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Isoxazolopyrimidines as Novel ΔF508-CFTR Correctors

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Abstract

Using a cell-based high-throughput screen, we identified isoxazolo[5,4-*d*]pyrimidines as novel small-molecule correctors of the cystic fibrosis mutant protein ΔF508-CFTR. 22 Isoxazolo[5,4-*d*]pyrimidine analogues were synthesized and tested. Synthesis of the key intermediate, 5-amino-3-arylisoxazole-4-carboxamide, was accomplished by nitrile oxide cycloaddition to (2-amino-1-cyano-2-oxoethyl)sodium. Formation of 3-arylisoxazolo-[5,4-*d*]pyrimidin-4(5*H*)-one and chlorination gave 4-chloro-3-arylisoxazolo[5,4-*d*]pyrimidine. Finally, functionalization at C-4 of the pyrimidine ring by nucleophilic substitution gave the targeted isoxazolo[5,4-*d*]pyrimidines. Six of the reported analogues had low micromolar potency for increasing halide transport in ΔF508-CFTR cells.

Keywords

cystic fibrosis; ΔF508-CFTR; corrector; isoxazolopyrimidine

Cystic fibrosis (CF) is a relatively common inherited disease caused by mutations in the CF transmembrane conductance regulator protein (CFTR). The most common CFTR mutation, deletion of phenylalanine at residue 508, ΔF508, results in a defective CFTR protein that fails to traffic to the plasma membrane and fails to activate as a chloride channel.[1–3] As a consequence a viscous mucus accumulates in the lung, which promotes bacterial growth and progressive deterioration of lung function.[4,5] Although CF patient care has improved considerably, there is no cure for CF. While available CF-relevant drugs target the various symptoms of CF, many current studies are focused on the discovery of compounds that restore normal ΔF508-CFTR processing, which are called correctors.[6–14]

Using screening methods we developed to discover bithiazoles as the first ΔF508-CFTR correctors,[14] we screened a new collection of 50,000 diverse, drug-like small molecules (from Chemdiv Inc., San Diego CA). The goal was to identify new corrector scaffolds as potential drug candidates. Screening and secondary verification studies indicated isoxazolopyrimidines as ΔF508-CFTR correctors. Fused pyrimidines have broad-ranging biological activities, including antibacterial,[15] CRF (corticotropin-releasing factor) antagonist,[16] antimalarial,[17] and antitumor activities.[18] While isoxazolopyrimidines are not well studied, pyrimidine-based synthesis and application studies have been the focus of numerous research groups, including Laitonjam et al.,[19] Badiger et al.,[20] Wentrup et al.,[21] Peinador et al.,[22] and Sankyo Co., Ltd., Japan.[23] Herein, we report the synthesis and ΔF508-

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CFTR corrector activity of twenty-two novel isoxazolopyrimidines. To the best of our knowledge, this is the first report of these heterocycles as ΔF508-CFTR correctors.

