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Fluorinated ΔF508-CFTR correctors and potentiators for PET imaging

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ABSTRACT

^{19}F-modified bithiazole correctors and phenylglycine potentiators of the ΔF508-CFTR chloride channel were synthesized and their function assayed in cells expressing human ΔF508-CFTR and a halide-sensitive fluorescent protein. Fluorine was incorporated into each scaffold using prosthetic groups for future biodistribution imaging studies using positron emission tomography (PET). The ΔF508-CFTR corrector and potentiator potencies of the fluorinated analogs were comparable to or better than those of the original compounds.

Cystic fibrosis (CF) is the most prevalent lethal hereditary disease among Caucasians, affecting approximately one in 2500 individuals.[1] The average life expectancy in CF is about 40 years. The principal cause of mortality in CF is deterioration of lung function caused by chronic lung infection.[2,3] Cystic fibrosis is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) protein, the most common being a deletion of phenylalanine at position 508 (ΔF508).[4,5] This mutation causes CFTR protein misfolding, resulting in retention and rapid degradation in the endoplasmic reticulum.[6–9]

Compounds have been synthesized that rescue defective ΔF508-CFTR protein processing (correctors) and defective chloride channel gating (potentiators).[10–12] Bithiazole **1** (corr-**4a**) and indolylacetamidophenylacetamide **2** (**PG-01**) are the benchmark corrector and potentiator studied here, respectively (Fig. 1). Structure–activity relationship studies based on corr-**4a** revealed that substitution of a 2-chloro-5-(*N*,*N*-dimethylamino)phenyl moiety for the 5-chloro-2-methoxyphenyl group improved water solubility (**3**; Fig. 1)[13] and that locking the bithiazole system into an s-*cis* conformation with a seven-membered ring (**3**; Fig. 1) improved corrector efficacy.[11]

We recently reported the synthesis of a fluorescently-labeled bithiazole corrector **4** (Fig. 1), which enabled the evaluation of its biodistribution in mice by fluorescence imaging.[14] Though this approach produced information about the in vivo handling of a functional CF corrector, the inclusion of a fluorophore label resulted in poor compound metabolic stability, limiting the time over which the non-modified compounds could be studied. Also, the limited penetration of light into tissues precluded non-invasive whole-body imaging.

To address these issues, we report here a first step to apply positron emission tomography (PET) imaging to non-invasively visualize the biodistribution of a bithiazole corrector and a phenylglycine potentiator.[15–17] Analogs suitable for PET imaging have the advantage of minimally modifying the original compound structure (replacing a H with an ^{18}F),[18] which increases the likelihood of maintaining activity while enabling in vivo monitoring of uptake and biodistribution. Indeed, imaging modalities can be relevant at all stages of CF drug development, with potential applications in animal models, tissue preparations, and human in vivo studies. Relevant imaging could enlighten at the basic mechanistic level leading to a better understanding CF pathophysiology, at the level

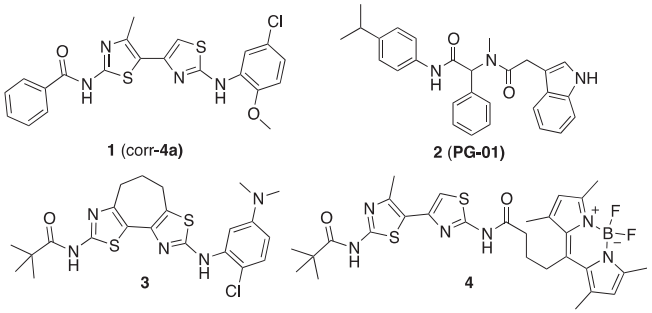

Figure 1. ΔF508-CFTR correctors (**1**/**3**/**4**) and potentiator (**2**).

