Creating an Antibacterial with in Vivo Efficacy: Synthesis and Characterization of Potent Inhibitors of the Bacterial Cell Division Protein FtsZ with Improved Pharmaceutical Properties

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3-Methoxybenzamide (1) is a weak inhibitor of the essential bacterial cell division protein FtsZ. Alkyl derivatives of 1 are potent antistaphylococcal compounds with suboptimal drug-like properties. Exploration of the structure—activity relationships of analogues of these inhibitors led to the identification of potent antistaphylococcal compounds with improved pharmaceutical properties.

Introduction

Multidrug-resistant strains of Gram-positive pathogens have evolved which are especially difficult to eradicate. In particular, the emergence and spread of multidrug-resistant Staphylococcus aureus is a serious health concern.1,2 Subsequently, there is a continuing need for alternative antibacterial agents, especially with novel mechanisms of action. The increasing use of methicillin resistant S. aureus screening with rapid diagnostics prior to elective hospital admissions means that a selective antistaphylococcal agent would be a useful addition to the clinician’s armory.

Cell division has been of considerable interest to the pharmaceutical industry as a target because it involves a group of well-conserved proteins that are all essential for the viability of a wide range of bacteria, and their activities are distinct from those of the proteins involved in mammalian cell division.4,5 FtsZ is an essential guanosine triphosphatase that undergoes GTP-dependent polymerization at midcell and assembles to form the Z-ring. When bacteria divide, FtsZ recruits other cell division proteins to synthesize the septum that enables the daughter cells to separate. FtsZ is structurally and functionally homologous to mammalian β-tubulin, which has been successfully exploited for cancer therapy.6–8 This suggests that FtsZ may also be amenable to inhibitor development.

Several compounds have been reported to block bacterial cell division through inhibition of FtsZ.4,9,10 Many of these reported inhibitors were explored, and 3-methoxybenzamide (compound 1) was found to be the most attractive for development into an antibacterial agent. Recently, we reported the identification of a potent derivative of 1, PC190723 (Figure 1, compound 2), that inhibits FtsZ, resulting in enlargement of the bacterial cells (Figure 2) and killing of staphylococci in vivo.11

The early structure—activity relationships (SAR) leading to the synthesis of potent 2,6-difluoro-3-alkyloxybenzamide FtsZ inhibitors from 1 has been published.12 These 2,6-difluoro-3-alkyloxybenzamides are 8000× more potent than 112 and are excellent reagents to explore bacterial cell biology. To be clinically efficacious, a compound must have appropriate physicochemical properties13 so that it is absorbed, distributed, and not extensively metabolized or rapidly excreted. The 2,6-difluoro-3-alkyloxybenzamides have suboptimal drug-like properties, such as absorption, distribution, metabolism, or excretion (ADME) properties, so the objective was to improve the pharmaceutical profile of these FtsZ inhibitors while retaining the on-target antistaphylococcal activity to create molecules suitable for preclinical development.

The SAR and the process used to create 2, a compound with attractive in vivo pharmacology, from the 2,6-difluoro-3-alkyloxybenzamide FtsZ inhibitors that have antibacterial activity, but suboptimal drug-like properties, are described here.

Chemistry

The routes to the target 3-substituted 2,6-difluoro-benzamide analogues are concise, straightforward, and are described below. The commercially available 2,6-difluoro-3-methoxybenzamide (3) was demethylated to the phenol (4) via treatment with boron tribromide in dichloromethane. The synthesis of most final compounds was achieved via alkylation of 4 with an alkyl halide in the presence of potassium carbonate with dimethylformamide as solvent (Schemes 1 and 2).

In the case of compounds 6j and 6k, the alkylation of 4 with the corresponding alcohols was performed under Mitsunobu reaction conditions, using triphenyl phosphine and disopropyl azodicarboxylate (DIAD) in tetrahydrofuran (THF) (Scheme 2).

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Abbreviations: ADME, absorption, distribution, metabolism and excretion; APCI, atmospheric pressure chemical ionization; AUC, area under the curve; BSA, bovine serum albumin; CFU, colony forming units; DIAD, diisopropyl azodicarboxylate; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; ESI, electrospray ionization; GTP, guanosine triphosphate; HPLC, high pressure liquid chromatography; IP, intraperitoneal; IV, intravenous; LC, liquid chromatography; MIC, minimal inhibitory concentration; MS, mass spectrometry; NMR, nuclear magnetic resonance; PO, per oral; PK, pharmacokinetics; R, retention time; SAR, structure—activity relationship; SC, subcutaneous; THF, tetrahydrofuran; TLC, thin layer chromatography; UV, ultraviolet.

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A subseries based on the 5-substituted benzothiazol-2-yl methoxy group was accessed by alkylation with a wide range of 5-substituted-2-halomethyl-benzothiazoles (Scheme 3). Further analogues were accessed by standard modification of several 5-position substituents.

Similarly, a small series of substituted thiazolopyridines was accessed by alkylation with substituted halomethyl-thiazolopyridines and subsequent standard modification (Scheme 6).