Our synthetic route, illustrated in Scheme 1, began with hydroximoyl chloride formation from aryl aldehyde **1** in two steps in quantitative yield.[24] Hydroximoyl chlorides are well-established nitrile oxide precursors (via dehydrochlorination) that readily participate in 1,3-diploar cycloadditions. Adapting a protocol reported by Rajagopalan and Talaty,[25] hydroximoyl chloride **3** was dissolved in ethanol and added dropwise to an ethanol suspension of (2-amino-1-cyano-2-oxoethyl)sodium to produce 5-amino-3-arylisoxazole-4-carboxamide **4** (35–89% yield). Next, carboxamide **4** and triethyl orthoformate were dissolved in acetic anhydride, and the solution was refluxed for 2.5 hours to give 3-isoxazolo[5,4-*d*]pyrimidin-4(5*H*)-one **5** in 48–75% yield. Pyrimidinone **5** was treated with phosphorous oxychloride (0.83 equiv) in dry acetonitrile (alternatively, toluene can be used as solvent) at reflux in the presence of *N, N*-dimethylaniline (2 equiv) for 3 hours in a sealed tube under a N_2 atmosphere to afford 4-chloropyrimidine **6**.[26] The chlorination of **5a** to **6a** was also attempted with excess phosphorous oxychloride as described by Rajagopalan and Talaty.[25] However, after washing with water, only starting material **5a** was recovered suggesting that, under acidic conditions, water displaces the chloride to reverse the transformation (e.g., **6a** → **5a**). We also found that treating chloropyrimidine **6a** with 2,3-dimethylaniline under reflux in methanol for 24 hours in the presence of K_2CO_3 failed to deliver isoxazolopyrimidine **7** (4-chloro- → 4-hydroxypyrimidine occurred instead). Fortunately, target molecules **7–26** were obtained by refluxing an isopropanol solution of **6** with the appropriate aniline (2 equiv) in the presence of a catalytic amount of HCl gas.[27]

Twenty isoxazolo[5,4-*d*]pyrimidine analogues (**7–26**) were synthesized using the method outlined in Scheme 1 by varying R^1 (four inputs) and R^2 (five inputs). ΔF508-CFTR corrector data for six active compounds are summarized in Table 1. Compounds were tested using a cell-based fluorimetry assay of iodide influx as described previously.[30] Corrector activity of each compound was verified by the lack of compound effect on FRT-null cells (not expressing ΔF508-CFTR) and by inhibition of the increased iodide influx by the thiazolidinone CFTR inhibitor $CFTR_{inh}$-172. Compound **7** exhibited a greater V_{max} value than *s-cis* locked bithiazole **27** reported in our earlier studies.[14] Compounds **10–14** also had comparable corrector activities (Table 1). An SAR trend is observed when the location of substituent R on the phenyl ring of the isoxazole portion of the molecule is varied. For instance, when R^1 = 3-Cl or 2-OMe, corrector activity is diminished. Of the isoxazolopyrimidines tested, only those with a *para* substituent on the isoxazole phenyl moiety (4-F and 4-Cl) were active. This dramatic change in corrector activity in the 4-F or 4-Cl versus the 3-Cl or 2-OMe suggests that the aldehyde reagent employed in Scheme 1 is best fixed as 4-chloro- or 4-fluorobenzaldehyde. When R^2 is fixed as 2,3-dimethyl (e.g., employing 3,4-dimethylaniline in Scheme 1), isoxazolopyrimidines **7** and **12** had comparable IC_{50} values, although the V_{max} of **7** is much larger. The best IC_{50} value was obtained when 3-methoxyaniline (**13**) was employed.

The hydrogen bond donor/acceptor profile of these isoxazolopyrimidines was then considered. For example, we investigated changing the NH of isoxazolopyrimidine **7** to an NMe (Figure 1; **28** was prepared by N-methylating **7** with iodomethane in methanol + NaH). We also modified **7** (starting from **6a**) by replacing the 2,3-dimethylaniline moiety with a 3-methylpyridin-2-amine moiety to give analogue **29**. When comparing the LogP values of compound **7**, **28**, and **29** (Figure 1), there is no significant difference between **7** and **28**, while **29** has a lower LogP value (**7**: LogP 5.4813; **28**: LogP 5.7171; **29**: LogP 4.3731; data generated with ChemPropPro 8.03). Unfortunately, isoxazolopyrimidines **28** and **29** showed little corrector activity compared to **7**.

In summary, a collection of 4-aniline substituted derivatives of isoxazole[5,4-*d*]pyrimidines was synthesized. The synthetic protocol outlined in Scheme 1 is suitable to high-volume combinatorial analogue generation. The six isoxazolopyrimidine-fused heterocycles listed in Table 1 demonstrate corrector activity with low micromolar potency. Isoxazolopyrimidines might thus serve as novel correctors to probe defective ΔF508-CFTR cellular processing and for further preclinical development.