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of assessing the potential of new correctors or potentiators, at the effectiveness of treatment level, and/or at the level of measuring function to better drug delivery.[19]

PET produces images through the detection of positron emitting radioisotopes. The most commonly used radionucleotide is fluorine-18 due to its relatively long half-life ($t_{1/2}$ = 110 min). Other elements, such as carbon (^{11}C $t_{1/2}$ = 20.4 min), nitrogen (^{13}N $t_{1/2}$ = 9.97 min), and oxygen (^{15}O $t_{1/2}$ = 2.04 min), have much shorter half-lives. To address the need for new disease-specific probes, prosthetic groups[18] have been developed for the rapid incorporation of radioisotopes into the specific tracers. A variety of amine-reactive prosthetic groups have been developed, the most commonly being [^{18}F]*N*-succinimidyl-4-fluorobenzoate (**5**; Fig. 2).[20] Other ^{18}F-acylating agents include the carboxylate reactive [^{18}F]4-fluoroaniline prosthetic group (**6**; Fig. 2).[21] A strategy that has received considerable attention is the use of click chemistry to incorporate small molecular weight ^{18}F-labeled alkynes such as [^{18}F]5-fluoroalkyne (**7**; Fig. 2) into azide substituted peptides.[22] Copper(I)-catalyzed 1,2,3-triazole formation is fast, chemoselective, and performed under mild conditions.[22]

As a first step in enabling CF-relevant PET studies, we report here the design, synthesis, and ΔF508-CFTR activity of two ^{19}F-labeled correctors (**8** and **9**; Fig. 3) and two ^{19}F-labeled potentiators (**10** and **11**; Fig. 3). Since ^{18}F has a 110 min half-life, late-stage introduction of the fluorinated moiety is essential and, consequently, targets **8–11** were designed with the aforementioned ^{18}F-prosthetic groups in mind.

Targeting fluorobenzoyl analogs ^{19}F-**8** and ^{19}F-**9** (Scheme 1), the first synthetic step was the condensation of 3-chloropentane-2,4-dione with thiourea in refluxing ethanol over 12 h to afford aminothiazole **12** in 95% yield.[11] The acetyl group of **12** was subsequently α-brominated with bromine in acetic acid to produce **13** in 86% yield. Aminothiazole building block **15** was obtained in 79% yield by the condensation of **13** with 1-(2-chloro-5-dimethylaminophenyl)thiourea (**14**) in refluxing ethanol. The final step in the synthesis of ^{19}F-bithiazole **8** proved to be more challenging than expected as numerous attempts to couple it with fluorobenzoic acid by amide-coupling condensation with HATU, EDC, or CDI proved unsuccessful. Eventually, bithiazole **15** was acylated with 4-fluorobenzoyl chloride (prepared by treatment of 4-fluorobenzoic acid with oxalyl chloride) in the presence of triethylamine at room temperature (10 min) to afford ^{19}F-**8**.

Given these difficulties, a new strategy was developed for the synthesis of fluorinated bithiazole **9**. Work began here with the CDI-mediated coupling of aminothiazole **12** with 4-fluorobenzoic acid. Thiazole **17** was markedly easier to purify than **8**. Bromination of **17** proved significantly more difficult than **12**, requiring the use of a stronger acidic medium (HBr vs AcOH) with pyridinium tribromide to afford **18** (91% yield). The final reaction involved condensation of **18** with 1-(5-chloro-2-methoxyphenyl)thiourea (**19**), delivering ^{19}F-**9** in 76% yield.

Yield and purification difficulties were due to the poor solubility of aminobithiazole intermediate **15** as well as the corresponding fluorinated bithazoles **8** and **9**. Because [^{18}F]*N*-succinimidyl-4-fluorobenzoate (^{18}F-SFB) would be used in picomolar quantities for the final coupling to aminobithiazole intermediates, the reaction solution would be quite dilute and solubility is not expected to be a significant issue.

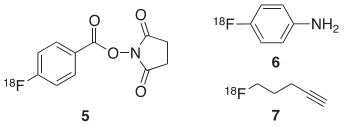


Figure 2. Potential ^{18}F-prosthetics.

