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Research paper

Identification of 6,9-dihydro-5*H*-pyrrolo[3,2-*h*]quinazolines as a new class of F508del-CFTR correctors for the treatment of cystic fibrosis

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ABSTRACT

Although substantial advances have been obtained in the pharmacological treatment of cystic fibrosis (CF) with the approval of Kaftrio, a combination of two correctors (VX-661, VX-445) and one potentiator (VX-770), new modulators are still needed to rescue F508del and other CFTR mutants with trafficking defects. We have previously identified PP compounds based on a tricyclic core as correctors with high efficacy in the rescue of F508del-CFTR on native epithelial cells of CF patients, particularly in combination with class 1 correctors (VX-809, VX-661). Compound PP028 was found as a lead candidate for the high rescue of F508del-CFTR and used for mechanistic insight indicating that PP028 behaves as a class 3 corrector, similarly to VX-445.

From the exploration of the chemical space around the hit structure, based on iterative cycles of chemical synthesis and functional testing, the class of 6,9-dihydro-5*H*-pyrrolo [3,2-*h*]quinazolines with corrector activity was discovered. Within a series of 38 analogues, two derivatives emerged as promising candidates and used for further insight to assess the mechanism of action. Both compounds, decorated with a benzensulfonylamino group at the pyrimidine moiety, were able to generate a dose-dependent increase in CFTR function, particularly in the presence of VX-809. Half-effective concentrations (EC_{50}) were in the single digit micromolar range and decreased in the presence of VX-809 thus indicating a synergistic interaction with class 1 correctors. Synergy was also observed with corr-4a (class 2 corrector) but not with VX-445 and PP028 (class 3 correctors) indicating that the new compounds behave as class 3 correctors. These results suggest that tricyclic pyrrolo-quinazolines interact with CFTR at a site different from that of VX-809 and represent a novel class of CFTR correctors suitable for combinatorial pharmacological treatments for the basic defect in CF.

1. Introduction

Cystic fibrosis (CF) is a common autosomal recessive disease that mainly affects the Caucasian population [1]. CF is a complex multiorgan disorder caused by loss-of-function mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene on the long arm of chromosome 7. This gene encodes for a cAMP-dependent chloride and bicarbonate ion channel that is expressed at the apical membrane of various types of epithelial cells [1–3]. CFTR protein plays a key role in transepithelial ion/fluid transport and in modulating the volume and pH of airway surface fluid and other epithelial secretions. As a result, defective CFTR protein function causes a broad range of symptoms, which include airway obstruction by thickened mucous leading to serious lung infections, pancreatic insufficiency, liver damage, high sweat chloride concentration, malabsorption with malnutrition, and infertility [1–3]. The CFTR protein consists of two membrane domains (MSD1 and MSD2) that form an anion-selective pore, two nucleotide domains (NBD1 and NBD2) that contain ATP-binding sites and a regulatory (R) region that is the site for cAMP-dependent phosphorylation. To date, more than 2000 mutations in the CFTR gene have been

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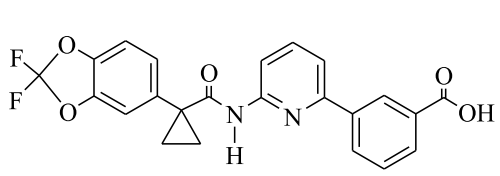
identified, although pathogenicity has only been confirmed for about 400 mutations [4,5]. The most common mutation is the deletion of phenylalanine at position 508 (F508del), that is present in 80–85 % of the worldwide CF population [5]. F508del causes misfolding of the mutant protein, which leads to a reduced stability of F508del-CFTR, retention of the mutant channel at the endoplasmic reticulum (ER), and premature degradation by the ubiquitin-proteasome system, resulting in a reduced expression of the channel at the plasma membrane [6–8]. The therapeutic approach to CF involves the use of small molecules, named CFTR modulators, which target specific defects triggered by mutations in the CFTR gene. Based on the mechanism of action, these modulators are classified as potentiators (which increase the time spent by the CFTR channel in the open state), and correctors (which promote CFTR protein folding, processing, and trafficking to the plasma membrane) [5,9,10].

The use of small molecules named correctors, such as VX-809 (lumacaftor), VX-661 (tezacaftor), and VX-445 (elexacaftor) (Fig. 1) [11,12], and potentiators, such as VX-770 (ivacafor) [13,14], can target the misfolding and instability caused by F508del allowing the CFTR protein to reach the plasma membrane and overcome the channel-gating defect. Three classes of correctors have been identified: C1 correctors (VX-809 and VX-661), which interact with TMD1; C2 correctors, (bithiazole corr-4a and compound 3151), which act on the second half of the CFTR protein; C3 correctors (4172 and VX-445), which are postulated to improve the stability of NBD1 [15].

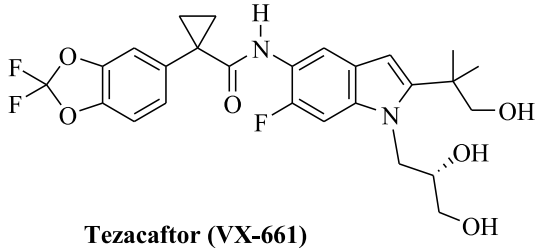
Four CFTR modulating therapies, developed by Vertex Pharmaceuticals, are now available for the treatment of CF patients. Ivacaftor (trade name Kalydeco®) effective in treating G551D-CFTR and 37 other gating mutations; lumacaftor-ivacaftor combination (trade name Orkambi®) effective in treating F508del homozygous subjects [16]; tezacaftor-ivacaftor combination (trade name Symdeko®/Symkevi®) effective in treating homozygous F508del subjects [17], or CF patients with F508del and a residual function allele; and elexacaftor-tezacaftor-ivacaftor (trade name Trikafta in US and Kaftrio in Europe) [12], which shows promising benefits for around 90 % of CF patients, including those homozygous for F508del and those with F508del and a second minimal-function mutation. Nevertheless, for about 10 % of CF patients with rare, or hard-to-treat mutation, no therapies are available [18]. So, the search of new drug candidates that possess different mechanism of action or better characteristic is still required.

Tricyclic molecules have been demonstrated to influence CFTR synthesis and/or function. For example, psoralens are linear furocumarins which showed the ability to potentiate CFTR channel activity, while 4,6,4′-trimethylangelicin (TMA) is an angular furocumarin, which showed both potentiator and corrector activity [19].

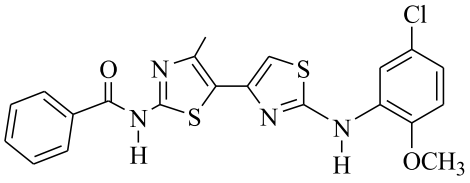
Our previous work on biologically active tricyclic compounds, containing a pyrrole ring, led to the synthesis of nearly 200 molecules [20–25]. We decided to test those compounds for their activity as CFTR correctors. Compound PP007 (Fig. 2) emerged as the only active derivative. Interestingly, PP007 was able to functionally rescue F508del-CFTR, particularly in combination with VX-809, a C1 corrector. A small set of modifications of PP007 led to the identification of PP008 (Fig. 2), which confirmed its activity in primary bronchial epithelial cells showing the ability of synergizing with C1 correctors. Modifications of PP008 afforded PP028, in which the methyl group of the benzyl



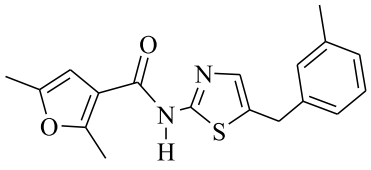
Lumacaftor (VX-809)
Class 1 corrector



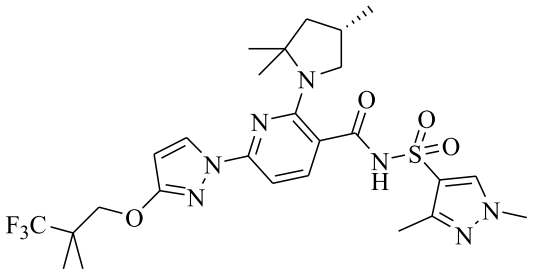
Tezacaftor (VX-661)
Class 1 corrector



Corr-4a
Class 2 corrector



3151
Class 2 corrector



Elexacaftor (VX-445)
Class 3 corrector

Fig. 1. Structures of class 1,2 and 3 correctors of the CFTR protein.

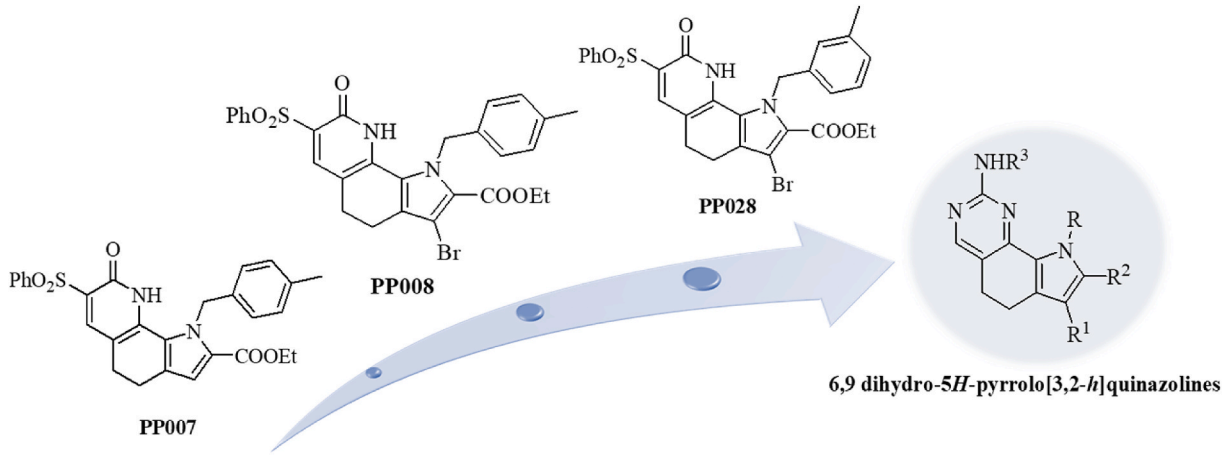


Fig. 2. Ethyl 7-(benzenesulfonyl)-1-(4-methylbenzyl)-8-oxo-4,5,8,9-tetrahydro-1*H*-pyrrolo [3,2-*h*]quinoline-2-carboxylate (PP007), ethyl 7-(benzenesulfonyl)-3-bromo-1-[(4-methylphenyl)methyl]-8-oxo-4,5,8,9-tetrahydro-1*H*-pyrrolo [3,2-*h*]quinoline-2-carboxylate (PP008), ethyl 3-bromo1-(3-methylbenzyl)-8-oxo-7-(phenylsulfonyl)-4,5,8,9-tetrahydro-1*H*-pyrrolo [3,2-*h*]quinoline-2-carboxylate (PP028), 6,9-dihydro-5*H*-pyrrolo [3,2-*h*]quinazolines.

moiety was moved from position 4 to position 3. Derivative PP028 (Fig. 2) became our reference candidate, based on its activity profile, improved with respect to PP008, and the ability to synergize with VX-809 [26].

Further insight on the mechanism of action, using PP028 in different combinations with a C2 corrector (3151) or C3 correctors (4172 and VX-445), showed its ability to synergize with 3151 but not with 4172 and VX-445, indicating that it behaves as a C3 corrector. Moreover, we observed that PP028 is able to stabilize F508del-CFTR protein only when the MSD2 is included, similarly to VX-445 [26]. This last result suggests that C3 correctors interact with this CFTR domain rather than with NBD1.

In our exploration of the chemical space around PP compounds, we were attracted by the replacement of the pyridine ring with a pyrimidine, thus generating pyrrolo [3,2-*h*]quinazolines (Fig. 2), as pyrimidines and their condensed analogues constitute the core structure of several bioactive compounds [27–33]. Therefore, we undertook the synthesis of a set of 6,9-dihydro-5*H*-pyrrolo [3,2-*h*]quinazolines with the aim of expanding the diversity of our tricyclic compound library and assessing their potential activity as CFTR correctors.

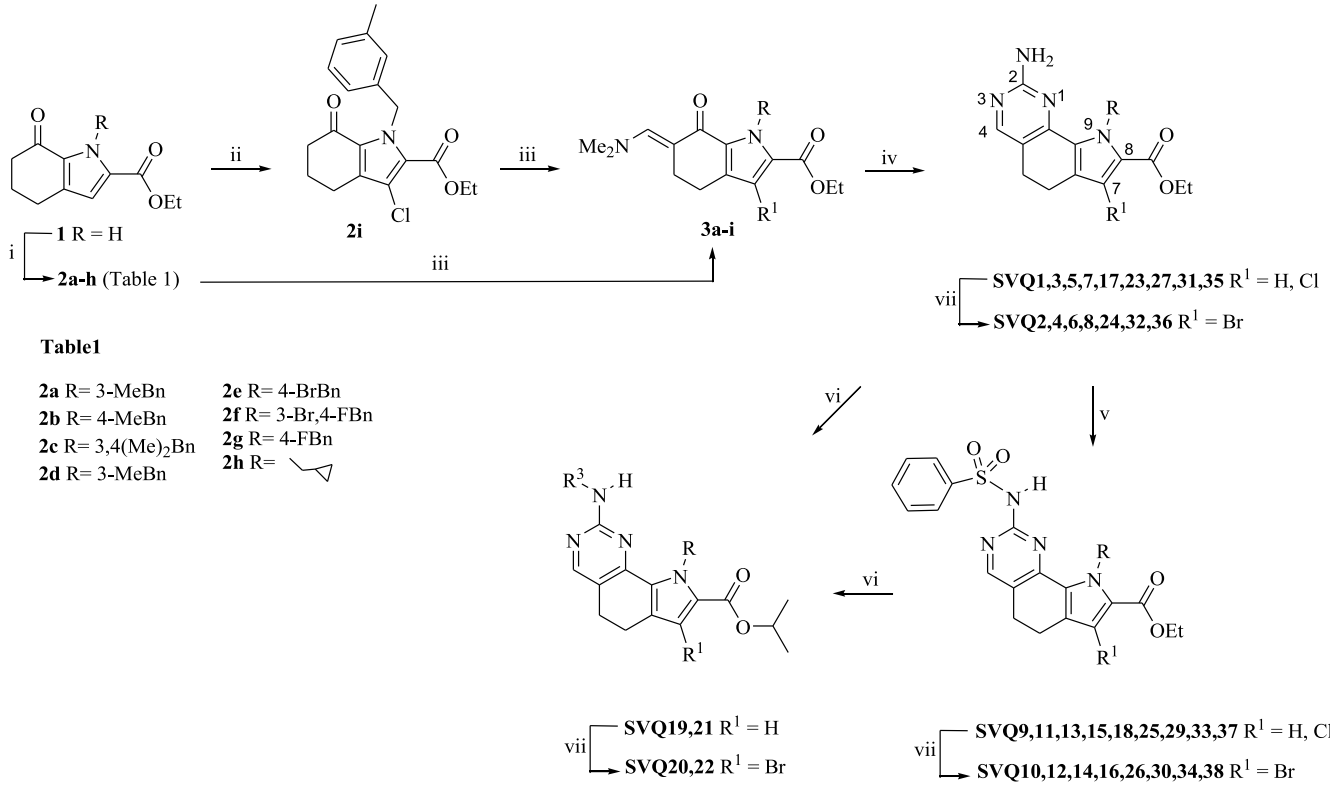


Table1

2a R= 3-MeBn	**2e** R= 4-BrBn
2b R= 4-MeBn	**2f** R= 3-Br,4-FBn
2c R= 3,4(Me)$_2$Bn	**2g** R= 4-FBn
2d R= 3-MeBn	**2h** R=

Scheme 1. Synthesis of 6,9-dihydro-5*H*-pyrrolo [3,2-*h*] quinazoline-8-carboxylate (SVQ1-38). (i) NaH, DMF, 0 °C to rt, 90 min then substituted arylalkyl halides, 0 °C to rt 5–24 h; (ii) for 2a: acetic acid, NCS, 60 °C, 6 h; (iii) TBDMAM (1: 3), toluene, reflux 2–16 h; (iv) guanidine nitrate, MeONa, ethanol, reflux 2–24 h; (v) benzene ulfonyl chloride, pyridine, rt, 24 h; (vi) for SVQ3 and SVQ11: a) KOH, ethanol, reflux 5–8 h; b) *i*-propanol, EDC, DMAP, rt, 16 h; (vii) Br_2 (1:2), dichloromethane, rt, 24 h.