4-Fluorobenzaldehyde Oxime (2a)

To a stirred solution of hydroxylamine·HCl (1.85 g, 26.6 mmol) in THF–EtOH–H_2O (30 mL:75 mL:15 mL) was added 4-fluorobenzaldehyde (**1a**, 3.0 g, 24.2 mmol), and the mixture was stirred at r.t. for 25 min at which time EtOH and THF were removed in vacuo. The residue was extracted with Et_2O (3 × 30), washed with brine, dried over anhyd Na_2SO_4, and filtered. Evaporation of the solvent afforded **2a** (3.36 g, 100%), which was used in the next step without further purification. ^{1}H NMR matches the literature data;[28] ESI-MS: *m/z* = 139.99 $[M + H]^+$. Oximes **2b–d** were prepared following analogous procedures with or without NaOAc.

4-Fluoro-*N*-hydroxybenzimidoyl Chloride (3a)

To a stirred solution of NCS (3.55 g, 26.6 mmol) in DMF–CH_2Cl_2 (20 mL:120 mL) was added dropwise a CH_2Cl_2 (120 mL) solution of pyridine (200 μL, 2.42 mmol), Et_3N (3.37 mL, 24.2 mmol), and 4-fluorobenzaldehyde oxime (**2a**: 3.36 g, 24.2 mmol). The solution was stirred at r.t. for 12 h at which time it was washed with H_2O (5 × 100 mL) and concentrated to afford **3a** (4.2 g, 100%). ^{1}H NMR matches the literature data.[28] Hydroximoyl chloride **3b–d** were prepared following analogous procedures.

5-Amino-3-(4-fluorophenyl)isoxazole-4-carboxamide (4a)

A freshly prepared NaOEt in EtOH solution, made at r.t. from Na metal (977 mg, 42.5 mmol) in abs. EtOH (150 mL), was added to a stirred solution of 2-cyanoacetamide (3.57 g, 42.5 mmol) in abs. EtOH (50 mL) at 50 °C. To the resulting clear solution cooled to 0 °C was added dropwise a solution of hydroximoyl chloride **3a** (7.38 g, 42.5 mmol) in abs. EtOH (100 mL). The resulting suspension was stirred at r.t. for 30 min and then refluxed overnight. EtOH was removed in vacuo, and the resulting residue was washed with H_2O and recrystallized from MeOH to yield **4a** as light yellow crystals (2.91 g, 31%).[29a] Carboxamides **4b–d** were prepared following analogous procedures.

3-(4-Fluorophenyl)isoxazolo[5,4-*d*]pyrimidin-4(5*H*)-one (5a)

A mixture of **4a** (2.88 g, 13 mmol), triethyl orthoformate (2.16 mL, 13 mmol), and Ac_2O (15 mL) was refluxed overnight. The solution was cooled on ice, and the resulting precipitate was collected by filtration, washed with H_2O, and air dried to yield **5a** as an off white powder (2.05 g, 68%).[29b] Pyrimidinones **5b–d** were prepared following analogous procedures refluxing for from 2.5–12 h.

4-Chloro-3-(4-fluorophenyl)-4,5-dihydroisoxazolo[5, 4-*d*]pyrimidine (6a)

$POCl_3$ (167 μL, 1.79 mmol) was added to a solution of **5a** (0.54 g, 2.16 mmol), *N, N*-dimethylaniline (411 μL, 3.24 mmol), and Et_3N·HCl (0.6 g, 4.33 mmol) in dry MeCN (1.7 mL) under N_2 in a sealable tube. The mixture was refluxed for 3 h, cooled, and the MeCN was removed in vacuo. The resulting residue was subjected to flash column chromatography purification (hexane–EtOAc = 9:1) to provide **6a** as off-white crystals (480 mg, 92%).[29c] Pyrimidines **6b–d** were prepared following analogous procedures.