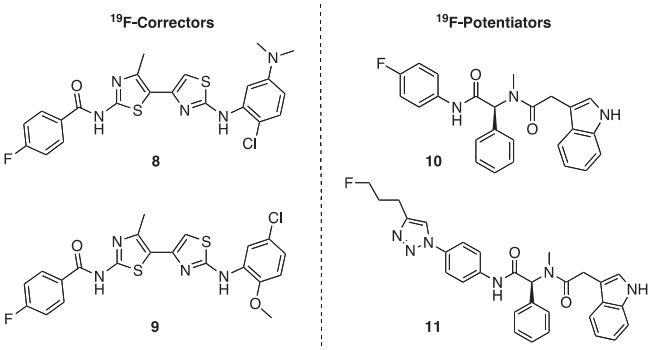
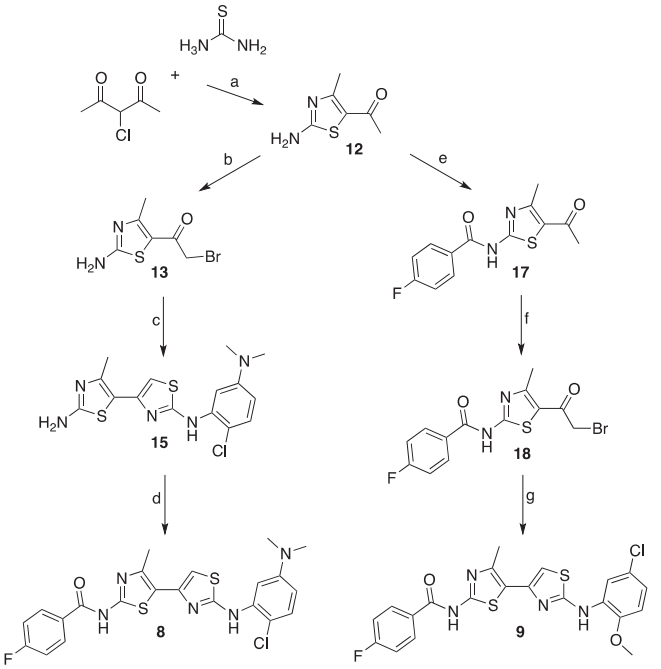


Figure 3. Fluorine-containing corrector and potentiator analogs.



Scheme 1. Synthesis of fluorinated corrector analogs. Reagents and conditions: (a) EtOH, reflux; (b) Br_2, AcOH; (c) 1-(2-chloro-5-dimethylaminophenyl)thiourea (**14**), EtOH, reflux; (d) (i) 4-fluorobenzoic acid, CH_2Cl_2, oxalyl chloride, (ii) 4-fluorobenzoyl chloride (**16**), CH_2Cl_2, TEA; (e) 4-fluorobenzoic acid, CDI, DMF, 100 °C; (f) $PyrH^+Br_3^-$, 33% HBr in AcOH; (g) 1-(5-chloro-2-methoxyphenyl)thiourea (**19**), EtOH, reflux.

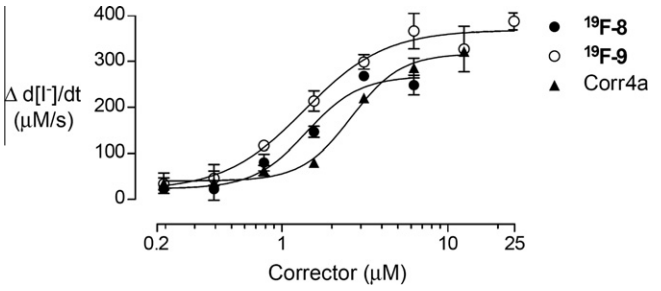
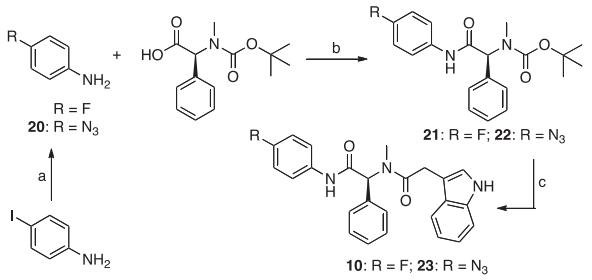


Figure 4. Concentration-dependence of ΔF508-CFTR corrector activity of ^{19}F-**8** and ^{19}F-**9** compared to corr-**4a**.