2. Results and discussion

2.1. Chemistry

Our general approach to the synthesis of 6,9-dihydro-5*H*-pyrrolo [3,2-*h*]quinazolines ring system started from the preparation of 1,4,5,6-tetrahydro-7*H*-indol-7-one 1 as proper building block to achieve the tricyclic framework (Scheme 1). The latter was prepared following a multistep sequence as previously reported by us [34].

Functionalization of the pyrrole nitrogen was achieved using classical procedures in *N,N*-dimethylformamide (DMF) with sodium hydride as the base, followed by nucleophilic substitution with the suitable aralkyl halides. Thus, ketone 1 was reacted with 3-methylbenzyl bromide, 4-methylbenzyl chloride, 3,4-dimethylbenzyl chloride, 3-bromobenzyl bromide, 4-bromobenzyl bromide, 3-bromo 4-fluorobenzyl bromide, cyclopropylmethyl bromide or 4-fluorobenzyl chloride in the presence of sodium hydride producing the corresponding *N*-substituted derivatives 2a-h with good to excellent yields (60–84 %) (Scheme 1, Table 1).

Functionalization in α position to the carbonyl of ketones 2 was obtained by direct introduction of the enamino group using acetal amide *tert*-butoxy-bis(dimethylamino)methane (TBDMAM) in refluxing toluene, leading to the desired enaminoketones 3, which were used as crude products in the next step (Scheme 1).

Annelation of the pyrimidine ring was achieved by reacting the enaminoketones 3 with guanidine nitrate in the presence of sodium methoxide as the base, to obtain the 2-amino substituted pyrrolo [3,2-*h*] quinazolines SVQ1,3,5,7,23,27,31,35 (60–92 %) (Scheme 1, Table 2).

To evaluate the effect on the corrector activity of the presence of a chlorine atom in position 7 of the tricyclic system, we synthetized SVQ17 (Table 2), bearing the same substituent as PP028 at the pyrrole nitrogen. Thus, compound 2a was subjected to chlorination using *N*-chlorosuccinimide (NCS) in glacial acetic acid allowing the isolation of ketone 2i (65 %) (Scheme 1, Table 2). The latter was converted into the corresponding enaminoketone 3i, which was in turn reacted with guanidine nitrate to afford SVQ17 (94 %) (Scheme 1, Table 2)

Compounds SVQ1,3,5,7,17,23,27,31,35 were subjected to sulfonylation at the amino group generating compounds SVQ9,11,13,15,18,25,29, 33,37 (70–92 %) (Scheme 1, Table 2).

Finally, conversion of the ethyl ester in position 8 of compounds SVQ3 and SVQ11, bearing a 3-methylbenzyl substituent at the pyrrole nitrogen, to isopropyl ester SVQ19,21 was obtained in two steps. Hydrolysis in basic media in ethanol generated the corresponding carboxylic acids which were directly converted into isopropyl ester by reaction with *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) in isopropanol in the presence of 4-dimethylaminopyridine (DMAP) (75–89 %) (Scheme 1, Table 2).

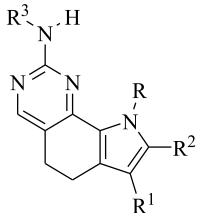
All the above-mentioned tricyclic compounds (R^1=H) were then subjected to bromination at the pyrrole ring providing the corresponding bromo derivatives SVQ2,4,6,8,10,12,14,16,20,22,24,26,28,30,32, 34,36,38 (60–88 %) (Scheme 1, Table 2).

2.2. Biology

All compounds (Table 2) were tested in a CFTR corrector assay that was carried out on CFBE41o-cells expressing F508del-CFTR. Such cells also express the halide-sensitive yellow fluorescent protein (HS-YFP). CFTR-dependent iodide influx causes HS-YFP fluorescence quenching. Hence, the quenching rate is proportional to the amount of functional CFTR channels in the plasma membrane and therefore reflects the efficacy of correctors in rescuing mutant CFTR from intracellular compartments. Cells were incubated for 24 h with compounds at 1 and 10 μM. Treatment was also done in combination with VX-809 (1 μM), a C1 corrector. After treatment, cells were further stimulated with forskolin and genistein to fully activate CFTR in the plasma membrane and promote anion transport.

Within the entire class of thirty eight pyrrolo [3,2-*h*]quinazoline derivatives equally distributed between amino and benzensulfonylamino analogues, referring to the 2 position of the final scaffold, compounds SVQ11, 12, 14, 16, 18, 22, 26, 29, 30, 34 showed activity as

Table 2
6,9-Dihydro-5*H*-pyrrolo[3,2-*h*]quinazolines SVQ1-38.



CPD	R	R^1	R^2	R^3	CPD	R	R^1	R^2	R^3
SVQ1	4-MeBn	H	COOEt	H	**SVQ20**	3-MeBn	Br	COO*i*Pr	H
SVQ2	4-MeBn	Br	COOEt	H	**SVQ21**	3-MeBn	H	COO*i*Pr	SO_2Ph
SVQ3	3-MeBn	H	COOEt	H	**SVQ22**	3-MeBn	Br	COO*i*Pr	SO_2Ph
SVQ4	3-MeBn	Br	COOEt	H	**SVQ23**	3-BrBn	H	COOEt	H
SVQ5	3,4-$(Me)_2$Bn	H	COOEt	H	**SVQ24**	3-BrBn	Br	COOEt	H
SVQ6	3,4-$(Me)_2$Bn	Br	COOEt	H	**SVQ25**	3-BrBn	H	COOEt	SO_2Ph
SVQ7	4-BrBn	H	COOEt	H	**SVQ26**	3-BrBn	Br	COOEt	SO_2Ph
SVQ8	4-BrBn	Br	COOEt	H	**SVQ27**	3-Br,4-FBn	H	COOEt	H
SVQ9	4-MeBn	H	COOEt	SO_2Ph	**SVQ28**	3-Br,4-FBn	Br	COOEt	H
SVQ10	4-MeBn	Br	COOEt	SO_2Ph	**SVQ29**	3-Br,4-FBn	H	COOEt	SO_2Ph
SVQ11	3-MeBn	H	COOEt	SO_2Ph	**SVQ30**	3-Br,4-FBn	Br	COOEt	SO_2Ph
SVQ12	3-MeBn	Br	COOEt	SO_2Ph	**SVQ31**	Me-cycloPr	H	COOEt	H
SVQ13	3,4-$(Me)_2$Bn	H	COOEt	SO_2Ph	**SVQ32**	Me-cycloPr	Br	COOEt	H
SVQ14	3,4-$(Me)_2$Bn	Br	COOEt	SO_2Ph	**SVQ33**	Me-cycloPr	H	COOEt	SO_2Ph
SVQ15	4-BrBn	H	COOEt	SO_2Ph	**SVQ34**	Me-cycloPr	Br	COOEt	SO_2Ph
SVQ16	4-BrBn	Br	COOEt	SO_2Ph	**SVQ35**	4-FBn	H	COOEt	H
SVQ17	3-MeBn	Cl	COOEt	H	**SVQ36**	4-FBn	Br	COOEt	H
SVQ18	3-MeBn	Cl	COOEt	SO_2Ph	**SVQ37**	4-FBn	H	COOEt	SO_2Ph
SVQ19	3-MeBn	H	COO*i*Pr	H	**SVQ38**	4-FBn	Br	COOEt	SO_2Ph

correctors, particularly when combined with VX-809 (Fig. 3). Interestingly, all compounds belong to the 2-benzensulfonylamino series, indicating the importance of this group to obtain activity and confirming our previous observations on the PP class of compounds. The best results were obtained for the 3-methylbenzyl derivative SVQ18 and the 3-bromobenzyl one SVQ26. Both compounds belong to the benzensulfonylamino series bearing a halogen atom at the 7 position of the scaffold, i.e. chlorine (SVQ18) and bromine (SVQ26), respectively. From the SAR analysis it is highlighted that the presence of the 3-methylbenzyl group at the pyrrole nitrogen is important for the activity considering that it can be found in four out of ten active compounds, SVQ11, 12, 18, and 22, characterized also by the presence of a hydrogen (SVQ11), bromine (SVQ12,22) or chlorine atoms (SVQ18) in position 7 and an 8-ethyl or 8-isopropyl carboxylate groups.

The pyrrole *N*-substitution with the 3-bromobenzyl group produced activity only in the case SVQ26, which maintained the decoration of the pyrrole ring with a bromine atom and an ethoxycarbonyl groups in positions 7 and 8, respectively, whereas the absence of the bromine led a loss of activity (SVQ25).

Moving the methyl (SVQ9,10) or bromine (SVQ15,16) in position 4

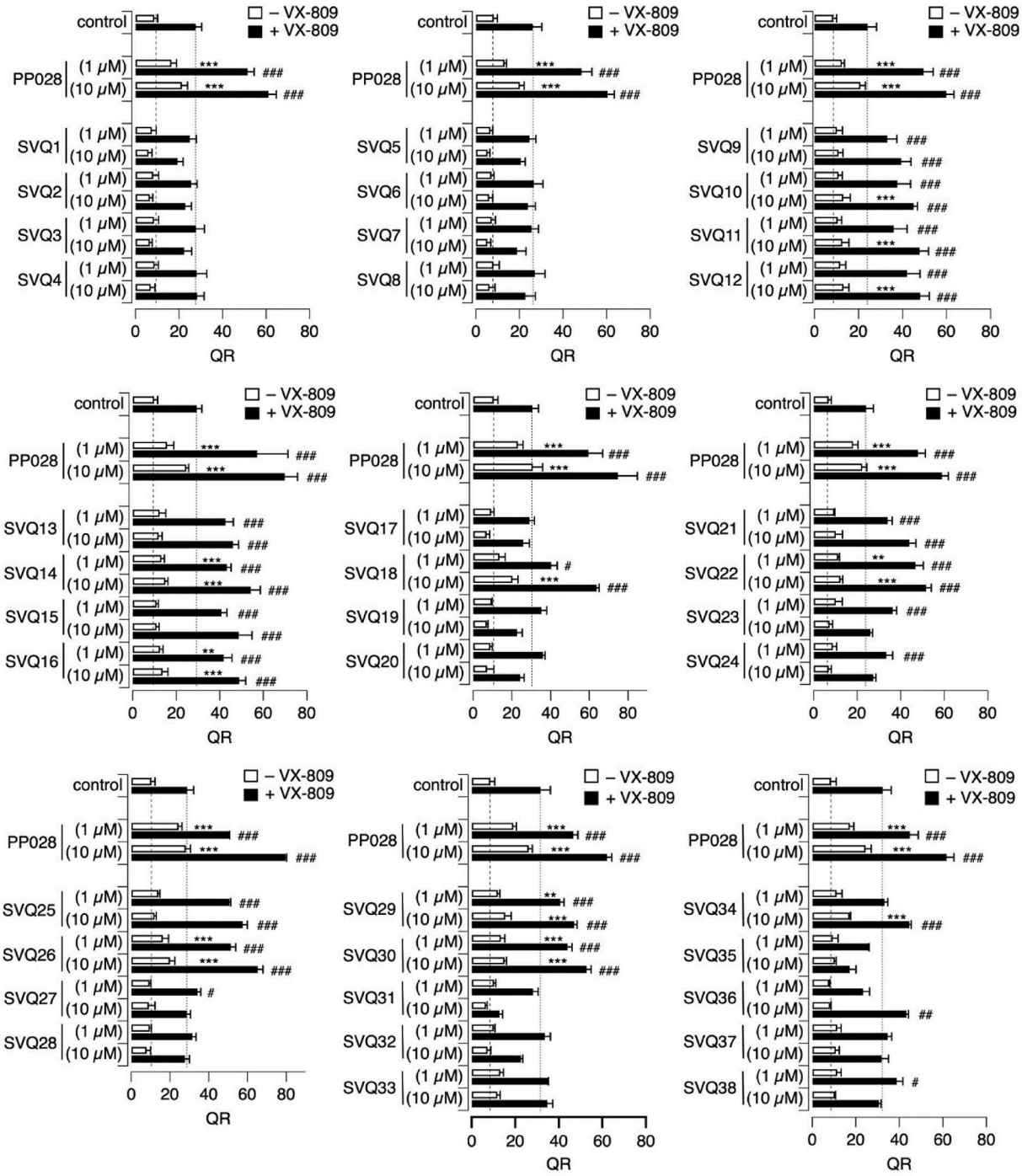


Fig. 3. Corrector activity of SVQ compounds. The bar graphs report CFTR activity as quenching rate (QR) determined with the HS-YFP assay. Each graph reports the results obtained for a panel of SVQ compounds in comparison with PP028. Each compound was tested at 1 and 10 μM, in the presence/absence of VX-809 (1 μM). Data are mean ± SD from 6 experiments. **, $p < 0.01$; ***, $p < 0.001$ vs. vehicle alone. #, $p < 0.05$; ##, $p < 0.01$; ###, $p < 0.001$ vs. VX-809 (ANOVA with Dunnett's post-hoc test).

led to inactive compounds. The same applies for the 4-fluorobenzyl derivatives SVQ34 and SVQ38, whereas an additional methyl (SVQ14) or fluorine (SVQ29,30) in α position to the 3-bromo benzyl group are tolerated although with a slight decrease of the activity. The *N*-cyclopropylmethyl derivatives (SVQ33,34) did not show any corrector activity.

The most active compounds, SVQ18 and SVQ26, were retested at multiple concentrations, with/without VX-809, to generate dose-response relationships (Fig. 4). For comparison, a dose-response relationship was also generated for PP028. Cell incubation with SVQ18 and SVQ26 generated a dose-dependent increase in CFTR function, particularly in the presence of VX-809. The half-effective concentration (EC_{50}) for SVQ18 and SVQ26 was comparable: 3.14 and 3.08 μM, respectively. Interestingly, as previously reported for PP028 and other analogues, the EC_{50} decreased in the presence of VX-809 (1.88 μM for SVQ18, 2.12 μM for SVQ26). This behaviour was confirmed in our retest of PP028: 0.75 μM without VX-809, 0.45 μM with VX-809. Regarding synergy with the C1 corrector, SVQ18, SVQ26, and PP028 increased CFTR activity by 2.1-, 2.3-, and 2.4-fold over VX-809 (1 μM) alone.

In our previous study, we found that PP028 acts with a C3 mechanism of action, based on the results obtained by treating CFBE41o-cells with a combination of our compound and other correctors [26]. We conducted the same study also for SVQ18 and SVQ26 (Fig. 5). These compounds were tested at 10 μM in the presence or absence of corr-4a (10 μM), as a C2 corrector, or VX-445 (5 μM), as a C3 corrector. PP028 was also included for comparison. SVQ18 and SVQ26, as well as PP028, showed synergy with corr-4a but not with VX-445 (Fig. 5). From these results we can postulate that compounds SVQ18 and SVQ26, similarly to VX-445, interact with CFTR at a site different from that of VX-809.

