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Discovery of 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid diamides that increase CFTR mediated chloride transport

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Abstract—A series of 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid diamides that increase chloride transport in cells expressing mutant cystic fibrosis transmembrane conductance regulator (CFTR) protein has been identified from our compound library. Analoging efforts and the resulting structure–activity relationships uncovered are detailed. Compound potency was improved over 30-fold from the original lead, yielding several analogs with EC_{50} values below 10 nM in our cellular chloride transport assay.

Cystic fibrosis (CF) is the most common lethal genetic disease among the Caucasian population, affecting more than 30,000 people in the US alone.[1] The underlying cause of the disease is a mutation in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) protein.[2] This cAMP-gated chloride channel is expressed in the apical membrane of epithelial cells in the airways, pancreas, intestines, and testes.[3] The most common CFTR mutation in CF is the deletion of phenylalanine 508 (ΔF508).[4] ΔF508 mutant protein fails to fold properly and is largely recognized and destroyed by cellular quality control machinery. A small amount of mutant CFTR reaches the apical membrane and remains capable of ion transport, although the efficiency of this process is reduced significantly.[5] This ultimately results in a variety of complications due to decreased water transport, most notably, chronic infection and inflammation of the airways leading to progressive deterioration of lung function, the primary cause of mortality in CF patients.

One potential therapeutic approach to CF is to identify agents which can enhance the limited chloride transport capability of mutant CFTR. This may be achieved by inducing more of the defective protein to fold properly and traffick to the cell surface (e.g., via chemical chaperones) or by increasing the activity of the channel once in place at the membrane. The former class of bioactives has been termed CFTR correctors and the latter, CFTR activators or openers.[6] Agents operating via either mechanism can be detected using one of several cell-based fluorescence assays measuring chloride efflux. To date, several classes of small molecule compounds have been identified as CFTR correctors or activators, including flavonoids,[7] xanthines,[8] benzoquinoliziniums,[9] benzimidazolones,[10] and isoxazoles.[11] This paper describes our discovery and subsequent medicinal chemistry investigation of a novel class of CFTR modulators, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Tic) diamides. While the CFTR activity of these compounds is apparent even at low concentrations (<10 nM), their mechanism of action has yet to be elucidated.

Our CF drug discovery program was powered by a high-throughput screen, which utilized C127 murine mammary epithelial cells stably transfected with recombinant ΔF508 CFTR.[12] Chloride efflux was measured by monitoring the fluorescence of a halide sensitive dye, MQAE (*N*-(ethoxycarbonylmethyl)-6-methoxyquinolinium bromide), and normalized by scaling between the relative responses of untreated cells (0%) and cells maintained at low temperature (28–30 °C; 100%), a condition which is known to increase the level of functional ΔF508 CFTR.[13] Analogs of our lead compounds were tested at multiple concentrations to generate dose–response

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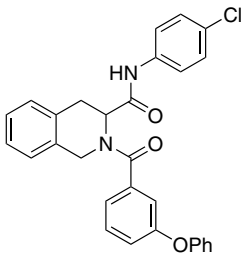
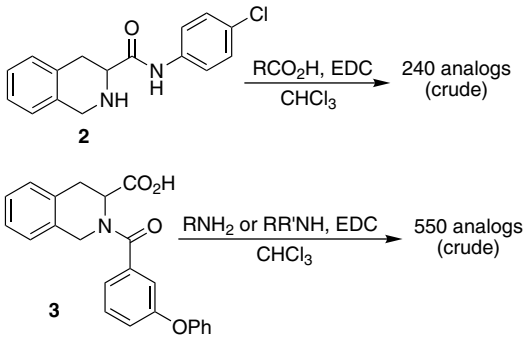
curves and associated EC_{50} values. The peak level of activity from this curve is referred to here as a 'maximum response' percentage (MR).