The ΔF508-CFTR corrector activity of ^{19}F-**8** and ^{19}F-**9** was assayed using a cell-based fluorescence assay in Fischer rat thyroid (FRT) cells co-expressing human ΔF508-CFTR and the halide-sensitive fluorescent protein YFP-H148Q/I152L.[11] The concentration-



Scheme 2. Synthesis of fluorinated potentiator ^{19}F-**10** and azido intermediate **23**. Reagents and conditions: (a) CuI, NaN_3, L-proline, NaOH, DMSO, 60 °C; (b) EDC, HOBt, DMF, DCM, 0 °C to rt; (c) (i) TFA, DCM, (ii) EDC, DMAP, DCM, DMF, 0 °C to rt.

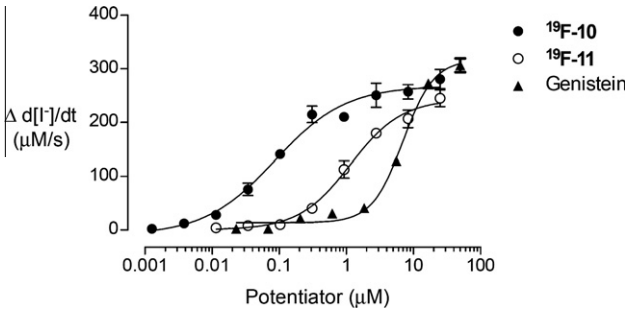


Figure 5. Concentration-dependence of ΔF508-CFTR potentiator activity of ^{19}F-**10** and ^{19}F-**11** compared to genistein.

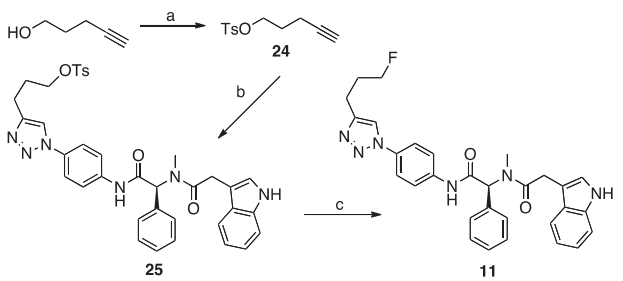
dependence data show slightly greater potency of ^{19}F-**8** and ^{19}F-**9**, compared to benchmark corrector corr-**4a**, as shown in Figure 4. EC_{50} values (in μM) were: **8** = 1.4; **9** = 1.4; corr-**4a** = 2.3].

Synthesis of fluoroaniline potentiator ^{19}F-**10** (Scheme 2) began with an EDC-mediated coupling of 4-fluoroaniline to Boc-*N*-methyl-L-phenylglycine (Scheme 2).[10,23] Boc-protected **21**, obtained in 61% yield, was then deprotected by treatment with TFA and subsequent EDC-activated coupling with 3-indole acetic acid and DMAP gave ^{19}F-**10** in 72% yield over two steps.

Synthesis of 4-(3-fluoropropyl)-1*H*-1,2,3-triazole analog **11** began with the synthesis of 4-azidoaniline via a CuI-catalyzed, proline-promoted coupling reaction[24] followed by an EDC-mediated condensation of **20** (Scheme 2) with Boc-*N*-methyl-L-phenylglycine to afford **22** (**20**, 74%; and **22**, 97%, respectively). Subsequent Boc removal (TFA) followed by treatment with EDC-activated 3-indole acetic acid and DMAP afforded azido phenylglycine intermediate **23** (90% yield).

