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Bioorganic & Medicinal Chemistry Letters 16 (2006) 537–540
Bioorganic & Medicinal Chemistry Letters

Sulfamoyl-4-oxoquinoline-3-carboxamides: Novel potentiators of defective ΔF508-cystic fibrosis transmembrane conductance regulator chloride channel gating

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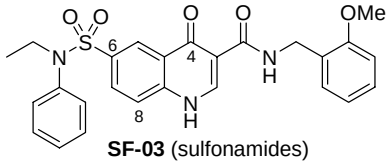
Received 19 September 2005; revised 17 October 2005; accepted 18 October 2005
Available online 8 November 2005

Abstract—The synthesis of a small collection of sulfamoyl-4-oxoquinoline-3-carboxamides is described for use as correctors of defective gating of the ΔF508-cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel. Several compounds with submicromolar potency were obtained. *N*-Ethyl 6-(ethylphenylsulfamoyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (**7b**) was found to be the most effective sulfonamide corrector of defective ΔF508-CFTR gating.

The ΔF508 mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel causes defects in channel gating and cellular processing leading to the disease cystic fibrosis (CF).[1] Focusing on the identification of correctors of defective gating, referred to as 'potentiators', we recently reported the results of screening a diverse 50,000 compound small molecule library with an iodide uptake assay in epithelial cells coexpressing ΔF508-CFTR and a fluorescent halide indicator (yellow fluorescent protein-H148Q/I152L) after ΔF508-CFTR rescue by 24 h culture at 27 °C.[2] Two novel classes of potentiators with submicromolar potency were obtained (Fig. 1): a sulfonamide {cf. 6-(ethylphenylsulfamoyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid 2-methoxybenzylamide; **SF-03**} and a phenylglycine {cf. 2-[(2-1*H*-indol-3-yl-acetyl)methylamino]-*N*-(4-isopropylphenyl)-2-phenylacetamide; **PG-01**} class.[2] As the first step in a hit-to-lead study of the sulfonamide class, we report here the preparation and CFTR potentiation profile of a 16 compound collection of 6- and 8-sulfamoyl-4-oxo-1,4-dihydroquinoline-3-carboxamides.



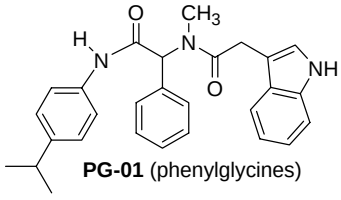


Figure 1. Novel correctors of defective human ΔF508-CFTR gating.

The synthetic aspect of our investigation started with an attempt to implement the chemistry outlined in Scheme 1. From the outset, there were two attractive advantages with this route to sulfamoyl-4-oxoquinoline-3-carboxamides. The first is that the key Gould–Jacob cyclization reaction[3] to quinolone **2** would be performed on a relatively electron-rich aniline derivative (vis-à-vis electron-deficient **11** in Scheme 2) and the second is that the two amide diversities would be introduced at a late stage—a fact that would allow one simple core structure (e.g., **3**) to lead to the entire collection of targeted compounds. Unfortunately, while conversion of aniline