Screening of our in-house compound library, which was prepared and tested as mixtures (generally 100 theoretical compounds per sample), yielded several hits. Individual active compounds were identified via a stepwise deconvolution/retesting process and confirmed by determining EC_{50} values for the final purified and characterized compounds. This effort yielded several interesting actives including the 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Tic) diamide, **1**.



1
EC_{50}: 294 nM
Max. Resp. 72%

Analog preparation around this initial lead was carried out in two stages. First, a large array of single, unpurified Tic diamides was prepared in one step (amide coupling) from one of two purified Tic precursors (Scheme 1). While the products were not subjected to purity analysis, pilot reactions for this simple conversion indicated yields were generally robust. The objective of this exercise was to quickly survey the structural space around compound **1** and identify, if evident, general SAR trends. This information was used to guide our second, more deliberate, phase of analoging, where selected target molecules were purified and characterized prior to biological testing. The structures of the crude array analogs are not detailed here, but discussed generally with regards to SAR findings (vide infra). The structures of the purified analogs are shown in the tables that follow.



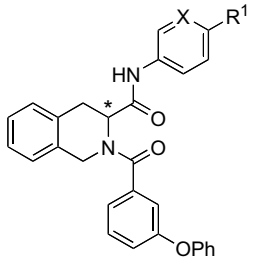
Scheme 1.

Crude analog arrays were generated utilizing standard EDC (1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide) amidation chemistry.[14] Intermediate **2** was coupled with a collection of 240 commercially available carboxylic acids, including alkyl, aryl, and arylalkyl types with substituents of varying size and polarity. Likewise, intermediate **3** was coupled to a diverse set of 550 primary and secondary amines. After confirming that neither starting material was active in the CFTR assay, analog data was examined to reveal several strong SAR trends. It was found that only a select group of benzoic acids coupled with amine **2** to yield diamides of significant activity, specifically those featuring moderately polar to non-polar substituents in 3-position. The most potent of these was the original acid component of **1**, 3-phenoxybenzoic acid. Lesser activity was observed with a small number of 3,4-disubstituted benzoic acids. Alkanoic acids, substituted or otherwise, did not yield products of significant activity. In the case of the analogs prepared from acid **3**, activity spiked sharply around a certain set of arylamine inputs. While alkyl and secondary amines produced only a few weakly active diamides, anilines bearing non-polar to moderately polar substituents in the 4-position gave robust activity. In particular, several 4-alkylanilines afforded potent analogs, though activity seemed to be concentrated in the straight chain variants. Anilines bearing other modestly polar 4-substituents (halides, alkoxides, esters, *N*-alkyl-*N*-acylamino, etc.) returned products with varying levels of notable activity. This gross survey of structural preferences seemed to indicate that SAR in the Tic diamide series is fairly sharp with activity tightly clustered around the structure of **1**. In light of this finding, subsequent analoging efforts focused on compounds, which mirrored the positional and electronic substitution patterns of this lead compound.

The purified Tic diamides prepared featured changes to both sidechains of compound **1**.[15] Many of the requisite amines and carboxylic acids were commercially available; the remainder were prepared by simple, known methods (syntheses not shown). Initial analogs were prepared as racemates, but, as efforts continued, single enantiomers were examined. Activity was found to reside exclusively in the (*S*)-isomer and later analogs were prepared only in this form.

Structures and data for aniline modified compounds are shown in Table 1. Halogen substituents, as demonstrated by compounds **4**–**7**, are generally acceptable in the 4-position. The favorability of straight chain alkyl groups is confirmed by analogs **8**–**12**. Interestingly, there appears to be a discernible preference in chain length, with activity peaking at five carbons and falling off in either direction. Branching, even four carbons out from the aromatic ring, continues to have a detrimental effect on activity as exemplified by compound **13**. Alkoxy groups are also acceptable in the 4-position (e.g., **14**, **15**), although activity is reduced relative to alkyl variants. Polar amino groups, as anticipated, are not tolerated (e.g., **16**). Although hinted at in our crude arrays, the activity of *N*-alkyl-acetanilide compounds **17** and **18** and the pyridine variants **19**–**21** is pleasantly surprising. In the end,

