Hill coefficient.

4.3.3. Biochemical analysis of CFTR expression pattern

CFBE41o- cells stably expressing mutant CFTR and HS-YFP were grown to confluence on 60-mm diameter dishes and treated for 24 h with test compounds or vehicle alone. After 24 h cells were lysed in RIPA buffer containing a complete protease inhibitor (Roche). Cell lysates were subjected to centrifugation at 12000 rpm at 4°C for 10min. Supernatant protein concentration was calculated using the BCA assay (Euroclone) following the manufacturer's instructions. Equal amounts of protein (10 μg) were separated onto gradient (4-15%) Criterion TGX Precast gels (Bio-rad laboratories Inc.), transferred to nitrocellulose membrane with Trans-Blot Turbo system (Bio-rad Laboratories Inc.) and analyzed by Western blotting. Proteins were detected using monoclonal anti-CFTR (596, Cystic Fibrosis Foundation Therapeutics, University of North Carolina, Chapel Hill) or mouse monoclonal anti-GAPDH (cl.6C5; Santa Cruz Biotechnology, Inc) followed by horseradish peroxidase (HRP)-conjugated anti-mouse IgG (Abcam), and subsequently visualized by chemiluminescence using the SuperSignal West Femto Substrate (Thermo Scientific). Chemiluminescence was monitored using the Molecular Imager ChemiDoc XRS System. Images were analyzed with ImageJ software (National Institutes of Health). Bands were analyzed as ROI, normalized against the GAPDH loading control. Data are presented as mean ± SEM of independent experiments.

Author contributions

The manuscript was written through contributions of all authors. NL performed synthesis, purification, characterization of the new compounds, EC conceived, performed and interpreted all computational studies discussing the results with PF and EM at any stage of the project, draft the manuscript, MA synthesised a number of compounds, BT determined and interpreted NMR spectra, EP, VT and NP performed biological studies, LJVG contributed analytical tools. MP performed HPLC purification, AS and GD performed mass spectrometry characterization of all compounds, PF supervised and discussed the computational studies and their implications and commented on the manuscript at all stages, EM conceived the compounds, drawn their SAR, designed, coordinated and supervised the whole project, and critically revised the manuscript.

All authors have given approval to the final version of the manuscript.

Conflicts of interest: none

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Appendix A. Supporting Information

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Figure Captions

Scheme 1. Reagents and conditions (a) NBS, TMS-OTf, acetonitrile, 40°C, 3-24h (b) thiourea, EtOH absolute, reflux, 2h-24h. (c) HBTU, DIPEA, DMF, 50°C, 14h to 24h. For complete structure see Table 2.

Scheme 2. Reagents and conditions (a) thiourea, I_2, MW(100W), 140°C, 5-10 min (b) **1a,** HBTU,DIPEA, DMF, 50°C, 20h.

Scheme 3. Reagents and conditions (a) (i) CuCN, DMF , reflux, 8h, (ii) $FeCl_3$,HCl 12N, 25°C,ON (b) (i) KOH, EtOH absolute, reflux, 2h (ii) H_2SO_4, EtOH absolute, 25°C, 4h then 40°C,16h (c) NBS, TMS-OTf, acetonitrile, 40°C, 6h (d) thiourea, EtOH absolute, reflux, 8h (e) HBTU,DIPEA, DMF, 50°C, 24h (f) NaOH 1N:acetonitrile 1:1, 40°C, 24h.

Scheme 4 Reagents and conditions (a) NBS, TMS-OTf, acetonitrile, 40°C, 5h (d) thiourea, EtOH absolute, reflux, 4h (c) HBTU,DIPEA, DMF, 50°C, 20h (d) NaOH 1N:acetonitrile 1:1, 40°C, 16h.

Scheme 5 Reagents and conditions (a) HBTU, DIPEA, DMF, 60°C, 10h for **7a** and 16h for **7b.**