N-(2,3-Dimethylphenyl)-3-(4-fluorophenyl)-4,5-dihydroisoxazolo[5,4-*d*]pyrimidin-4-amine (7)

A mixture of **6a** (111 mg, 0.443 mmol), 2,3-dimethylbenzenamine (108 μL, 0.885 mmol), and catalytic HCl (g) in 2-PrOH (2 mL) was sealed in a tube and refluxed overnight. Cooling on ice produced white crystals which were collected by filtration and washed with cold 2-PrOH to afford **7** (112 mg, 76%).[29d] Isoxazolopyrimidines **8–26** and **29** were prepared following analogous procedures.

N-(2,3-dimethylphenyl)-3-(4-fluorophenyl)-4,5-dihydroisoxazolo[5,4-*d*]pyrimidin-4-amine (28)

To a solution of **7** (95 mg, 0.284 mmol) in DMF (3 mL) was added 60% NaH (26 mg, 0.643 mmol) in mineral oil. The suspension was stirred at r.t. for 30 min; MeI (40 μL, 0.643 mmol) was added and the mixture stirred at r.t. overnight. The resulting mixture was washed with H_2O (3 × 10 mL), extracted with EtOAc (10 mL), washed with brine, dried over anhyd Na_2SO_4, and filtered. Removal of EtOAc afforded crude **28** which was subjected to flash chromatography (hexanes–EtOAc = 9:1 → 4:1) to deliver pure **28** (58 mg, 61%).[29e]

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References and Notes

1. Riordan JR. Annu. Rev. Biochem. 2008; 77:701. [PubMed: 18304008]
2. Kunzelmann K, Nitschke R. Exp. Nephrol. 2000; 8:332. [PubMed: 11014930]
3. Kleizen B, Braakman I, de Jonge HR. Eur. J. Cell Biol. 2000; 79:544. [PubMed: 11001491]
4. Tarran R, Button B, Boucher RC. Annu. Rev. Physiol. 2006; 68:543. [PubMed: 16460283]
5. Verkman AS, Song Y, Thiagarajah JR. Am. J. Physiol. Cell Physiol. 2003; 284:C2. [PubMed: 12475759]
6. Van Goor F, Hadida S, Grootenhuis PD, Burton B, Cao D, Neuberger T, Turnbull A, Singh A, Joubran J, Hazlewood A, Zhou J, McCartney J, Arumugam V, Decker C, Yang J, Young C, Olson ER, Wine JJ, Frizzell RA, Ashlock M, Negulescu P. Proc. Natl. Acad. Sci. U.S.A. 2009; 106:18825. [PubMed: 19846789]
7. Verkman AS, Galietta LJ. Nat. Rev. Drug Discov. 2009; 8:153. [PubMed: 19153558]
8. Van Goor F, Straley KS, Cao D, González J, Hadida S, Hazlewood A, Joubran J, Knapp T, Makings LR, Miller M, Neuberger T, Olson E, Panchenko V, Rader J, Singh A, Stack JH, Tung R, Grootenhuis PD, Negulescu P. Am. J. Physiol. 2006; 290:L1117.
9. Verkman AS, Lukacs GL, Galietta LJV. Curr. Pharm. Des. 2006; 12:2235. [PubMed: 16787252]
10. Rosser MFN, Grove DE, Cry DM. Curr. Chem. Biol. 2009; 3:420.
11. Pedemonte N, Lukacs GL, Du K, Caci E, Zegarra-Moran O, Galietta LJV, Verkman AS. J. Clin. Invest. 2005; 115:2564. [PubMed: 16127463]
12. Pedemonte N, Sonawane ND, Taddei A, Hu J, Zegarra-Moran O, Suen YF, Robins LI, Dicus CW, Willenbring D, Nantz MH, Kurth MJ, Galietta LJV, Verkman AS. Mol. Pharmacol. 2005; 67:1797. [PubMed: 15722457]
13. Yoo CL, Yu GJ, Yang B, Robins LI, Verkman AS, Kurth MJ. Bioorg. Med. Chem. Lett. 2008; 18:2610. [PubMed: 18394886]
14. Yu GJ, Yoo CL, Yang B, Lodewyk MW, Meng L, El-Idreesy TT, Fettinger JC, Tantillo DJ, Verkman AS, Kurth MJ. J. Med. Chem. 2008; 51:6044. [PubMed: 18788728]