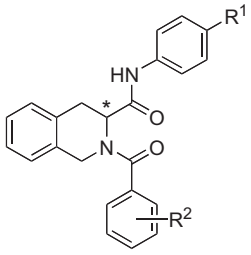
Table 1. Amine modified Tic diamides



Compd	*R/S	R^1	X	EC_{50} (nM)	MR[a] (%)
4	*R/S*	F	CH	202	92
5	*S*	Cl	CH	119	61
6	*R/S*	Br	CH	292	65
7	*R/S*	I	CH	314	67
8	*R/S*	*n*-Butyl	CH	202	68
9	*R/S*	*n*-Pentyl	CH	61	72
10	*S*	*n*-Pentyl	CH	23	75
11	*R/S*	*n*-Hexyl	CH	100	75
12	*R/S*	*n*-Heptyl	CH	230	63
13	*R/S*	4-Me-Pentyl	CH	160	95
14	*R/S*	OEt	CH	420	39
15	*R/S*	OBu	CH	145	64
16	*R/S*	$OCH_2CH_2N(CH_3)_2$	CH	NA	NA
17	*R/S*	*N*-Acetyl-*N*-butyl-amino	CH	150	66
18	*R/S*	*N*-Acetyl-*N*-pentyl-amino	CH	58	71
19	*R/S*	H	N	197	41
20	*R/S*	Cl	N	119	66
21	*S*	*n*-Pentyl	N	62	53

[a] Maximum response relative to cold temperature control (100%).

Table 2. Benzoic acid modified Tic diamides



Compd	*R/S	R^1	R^2	EC_{50} (nM)	MR[a] (%)
22	*R/S*	Cl	2-OPh	338	55
23	*R/S*	Cl	4-OPh	NA	NA
24	*R/S*	Cl	3-Bz	336	82
25	*R/S*	Cl	3-Bn	291	67
26	*R/S*	Cl	3-SPh	394	55
27	*R/S*	Cl	3-(4-(OMe)OPh)	167	92
28	*R/S*	Cl	3-(4-(CF_3)OPh)	282	69
29	*R/S*	Cl	3-(4-(F)OPh)	590	70
30	*R/S*	Cl	3-(3,4-$(Cl)_2$OPh)	NA	NA
31	*R/S*	Cl	3-O-(2-pyridyl)	268	74
32	*S*	*n*-Pn	H	NA	NA
33	*R/S*	*n*-Pn	3-OH	NA	NA
34	*S*	*n*-Pn	3-OMe	176	93
35	*S*	*n*-Pn	3-OEt	26	73
36	*S*	*n*-Pn	3-O-*i*-Pr	6.3	69
37	*S*	*n*-Pn	3-O-*t*-Bu	10	78
38	*S*	*n*-Pn	3-O-Cyclopentyl	15	74
39	*S*	*n*-Pn	3-O-Cyclohexyl	20	71
40	*S*	*n*-Pn	3-O-(*N*-Me-piperidin-4-yl)	NA	NA
41	*S*	*n*-Pn	3-Cl	51	52
42	*S*	*n*-Pn	3,4-Cl_2	82	40
43	*S*	*n*-Pn	3-OPh-4-OMe	12	64
44	*S*	*n*-Pn	3-O-*i*-Pr-4-OMe	3.5	72
45	*S*	*n*-Pn	3-CH_2 ($N(Me)_2$)-4-OMe	NA	NA
46	*S*	*n*-Pn	3-O-*i*-Pr-4-CH_2 ($N(Me)_2$)	NA	NA

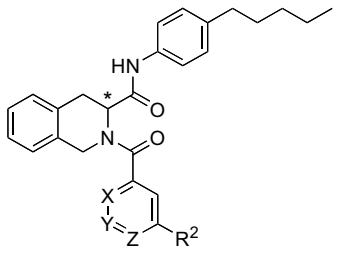
[a] Maximum response relative to cold temperature control (100%).

however, the optimal substituent for the aniline unit appears to be the 4-*n*-pentyl chain and all our later analogs feature this group. This change results in a fivefold improvement in potency versus the lead compound **1**.