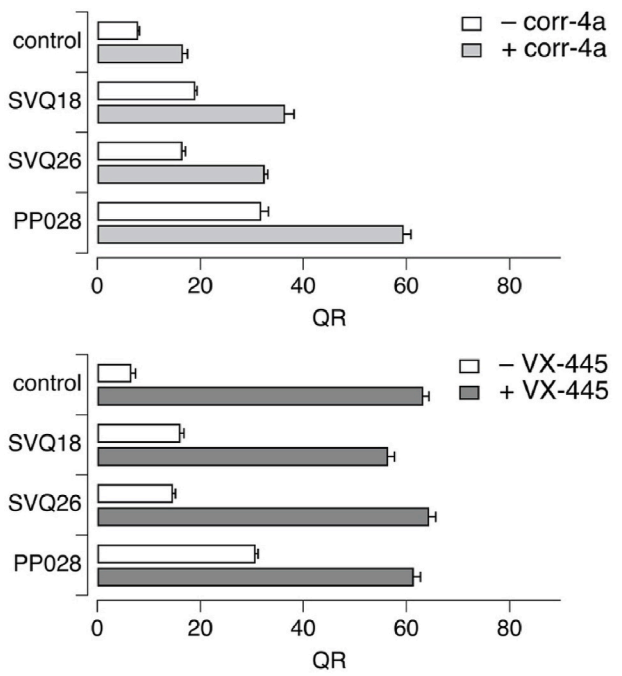


Fig. 5. Effect of compound combinations. The graphs report the activity of CFTR measured with the HS-YFP assay in cells treated with vehicle (control), SVQ18 (10 μM), SVQ26 (10 μM), or PP028 (10 μM) with/without corr-4a (5 μM) or VX-445 (5 μM). The bars report the mean ± SD of 4 experiments.

3. Conclusions

Pharmacological correctors of mutant CFTR are an effective therapeutic solution for CF patients. Besides targeting F508del mutation, correctors may also be beneficial for other types of CF mutations characterized by protein misfolding and mistrafficking. However, differences in the response to correctors are expected among mutations localized in different regions of CFTR. Therefore, the search for novel CFTR corrector classes, having a different type of interaction with CFTR protein, enhances the therapeutic options with the possible development of drugs with optimized efficacy for specific groups of CF mutations. Quinazolines are valuable heterocycles in medicinal chemistry, as they are highly represented as the pharmacophore unit of many drug candidates. They show a variety of pharmacological properties and offer a high degree of structural diversity as a useful tool for developing new therapeutic agents. Continuing our research line on CFTR modulators, we have described a series of pyrrolo [3,2-*h*]quinazolines as a new class of F508del-CFTR correctors. In particular, compounds SVQ18 and SVQ26 were identified as promising candidates, leading to rescue of the F508del-CFTR chloride channel function. Although these compounds did not show improved potency and efficacy compared to the parent PP028 compound, they synergized with VX-809 and corr-4a but not with VX-445, suggesting that they also behave as class 3 correctors. These results indicate that tricyclic pyrrolo-quinazolines represent a novel class of CFTR modulators that interact with CFTR at a site different from that of VX-809 similarly to VX-445 and PP028. This is an important point that expands the panel of tricyclic heterocycles in our hands acting as CFTR correctors, with a mechanism that probably involves interaction with the MSD2 [26]. Structural tuning is currently ongoing to discover improved derivatives in terms of potency/efficacy and drug-like properties. Thus, in the course of our search for more effective and potent compounds, the pyrrole quinazoline structural pattern seems to preserve the activity, prompting further investigation into its relationship with the modulation of CFTR activity. Lead molecules will be modified by iterative cycles of chemical synthesis evaluating the corrector activity and ADME/Tox properties to address a multiparametric optimization. In particular, an initial evaluation of the

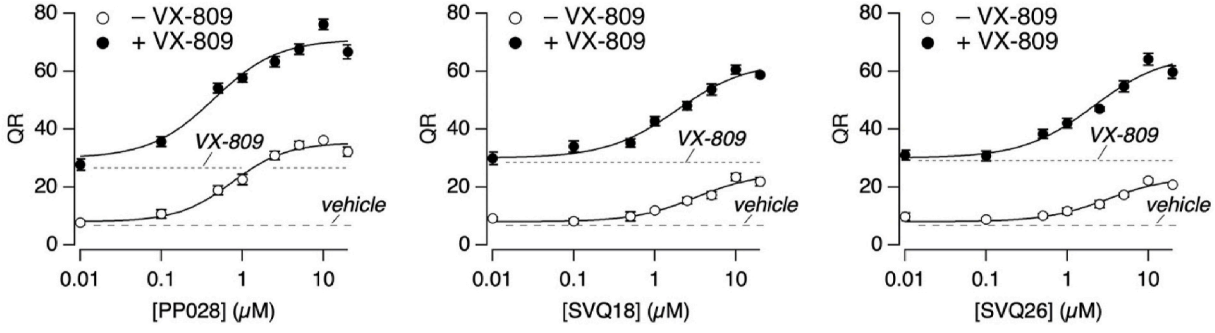


Fig. 4. Potency and efficacy of compounds as CFTR correctors. The graphs report dose-response relationships for PP028, SVQ18, and SVQ26 tested at multiple concentrations (0.01–20 μM) in the presence/absence of VX-809 (1 μM). Data (mean ± SD of 6 experiments) were fitted with the Hill equation to derive EC_{50} and maximal effect. Dashed and dotted lines indicate activity with vehicle (DMSO) or VX-809, respectively.

drug-like physical properties, such as chemical and metabolic stability in human plasma and liver microsomes, permeability and solubility will provide the basis for further optimization to achieve the best compromise between corrector activity on mutant CFTR and drug-likeness.

The expansion of the SAR within this family of compounds may lead to compounds with improved ability to rescue F508del and other CFTR mutants with trafficking defects, suitable for combinatorial pharmacological treatments. These results, along with a deeper understanding of their interaction with specific domains of CFTR protein, may help to design innovative correctors for new combinatorial treatments to achieve maximal rescue of mutant CFTR to treat the basic defect in CF.

With this manuscript, we aim to provide new information on class 3 correctors to be used in new combinatorial treatments and broaden the spectrum of structural optimization strategies, thus offering fresh insights into future drug development.

4. Experimental section

4.1. Chemistry. Synthesis and characterization

All melting points were taken on a Büchi melting point M − 560 apparatus. IR spectra were determined in bromoform with a Shimadzu FT/IR 8400 S spectrophotometer. ^{1}H and ^{13}C NMR spectra were measured at 200 and 50.0 MHz, respectively, in DMSO-d_6 or $CDCl_3$ solution using a Bruker Avance II series 200 MHz spectrometer. Column chromatography was performed with Merck silica gel (230−400 mesh ASTM) or a Büchi Sepacor chromatography module (prepacked cartridge system). Elemental analyses (C, H, N) were within ±0.4 % of theoretical values and were performed with a VARIO EL III elemental analyzer. The purity of all the tested compounds was >95 %, determined by HPLC (Agilent 1100 series).

Compounds **1**, **2b** and **3b** were prepared according to our published procedures [34].

4.1.1. General procedure for the synthesis of 1-substituted-1,4,5,6-tetrahydro-7H-indolones (2a,c-h)

To a solution ketone **1** (15 mmol) in anhydrous DMF (20 mL), NaH (0.64 g, 16 mmol) was added at 0 °C and the reaction mixture was stirred at room temperature for 1 h. Then the suitable aralkyl halide (16 mmol) was added at 0 °C. The reaction mixture was stirred at room temperature until the reaction was complete (TLC). Then the reaction mixture was poured onto crushed ice and the precipitate was filtered off and dried. In the absence of a precipitate, the solution was extracted with DCM (3 x 30 mL). The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified using a chromatography column (petroleum ether: ethyl acetate 9 : 1).

*4.1.1.1. Ethyl 1-(3-methylbenzyl)-7-oxo-4,5,6,7-tetrahydro-1H-indole-2-carboxylate (**2a**).* This compound was obtained from reaction of compound **1** with 3-methylbenzyl chloride after 24 h. Colourless oil; yield: 65 %; IR (cm^{-1}): 1711 (CO), 1656 (CO); ^{1}H NMR ($CDCl_3$, 200 MHz, ppm): 1.30 (t, 3H, $J = 7.1$ Hz, CH_3), 2.01–2.14 (m, 2H, CH_2), 2.28 (s, 3H, CH_3), 2.53 (t, 2H, $J = 6.0$ Hz, CH_2), 2.76 (t, 2H, $J = 6.0$ Hz, CH_2), 4.26 (q, 2H, $J = 7.1$ Hz, CH_2), 6.08 (s, 2H, CH_2), 6.80–7.16 (m, 4H, H-3, H-2′, H-4′, H-5′ and H-6’); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): 14.2, 21.5, 23.7, 24.6, 40.2, 49.3, 60.7, 115.1, 123.5, 127.3, 127.7, 128.1, 128.2, 130.3, 135.6, 137.8, 138.8, 160.9, 190.5. Anal calcd for $C_{19}H_{21}NO_3$: C, 73.29; H, 6.80; N, 4.50. Found: C, 73.14; H, 6.53; N, 4.69.

*4.1.1.2. Ethyl 1-(3,4-dimethylbenzyl)-7-oxo-4,5,6,7-tetrahydro-1H-indole-2-carboxylate. (**2c**).* This compound was obtained from reaction of compound **1** with 3,4-dimethylbenzyl chloride after 24 h. Light yellow oil; yield: 60 %; IR (cm^{-1}): 1710 (CO), 1654 (CO); ^{1}H NMR (DMSO-d_6, 200 MHz, ppm): 1.23 (t, 3H, $J = 6.0$ Hz, CH_3), 1.92–2.04 (m, 2H, CH_2), 2.13 (s, 6H, 2 x CH_3), 2.47 (t, 2H, $J = 6.0$ Hz, CH_2), 2.73 (t, 2H, $J = 6.0$ Hz, CH_2), 4.21 (q, 2H, $J = 6.0$ Hz, CH_2), 5.93 (s, 2H, CH_2), 6.60 (d, 1H, $J = 8.0$ Hz, Ar), 6.79–6.82 (m, 2H, Ar), 6.99 (d, 1H, $J = 8.0$ Hz, Ar); ^{13}C NMR (DMSO-d_6, 50 MHz, ppm): 14.0, 18.9, 19.5, 22.9, 24.1, 39.6, 48.3, 60.5, 115.0, 123.3, 127.1, 127.3, 129.4, 129.7, 134.6, 135.3, 135.9, 136.1, 160.2, 190.0. Anal calcd for $C_{20}H_{23}NO_3$: C, 73.82; H, 7.12; N, 4.30. Found: C, 73.75; H, 6.95; N, 4.59.

*4.1.1.3. Ethyl 1-(3-bromobenzyl)-7-oxo-4,5,6,7-tetrahydro-1H-indole-2-carboxylate (**2d**).* This compound was obtained from reaction of compound **1** with 3-bromobenzyl chloride after 24 h. Colorless oil; yield: 75 %; IR (cm^{-1}): 1709 (CO), 1654 (CO); ^{1}H NMR ($CDCl_3$, 200 MHz, ppm): 1.33 (t, 3H, $J = 7.1$ Hz, CH_3), 2.06–2.14 (m, 2H, CH_2), 2.55 (t, 2H, $J = 6.1$ Hz, CH_2), 2.79 (t, 2H, $J = 6.1$ Hz, CH_2), 4.29 (q, 2H, $J = 7.1$ Hz, CH_2), 6.09 (s, 2H, CH_2), 6.83 (s, 1H, H-3), 7.03 (d, 1H, $J = 7.8$ Hz, H-6’), 7.13 (t, 1H, $J = 7.8$ Hz, H-5′), 7.21 (s, 1H, H-2′), 7.33 (d, 1H, $J = 7.8$ Hz, H-4’); ^{13}C NMR ($CDCl_3$, 50 MHz, ppm): 14.2, 23.6, 24.5, 40.1, 48.8, 60.8, 115.3, 115.4, 122.5, 125.4, 127.9, 129.7, 129.9, 130.1, 135.8, 141.1, 160.8, 190.6. Anal calcd for $C_{18}H_{18}BrNO_3$: C, 57.46; H, 4.82; N, 3.72. Found: C, 57.29; H, 4.97; N, 3.88.

*4.1.1.4. Ethyl 1-[(4-bromophenyl)methyl]-7-oxo-4,5,6,7-tetrahydro-1H-indole-2-carboxylate (**2e**).* This compound was obtained from reaction of compound **1** with 4-bromobenzyl chloride after 24 h. White solid; yield: 78 %; m.p.: 75–76 °C; IR (cm^{-1}): 1715 (CO), 1658 (CO); ^{1}H NMR (DMSO-d_6, 200 MHz, ppm): 1.21 (t, 3H, $J = 7.1$ Hz, CH_3), 1.90–2.05 (m, 2H, CH_2), 2.47 (t, 2H, $J = 5.8$ Hz, CH_2), 2.73 (t, 2H, $J = 5.8$ Hz, CH_2), 4.20 (q, 2H, $J = 7.1$ Hz, CH_2), 5.94 (s, 2H, CH_2), 6.85 (s, 1H, H-3), 6.91 (d, 2H, $J = 8.3$ Hz, H-2′ and H-6′), 7.47 (d, 2H, $J = 8.3$ Hz, H-3′ and H-5’); ^{13}C NMR (DMSO-d_6, 50 MHz, ppm): 14.0, 22.8, 24.1, 39.5, 48.3, 60.6, 115.1, 119.9, 127.0, 128.3 (2 x C), 129.7, 131.2 (2 x C), 135.5, 138.2, 161.1, 190.1. Anal calcd for $C_{18}H_{18}BrNO_3$: C, 57.46; H, 4.82; N, 3.72. Found: C, 57.33; H, 4.69; N, 3.84.

*4.1.1.5. Ethyl 1-(3-bromo-4-fluorobenzyl)-7-oxo-4,5,6,7-tetrahydro-1H-indole-2-carboxylate (**2f**).* This compound was obtained from reaction of compound **1** with 3-bromo,4-fluorobenzyl chloride after 24 h. Colorless oil; yield: 84 %; IR (cm^{-1}): 1711 (CO), 1656 (CO); ^{1}H NMR (DMSO-d_6, 200 MHz, ppm): 1.21 (t, 3H, $J = 7.1$ Hz, CH_3), 1.94–2.01 (m, 2H, CH_2), 2.47 (t, 2H, $J = 6.0$ Hz, CH_2), 2.72 (t, 2H, $J = 6.0$ Hz, CH_2), 4.20 (q, 2H, $J = 7.1$ Hz, CH_2), 5.92 (s, 2H, CH_2), 6.84 (s, 1H, H-3), 6.93–6.97 (m, 1H, Ar), 7.27 (t, 1H, $J = 8.7$ Hz, Ar), 7.35 (dd, 1H, $J = 6.7$, 2.2 Hz, Ar); ^{13}C NMR (DMSO-d_6, 50 MHz, ppm): 14.5, 23.3, 24.5, 39.7, 48.2, 61.1, 108.2 (d, $J_{C3'\text{-}F} = 15.8$ Hz), 113.5, 115.7, 117.1 (d, $J_{C5'\text{-}F} = 16.5$ Hz), 127.5, 128.0, 131.7, 136.2, 137.3, 157.7 (d, $J_{C4'\text{-}F} = 181.5$ Hz), 160.6, 190.7. Anal calcd for $C_{18}H_{17}BrFNO_3$: C, 54.84; H, 4.35; N, 3.55. Found: C, 54.99; H, 4.18; N, 3.37.