15. Burch HA, Benjamin LE, Russell HE, Freedman R. J. Med. Chem. 1974; 17:451. [PubMed: 4151508]
16. Frietze, WE. Chem. Abstr WO 2000011003. 2000. p. 180596**2000**;
17. Hynes JB, Gratz RF, Ashton WT. J. Med. Chem. 1972; 15:1332. [PubMed: 4629015]
18. Taylor EC, Patel HH. Tetrahedron. 1992; 48:8089.
19. Thokchom HS, Nongmeikapam AD, Laitonjam WS. Can. J. Chem. 2005; 83:1056.
20. Adhikari VA, Savalgi VP, Badiger VV. Curr. Sci. 1988:703.
21. Kappe CO, Flammang R, Wentrup C. Heterocycles. 1994; 63:1615.
22. Quintela JM, Peinador C. Trends Heterocycl. Chem. 2006; 11:33.
23. Chem. Abstr. 1984; 101:110940. JP 59036683, **1984**;
24. Dixon SM, Milinkevich KA, Fujii J, Liu R, Yao N, Lam KS, Kurth MJA. J. Comb. Chem. 2007; 9:143. [PubMed: 17206843]
25. Rajagopalan P, Talaty CN. Tetrahedron. 1967; 23:3541.
26. Connolly DJ, Lacey PM, McCarthy M, Saunders CP, Carroll A-M, Goddard R, Guiry PJ. J. Org. Chem. 2004; 69:6572. [PubMed: 15387579]
27. Fernandes C, Oliveira C, Gano L, Bourkoula A, Pirmettis I, Santos I. Bioorg. Med. Chem. 2007; 15:3974. [PubMed: 17449254]
28. Di Nunno L, Vitale P, Scilimati A, Simone L, Capitelli F. Tetrahedron. 2007; 63:12388.
29. **Representative Spectral Data** (a) Compound **4a**: ^{1}H NMR (600 MHz, DMSO): δ = 7.65 (br s, 2 H), 7.63–7.58 (m, 2 H), 7.38–7.32 (m, 2 H). ^{13}C NMR (15 MHz, DMSO): δ = 171.70, 164.11, 163.85, 162.22, 159.78, 131.09, 131.04, 125.46, 125.44, 115.93, 115.79, 86.79. ESI-MS: *m/z* = 222.08 $[M + H]^{+}$. (b) Compound **5a**: ^{1}H NMR (300 MHz, DMSO): δ = 13.17 (br s, 1 H), 8.46 (s, 1 H), 8.38 (m, 2 H), 7.42 (m, 2 H). ESI-MS: *m/z* = 232.06 $[M + H]^{+}$. (c) Compound **6a**: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 9.02 (s, 1 H), 7.92–7.77 (m, 2 H), 7.28 (m, 2 H). ^{13}C NMR (150 MHz, $CDCl_3$): δ = 175.19, 165.60, 164.12, 158.07, 157.03, 156.72, 132.17, 132.11, 122.57, 116.39, 116.24, 110.62, 77.37, 77.16, 76.95. (d) Compound **7**: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.59 (s, 1 H), 7.85–7.69 (m, 2 H), 7.45 (d, *J* = 7.9 Hz, 1 H), 7.38–7.29 (m, 2 H), 7.16 (t, *J* = 7.7 Hz, 1 H), 7.10 (d, *J* = 7.5 Hz, 1 H), 6.81 (br s, 1 H), 2.31 (s, 3 H), 2.03 (s, 3 H). ^{13}C NMR (150 MHz, $CDCl_3$): δ = 176.12, 165.31, 163.63, 159.45, 157.18, 156.18, 138.28, 134.79, 131.06, 130.70, 130.64, 128.85, 126.29, 124.84, 123.31, 117.45, 117.30, 95.73, 20.71, 14.21. ESI-MS: *m/z* = 335.10 $[M + H]^{+}$. (e) Compound **28**: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.67 (s, 1 H), 6.79 (m, 7 H), 6.56 (br s, 1 H), 3.35 (s, 3 H), 2.00 (s, 2 H), 1.96 (s, 3 H). ^{13}C NMR (150 MHz, $CDCl_3$): δ = 176.07, 163.97, 162.25, 160.25, 157.52, 144.82, 139.36, 133.03, 130.43, 129.88, 127.08, 123.56, 114.55, 96.77, 40.97, 20.28, 15.68. ESI-MS: *m/z* = 349.12 $[M + H]^{+}$.
30. **General Procedure for Bioassays – Δ508-CFTR Corrector Activity Assay** Assays were performed by utilizing FRT epithelial cells stably coexpressing human ΔF508-CFTR and the high-sensitivity halide-sensing fluorescent protein YFP-H148Q/I152L used as described previously.[11] Cells were grown at 37 °C (95% air/5% CO_2) for 24 h and then incubated for 16–20 h with 50 μL of medium containing the test compound. At the time of the assay, cells were washed with PBS and then incubated with PBS containing forskolin (20 μM) and genistein (50 μM) for 20 min. Measurements were carried out using FLUOstar fluorescence plate readers (Optima; BMG LABTECH Gmbh), each equipped with 500 ± 10 nm excitation and 535 ± 15 nm emission filters (Chroma Technology Corp.). Each well was assayed individually for I^- influx by recording fluorescence continuously (200 ms per point) for 2 s (baseline) and then for 12 s after rapid (<1 s) addition of 165 μL PBS in which 137 mM Cl^- was replaced by I^-. I^- influx was computed by fitting the final 11.5 s of the data to an exponential for extrapolation of initial slope All experiments contained negative control (DMSO vehicle) and positive control corr-4a[11] ({*N*-[2-(5-chloro-2-methoxyphenylamino)-4′-methyl-4,5′-bithiazol-2′-yl]benzamide}). Background I^- influx (from DMSO control) was subtracted to report the increase in I^- influx in Table 1.