Keywords: Cystic fibrosis; CFTR; Activator; Potentiator; Sulfonamide.
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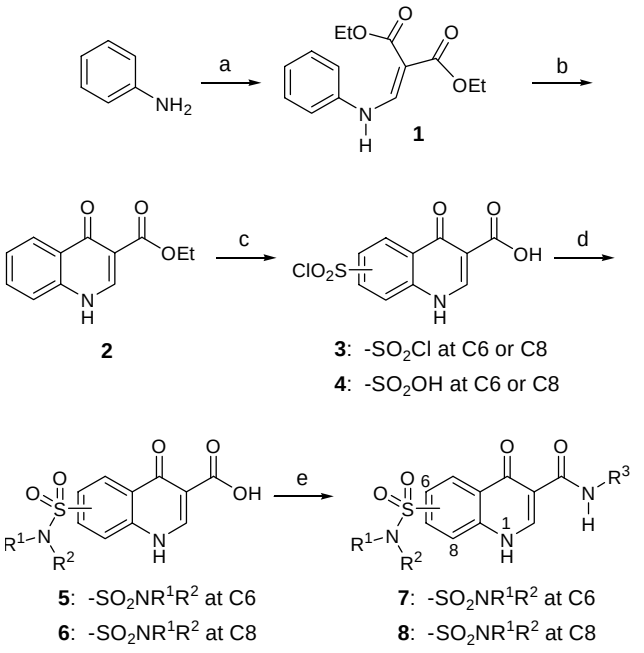
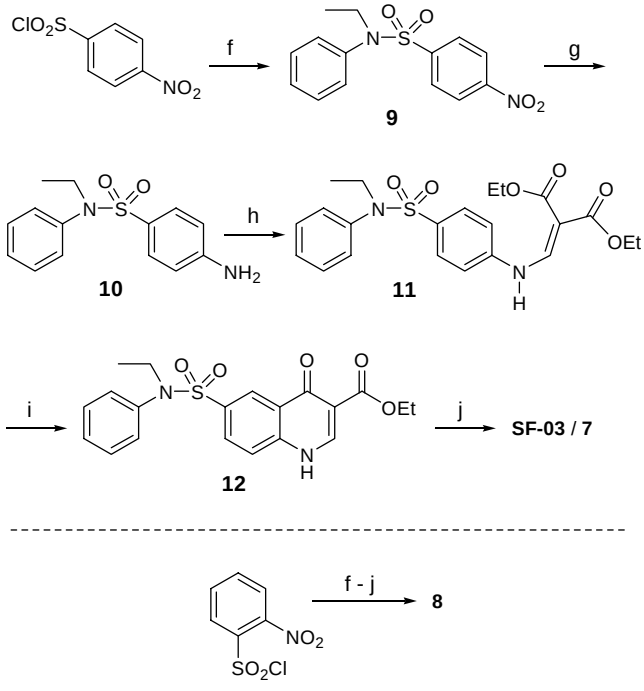
doi:10.1016/j.bmcl.2005.10.050



Scheme 1. Synthesis of 6- and 8-sulfamoyl-4-oxoquinoline-3-carboxamides. Reagents and conditions: (a) Diethyl ethoxymethylenemalonate, 140 °C, 2 h, 95%. (b) Cat. *p*-chlorobenzoic acid, Ph_2O, 250 °C, 2 h, μW, 45–60%. (c) Chlorosulfonic acid, 140 °C, 3.5 h, variable yield. (d) R^1R^2NH, TEA, THF, 0 °C, 1 h, ~90%. (e) Ethylchloroformate, TEA, R^3NH_2, THF, ~50%.[4]



Scheme 2. Synthesis of **SF-03** and **7/8**. Reagents and conditions: (f) *N*-Ethyl aniline, TEA, 0 °C, 1 h, 90%. (g) $SnCl_2 \cdot 2H_2O$, THF, EtOH, overnight, rt, 75%. (h) Diethyl ethoxymethylenemalonate, 140 °C, 1 h, 95%. (i) Cat. *p*-chlorobenzoic acid, Ph_2O, 250 °C, 2 h, μW, 45%. (j) *o*-Methoxybenzylamine, neat, 180 °C, 1/2 h, μW, 35%.

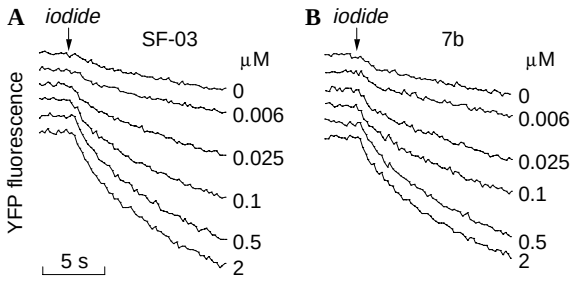


Figure 2. Dose–response analysis of ΔF508-CFTR potentiator activity of **SF-03** (A) and its analog **7b** (B). Data were obtained in ΔF508-CFTR-expressing FRT cells after low temperature rescue for 20 h and with 20 μM forskolin (0.5 μM used for wild type) for 15 min. CFTR-dependent I^- influx was measured from the time course of decreasing cellular YFP fluorescence (for a detailed description of methods, see Ref. 2).