*4.1.1.6. Ethyl 1-[(4-fluorophenyl)methyl]-7-oxo-4,5,6,7-tetrahydro-1H-indole-2-carboxylate (**2g**).* This compound was obtained from reaction of compound **1** with 4-fluorobenzyl chloride after 24 h. White solid; yield: 62 %; m.p.: 72–73 °C; IR (cm^{-1}): 1726 (CO), 1658 (CO); ^{1}H NMR (DMSO-d_6, 200 MHz, ppm): 1.22 (t, 3H, $J = 7.1$ Hz, CH_3), 1.89–2.07 (m, 2H, CH_2), 2.47 (t, 2H, $J = 5.8$ Hz, CH_2), 2.72 (t, 2H, $J = 5.8$ Hz, CH_2), 4.20 (q, 2H, $J = 7.1$ Hz, CH_2), 5.96 (s, 2H, CH_2), 6.83 (s, 1H, H-3), 7.00–7.12 (m, 4H, H-2′, H-3′, H-5′ and H-6′); ^{13}C NMR (DMSO-d_6, 50 MHz, ppm): 14.4, 23.3, 24.5, 40.0, 48.5, 61.0, 115.4, 115.6 (2 x d, $J_{C3'\text{-}F} = 16.2$ Hz), 127.5, 128.7 (2 x d, $J_{C2'\text{-}F} = 8.2$ Hz), 130.1, 135.3, 136.0, 160.0, 161.9 (d, $J_{C4'\text{-}F} = 191.5$ Hz), 190.6. Anal calcd for $C_{18}H_{18}FNO_3$: C, 68.56; H, 5.75; N, 4.44. Found: C, 68.69; H, 5.51; N, 4.31.

*4.1.1.7. Ethyl 1-(cyclopropylmethyl)-7-oxo-4,5,6,7-tetrahydro-1H-indole-2-carboxylate (**2h**).* This compound was obtained from reaction of compound **1** with (chloromethyl)cyclopropane after 5 h. Yellow oil; yield: 60 %; IR (cm^{-1}): 1715 (CO), 1664 (CO); ^{1}H NMR (DMSO-d_6, 200 MHz, ppm): 0.31–0.38 (m, 4H, 2 x CH_2), 1.14–1.32 (m, 4H, CH_3 and

CH), 1.91–2.03 (m, 2H, CH_2), 2.48 (t, 2H, $J = 6.1$ Hz, CH_2), 2.70 (t, 2H, $J = 6.1$ Hz, CH_2), 4.26 (q, 2H, $J = 7.1$ Hz, CH_2), 4.64 (d, 2H, $J = 7.1$ Hz, CH_2), 6.75 (s, 1H, H-3); ^{13}C NMR (DMSO-d_6, 50 MHz, ppm): 12.8 (2 x C), 14.0, 22.9, 24.1, 39.7, 49.1, 60.5, 114.7, 126.7, 129.4, 135.2, 160.5, 190.0. Anal calcd for $C_{15}H_{19}NO_3$: C, 68.94; H, 7.33; N, 5.36. Found: C, 69.08; H, 7.21; N, 5.24.

*4.1.2. Preparation of ethyl 3-chloro-1-(3-methylbenzyl)-7-oxo-4,5,6,7-tetrahydro-1H-indole-2-carboxylate **(2i)***

To a solution of **2a** (4.5 mmol) in acetic acid (12 mL) NCS (4.5 mmol) was added and the reaction mixture was stirred at 60 °C for 6 h. After cooling, the reaction mixture was poured onto crushed ice and the aqueous solution was extracted with ethyl acetate (3 x 50 mL). The organic layers were dried over Na_2SO_4 and the solvent removed under reduced pressure. The crude product was purified using a chromatography column (petroleum ether: ethyl acetate 95 : 5). Colourless oil; yield: 65 %; IR (cm^{-1}): 1710 (CO), 1657 (CO); ^{1}H NMR (DMSO-d_6, 200 MHz, ppm): 1.20 (t, 3H, $J = 7.1$ Hz, CH_3), 2.00–2.08 (m, 2H, CH_2), 2.24 (s, 3H, CH_3), 2.53 (t, 2H, $J = 6.0$ Hz, CH_2), 2.69 (t, 2H, $J = 6.0$ Hz, CH_2), 4.24 (q, 2H, $J = 7.1$ Hz, CH_2), 5.94 (s, 2H, CH_2), 6.67 (d, 1H, $J = 7.6$ Hz, H-4′), 6.82 (s, 1H, H-2′), 7.03 (d, 1H, $J = 7.5$ Hz, H-6′), 7.16 (t, 1H, $J = 7.6$ Hz, H-5'); ^{13}C NMR (DMSO-d_6, 50 MHz, ppm): 14.3, 21.4, 21.5, 23.6, 49.7, 61.6, 116.3, 123.4, 127.1, 124.3, 128.12, 128.2, 128.8, 133.7, 137.9, 138.6, 159.8, 190.3. Anal calcd for $C_{19}H_{20}ClNO_3$: C, 65.99; H, 5.83; N, 4.05. Found: C, 66.11; H, 5.98; N, 3.89.

*4.1.3. General procedure for the synthesis of 1-substituted 6-[(dimethylamino)methylidene]-1,4,5,6- tetrahydro-7H-indole-7-ones **(3a-i)***

To a solution of **2a-i** (1.3 mmol) in anhydrous toluene (3 mL) TBDMAM (0.80 mL, 3.9 mmol) was added and the reaction mixture was heated at reflux up to completeness (TLC). After cooling, the solvent was removed under reduced pressure. The crude product was crystallized from diethyl ether or use in the next step without further purification.

*4.1.3.1. Ethyl 6-[(dimethylamino)methylidene]-1-(3-methylbenzyl)-7-oxo-4,5,6,7-tetrahydro-1H-indole-2-carboxylate **(3a)***. This compound was obtained by reaction of **2a** after 3 h. Dark brown solid; yield: 85 %; m.p.: 118–119 °C; IR (cm^{-1}): 1704 (CO), 1634 (CO); ^{1}H NMR (DMSO-d_6, 200 MHz, ppm):1.21 (t, 3H, $J = 7.1$ Hz, CH_3), 2.22 (s, 3H, CH_3), 2.61 (t, 2H, $J = 6.6$ Hz, CH_2), 2.85 (t, 2H, $J = 6.6$ Hz, CH_2), 3.07 (s, 6H, 2 x CH_3), 4.17 (q, 2H, $J = 7.1$ Hz, CH_2), 6.11 (s, 2H, CH_2), 6.65 (d, 1H, $J = 7.5$ Hz, Ar), 6.78–6.80 (m, 2H, H-3 and Ar), 6.98 (d, 1H, $J = 7.5$ Hz, Ar), 7.15 (t, 1H, $J = 7.5$ Hz, Ar), 7.38 (s, 1H, CH); ^{13}C NMR (DMSO-d_6, 50 MHz, ppm):14.1, 21.1, 22.4, 24.0, 43.3 (2 x C), 48.1, 59.9, 103.5, 114.7, 123.0, 125.0, 126.6, 127.2, 128.1, 130.1, 131.9, 137.1, 139.7, 149.1, 160.3, 178.6. Anal calcd for $C_{22}H_{26}N_2O_3$: C, 72.11; H, 7.15; N, 7.64. Found: C, 71.97; H, 7.29; N, 7.76.

*4.1.3.2. Ethyl 6-[(dimethylamino)methylidene]-1-[(3,4-dimethylphenyl)methyl]-7-oxo-4,5,6,7-tetrahydro-1H-indole-2-carboxylate **(3c)***. This compound was obtained by reaction of **2c** after 3 h and used in the next step without further purification.

*4.1.3.3. Ethyl 6-[(dimethylamino)methylidene]-1-(3-bromobenzyl))-7-oxo-4,5,6,7-tetrahydro-1H-indole-2-carboxylate **(3d)***. This compound was obtained by reaction of **2d** after 4 h. Dark brown oil; yield: 75 %; IR (cm^{-1}): 1707 (CO), 1636 (CO); ^{1}H NMR (DMSO-d_6, 200 MHz, ppm):1.21 (t, 3H, $J = 7.1$ Hz, CH_3), 2.62 (t, 2H, $J = 6.6$ Hz, CH_2), 2.85 (t, 2H, $J = 6.6$ Hz, CH_2), 3.07 (s, 6H, 2 x CH_3), 4.17 (q, 2H, $J = 7.1$ Hz, CH_2), 6.10 (s, 2H, CH_2), 6.80 (s, 1H, H-3), 6.93 (d, 1H, $J = 7.8$ Hz, Ar), 7.15 (s, 1H, Ar), 7.23 (d, 1H, $J = 7.8$ Hz, Ar), 7.38–7.40 (m, 2H, Ar and CH); ^{13}C NMR (DMSO-d_6, 50 MHz, ppm): 14.5, 22.8, 24.4, 43.8 (2 x C), 48.9, 60.5, 103.8, 115.4, 122.0, 125.3, 125.6, 129.3, 129.9, 130.7, 130.9, 132.3, 143.0, 149.8, 160.7, 179.0. Anal calcd for $C_{21}H_{23}BrN_2O_3$: C, 58.48; H, 5.37; N, 6.49. Found: C, 58.61; H, 5.49; N, 6.27.

*4.1.3.4. Ethyl 1-[(4-bromophenyl)methyl]-6-[(dimethylamino)methylidene]-7-oxo-4,5,6,7-tetrahydro-1H-indole-2-carboxylate **(3e)***. This compound was obtained by reaction of **2e** after 16 h. Light brown solid; yield: 93 %; m.p.: 142–143 °C; IR (cm^{-1}): 1710 (CO), 1635 (CO); ^{1}H NMR (DMSO-d_6, 200 MHz, ppm): 1.20 (t, 3H, $J = 7.1$ Hz, CH_3), 2.60 (t, 2H, $J = 6.7$ Hz, CH_2), 2.84 (t, 2H, $J = 6.7$ Hz, CH_2), 3.06 (s, 6H, 2 x CH_3), 4.16 (q, 2H, $J = 7.1$ Hz, CH_2), 6.07 (s, 2H, CH_2), 6.78 (s, 1H, H-3), 6.91 (d, 2H, $J = 8.3$ Hz, H-2′ and H-6′), 7.38 (s, 1H, CH), 7.45 (d, 2H, $J = 8.3$ Hz, H-3′ and H-5'); ^{13}C NMR (DMSO-d_6, 50 MHz, ppm): 14.1, 22.3, 24.0, 43.3 (2 x C), 47.8, 60.0, 103.4, 114.9, 119.6, 124.8, 128.4 (2 x C), 130.2, 131.1 (2 x C), 131.9, 139.1, 149.3, 160.3, 178.5. Anal calcd for $C_{21}H_{23}BrN_2O_3$: C, 58.48; H, 5.37; N, 6.49. Found: C, 58.61; H, 5.29; N, 6.33.

*4.1.3.5. Ethyl 6-[(dimethylamino)methylidene]-1-(3-bromo-4-fluorobenzyl))-7-oxo-4,5,6,7-tetrahydro-1H-indole-2-carboxylate **(3f)***. This compound was obtained by reaction of **2f** after 2 h and used in the next step without further purification.

*4.1.3.6. Ethyl 6-[(dimethylamino)methylidene]-1-[(4-fluorophenyl)methyl]-7-oxo-4,5,6,7-tetrahydro-1H-indole-2-carboxylate **(3g)***. This compound was obtained by reaction of **2g** after 5 h and used in the next step without further purification.

*4.1.3.7. Ethyl 1-(cyclopropylmethyl)-6-[(dimethylamino)methylidene]-7-oxo-4,5,6,7-tetrahydro-1H-indole-2-carboxylate **(3h)***. This compound was obtained by reaction of **2h** after 2 h and used in the next step without further purification.

*4.1.3.8. Ethyl 6-[(dimethylamino)methylidene]-3-chloro-1-(3-methylbenzyl)-7-oxo-4,5,6,7-tetrahydro-1H-indole-2-carboxylate **(3i)***. This compound was obtained by reaction of **2i** after 16 h and used in the next step without further purification.

*4.1.4. General procedure for the synthesis of ethyl 2-amino-9-substituted-6,9-dihydro-5H-pyrrolo [3,2-h]quinazoline-8-carboxylate **(SVQ1,3,5,7,17,23,27,31,35)***

To a suspension of MeONa (1.08 g, 20 mmol) in anhydrous ethanol (15 mL), guanidine nitrate (1.22 g, 10 mmol) and a solution of the suitable enaminoketons **3** (2 mmol) in anhydrous ethanol (20 mL) were added. The reaction mixture was heated at reflux up to completeness. Then the reaction mixture was poured onto crushed ice and the precipitate was filtered off, dried and purified using a chromatography column (dichloromethane: ethyl acetate 9 : 1).

*4.1.4.1. Ethyl 2-amino-9-[(4-methylphenyl)methyl]-6,9-dihydro-5H-pyrrolo [3,2-h]quinazoline-8-carboxylate **(SVQ1)***. This compound was obtained by reaction of **3b** after 6 h. White solid; yield: 85 %; m.p.: 171–172 °C; IR (cm^{-1}): 3416–3313 (NH_2), 1699 (CO); ^{1}H NMR (DMSO-d_6, 200 MHz, ppm): 1.23 (t, 3H, $J = 7.1$ Hz, CH_3), 2.20 (s, 3H, CH_3), 2.64 (s, 4H, 2 x CH_2), 4.18 (q, 2H, $J = 7.1$ Hz, CH_2), 6.37–6.43 (m, 4H, CH_2 and NH_2), 6.84 (s, 1H, H-7), 6.90 (d, 2H, $J = 8.0$ Hz, H-3′and H-5′), 7.03 (d, 2H, $J = 8.0$ Hz, H-2′and H-6′), 8.05 (s, 1H, H-4); ^{13}C NMR (DMSO-d_6, 50 MHz, ppm): 14.1, 20.5, 21.1, 24.3, 48.1, 59.8, 115.8, 116.5, 125.0, 126.3 (2 x C), 127.3, 128.8 (2 x C), 130.5, 135.8, 136.5, 154.7, 156.0, 160.2, 161.9. Anal calcd for $C_{21}H_{22}N_4O_2$: C, 69.59; H, 6.12; N, 15.46. Found: C, 69.78; H, 6.01; N, 15.32.

*4.1.4.2. Ethyl 2-amino-9-[(3-methylphenyl)methyl]-6,9-dihydro-5H-pyrrolo [3,2-h]quinazoline-8-carboxylate **(SVQ3)***. This compound was obtained by reaction of **3a** after 3 h. Brown solid; yield: 92 %; m.p.: 149–150 °C; IR (cm^{-1}): 3461–3416 (NH_2), 1697 (CO); ^{1}H NMR (DMSO-d_6, 200 MHz, ppm): 1.23 (t, 3H, $J = 7.0$ Hz, CH_3), 2.20 (s, 3H,

CH_3), 2.65 (s, 4H, 2 x CH_2), 4.18 (q, 2H, $J = 7.0$ Hz, CH_2), 6.42 (s, 4H, CH_2 and NH_2), 6.69–7.14 (m, 5H, H-7, H-2′, H-4′, H-5′ and H-6'), 8.05 (s, 1H, H-4); ^{13}C NMR (DMSO-d_6, 50 MHz, ppm): 14.1, 21.0, 21.1, 24.3, 48.4, 59.8, 115.9, 116.5, 123.2, 125.0, 126.9, 127.2, 127.3, 128.2, 130.7, 137.1, 139.5, 154.7, 156.2, 160.2, 162.0. Anal calcd for $C_{21}H_{22}N_4O_2$: C, 69.59; H, 6.12; N, 15.46. Found: C, 69.46; H, 6.24; N, 15.32.

*4.1.4.3. Ethyl 2-amino-9-[(3,4-dimethylphenyl)methyl]-6,9-dihydro-5H-pyrrolo [3,2-h]quinazoline-8-carboxylate (**SVQ5**).* This compound was obtained by reaction of **3c** after 2 h. White solid; yield: 82 %; m.p.: 157–158 °C; IR (cm^{-1}): 3513–3410 (NH_2), 1703 (CO); ^{1}H NMR (DMSO-d_6, 200 MHz, ppm): 1.24 (t, 3H, $J = 7.0$ Hz, CH_3), 2.10 (s, 6H, 2 x CH_3), 2.64 (s, 4H, 2 x CH_2), 4.19 (q, 2H, $J = 7.0$ Hz, CH_2), 6.38–6.42 (m, 4H, CH_2 e NH_2), 6.65 (d, 1H, $J = 7.6$ Hz, H-6′), 6.84 (s, 1H, H-7 and H-2′), 6.96 (d, 1H, $J = 6.5$ Hz, H-5'), 8.05 (s, 1H, H-4); ^{13}C NMR (DMSO-d_6, 50 MHz, ppm): 14.1, 18.9, 19.5, 20.1, 24.3, 48.1, 59.8, 115.8, 116.4, 123.6, 124.9, 127.1, 127.6, 129.3, 130.6, 134.5, 135.7, 136.8, 154.6, 156.1, 160.2, 161.9. Anal calcd for $C_{22}H_{24}N_4O_2$: C, 70.19; H, 6.43; N, 14.88. Found: C, 70.07; H, 6.56; N, 15.01.