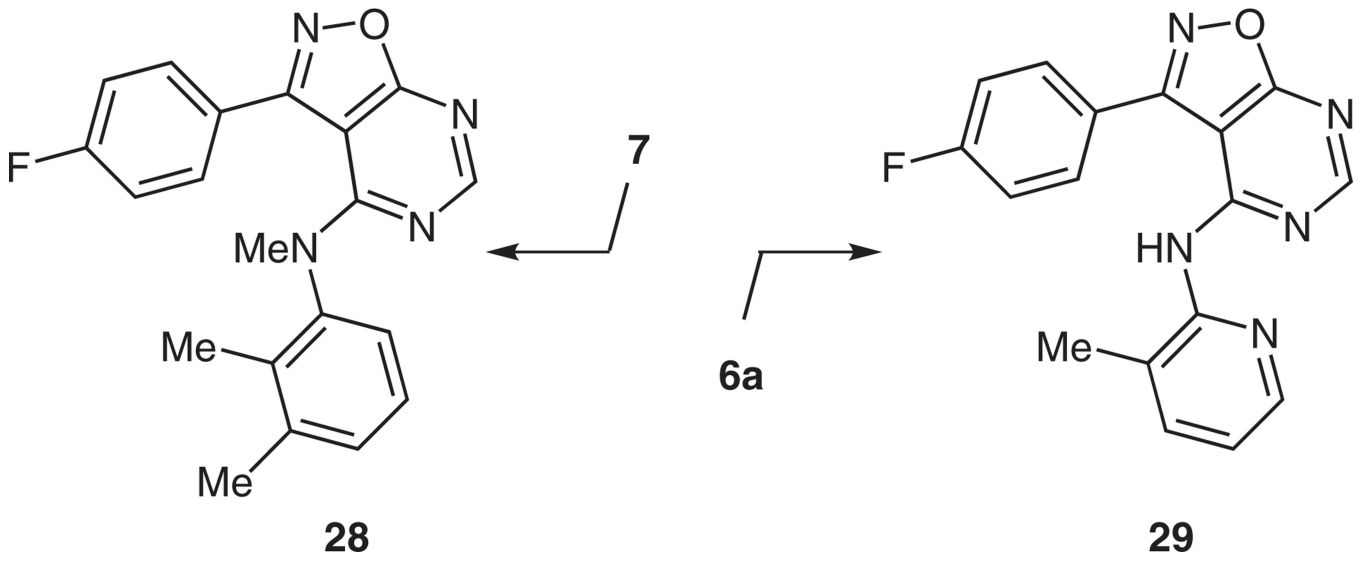