Analogs featuring changes to the acid component are shown in Tables 2 and 3. Although the 2-phenoxybenzoyl analog of **1** does exhibit reasonable activity, compounds **22** and **23** confirm the preference for substitution at the 3-position. There is considerable flexibility in the form of the 3-substituent, however. A variety of linker modified (e.g., **24**–**26**) and phenoxy substituted compounds (e.g., **27**–**29**) return very respectable, and sometimes improved, activity. At the same time, the dichloro analog **30** demonstrates that there are limits to acceptable substitution. Cutting back the 3-substituent to either hydrogen or hydroxyl is not allowed (e.g., **32**, **33**), although smaller ethers at this position do result in good activity (i.e., the methoxy group of **34**). From this point the 3-substituent was expanded into several larger alkoxy and cycloalkoxy variants (e.g., **35**–**39**), all of which exhibited impressive potency. The one exception was compound **40**, which contains an embedded tertiary amine. Halogens, as exemplified by analogs **41** and **42**, are also acceptable substituents, although activity suffers somewhat relative to alkoxy analogs. Another key observation was that a small 4-substituent (i.e., methoxy) can augment the potency of a 3-substituent. This finding led to our most potent analog, compound **44**, which boasts a near sevenfold increase in activity versus the corresponding phenoxy derivative. As a final area of investigation, we examined aza analogs of selected compounds (Table 3). Compounds **47**–**53** clearly demonstrate this is an acceptable modification.

Finally, as an initial attempt to characterize the activity of Tic diamides, a series of assay timing experiments was carried out. It was determined that an exposure time on cells of 17–24 h was required for maximal compound activity. Half-maximal activity was observed after a 6–8 h incubation period. Because agents directly targeting membrane bound CFTR (activators) should, in general, have a rapid onset of action, this observation seems to be more consistent with a corrector mechanism, though additional studies will be needed to confirm this hypothesis. Furthermore, it appears that the activity of these compounds extends beyond the ΔF508 form of CFTR. In an assay analogous to our high-throughput screen, compound **1** produced a robust enhancement of chloride efflux in C127 cells stably transfected with wild-type protein. If Tic diamides do, indeed, act to promote ΔF508 CFTR trafficking, the same mechanism may apply to

Table 3. Azine carboxylic acid modified Tic diamides



Compd	*R/S	R^2	X/Y/Z	EC_{50} (nM)	MR[a] (%)
47	*S*	OPh	N/CH/CH	20	85
48	*S*	O-*i*-Pr	N/CH/CH	6.0	74
49	*S*	O-Cyclohexyl	N/CH/CH	9.4	83
50	*S*	Cl	N/CH/CH	79	55
51	*S*	O-Cyclohexyl	CH/N/CH	35	71
52	*S*	O-*i*-Pr	CH/N/CH	16	55
53	*S*	O-*i*-Pr	CH/CH/N	11	51

[a] Maximum response relative to cold temperature control (100%).

the wild-type protein, which is also known to be fairly inefficiently processed, with only an estimated 20–25% of the immature protein reaching the cell surface.[16]

In summary, screening of our in-house compound library led to the discovery of a novel class of CFTR modulators capable of stimulating chloride secretion, Tic diamides. Subsequent analoging was carried out in two stages: rapid array synthesis (unpurified analogs) was used to quickly and broadly assess SAR around the lead while a more focused, traditional medicinal chemistry effort followed. By optimizing both the acid and amine regions of the lead compound, we were able to improve CFTR activity of the series more than 30-fold.