*4.1.4.4. Ethyl 2-amino-9-[(4-bromophenyl)methyl]-6,9-dihydro-5H-pyrrolo [3,2-h]quinazoline-8-carboxylate (**SVQ7**).* This compound was obtained by reaction of **3e** after 5 h. White solid; yield: 74 %; m.p.: 168–169 °C; IR (cm^{-1}): 3513–3410 (NH_2), 1697 (CO); ^{1}H NMR (DMSO-d_6, 200 MHz, ppm): 1.23 (t, 3H, $J = 7.0$ Hz, CH_3), 2.66 (s, 4H, 2 x CH_2), 4.18 (q, 2H, $J = 7.0$ Hz, CH_2), 6.35–6.42 (m, 4H, CH_2 and NH_2), 6.87 (s, 1H, H-7), 6.98 (d, 2H, $J = 8.3$ Hz, H-2′ and H-6′), 7.44 (d, 2H, $J = 8.3$ Hz, H-3′ and H-5'), 8.06 (s, 1H, H-4); ^{13}C NMR (DMSO-d_6, 50 MHz, ppm): 14.1, 21.1, 24.3, 48.1, 59.9, 115.9, 116.5, 119.8, 124.8, 127.4, 128.6 (2 x C), 130.4, 131.1 (2 x C), 138.9, 154.4, 156.2, 160.1, 161.9. Anal calcd for $C_{20}H_{19}BrN_4O_2$: C, 56.22; H, 4.48; N, 13.11. Found: C, 56.10; H, 4.51; N, 12.98.

*4.1.4.5. Ethyl 2-amino-7-chloro-9-(3-methylbenzyl)-6,9-dihydro-5H-pyrrolo [3,2-h]quinazoline-8-carboxylate (**SVQ17**).* This compound was obtained by reaction of **3i** after 8 h. Dark brown solid; yield: 94 %; m.p.: 138–139 °C; IR (cm^{-1}): 3472–3406 (NH_2), 1699 (CO); ^{1}H NMR (DMSO-d_6, 200 MHz, ppm): 1.23 (t, 3H, $J = 7.0$ Hz, CH_3), 2.22 (s, 3H, CH_3), 2.65–2.70 (m, 4H, 2 x CH_2), 4.23 (q, 2H, $J = 7.0$ Hz, CH_2), 6.43 (s, 2H, NH_2), 6.51 (s, 2H, CH_2), 6.73 (s, 1H, Ar), 6.89 (s, 1H, Ar), 6.99–7.02 (m, 1H, Ar), 7.11–7.16 (m, 1H, Ar), 8.13 (s, 1H, H-4); ^{13}C NMR (DMSO-d_6, 50 MHz, ppm): 14.5, 19.5, 21.5, 24.1, 49.6, 60.9, 117.0, 117.4, 122.1, 123.7, 126.0, 127.5, 128.1, 128.8, 129.6, 137.8, 139.4, 154.4, 157.3, 159.9, 162.5. Anal calcd for $C_{21}H_{21}ClN_4O_2$: C, 63.55; H, 5.33; N, 14.12. Found: C, 63.69; H, 5.21; N, 14.02.

*4.1.4.6. Ethyl 2-amino-9-(3-bromobenzyl)-6,9-dihydro-5H-pyrrolo [3,2-h]quinazoline-8-carboxylate (**SVQ23**).* This compound was obtained by reaction of **3d** after 24 h. White solid; yield: 70 %; m.p.: 167–168 °C; IR (cm^{-1}): 3509–3415 (NH_2), 1696 (CO); ^{1}H NMR (DMSO-d_6, 200 MHz, ppm): 1.23 (t, 3H, $J = 7.1$ Hz, CH_3), 2.67 (s, 4H, 2 x CH_2), 4.20 (q, 2H, $J = 7.1$ Hz, CH_2), 6.44 (s, 4H, CH_2 and NH_2), 6.89 (s, 1H, H-7), 6.97 (d, 1H, $J = 7.7$ Hz, Ar), 7.19–7.26 (m, 2H, Ar), 7.37 (d, 1H, $J = 7.0$ Hz, Ar), 8.06 (s, 1H, H-4); ^{13}C NMR (DMSO-d_6, 50 MHz, ppm): 14.6, 21.5, 24.7, 48.5, 60.4, 116.5, 116.9, 122.0, 125.3, 125.8, 127.8, 129.7, 130.1, 130.9, 131.0, 142.7, 154.9, 156.8, 160.7, 162.4. Anal calcd for $C_{20}H_{19}BrN_4O_2$: C, 56.22; H, 4.48; N, 13.11. Found: C, 56.38; H, 4.67; N, 12.76.

*4.1.4.7. Ethyl 2-amino-9-(3-bromo-4-fluorobenzyl)-6,9-dihydro-5H-pyrrolo [3,2-h]quinazoline-8-carboxylate (**SVQ27**).* This compound was obtained by reaction of **3f** after 6 h. White solid; yield: 60 %; m.p.: 165–166 °C; IR (cm^{-1}): 3522–3424 (NH_2), 1698 (CO); ^{1}H NMR (DMSO-d_6, 200 MHz, ppm): 1.24 (t, 3H, $J = 7.0$ Hz, CH_3), 2.66 (s, 4H, 2 x CH_2), 4.21 (q, 2H, $J = 7.0$ Hz, CH_2), 6.40 (s, 4H, CH_2 and NH_2), 6.87 (s, 1H, H-7), 7.05–7.07 (m, 1H, Ar), 7.25 (t, 1H, $J = 8.6$ Hz, Ar), 7.45 (d, 1H, $J = 6.3$ Hz, Ar), 8.06 (s, 1H, H-4); ^{13}C NMR (DMSO-d_6, 50 MHz, ppm): 14.6, 21.5, 24.7, 47.9, 60.5, 108.1 (d, $J_{C3'\text{-}F} = 20.3$ Hz), 116.5, 116.9, 117.0 (d, $J_{C5'\text{-}F} = 22.5$ Hz), 125.2, 127.8, 128.3, 130.7, 132.1, 138.0, 154.8, 156.8, 157.6 (d, $J_{C4'\text{-}F} = 242.3$ Hz), 160.7, 162.5. Anal calcd for $C_{20}H_{18}BrFN_4O_2$: C, 53.95; H, 4.07; N, 12.58. Found: C, 54.07; H, 3.95; N, 12.71.

*4.1.4.8. Ethyl 2-amino-9-(cyclopropylmethyl)-6,9-dihydro-5H-pyrrolo [3,2-h]quinazoline-8-carboxylate (**SVQ31**).* This compound was obtained by reaction of **3h** after 6 h. Grey solid; yield: 74 %; m.p.: 123–124 °C; IR (cm^{-1}): 3497–3389 (NH_2), 1695 (CO); ^{1}H NMR (DMSO-d_6, 200 MHz, ppm): 0.30–0.41 (m, 4H, 2 x CH_2), 1.24–1.31 (m, 4H, CH_3 and CH), 2.65 (s, 4H, 2 x CH_2), 4.24 (q, 2H, $J = 7.0$ Hz, CH_2), 5.07 (d, 2H, $J = 7.1$ Hz, CH_2), 6.47 (s, 2H, NH_2), 6.79 (s, 1H, H-7), 8.07 (s, 1H, H-4); ^{13}C NMR (DMSO-d_6, 50 MHz, ppm): 3.6 (2 x C), 14.7, 21.5, 21.6, 24.9, 49.2, 60.3, 115.9, 117.1, 125.3, 127.6, 130.5, 155.7, 155.9, 160.9, 162.0. Anal calcd for $C_{17}H_{20}N_4O_2$: C, 65.37; H, 6.45; N, 17.94. Found: C, 65.53; H, 6.31; N, 18.07.

*4.1.4.9. Ethyl 2-amino-9-(4-fluorobenzyl)-6,9-dihydro-5H-pyrrolo [3,2-h] quinazoline-8-carboxylate (**SVQ35**).* This compound was obtained by reaction of **3g** after 16 h. Light brown solid; yield: 65 %; m.p.: 130–131 °C; IR (cm^{-1}): 3514–3416 (NH_2), 1697 (CO); ^{1}H NMR (DMSO-d_6, 200 MHz, ppm): 1.24 (t, 3H, $J = 7.0$ Hz, CH_3), 2.65 (s, 4H, 2 x CH_2), 4.21 (q, 2H, $J = 7.0$ Hz, CH_2), 6.43 (s, 4H, CH_2 and NH_2), 6.86 (s, 1H, H-7), 7.08–7.10 (m, 4H, H-2′, H-3′, H-5′and H-6′), 8.06 (s, 1H, H-4); ^{13}C NMR (DMSO-d_6, 50 MHz, ppm): 14.6, 21.6, 24.8, 48.3, 60.4, 115.4 (2 x d, $J_{C3'\text{-}F} = 21.0$ Hz), 116.4, 117.0, 125.4, 127.8, 129.0 (2 x d, $J_{C2'\text{-}F} = 8.3$ Hz), 130.9, 136.1, 155.0, 156.7, 160.7, 161.5 (d, $J_{C4'\text{-}F} = 240.8$ Hz), 162.5. Anal calcd for $C_{20}H_{19}FN_4O_2$: C, 65.56; H, 5.23; N, 15.29. Found: C, 65.42; H, 5.12; N, 15.39.

*4.1.5. General procedure for the synthesis of 2-[(benzenesulfonyl)amino]-9substituted-6,9-dihydro-5H-pyrrolo [3,2-h]quinazoline-8-carboxylate (**SVQ9,11,13,15,18,25,29,33,37**)*

To a solution of suitable tricyclic compounds **SVQ** (0.67 mmol) in anhydrous pyridine (1.8 mL), benzensulfonyl chloride (0.17 mL, 1.3 mmol) was added and the reaction mixture was stirred at room temperature for 24 h. Then the reaction mixture was poured onto crushed ice and the precipitate was filtered off, dried and purified using a chromatography column (dichloromethane: ethyl acetate 95 : 5).

*4.1.5.1. Ethyl 2-[(benzenesulfonyl)amino]-9-[(4-methylphenyl)methyl]-6,9-dihydro-5H-pyrrolo [3,2-h]quinazoline-8-carboxylate (**SVQ9**).* This compound was obtained by reaction of **SVQ1**. White solid; yield: 82 %; m.p.: 218–219 °C; IR (cm^{-1}): 3422 (NH), 1709 (CO); ^{1}H NMR (DMSO-d_6, 200 MHz, ppm): 1.22 (t, 3H, $J = 7.1$ Hz, CH_3), 2.20 (s, 3H, CH_3), 2.69 (s, 4H, 2 x CH_2), 4.18 (q, 2H, $J = 7.1$ Hz, CH_2), 6.31 (s, 2H, CH_2), 6.77–6.84 (m, 3H, H-7, H-3′ and H-5′), 7.01 (d, 2H, $J = 7.9$ Hz, H-2′and H-6′), 7.48–7.56 (m, 3H, H-3″, H-4″ and H-5″), 7.86 (d, 2H, $J = 7.1$ Hz, H-2″ and H-6″), 8.26 (s, 1H, H-4), 11.82 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 50 MHz, ppm): 14.6, 20.0, 21.5, 24.8, 48.7, 60.5, 116.4, 117.3 (2 x C), 124.0, 125.7 (2 x C) 128.0 (2 x C), 128.1 (2 x C), 129.9 (2 x C), 131.0, 135.2, 136.4, 136.5, 155.3, 156.4, 160.0 (2 x C), 162.2. Anal calcd for $C_{27}H_{26}N_4O_4S$: C, 64.52; H, 5.21; N, 11.15. Found: C, 64.65; H, 5.09; N, 11.02.

*4.1.5.2. Ethyl 2-[(benzenesulfonyl)amino]-9-[(3-methylphenyl)methyl]-6,9-dihydro-5H-pyrrolo [3,2-h]quinazoline-8-carboxylate (**SVQ11**).* This compound was obtained by reaction of **SVQ3**. Yellow solid; yield: 92 %; m.p.: 201–202 °C; IR (cm^{-1}): 3399 (NH), 1709 (CO); ^{1}H NMR (DMSO-d_6, 200 MHz, ppm): 1.21 (t, 3H, $J = 7.0$ Hz, CH_3), 2.19 (s, 3H, CH_3), 2.69 (s, 4H, 2 x CH_2), 4.17 (q, 2H, $J = 7.0$ Hz, CH_2), 6.33 (s, 2H,

CH_2), 6.62 (d, 1H, J = 7.5 Hz, Ar), 6.78–6.85 (m, 2H, H-7 and Ar), 6.94–7.12 (m, 2H, Ar), 7.44–7.56 (m, 3H, H-3″, H-4″ and H-5″), 7.81 (d, 2H, J = 6.5 Hz, H-2″ and H-6″), 8.26 (s, 1H, H-4), 11.81 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 50 MHz, ppm): 13.0, 20.4, 20.0, 24.2, 48.4, 60.1, 115.9, 122.0, 122.8, 122.9, 126.6 (2 x C), 126.7, 127.3, 128.2, 128.9 (2 x C), 129.6, 132.6, 137.1, 139.4, 140.2, 140.8, 154.5, 155.1, 155.3, 160.0. Anal calcd for $C_{27}H_{26}N_4O_4S$: C, 64.52; H, 5.21; N, 11.15. Found: C, 64.40; H, 5.33; N, 11.02.

*4.1.5.3. Ethyl 2-[(benzenesulfonyl)amino]-9-[(3,4-dimethylphenyl)methyl]-6,9-dihydro-5H-pyrrolo [3,2-h]quinazoline-8-carboxylate (**SVQ13**).* This compound was obtained by reaction of **SVQ5**. Pale yellow solid; yield: 73 %; m.p.: 239–240 °C; IR (cm^{-1}): 3371 (NH), 1703 (CO); ^{1}H NMR (DMSO-d_6, 200 MHz, ppm): 1.22 (t, 3H, J = 7.1 Hz, CH_3), 2.09 (s, 3H, CH_3), 2.11 (s, 3H, CH_3), 2.68 (s, 4H, 2 x CH_2), 4.18 (q, 2H, J = 7.1 Hz, CH_2), 6.29 (s, 2H, CH_2), 6.53 (d, 1H, J = 7.5 Hz, H-6′), 6.75 (s, 1H, H-7), 6.84 (s, 1H, H-2′), 6.94 (d, 1H, J = 7.5 Hz, H-5″), 7.44–7.60 (m, 3H, H-3″, H-4″ and H-5″), 7.86 (d, 2H, J = 6.8 Hz, H-2″ and H-6″), 8.26 (s, 1H, H-4), 11.81 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 50 MHz, ppm): 14.1, 18.9, 19.5, 20.4, 24.3, 48.0, 60.1, 115.9, 121.9, 122.0, 123.2, 126.7 (2 x C), 127.4, 128.9 (2 x C), 129.3, 129.6, 132.6, 134.5, 135.7, 136.8, 140.8, 153.1, 154.5, 155.1, 155.3, 160.0. Anal calcd for $C_{28}H_{28}N_4O_4S$: C, 65.10; H, 5.46; N, 10.85. Found: C, 65.23; H, 5.32; N, 10.80.