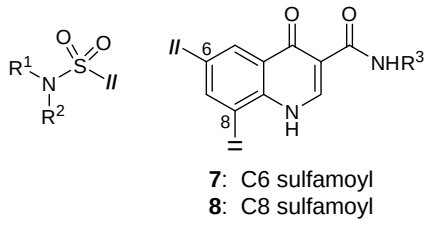


Figure 3. Target sulfamoyl-4-oxoquinoline-3-carboxamides.

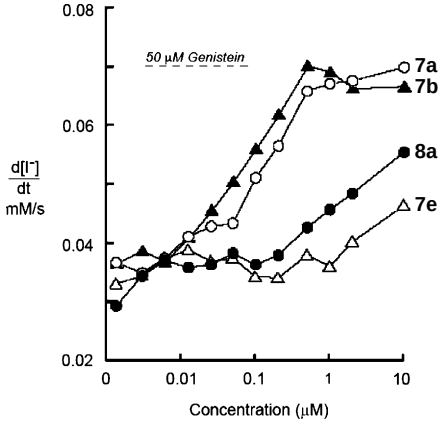


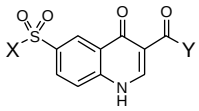
Figure 4. Dose–response analysis of indicated **SF-03** analogs. The ordinate is the rate of iodide influx mediated by ΔF508-CFTR.

to 4-oxo-1,4-dihydroquinoline-3-carboxylate **2** was uneventful, the chlorosulfonylation reaction (**2** → **3**; 3:1::C6:C8) proved quite difficult to reliably perform, generally giving the sulfonic acid analog (**4**) as the major product. Attempts to convert this sulfonic acid to the corresponding chlorosulfonic acid (for example, PCl_5 treatment) led to an intractable mixture. Thus, while we were able to convert **4** to several sulfamoyl-4-oxo-quinoline-3-carboxamides, the chemistry of Scheme 1 proved too unreliable for our needs. However, as a side note, we found that the regioisomeric product mixtures of reactions c and d could be carried forward without separation because isomer separation is easily affected by a simple flash column purification after reaction e. Indeed, in both cases [see Table 1: **7b/8b** with R^1 = phen-

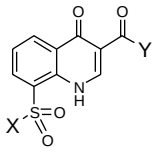
yl, $R^2 = R^3$ = ethyl; **7c**/**8c** with R^1 = phenyl, R^2 = ethyl, and R^3 = (3-imidazol-1-yl)propyl], the C6 sulfamoyl product (**7**) is significantly more polar than the C8 sulfamoyl product (**8**)—presumably because of an intramolecular H-bond between N1 and the sulfamoyl moiety in **8** but not **7**.

Given these difficulties with **2** → **4**, we turned to an investigation of the chemistry outlined in Scheme 2 and began this effort with a synthesis of hit compound **SF-03**. Starting from commercial 4-nitrobenzene sulfonyl chloride, straightforward sulfonamide formation and nitro reduction delivered 4-amino-*N*-ethyl-*N*-phenylbenzene sulfonamide (**10**). Aminomethylenemalonate formation (**10** → **11**) set the stage for the Gould–Jacob cyclization reaction[3] to quinolone **12**. Building upon the reports by Dave and co-workers,[5] we found that microwave irradiation of a phenyl ether solution of **11** containing catalytic *p*-chloro-

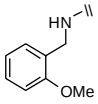
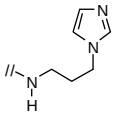
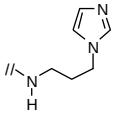
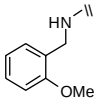
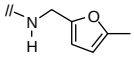
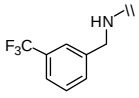
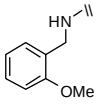
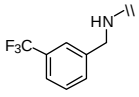
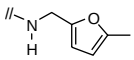
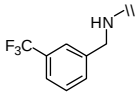
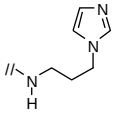
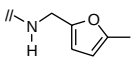
Table 1. 6- and 8-Sulfamoyl-4-oxoquinoline-3-carboxamides studied