The synthesis of 5-fluoroalkyne for the triazole-forming cycloaddition with azido-phenylglycine intermediate **23** proved to be unsuccessful. Initially, we focused on utilizing diethylaminosulfur trifluoride (DAST) for the conversion of the alcohol moiety of 4-pentyne-1-ol into an alkyl fluoride.[25] Unfortunately, no fluorinated alkyne was isolated. Das et al. reported that ionic liquids can be used in an improved purification procedure of the dehydroxy-fluorination reaction with DAST; again, no desired product was isolated.[26] Likewise, a modified procedure employing tosylate **24** (synthesized by the treatment of 4-pentyn-1-ol with *p*-toluenesulfonyl chloride and triethylamine[27]) and nucleophilic fluoride [KF plus 18-Crown-6 or $Bu_4N^+F^-(t\text{BuOH})_4$[28]] was unsuccessful.

With this as a backdrop, tosylate **24** was clicked to azido intermediate **23** in a Huisgen copper-catalyzed 1,3-dipolar cycloaddition to afford **25** in 85% yield (Scheme 3). Finally, potentiator analog ^{19}F-**11** was obtained by treatment of **25** with $Bu_4N^+F^-(t\text{-BuOH})_4$ (**26**) in 31% yield.



Scheme 3. Synthesis of fluorinated potentiator ^{19}F-**11**. Reagents and conditions: (a) TsCl, TEA, DCM, 0 °C to rt; (b) Na ascorbate, $CuSO_4$, **23**, DCM, *t*BuOH, H_2O; (c) (i) $Bu_4N^+F^-{\cdot}H_2O$, *t*BuOH, hexane, 90 °C, 30 min forms $Bu_4N^+F^-(t\text{BuOH})_4$ (**26**), (ii) **25**, **26**, ACN, 70 °C, 1 h.

The ΔF508-CFTR potentiator activity of ^{19}F-**10** and ^{19}F-**11** was assayed in FRT cells co-expressing ΔF508-CFTR and YFP-H148Q/I152L after rescue of ΔF508-CFTR by 24 h culture at 27 °C.[11,12] Fluorinated phenylglycine analogs had EC_{50} of 0.09 μM (^{19}F-**10**) and 1.1 μM (^{19}F-**11**), comparable to that of 0.3 μM reported previously for PG-01,[10] and much better than that of 7.0 μM for benchmark potentiator genistein (Fig. 5).

The retained corrector activity of ^{19}F-**8** and ^{19}F-**9** and potentiator activity of ^{19}F-**10** is likely the consequence of the enhanced binding interactions of fluorine.[29] Indeed, fluorine is found in approximately 5–15% of drugs that have come to market over the past 50 years.[29] Fluorine has also been shown to improve metabolic stability and generally enhance physicochemical properties,[29] features which would be useful in future in vivo studies.

In conclusion, two active fluorinated corrector analogs and two active fluorinated potentiator analogs were synthesized and the introduction of a fluorine atom was found to improve potency. Both fluorinated correctors (^{19}F-**8** and ^{19}F-**9**) and a fluorinated potentiator (^{19}F-**10**) showed improved or comparable activity to the original compounds. These active, fluorinated analogs are potentially useful for non-invasive PET imaging of in vivo compound uptake and biodistribution. Given the short half-life of ^{19}F ($t_{1/2}$ = 110 min), the next step en route to PET studies with these compounds will be to develop synthetic routes to ^{18}F-**8**, ^{19}F-**9**, and ^{19}F-**10** where the ^{19}F moiety is introduced in the last synthetic step. Current efforts are focused on developing viable and high-yielding routes to ^{18}F-**8** from N^2-(2-chloro-5-(dimethylamino)phenyl)-4′-methyl-[4,5′-bithiazole]-2,2′-diamine (**15**) + **7** and ^{18}F-**10** from (*S*)-2-(2-(1*H*-indol-3-yl)-*N*-methylacetamido)-2-phenylacetic acid + **6**.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.12.128.

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