*4.1.5.4. Ethyl 2-[(benzenesulfonyl)amino]-9-[(4-bromophenyl)methyl]-6,9-dihydro-5H-pyrrolo [3,2-h]quinazoline-8-carboxylate (**SVQ15**).* This compound was obtained by reaction of **SVQ7**. White solid; yield: 79 %; m.p.: 208–209 °C; IR (cm^{-1}): 3399 (NH), 1709 (CO); ^{1}H NMR (DMSO-d_6, 200 MHz, ppm): 1.21 (t, 3H, J = 7.0 Hz, CH_3), 2.70 (s, 4H, 2 x CH_2), 4.18 (q, 2H, J = 7.0 Hz, CH_2), 6.31 (s, 2H, CH_2), 6.77–6.89 (m, 3H, H-7 and Ar), 7.41–7.52 (m, 5H, Ar), 7.83 (d, 2H, J = 6.0 Hz, Ar), 8.27 (s, 1H, H-4), 11.82 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 50 MHz, ppm): 14.0, 20.4, 24.2, 47.9, 60.2, 116.1, 119.8, 122.0, 122.1, 126.4, 128.6 (2 x C), 128.2 (2 x C), 128.9 (2 x C), 129.4, 131.1 (2 x C), 132.6, 138.8, 140.7, 149.0, 155.1 (2 x C), 159.8. Anal calcd for $C_{26}H_{23}BrN_4O_4S$: C, 55.03; H, 4.09; N, 9.87. Found: C, 55.26; H, 3.98; N, 9.76.

*4.1.5.5. Ethyl 2-[(benzenesulfonyl)amino]-7-chloro-9-[(3-methylphenyl) methyl]-6,9-dihydro-5H-pyrrolo [3,2-h]quinazoline-8-carboxylate (**SVQ18**).* This compound was obtained by reaction of **SVQ17**. Yellow solid; yield: 87 %; m.p.: 217–218 °C; IR (cm^{-1}): 3376 (NH), 1710 (CO); ^{1}H NMR (DMSO-d_6, 200 MHz, ppm): 1.19 (t, 3H, J = 7.1 Hz, CH_3), 2.19 (s, 3H, CH_3), 2.62 (t, 2H, J = 7.6 Hz, CH_2), 2.77 (t, 2H, J = 7.6 Hz, CH_2), 4.19 (q, 2H, J = 7.1 Hz, CH_2), 6.28 (s, 2H, CH_2), 6.59 (d, 1H, J = 7.7 Hz, Ar), 6.77 (s, 1H, Ar), 6.98 (d, 1H, J = 7.5 Hz, Ar), 7.09 (t, 1H, J = 7.6 Hz, Ar), 7.45 (t, 2H, J = 7.4 Hz, H-3″ and H-5″), 7.53 (t, 1H, J = 7.4 Hz, H-4″), 7.82 (d, 2H, J = 7.4 Hz, H-2″ and H-6″), 8.32 (s, 1H, H-4), 11.77 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 50 MHz, ppm): 14.4, 18.9, 21.5, 24.0, 49.6, 61.1, 117.4, 123.4, 123.5, 125.9, 127.2, 127.3, 127.5, 128.0, 128.1, 128.6, 128.8, 129.4, 133.2, 137.8, 139.3, 141.0, 154.8, 155.6, 159.6. Anal calcd for $C_{27}H_{25}ClN_4O_4S$: C, 60.39; H, 4.69; N, 10.43. Found: C, 60.25; H, 4.57; N, 10.54.

*4.1.5.6. Ethyl 2-[(benzenesulfonyl)amino]-9-(3-bromobenzyl)-6,9-dihydro-5H-pyrrolo [3,2-h]quinazoline-8-carboxylate (**SVQ25**).* This compound was obtained by reaction of **SVQ23**. White solid; yield: 70 %; m.p.: 237–238 °C; IR (cm^{-1}): 3401 (NH), 1702 (CO); ^{1}H NMR (DMSO-d_6, 200 MHz, ppm): 1.20 (t, 3H, J = 7.0 Hz, CH_3), 2.69–2.77 (m, 4H, 2 x CH_2), 4.18 (q, 2H, J = 7.0 Hz, CH_2), 6.36 (s, 2H, CH_2), 6.81–6.87 (m, 2H, H-7 and Ar), 7.16–7.20 (m, 2H, Ar), 7.37 (d, 1H, J = 7.5 Hz, Ar), 7.47–7.55 (m, 3H, Ar), 7.86 (d, 2H, J = 7.5 Hz, Ar), 8.26 (s, 1H, H-4), 11.85 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 50 MHz, ppm): 14.5, 20.9, 24.7, 48.4, 60.7, 116.6, 122.0, 122.5, 125.0, 125.4, 126.9, 127.2 (2 x C), 129.5 (2 x C), 129.6, 129.9, 130.1, 131.0, 133.1, 141.2, 142.6, 148.7, 155.6, 160.4. Anal calcd for $C_{26}H_{23}BrN_4O_4S$: C, 55.03; H, 4.09; N, 9.87. Found: C, 54.93; H, 4.19; N, 9.99.

*4.1.5.7. Ethyl 2-[(benzenesulfonyl)amino]-9-(3-bromo-4-fluorobenzyl)-6,9-dihydro-5H-pyrrolo [3,2-h]quinazoline-8-carboxylate (**SVQ29**).* This compound was obtained by reaction of **SVQ27**. White solid; yield: 89 %; m.p.: 237–238 °C; IR (cm^{-1}): 3380 (NH), 1712 (CO); ^{1}H NMR (DMSO-d_6, 200 MHz, ppm): 1.22 (t, 3H, J = 7.0 Hz, CH_3), 2.68–2.70 (m, 4H, 2 x CH_2), 4.19 (q, 2H, J = 7.0 Hz, CH_2), 6.31 (s, 2H, CH_2), 6.87 (s, 2H, H-7 and Ar), 7.23 (t, 1H, J = 8.6 Hz, Ar), 7.37 (d, 1H, J = 6.2 Hz, Ar), 7.47–7.56 (m, 3H, H-3″, H-4″ and H-5″), 7.86 (d, 2H, J = 7.2 Hz, H-2″ and H-6″), 8.27 (s, 1H, H-4), 11.86 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 50 MHz, ppm): 14.5, 20.9, 24.6, 47.8, 60.7, 108.1 (d, $J_{C3'\text{-}F}$ = 21.0 Hz), 116.6, 117.1 (d, $J_{C5'\text{-}F}$ = 21.8 Hz), 122.5, 126.8, 127.1 (2 x C), 127.8, 127.9, 129.4 (2 x C), 129.6, 129.8, 131.9, 133.1, 137.8, 137.9, 141.2, 155.6, 157.6 (d, $J_{C4'\text{-}F}$ = 242.3 Hz), 160.4. Anal calcd for $C_{26}H_{22}BrFN_4O_4S$: C, 53.34; H, 3.79; N, 9.57. Found: C, 53.26; H, 3.64; N, 9.71.

*4.1.5.8. Ethyl 2-[(benzenesulfonyl)amino]-9-(cyclopropylmethyl)-6,9-dihydro-5H-pyrrolo [3,2-h]quinazoline-8-carboxylate (**SVQ33**).* This compound was obtained by reaction of **SVQ31**. Off-white solid; yield: 74 %; m.p.: 234–235 °C; IR (cm^{-1}): 3374 (NH), 1697 (CO); ^{1}H NMR (DMSO-d_6, 200 MHz, ppm): 0.27–0.38 (m, 4H, 2 x CH_2), 1.10 (s, 1H, CH), 1.29 (t, 3H, J = 7.0 Hz, CH_3), 2.63–2.73 (m, 4H, 2 x CH_2), 4.26 (q, 2H, J = 7.0 Hz, CH_2), 4.95 (d, 2H, J = 7.1 Hz, CH_2), 6.78 (s, 1H, H-7), 7.59 (s, 3H, H-3″, H-4″ and H-5″), 7.92 (s, 2H, J = 7.1 Hz, H-2″ and H-6″), 8.27 (s, 1H, H-4), 11.69 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 50 MHz, ppm): 3.6 (2 x C), 13.9, 14.6, 21.0, 24.8, 49.2, 60.6, 116.0, 122.3, 125.9, 126.7, 127.1 (2 x C), 129.2, 129.5 (2 x C), 129.7, 133.1, 141.6, 155.6, 156.1, 160.7. Anal calcd for $C_{23}H_{24}N_4O_4S$: C, 61.05; H, 5.35; N, 12.38. Found: C, 60.93; H, 5.16; N, 12.52.

*4.1.5.9. Ethyl 2-[(benzenesulfonyl)amino]-9-(4-fluorobenzyl)-6,9-dihydro-5H-pyrrolo [3,2-h]quinazoline-8-carboxylate (**SVQ37**).* This compound was obtained by reaction of **SVQ35**. White solid; yield: 79 %; m.p.: 220–221 °C; IR (cm^{-1}): 3398 (NH), 1709 (CO); ^{1}H NMR (DMSO-d_6, 200 MHz, ppm): 1.22 (t, 3H, J = 6.9 Hz, CH_3), 2.68–2.71 (m, 4H, 2 x CH_2), 4.19 (q, 2H, J = 6.9 Hz, CH_2), 6.32 (s, 2H, CH_2), 6.86 (s, 1H, H-7), 6.97–7.09 (m, 4H, H-2′, H-3′, H-5′ and H-6′), 7.50–7.59 (m, 3H, H-3″, H-4″ and H-5″), 7.87 (d, 1H, J = 7.1 Hz, H-2″ and H-6″), 8.28 (s, 1H, H-4), 11.83 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 50 MHz, ppm): 14.5. 20.9, 24.7, 40.8, 48.2, 60.6, 115.5 (d, $J_{C3'\text{-}F}$ = 21.0 Hz), 116.6, 122.5, 127.0, 127.1 (2 x C), 128.7 (d, $J_{C2'\text{-}F}$ = 8.3 Hz), 129.4 (2 x C), 129.6, 129.9, 133.1, 135.9, 136.0, 141.3, 155.6, 160.5, 161.5 (d, $J_{C4'\text{-}F}$ = 240.8 Hz). Anal calcd for $C_{26}H_{23}FN_4O_4S$: C, 61.65; H, 4.58; N, 11.06. Found: C, 61.78; H, 4.44; N, 10.91.

*4.1.6. General procedure for the preparation of propan-2-yl 2-amino-9-substituted-6,9-dihydro-5H-pyrrolo [3,2-h]quinazoline-8-carboxylate (**SVQ19,21**)*

To a solution of KOH (0.5 mmol) in EtOH (5 mL), a solution of **SVQ3** or **SVQ11** (0.1 mmol) in EtOH (8 mL) was added and the reaction mixture was heated at reflux for 5 h (**SVQ3**) and 8 h (**SVQ11**). After cooling, the solvent was removed under reduced pressure. The residue was added of water and the solution was acidified with HCl 6 M. Then the formed precipitate was filtered off and dried to give the crude acid derivative which was taken up immediately onto next step.

To a solution of the above acid derivatives (5.3 mmol) in 2-propanol (50 mL), DMAP (2.59 g, 21.2 mmol) and EDC (1.65 g, 10.6 mmol) were added and the reaction mixture was stirred at room temperature for 16 h. Then the solvent was removed under reduced pressure and water and crushed ice were added. The precipitate was filtered off, dried and recrystallised from diethyl ether.

*4.1.6.1. Propan-2-yl 2-amino-9-(3-methylbenzyl)-6,9-dihydro-5H-pyrrolo [3,2-h]quinazoline-8-carboxylate (**SVQ19**).* This compound was

obtained by reaction of **SVQ3**. Brown solid; yield: 89 %; m.p.: 154–155 °C; IR (cm^{-1}): 3432–3395 (NH_2), 1698 (CO); ^1H NMR (DMSO-d_6, 200 MHz, ppm): 1.22 (d, 6H, $J = 4.5$ Hz, 2 x CH_3), 2.21 (s, 3H, CH_3), 2.65 (s, 4H, 2 x CH_2), 5.02 (s, 1H, CH), 6.42 (s, 4H, CH_2 and NH_2), 6.72–7.11 (m, 5H, H-7, H-2′, H-4′, H-5′ and H-6'), 8.06 (s, 1H, H-4); ^{13}C NMR (DMSO-d_6, 50 MHz, ppm): 21.5, 21.6, 22.1 (2 x C), 24.9, 48.9, 67.7, 116.3, 117.0, 123.7, 126.0, 127.5, 127.6, 127.8, 128.6, 130.9, 137.6, 140.0, 155.2, 156.6, 160.3, 162.5. Anal calcd for $C_{22}H_{24}N_4O_2$: C, 70.19; H, 6.43; N, 14.88. Found: C, 69.98; H, 6.32; N, 15.02.

*4.1.6.2. Propan-2-yl 2-[(benzenesulfonyl)amino]-9-[(3-methylphenyl) methyl]-6,9-dihydro-5H-pyrrolo [3,2-h]quinazoline-8-carboxylate (**SVQ21**).* This compound was obtained by reaction of **SVQ11**. White solid; yield: 75 %; m.p.: 205–206 °C; IR (cm^{-1}): 3375 (NH), 1705 (CO); ^1H NMR (DMSO-d_6, 200 MHz, ppm): 1.20 (d, 6H, $J = 4.2$ Hz, 2 x CH_3), 2.20 (s, 3H, CH_3), 2.67–2.70 (s, 4H, 2 x CH_2), 4.93–5.09 (m, 1H, CH), 6.32 (s, 2H, CH_2), 6.61 (d, 1H, $J = 6.5$ Hz, Ar), 6.79–6.82 (m, 2H, H-7 and Ar), 6.98 (s, 1H, Ar), 7.06 – 7-11 (m, 1H, Ar), 7.48–7.55 (m, 3H, H-3″, H-4″ and H-5″), 7.87 (d, 2H, $J = 6.8$ Hz, H-2″ and H-6"), 8.26 (s, 1H, H-4), 11.79 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 50 MHz, ppm): 21.0, 21.5, 22.0 (2 x C), 24.8, 48.9, 68.1, 116.4, 122.4, 123.4, 127.2 (2 x C), 127.3, 127.7, 127.8, 128.6, 129.4 (2 x C), 129.5, 130.0, 133.1, 137.6, 140.0 (2 x C), 141.3, 155.6, 155.8, 160.1. Anal calcd for $C_{28}H_{28}N_4O_4S$: C, 65.10; H, 5.46; N, 10.85. Found: C, 65.26; H, 5.31; N, 10.99.

4.1.7. General procedure for the preparation of ethyl 2-substituted-7-bromo-9-[(4-methylphenyl)methyl]-6,9-dihydro-5H-pyrrolo [3,2-h] quinazoline-8-carboxylate
(SVQ2,4,6,8,10,12,14,16,20,22,24,26,28,30,32,34,36,38)

To a solution of suitable tricyclic compounds **SVQ** (0.22 mmol) in anhydrous DCM (20 ml), Br_2 (0.44 mmol, 0.02 mL) was added at 0 °C and the reaction mixture was stirred at room temperature for 24 h. Then the reaction mixture was evaporated under reduced pressure. The crude product was purified using a chromatography column (dichloromethane: ethyl acetate 95 : 5).