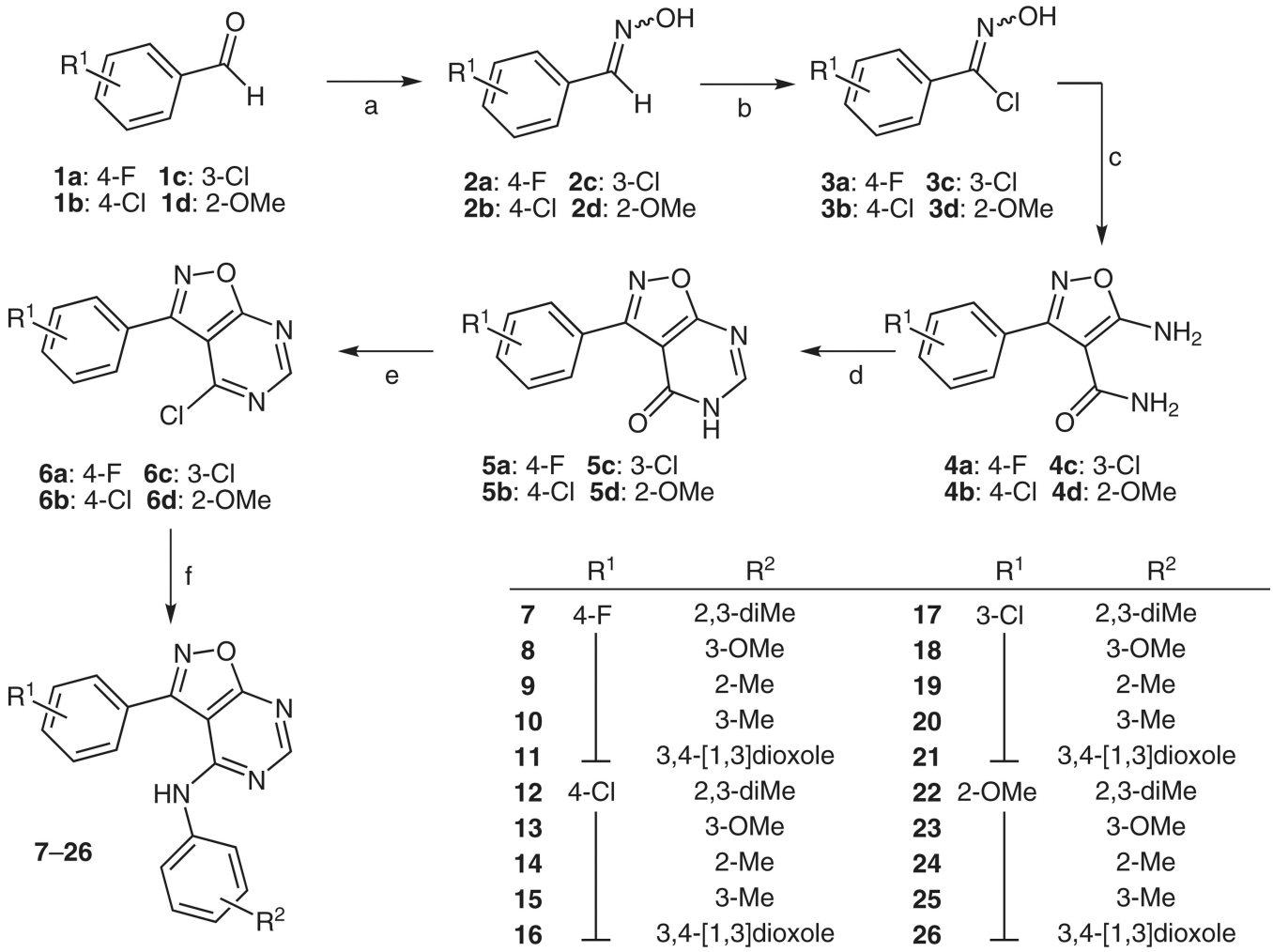