Acknowledgements

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12. Murine C127 cells expressing ΔF508 CFTR were grown in microtiter plates under a 37 °C, 5% CO_2 atmosphere (Dulbecco's Modified Eagle Medium supplemented with fetal bovine serum). After three days, the cultures were switched to serum-free medium and treated overnight with test compounds (final concentration, 12.5 μM) and MQAE (10 mM). The cells were then washed with a chloride-containing buffer to remove extracellular MQAE and incubated to quench intracellular MQAE fluorescence. Chloride buffer was replaced with nitrate containing buffer with added forskolin (10 μM) and isobutylmethylxanthine (100 μM). The buffers were adjusted to pH 7.4 and contained (mM): HEPES (10), K_2HPO_4 (2.4), KH_2PO_4 (0.6), $CaSO_4$ (1.0), $MgSO_4$ (1.0), NaCl (150), or $NaNO_3$ (150), glucose (25). CFTR chloride transport was determined by measuring the rate of increase in MQAE fluorescence over the first five minutes (excitation 360 nM, emission 460 nM). False positives (i.e., compounds which induce non-specific chloride leakage) were identified by measuring chloride flux in cells treated with nitrate containing buffer without added forskolin and isobutylmethylxanthine.
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14. The racemic intermediates **2** and **3** were prepared from commercially available *N*-(*tert*-butoxycarbonyl)-Tic and Tic methyl ester, respectively. EDC amidation of the former starting material with 4-chloroaniline followed by trifluoroacetic acid mediated amine deprotection afforded **2**. Amidation of the latter starting material with 3-phenoxybenzoic acid followed by hydrolysis of the ester furnished **3**. For **2**: ^{1}H NMR ($CDCl_3$) δ 9.42 (s, 1H), 7.65–7.53 (m, 2H), 7.36–7.04 (m, 6H), 4.12–3.97 (m, 2H), 3.68 (dd, J = 10.4, 5.4 Hz, 1H), 3.35 (dd, J = 16.3, 5.4 Hz, 1H), 2.90 (dd, J = 16.3, 10.4 Hz, 1H) ppm. For **3** (6:4 mixture of rotamers): ^{1}H NMR ($CDCl_3$) δ 7.44–6.78 (m, 13H), 5.29

(t, J = 5.7 Hz, 0.6H), 5.18 (d, J = 17.9 Hz, 0.4H), 4.81–4.73 (m, 0.4H), 4.62–4.47 (m, 1.6H), 3.30–3.15 (m, 1.6H), 3.13–3.01 (m, 0.4H) ppm.

15. As with intermediates **2** and **3**, preparation of purified Tic diamide analogs relied on standard aminoacid chemistry. Thus, racemic or L-*N*-(*tert*-butoxycarbonyl)-Tic was treated with the amine component and EDC. The resulting amide was *N*-deprotected with trifluoroacetic acid and then amide coupled to the carboxylic acid component. The final products were purified to homogeneity by column chromatography and deemed to be of 95% or greater purity by ^{1}H NMR. As example, compound **12** (8:2 mixture of rotamers): ^{1}H NMR ($CDCl_3$) δ 8.81 (s, 0.8H), 7.54 (br s, 0.2H), 7.51–6.74 (m, 17H), 5.27 (t, J = 6.4 Hz, 0.8H), 5.16 (d, J = 16.5 Hz, 0.2H), 4.85–4.74 (m, 0.2H), 4.72–4.52 (m, 1H), 4.44–4.31 (m, 0.8H), 3.65–3.52 (m, 0.8H), 3.51–3.37 (m, 0.2H), 3.19–3.03 (m, 1H), 2.55 (t, J = 7.6 Hz, 2H), 1.64–1.49 (m, 2H), 1.35–1.12 (m, 8H), 0.87 (t, J = 6.6 Hz, 3H) ppm.
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