*4.1.7.1. Ethyl 2-amino-7-bromo-9-[(4-methylphenyl)methyl]-6,9-dihydro-5H-pyrrolo [3,2-h]quinazoline-8-carboxylate (**SVQ2**).* This compound was obtained by reaction of **SVQ1**. White solid; yield: 87 %; m.p.: 140–141 °C; IR (cm^{-1}): 3608–3586 (NH_2), 1664 (CO); ^1H NMR (DMSO-d_6, 200 MHz, ppm): 1.25 (t, 3H, $J = 7.1$ Hz, CH_3), 2.21 (s, 3H, CH_3), 2.60–2.70 (m, 4H, 2 x CH_2), 4.26 (q, 2H, $J = 7.1$ Hz, CH_2), 6.41 (s, 2H, CH_2) 6.51 (s, 2H, NH_2), 6.89 (d, 2H, $J = 8.0$ Hz, H-3′and H-5′), 7.05 (d, 2H, $J = 8.0$ Hz, H-2′and H-6′), 8.11 (s, 1H, H-4); ^{13}C NMR (DMSO-d_6, 50 MHz, ppm): 13.9, 20.4, 20.6, 23.6, 49.0, 60.6, 103.7, 116.5, 123.2, 126.3 (2 x C), 126.5, 127.8, 128.9 (2 x C), 129.7, 136.0, 153.9, 156.8, 159.5, 161.9. Anal calcd for $C_{21}H_{21}BrN_4O_2$: C, 57.15; H, 4.80; N, 12.70. Found: C, 57.02; H, 4.92; N, 12.57.

*4.1.7.2. Ethyl 2-amino-7-bromo-9-[(3-methylphenyl)methyl]-6,9-dihydro-5H-pyrrolo [3,2-h]quinazoline-8-carboxylate (**SVQ4**).* This compound was obtained by reaction of **SVQ3**. Yellow solid; yield: 60 %; m.p.: 119–120 °C; IR (cm^{-1}): 3307–3193 (NH_2), 1697 (CO); ^1H NMR (DMSO-d_6, 200 MHz, ppm): 1.23 (t, 3H, $J = 7.0$ Hz, CH_3), 2.22 (s, 3H, CH_3), 2.58–2.72 (m, 4H, 2 x CH_2), 4.22 (q, 2H, $J = 7.0$ Hz, CH_2), 6.43 (s, 2H, CH_2), 6.49 (s, 2H, NH_2), 6.71 (d, 1H, $J = 7.6$ Hz, Ar), 6.88 (s, 1H, H-2′), 7.01 (d, 1H, $J = 7.6$ Hz, Ar), 7.12 (t, 1H, $J = 7.6$ Hz, H-5'), 8.11 (s, 1H, H-4); ^{13}C NMR (DMSO-d_6, 50 MHz, ppm): 14.4, 20.8, 21.5, 24.1, 49.8, 61.0, 104.2, 107.0, 123.7, 123.8, 127.5, 128.1, 128.3, 128.8, 130.2, 137.8, 139.4, 154.5, 157.1, 160.0, 162.4. Anal calcd for $C_{21}H_{21}BrN_4O_2$: C, 57.15; H, 4.80; N, 12.70. Found: C, 57.02; H, 4.93; N, 12.62.

*4.1.7.3. Ethyl 2-amino-7-bromo-9-[(3,4-dimethylphenyl)methyl]-6,9-dihydro-5H-pyrrolo [3,2-h]quinazoline-8-carboxylate (**SVQ6**).* This compound was obtained by reaction of **SVQ5**. Yellow solid; yield: 78 %; m.p.: 244–245 °C; IR (cm^{-1}): 3296–3188 (NH_2), 1703 (CO); ^1H NMR (DMSO-d_6, 200 MHz, ppm): 1.25 (t, 3H, $J = 7.0$ Hz, CH_3), 2.12 (s, 6H, 2 x CH_3), 2.69–2.80 (m, 4H, 2 x CH_2), 4.27 (q, 2H, $J = 7.0$ Hz, CH_2), 6.26 (s, 2H, CH_2), 6.68 (d, 1H, $J = 6.5$ Hz, H-6′), 6.88 (s, 1H, H-2′), 6.99 (d, 1H, $J = 6.5$ Hz, H-5'), 8.04 (s, 2H, NH_2), 8.19 (s, 1H, H-4); ^{13}C NMR (DMSO-d_6, 50 MHz, ppm): 13.8, 18.9, 19.4, 20.1, 23.2, 49.3, 61.8, 103.2, 117.0, 123.7, 126.9, 127.4, 127.7, 129.5, 132.6, 135.1, 135.6, 136.1, 144.4, 155.4, 159.0, 159.3. Anal calcd for $C_{22}H_{23}BrN_4O_2$: C, 58.03; H, 5.09; N, 12.30. Found: C, 58.26; H, 4.97; N, 12.17.

*4.1.7.4. Ethyl 2-amino-7-bromo-9-[(4-bromophenyl)methyl]-6,9-dihydro-5H-pyrrolo [3,2-h]quinazoline-8-carboxylate (**SVQ8**).* This compound was obtained by reaction of **SVQ7**. Brown solid; yield: 69 %; m.p.: 174–175 °C; I IR (cm^{-1}): 3519–3416 (NH_2), 1703 (CO); ^1H NMR (DMSO-d_6, 200 MHz, ppm): 1.23 (t, 3H, $J = 7.0$ Hz, CH_3), 2.66 (s, 4H, 2 x CH_2), 4.20 (q, 2H, $J = 7.0$ Hz, CH_2), 6.48 (4H, s, CH_2 and NH_2), 6.99 (d, 2H, $J = 8.3$ Hz, H-2′ and H-6′), 7.45 (d, 2H, $J = 8.3$ Hz, H-3′ and H-5'), 8.04 (s, 1H, H-4); ^{13}C NMR (DMSO-d_6, 200 MHz, ppm): 13.9, 23.7, 27.6, 49.6, 61.3, 99.1, 112.5, 122.8, 127.4, 128.7 (2 x C), 129.8, 131.2 (2 x C), 132.5, 134.4, 144.9, 156.8, 159.9, 162.9. Anal calcd for $C_{20}H_{18}Br_2N_4O_2$: C, 47.46; H, 3.58; N, 11.07. Found: C, 47.62; H, 3.41; N, 10.94.

*4.1.7.5. ethyl 2-[(benzenesulfonyl)amino]-7-bromo-9-[(4-methylphenyl) methyl]-6,9-dihydro-5H-pyrrolo [3,2-h]quinazoline-8-carboxylate (**SVQ10**).* This compound was obtained by reaction of **SVQ9**. Pale yellow solid; yield: 80 %; m.p.: 221–222 °C; IR (cm^{-1}): 3604 (NH), 1703 (CO); ^1H NMR (DMSO-d_6, 200 MHz, ppm): 1.23 (t, 3H, $J = 7.1$ Hz, CH_3), 2.21 (s, 3H, CH_3), 2.51 (t, 2H, $J = 7.0$ Hz, CH_2), 2.78 (t, 2H, $J = 7.0$ Hz, CH_2), 4.21 (q, 2H, $J = 7.1$ Hz, CH_2), 6.30 (s, 2H, CH_2), 6.76 (d, 2H, $J = 7.8$ Hz, H-3′ and H-5′), 7.03 (d, 2H, $J = 7.8$ Hz, H-2′ and H-6'), 7.43–7.59 (m, 3H, H-3″, H-4″ and H-5″), 7.85 (d, 1H, $J = 7.1$ Hz, H-2″ and H-6"), 8.33 (s, 1H, H-4), 11.86 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 50 MHz, ppm): 13.9, 19.8, 20.6, 23.6, 49.0, 60.8, 103.7, 122.3, 124.7, 126.0 (2 x C), 126.7 (2 x C), 128.7 (2 x C), 128.9 (2 x C), 129.0, 129.3, 132.7, 135.9, 136.1, 140.5, 154.4. 155.2, 156.1, 159.3. Anal calcd for $C_{27}H_{25}BrN_4O_4S$: C, 55.77; H, 4.33; N, 9.64. Found: C, 55.64; H, 4.43; N, 9.77.

*4.1.7.6. Ethyl 2-[(benzenesulfonyl)amino]-7-bromo-9-[(3-methylphenyl) methyl]-6,9-dihydro-5H-pyrrolo [3,2-h]quinazoline-8-carboxylate (**SVQ12**).* This compound was obtained by reaction of **SVQ11**. White solid; yield: 78 %; m.p.: 237–238 °C; IR (cm^{-1}): 3387 (NH), 1703 (CO); ^1H NMR (DMSO-d_6, 200 MHz, ppm): 1.21 (t, 3H, $J = 7.0$ Hz, CH_3), 2.20 (s, 3H, CH_3), 2.60 (t, 2H, $J = 6.5$ Hz, CH_2), 2.75–2.78 (t, 2H, $J = 6.5$ Hz, CH_2), 4.20 (q, 2H, $J = 7.0$ Hz, CH_2), 6.32 (s, 2H, CH_2), 6.60 (d, 1H, $J = 7.3$ Hz, Ar), 6.79 (s, 1H, H-2'), 6.97–7.14 (m, 2H, Ar), 7.43–7.55 (m, 3H, H-3″, H-4″ and H-5″), 7.85 (d, 2H, $J = 7.1$ Hz, H-2″ and H-6"), 8.33 (s, 1H, H-4), 11.87 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 50 MHz, ppm): 13.9, 19.8, 21.0, 23.6, 49.3, 60.8, 103.6, 122.3, 122.9, 124.7, 126.7 (2 x C), 126.8, 127.6, 128.3, 128.7, 128.9, 129.2 (2 x C), 132.7, 137.3, 138.8, 140.5, 154.4, 155.1, 159.3, 160.6. Anal calcd for $C_{27}H_{25}BrN_4O_4S$: C, 55.77; H, 4.33; N, 9.64. Found: C, 55.91; H, 4.21; N, 9.53.

*4.1.7.7. Ethyl 2-[(benzenesulfonyl)amino]-7-bromo-9-[(3,4-dimethylphenyl)methyl]-6,9-dihydro-5H-pyrrolo [3,2-h]quinazoline-8-carboxylate (**SVQ14**).* This compound was obtained by reaction of **SVQ13**. Orange solid; yield: 68 %; m.p.: 224–225 °C; IR (cm^{-1}): 3399 (NH), 1700 (CO); ^1H NMR (DMSO-d_6, 200 MHz, ppm): 1.24 (t, 3H, $J = 7.0$ Hz, CH_3), 2.11 (s, 3H, CH_3), 2.13 (s, 3H, CH_3), 2.70 (s, 4H, 2 x CH_2), 4.19 (q, 2H, $J = 7.0$ Hz, CH_2), 6.25 (s, 2H, CH_2), 6.53 (d, 1H, $J = 6.4$ Hz, H-6′), 6.84 (s, 1H, H-2′), 6.94 (d, 1H, $J = 6.4$ Hz, H-5′), 7.43–7.57 (m, 3H, H-3″, H-4″ and H-5″), 7.86 (d, 2H, $J = 6.6$ Hz, H-2″ and H-6"), 8.28 (s, 1H, H-4); ^{13}C NMR (DMSO-d_6, 50 MHz, ppm): 14.3, 19.4, 19.9, 20.3, 24.1, 49.5, 61.3, 104.0, 123.9, 127.2 (2 x C), 128.0, 129.5 (2 x C), 130.0, 120.7, 129.0, 129.2, 133.4, 135.4, 135.5, 136.4, 136.7, 140.6, 152.7, 153.9, 155.6,

159.9. Anal calcd for $C_{28}H_{27}BrN_4O_4S$: C, 56.47; H, 4.57; N, 9.41. Found: C, 56.59; H, 4.36; N, 9.29.

*4.1.7.8. Ethyl 2-[(benzenesulfonyl)amino]-7-bromo-9-[(4-bromophenyl) methyl]-6,9-dihydro-5H-pyrrolo [3,2-h]quinazoline-8-carboxylate (**SVQ16**).* This compound was obtained by reaction of **SVQ15**. White solid; yield: 76 %; m.p.: 204–205 °C; IR (cm^{-1}): 3393 (NH), 1700 (CO); ^1H NMR (DMSO-d_6, 200 MHz, ppm): 1.21 (t, 3H, $J = 7.1$ Hz, CH_3), 2.62 (t, 2H, $J = 6.7$ Hz, CH_2), 2.80 (t, 2H, $J = 6.7$ Hz, CH_2), 4.21 (q, 2H, $J = 7.1$ Hz, CH_2), 6.31 (s, 2H, CH_2), 6.85 (d, 2H, $J = 8.3$ Hz, Ar), 7.42–7.57 (m, 5H, Ar), 7.81 (d, 2H, $J = 8.3$ Hz, Ar), 8.35 (s, 1H, H-4), 11.86 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 50 MHz, ppm): 13.9, 19.8, 23.5, 48.9, 60.8, 103.9, 120.0, 124.3, 126.6 (2 x C),128.3 (2 x C), 128.6, 129.0 (2 x C), 129.4, 131.2 (2 x C), 132.9, 138.3, 139.2, 140.5, 149.7, 155.1, 156.1, 159.2. Anal calcd for $C_{26}H_{22}Br_2N_4O_4S$: C, 48.31; H, 3.43; N, 8.67. Found: C, 48.19; H, 3.55; N, 8.54.

*4.1.7.9. Propan-2-yl 2-amino-7-bromo-9-(3-methylbenzyl)-6,9-dihydro-5H-pyrrolo [3,2-h]quinazoline-8-carboxylate (**SVQ20**).* This compound was obtained by reaction of **SVQ19**. Dark yellow solid; yield: 88 %; m.p.: 157–158 °C; IR (cm^{-1}): 3451–3419 (NH_2), 1695 (CO); ^1H NMR (DMSO-d_6, 200 MHz, ppm): 1.13 (d, 6H, $J = 4.4$ Hz, 2 x CH_3), 2.09 (s, 3H, CH_3), 2.65–2.75 (m, 4H, 2 x CH_2), 4.93–5.05 (m, 1H, CH), 5.99–6.43 (m, 6H, CH_2, NH_2 and Ar), 6.94–7.00 (m, 1H, Ar), 7.48–7.53 (m, 1H, Ar), 8.13 (s, 1H, H-4); ^{13}C NMR (DMSO-d_6, 50 MHz, ppm): 20.8, 21.2, 21.8 (2 x C), 24.2, 51.2, 68.7, 104.3, 117.3, 118.1, 126.8, 128.0, 128.4, 129.7, 130.2, 132.5, 137.8, 138.5, 154.4, 157.2, 159.1, 162.2. Anal calcd for $C_{22}H_{23}BrN_4O_2$: C, 58.03; H, 5.09; N, 12.30. Found: C, 57.84; H, 4.92; N, 12.52.

*4.1.7.10. Propan-2-yl 2-[(benzenesulfonyl)amino]-7-bromo-9-[(3-methylphenyl)methyl]-6,9-dihydro-5H-pyrrolo [3,2-h]quinazoline-8-carboxylate (**SVQ22**).* This compound was obtained by reaction of **SVQ21**. White solid; yield: 85 %; m.p.: 258–259 °C; IR (cm^{-1}): 3382 (NH), 1711 (CO); ^1H NMR (DMSO-d_6, 200 MHz, ppm): 1.09 (d, 6H, $J = 3.8$ Hz, 2 x CH_3), 2.10 (s, 3H, CH_3), 2.66 (t, 2H, $J = 6.9$ Hz, CH_2), 2.85 (t, 2H, $J = 6.9$ Hz, CH_2), 4.90–4.98 (m, 1H, CH), 6.38 (s, 2H, CH_2), 7.00 (d, 1H, $J = 7.5$ Hz, Ar), 7.36–7.43 (m, 3H, Ar), 7.50–7.58 (m, 3H, H-3″, H-4″ and H-5″), 7.77 (d, 2H, $J = 6.5$ Hz, H-2″ and H-6″), 8.37 (s, 1H, H-4), 11.76 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 50 MHz, ppm): 20.3, 21.2, 21.6 (2 x C), 24.2, 51.0, 69.0, 118.7, 104.2, 123.4, 125.24, 126.9, 127.1 (2 x C), 128.0, 129.3 (2 x C), 129.5, 129.8, 132.7, 133.1, 137.8, 138.2, 138.6, 139.5, 141.1, 155.7, 159.0. Anal calcd for $C_{28}H_{27}BrN_4O_4S$: C, 56.47; H, 4.57; N, 9.41. Found: C, 56.59; H, 4.66; N, 9.28.