Figure 1.
N-Methyl and pyridyl analogues of **7**



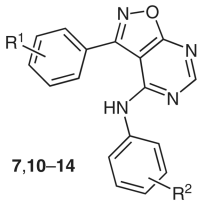
	R^1	R^2		R^1	R^2
7	4-F	2,3-diMe	**17**	3-Cl	2,3-diMe
8		3-OMe	**18**		3-OMe
9		2-Me	**19**		2-Me
10		3-Me	**20**		3-Me
11		3,4-[1,3]dioxole	**21**		3,4-[1,3]dioxole
12	4-Cl	2,3-diMe	**22**	2-OMe	2,3-diMe
13		3-OMe	**23**		3-OMe
14		2-Me	**24**		2-Me
15		3-Me	**25**		3-Me
16		3,4-[1,3]dioxole	**26**		3,4-[1,3]dioxole

Scheme 1.

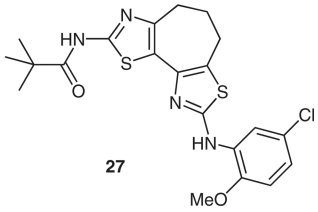
Reagents: (a) $NH_2OH{\cdot}HCl$, H_2O–EtOH–THF, NaOAc, 66–100%; (b) NCS, DMF–MeCl, Et_3N, pyridine (cat.), 60–95%; (c) i) 2-cyanoacetamide, NaOEt, EtOH, ii) **3**, EtOH, 35–89%; (d) triethyl orthoformate, Ac_2O, reflux, 2.5 h, 48–75%; (e) $POCl_3$ (0.83 equiv), *N, N*-dimethylaniline (2 equiv), $Et_3N{\cdot}HCl$ (2 equiv), MeCN, reflux under N_2, sealed tube, 2.5 h, 83–92%; (f) 2,3-dimethylaniline, *i*-PrOH, HCl (gas), 30–76%.

Table 1

IC_{50} and V_{max} Data for Active Isoxazolopyrimidines[a]



7,10–14



27

Compound	R^1	R^2	IC_{50} μM	V_{max} μM/s
7	4-F	2,3-diMe	3.9	161
10	4-F	3-Me	5.8	113
11	4-F	3,4-[1,3]dioxole	5.7	90
12	4-Cl	2,3-diMe	3.9	109
13	4-Cl	3-OMe	3.2	100
14	4-Cl	2-Me	6.9	103

[a]For comparison, IC_{50} and V_{max} of reference compound corr-4a[11] were 0.92 μM and 152 μM/s.