7



8

X	Y	d[I⁻]/d*t* (mM/s)	
		Compound: wild type ΔF508	Compound: wild type ΔF508
		**7a**: 1.15 ± 0.08** 0.07 ± 0.002**	**8a**: 0.70 ± 0.18** 0.06 ± 0.005**
		**7b**: 0.62 ± 0.02** 0.07 ± 0.002**	**8b**: 0.37 ± 0.003 0.04 ± 0.003*
		**7c**: 0.33 ± 0.03 0.03 ± 0.003	**8c**: 0.39 ± 0.04 0.03 ± 0.004
		**7d**: 0.39 ± 0.05 0.03 ± 0.005	**8d**: 0.34 ± 0.08 0.03 ± 0.006
		**7e**: 0.42 ± 0.10 0.05 ± 0.002**	**8e**: 0.40 ± 0.07 0.04 ± 0.003*
		**7f**: 0.45 ± 0.02** 0.04 ± 0.005*	**8f**: 0.30 ± 0.08 0.04 ± 0.003*
		—	**8g**: 0.23 ± 0.01 0.03 ± 0.004
		—	**8h**: 0.32 ± 0.02 0.04 ± 0.003*
		**7i**: 0.27 ± 0.06 0.03 ± 0.002	—
C_n = negative control C_p = positive control		**C_n**: 0.34 ± 0.01** 0.3 ± 0.002	**C_p**: 2.55 ± 0.36** 0.07 ± 0.002**

* $p < 0.05$.

** $p < 0.01$ compared with negative control (DMSO), mean ± SE, $n = 4$. Positive controls for wild type CFTR are 20 μM apigenin and for ΔF508-CFTR is 50 μM genistein.

benzoic acid dramatically facilitated cyclization to **12**, reducing the typical reaction time for optimal yields for this transformation from many hours[6] to ~2 h. Finally, microwave-assisted ester to amide conversion[7] delivered authentic **SF-03** with which we verified the initial ΔF508-CFTR rescue activity.

The ΔF508-CFTR potentiator activity of **SF-03** was verified in ΔF508-CFTR-expressing FRT cells after low temperature rescue (Fig. 2A). **SF-03** concentration-dependent I^- influx was seen from the time course of decreasing cellular YFP fluorescence, with 50% of maximum activity at ~0.1 μM **SF-03**.

With these data in hand, we set out to synthesize a small collection of sulfamoyl-4-oxoquinoline-3-carboxamides (**7** and **8**) with elements of diversity embracing the two amide moieties and the C6 (**7**) or C8 (**8**) placement of the sulfamoyl moiety (Fig. 3). As illustrated in Scheme 2, sulfamoyl placement was addressed by selecting either 4-nitrobenzene sulfonyl chloride (→**7**) or 2-nitrobenzene sulfonyl chloride (→**8**) as the starting material.

Employing the chemistries outlined in Schemes 1 and 2, we prepared the 15 analogs depicted in Table 1, which summarizes the rates of iodide influx for the analogs measured at 10 μM against wild type CFTR and at 10 μM against low temperature rescued ΔF508-CFTR (27 °C for 20 h to facilitate ΔF508-CFTR accumulation at the cell surface) ΔF508-CFTR potentiator activity of an **SF-03** analog **7b** is shown in Figure 2B. Figure 4 shows dose–response data for several of the ΔF508-CFTR potentiators. The most active compounds had V_{max} comparable to that of the reference flavone genistein at 50 μM, with activating potencies of under 0.1 μM.

These experiments provide an initial survey of the activity of **SF-03** and related compounds as correctors of defective human ΔF508-CFTR gating. Of the compounds tested, sulfonamide **7b** was found to be the most effective at correcting defective ΔF508-CFTR gating. With the exception of **7c**/**8c**, placement of the sulfonamide at C6 is superior to placement at C8. Additional work is warranted to continue optimization of this structural series and to further examine the potential role of these compounds in CF therapy.

Acknowledgments

We thank the National Institutes of Health (DK072517) and the National Science Foundation (CHE-0313888) for their generous support of this work.

References and notes

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