*4.1.7.11. Ethyl 2-amino-7-bromo-9-(3-bromobenzyl)-6,9-dihydro-5H-pyrrolo [3,2-h]quinazoline-8-carboxylate (**SVQ24**).* This compound was obtained by reaction of **SVQ23**. White solid; yield: 74 %; m.p.: 167–168 °C; IR (cm^{-1}): 3506–3421 (NH_2), 1701 (CO); ^1H NMR (DMSO-d_6, 200 MHz, ppm): 1.35 (t, 3H, $J = 7.1$ Hz, CH_3), 2.79 (s, 4H, 2 x CH_2), 4.32 (q, 2H, $J = 7.1$ Hz, CH_2), 5.12 (s, 2H, CH_2), 6.38 (s, 2H, NH_2), 6.98 (s, 1H, Ar), 7.12–7.17 (m, 1H, Ar), 7.26–7.34 (m, 2H, Ar), 8.11 (s, 1H, H-4); ^{13}C NMR (DMSO-d_6, 50 MHz, ppm): 14.7, 25.4, 28.5, 54.6, 61.5, 99.0, 112.1, 122.4, 127.1, 127.1, 129.7, 130.3, 131.4, 131.5, 132.3, 140.8, 144.8, 156.82 157.2, 162.1. Anal calcd for $C_{20}H_{18}Br_2N_4O_2$: C, 47.46; H, 3.58; N, 11.07. Found: C, 47.53; H, 3.41; N, 11.19.

*4.1.7.12. Ethyl 2-[(benzenesulfonyl)amino]-7-bromo-9-(3-bromobenzyl)-6,9-dihydro-5H-pyrrolo [3,2-h]quinazoline-8-carboxylate (**SVQ26**).* This compound was obtained by reaction of **SVQ25**. White solid; yield: 80 %; m.p.: 235–236 °C; IR (cm^{-1}): 3382 (NH), 1713 (CO); ^1H NMR (DMSO-d_6, 200 MHz, ppm): 1.22 (t, 3H, $J = 7.1$ Hz, CH_3), 2.63 (t, 2H, $J = 7.3$ Hz, CH_2), 2.80 (t, 2H, $J = 7.3$ Hz, CH_2), 4.22 (q, 2H, $J = 7.1$ Hz, CH_2), 6.35 (s, 2H, CH_2), 6.80 (d, 1H, $J = 7.9$ Hz, Ar), 7.17–7.24 (m, 2H, Ar), 7.39–7.59 (m, 4H, Ar), 7.84 (d, 2H, $J = 7.8$ Hz, Ar), 8.35 (s, 1H, H-4), 11.86 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 50 MHz, ppm): 14.4, 20.3, 24.0, 49.3, 61.3, 102.1, 104.4, 118.3, 121.0, 121.8, 122.0, 124.6, 125.4, 127.2 (2 x C), 129.1, 129.5 (2 x C), 129.6, 130.3, 131.1, 133.2, 140.9, 142.0, 155.6, 159.8. Anal calcd for $C_{26}H_{22}Br_2N_4O_4S$: C, 48.31; H, 3.43; N, 8.67. Found: C, 48.19; H, 3.55; N, 8.49.

*4.1.7.13. Ethyl 2-amino-7-bromo-9-(3-bromo-4-fluorobenzyl)-6,9-dihydro-5H-pyrrolo [3,2-h]quinazoline-8-carboxylate (**SVQ28**).* This compound was obtained by reaction of **SVQ27**. Light brown solid; yield: 81 %; m.p.: 126–127 °C; IR (cm^{-1}): 3489–3386 (NH_2), 1697 (CO); ^1H NMR (DMSO-d_6, 200 MHz, ppm): 1.25 (t, 3H, $J = 7.0$ Hz, CH_3), 2.61–2.75 (s, 4H, 2 x CH_2), 4.26 (q, 2H, $J = 7.0$ Hz, CH_2), 6.38 (s, 2H, CH_2), 6.66 (s, 2H, NH_2), 6.99–7.06 (m, 1H, Ar), 7.28 (t, 1H, $J = 8.7$ Hz, Ar), 7.49 (d, 1H, $J = 6.6$ Hz, Ar), 8.12 (s, 1H, H-4); ^{13}C NMR (DMSO-d_6, 50 MHz, ppm): 14.4, 20.8, 23.9, 48.8, 61.2, 104.4, 108.2 (d, $J_{C3'\text{-}F} = 21.0$ Hz), 117.1, 117.2 (d, $J_{C5'\text{-}F} = 22.5$ Hz),123.7, 128.2, 128.9, 129.7, 132.1, 137.5, 154.7, 156.1, 157.7 (d, $J_{C4'\text{-}F} = 237.8$ Hz), 160.0, 161.8. Anal calcd for $C_{20}H_{17}Br_2FN_4O_2$: C, 45.83; H, 3.95; N, 12.71. Found: C, 45.98; H, 4.07; N, 12.59.

*4.1.7.14. Ethyl 2-[(benzenesulfonyl)amino]-7-bromo-9-(3-bromo-4-fluorobenzyl)-6,9-dihydro-5H-pyrrolo [3,2-h]quinazoline-8-carboxylate (**SVQ30**).* This compound was obtained by reaction of **SVQ29**. White solid; yield: 81 %; m.p.: 221–222 °C; IR (cm^{-1}): 3398 (NH), 1708 (CO); ^1H NMR (DMSO-d_6, 200 MHz, ppm): 1.24 (t, 3H, $J = 7.1$ Hz, CH_3), 2.63 (t, 2H, $J = 8.2$ Hz, CH_2), 2.80 (t, 2H, $J = 8.2$ Hz, CH_2), 4.24 (q, 2H, $J = 7.1$ Hz, CH_2), 6.30 (s, 2H, CH_2), 6.86 (s, 1H, Ar), 7.25 (t, 1H, $J = 8.8$ Hz, Ar), 7.40 (d, 1H, $J = 5.6$ Hz, Ar), 7.47–7.59 (m, 3H, H-3″, H-4″ and H-5″), 7.85 (d, 2H, $J = 7.0$ Hz, H-2″ and H-6″), 8.35 (s, 1H, H-4), 11.85 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 50 MHz, ppm): 14.4, 20.3, 24.0, 48.7, 61.4, 104.4, 108.2 (d, $J_{C3'\text{-}F} = 21.8$ Hz), 117.2 (d, $J_{C5'\text{-}F} = 21.8$ Hz), 124.7, 126.1, 127.1 (2 x C), 127.5, 127.8, 127.2, 128.9, 129.5 (2 x C), 132.0, 133.2, 137.3, 137.4, 155.6, 157.7 (d, $J_{C4'\text{-}F} = 243.0$ Hz), 159.8. Anal calcd for $C_{26}H_{21}Br_2FN_4O_4S$: C, 47.01; H, 3.19; N, 8.43. Found: C, 47.19; H, 3.04; N, 8.31.

*4.1.7.15. Ethyl 2-amino-7-bromo-9-(cyclopropylmethyl)-6,9-dihydro-5H-pyrrolo [3,2-h]quinazoline-8-carboxylate (**SVQ32**).* This compound was obtained by reaction of **SVQ31**. Yellow solid; yield: 78 %; m.p.: 222–223 °C; IR (cm^{-1}): 3501–3412 (NH_2), 1699 (CO); ^1H NMR (DMSO-d_6, 200 MHz, ppm): 0.31–0.40 (m, 4H, 2 x CH_2), 1.22 (s, 1H, CH), 1.35 (t, 3H, $J = 7.0$ Hz, CH_3), 2.67 (s, 2H, CH_2), 2.82 (s, 2H, CH_2), 4.33–4.42 (m, 4H, CH_2 and NH_2), 5.00 (d, 2H, $J = 7.1$ Hz, CH_2), 8.22 (s, 1H, H-4); ^{13}C NMR (DMSO-d_6, 50 MHz, ppm): 3.7 (2 x C), 13.6, 14.4, 20.5, 23.7, 50.8, 61.9, 103.2, 117.8, 127.3, 127.7, 133.6, 143.0, 155.0, 160.1, 160.4. Anal calcd for $C_{17}H_{19}BrN_4O_2$: C, 52.19; H, 4.89; N, 14.32. Found: C, 52.02; H, 4.98; N, 14.19.

*4.1.7.16. Ethyl 2-[(benzenesulfonyl)amino]-7-bromo-9-(cyclopropylmethyl)-6,9-dihydro-5H-pyrrolo [3,2-h]quinazoline-8-carboxylate (**SVQ34**).* This compound was obtained by reaction of **SVQ33**. White solid; yield: 74 %; m.p.: 232–233 °C; IR (cm^{-1}): 3387 (NH), 11703 (CO); ^1H NMR (DMSO-d_6, 200 MHz, ppm): 0.25–0.31 (m, 4H, 2 x CH_2), 1.07 (s, 1H, CH), 1.33 (t, 3H, $J = 6.8$ Hz, CH_3), 2.55 (s, 2H, CH_2), 2.76 (s, 2H, CH_2), 4.33 (q, 2H, $J = 6.8$ Hz, CH_2), 4.97 (d, 2H, $J = 6.0$ Hz, CH_2), 7.52–7.59 (m, 3H, H-3″, H-4″ and H-5″), 7.93 (d, 2H, $J = 5.7$ Hz, H-2″ and H-6″), 8.32 (s, 1H, H-4), 11.89 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 50 MHz, ppm): 3.7 (2 x C), 13.9, 14.5, 20.3, 24.1, 50.2, 61.3, 103.6, 122.6, 124.8, 127.2 (2 x C), 128.8, 129.5 (2 x C), 129.6, 133.2, 141.3, 155.2, 155.6 (2 x C), 160.2. Anal calcd for $C_{23}H_{23}BrN_4O_4S$: C, 51.98; H, 4.36; N, 10.54. Found: C, 52.07; H, 4.51; N, 10.38.

*4.1.7.17. Ethyl 2-amino-7-bromo-9-(4-fluorobenzyl)-6,9-dihydro-5H-pyrrolo [3,2-h]quinazoline-8-carboxylate (**SVQ36**).* This compound was obtained by reaction of **SVQ35**. White solid; yield: 67 %; m.p.: 142–143

°C; IR (cm^{-1}): 3522–3421 (NH_2), 1695 (CO); ^1H NMR (DMSO-d_6, 200 MHz, ppm): 1.25 (bs, 3H, CH_3), 2.63 (bs, 2H, CH_2), 2.72 (bs, 2H, CH_2), 4.24 (bs, 2H, CH_2), 6.43 (s, 2H, CH_2), 6.57 (s, 2H, NH_2), 7.03–7.14 (m, 4H, H-2′, H-3′, H-5′and H-6′), 8.13 (s, 1H, H-4); ^{13}C NMR (DMSO-d_6, 50 MHz, ppm): 14.4, 20.9, 24.1, 49.2, 61.1, 104.3, 115.6 (2 x d, $J_{C3'\text{-}F}$ = 22.5 Hz), 117.1, 123.6, 128.6, 128.9 (2 x d, $J_{C2'\text{-}F}$ = 8.3 Hz), 130.0, 135.5, 135.6, 154.5, 156.8, 161.6 (d, $J_{C4'\text{-}F}$ = 240.0 Hz), 162.2. Anal calcd for $C_{20}H_{18}BrFN_4O_2$: C, 53.95; H, 4.07; N, 12.58. Found: C, 54.08; H, 3.93; N, 12.72.

*4.1.7.18. Ethyl 2-[(benzenesulfonyl)amino]-7-bromo-9-(4-fluorobenzyl)-6,9-dihydro-5H-pyrrolo [3,2-h]quinazoline-8-carboxylate (**SVQ38**).* This compound was obtained by reaction of **SVQ37**. White solid; yield: 75 %; m.p.: 221–222 °C; IR (cm^{-1}): 3394 (NH), 1702 (CO); ^1H NMR (DMSO-d_6, 200 MHz, ppm): 1.23 (t, 3H, J = 6.9 Hz, CH_3), 2.62 (s, 2H, CH_2), 2.80 (s, 2H, CH_2), 4.23 (q, 2H, J = 6.9 Hz, CH_2), 6.32 (s, 2H, CH_2), 6.97–7.11 (m, 4H, H-2′, H-3′, H-5′ and H-6′), 7.49–7.56 (m, 3H, H-3″, H-4″ and H-5″), 7.86 (d, 1H, J = 6.5 Hz, H-2″ and H-6″), 8.35 (s, 1H, H-4), 11.86 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 50 MHz, ppm): 14.4, 20.3, 24.0, 49.1, 61.3, 104.3, 115.6 (2 x d, $J_{C3'\text{-}F}$ = 21.0 Hz), 122.9, 125.0, 127.1 (2 x C), 128.7 (2 x d, $J_{C2'\text{-}F}$ = 8.3 Hz), 129.1, 129.5 (2 x C), 129.9, 133.2, 135.4, 141.1, 154.9, 155.7 (2 x C), 159.8, 161.6 (d, $J_{C4'\text{-}F}$ = 240.8 Hz). Anal calcd for $C_{26}H_{22}BrFN_4O_4S$: C, 53.34; H, 3.79; N, 9.57. Found: C, 53.22; H, 3.91; N, 9.72.

4.2. Biology

The CF bronchial epithelial cells, CFBE41o-, with stable expression of F508del-CFTR [35] were stably transfected with the halide-sensitive yellow fluorescent protein, HS-YFP [36,37]. Cells were cultured with MEM plus 10 % fetal calf serum, 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. For fluorescence-based measurement of CFTR activity, CFBE41o-cells were plated (50 000 cells/well) on clear-bottom 96-well black microplates (CLS3603, Corning). After 24 h after plating, cells were treated with test compounds (or vehicle, DMSO) at 37 °C for 24 h. At the time of assay, cells were washed three times with PBS (in mM: 137 NaCl, 2.7 KCl, 8.1 Na_2HPO_4, 1.5 KH_2PO_4, 1 $CaCl_2$, and 0.5 $MgCl_2$) to remove culture medium plus test compounds. Cells were then stimulated for 30 min with forskolin (20 μM) plus genistein (50 μM) in 60 μl PBS. The microplate was subsequently transferred to a microplate reader (FluoStar Galaxy; BMG Labtech) equipped with high-quality excitation (ET500/20X: 500 ± 10 nm) and emission (ET535/30 M: 535 ± 15 nm) filters for YFP (Chroma Technology). Assay consisted of a continuous 14-s fluorescence reading in each well, with injection at 2 s of 165 μl of an iodide-rich solution (modified PBS, with Cl^- replaced by I^-; final I^- concentration in the well: 100 mM). Fluorescence was read every 0.2 s, with 20 excitation flashes per time point. Data were normalized to the initial background-subtracted fluorescence. To determine fluorescence quenching rate (QR) reflecting the extent of I^- influx, the data points corresponding to the final 11 s of the fluorescence reading for each well were fitted with an exponential function (Igor software, Wavemetrics) to extrapolate initial slope (dF/dt).

CRediT authorship contribution statement

Marilia Barreca: Validation, Methodology, Investigation, Data curation. **Mario Renda:** Validation, Methodology, Investigation, Formal analysis, Data curation. **Virginia Spanò:** Writing – original draft, Conceptualization. **Alessandra Montalbano:** Writing – review & editing, Data curation. **Maria Valeria Raimondi:** Writing – review & editing, Formal analysis. **Stefano Giuffrida:** Methodology, Investigation. **Roberta Bivacqua:** Investigation, Formal analysis. **Tiziano Bandiera:** Writing – review & editing, Supervision. **Luis J.V. Galietta:** Writing – original draft, Project administration, Funding acquisition, Data curation, Conceptualization. **Paola Barraja:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: PAOLA BARRAJA reports financial support was provided by Italian Cystic Fibrosis Research Foundation. PAOLA BARRAJA, VIRGINIA SPANO', L. J. V. GALIETTA has patent pending to WO 2020104558 A1 20200528. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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