For the isomeric 4- and 6-substituted subseries of benzothiazoles, a different approach was used if the suitably substituted 2-halomethyl-benzothiazole was not available. 3-(Cyanomethoxy)-2,6-difluorobenzamide (19) was prepared via alkylation of 4 with chloroacetonitrile and served as a common intermediate for the synthesis of compounds 22a-e (Scheme 4). The 2-amino-1,3-benzothiazoles (20a-e) were converted to the required amino thiols (21a-e) by refluxing with KOH in aqueous 2-methoxyethanol. These in turn were condensed with 19 in EtOH at 120–150 °C to afford the desired 4-, 6- and 7-substituted benzothiazoles 22a–e.

Results and Discussion

The biological activities of the compounds were determined by measuring the minimal inhibitory concentrations (MICs) against S. aureus ATCC 29213 and by morphometric analysis using light microscopy to determine on-target activity, expressed by ballooning of the cocci.11,14 In line with CLSI approved standards, the reproducibility of the MIC test is within one 2-fold dilution of the actual end point.16 On-target activity was substantiated for selected compounds by generating compound-resistant strains; in all cases, a mutation was identified in the ftsZ gene (data not shown). Selected compounds with a suitable level of on-target activity were screened through a panel of assays to determine their in vitro ADME profiles. Compounds with drug-like in vitro ADME profiles were then characterized in vivo.

To improve the pharmaceutical properties of the 2,6-difluoro-3-alkoxybenzamide FtsZ inhibitors, a series of molecules where the alkyl substituent was replaced with a heterocycle, were synthesized (Scheme 2). Approximately 60 of this type of molecule were synthesized from commercial building blocks where the molecular weight of R1 was in the 100–200 Da range. Replacing the alkyl substituent with such heterocycles was designed to reduce the $\log P$, the number of rotatable bonds, and the plasma protein binding of the molecule as well as to introduce the potential for additional hydrogen bonding to the FtsZ protein.

The antibacterial activity against S. aureus and inhibition of cell division for a selection of this series of 3-substituted 2,6-difluorobenzamides (compounds 6a–k) is presented in Table 1. Several methoxy-linked heterocyclic derivatives demonstrated potent antistaphylococcal activity mediated through inhibition of cell division. The most potent of these, a benzothiazole compound 6g, was selected for additional exploration of the SAR. Analogues with substitutions in each of the available positions of the benzothiazole were prepared (Schemes 3, 4, 5). This series of substituted benzothiazoles were tested for inhibition of cell division and antibacterial activity against S. aureus (Table 2). The presence of a chloro substitution in the 4-, 6-, or 7-position of the benzothiazole (compounds 22a, 22b, and 24e) resulted in active compounds but with reduced...
antibacterial activity and potency in the cell division assay. Similarly, 4- and 5-methyl as well as 4-methoxy substitutions of the benzothiazole (compounds $22c$, $8f$, and $25$) resulted in less potent but active analogues. One compound, with a 5-methoxy substitution ($8h$), retained similar activity to the unsubstituted benzothiazole. From this set of substituted
benzothiazoles, two analogues, with 5-chloro or 5-phenyl substitutions, were found to have improved on-target antibacterial activity (8j and 8a) with MICs of 0.25 μg/mL.

Because improved activity was observed for the 5-chloro and 5-phenyl derivatives, further substitutions in the 5-position of the benzothiazole were explored next.

Table 3 summarizes the activity of a set of 5-substituted benzothiazoles. In contrast to polar substitutions, hydrophobic substitutions at the 5-position resulted in improved on-target activity compared to the unsubstituted benzothiazole. Derivatives with 5-propyl, 5-ethoxy, 5-bromo, and 5-trifluoromethyl substitutions all have submicrogram/mL MICs.

Table 1. *S. aureus* MICs and Cell Division Inhibitory Activity for Compounds 6a–k

| compd | R¹ group       | *S. aureus* MIC (μg/mL) | *S. aureus* MIC + 2% BSA (μg/mL) | cell division inhibition (μg/mL) | clogP |
|-------|----------------|-------------------------|----------------------------------|----------------------------------|-------|
| 1     | methoxy        | 2048                    | 4096                             | WT⁺                             | 1.1   |
| 6a    | hexyloxy       | 8                       | 32                               | 8                                | 3.3   |
| 6b    | (4-methyl-1,3-thiazol-2-yl)methoxy | 16                | 16                               | 16                               | 1.7   |
| 6c    | (5-methyl-1,3-thiazol-2-yl)methoxy | 256               | 256                              | WT                               | 1.7   |
| 6d    | (2-phenyl-1,3-thiazol-4-yl)methoxy | 4                  | 16                               | 4                                | 2.7   |
| 6e    | 2-(1H-pyrazol-1-yl)ethoxy | >256               | >256                             | WT                               | 1.7   |
| 6f    | 1,3-benzothiazol-2-yl-oxo | >256               | >256                             | WT                               | 2.8   |
| 6g    | 1,3-benzothiazol-2-ylmethoxy | 2                  | 8                                | 4                                | 2.2   |
| 6h    | quinolin-2-ylmethoxy | 16                  | 16                               | 16                               | 2.7   |
| 6i    | 1,3-benzoxazol-2-ylmethoxy | 128                | 256                              | 256                              | 2.3   |
| 6j    | (4-methylthiophen-2-yl)methoxy | 32                 | 128                              | 32                               | 2.7   |
| 6k    | (6-methylpyridin-2-yl)methoxy | 32                 | 64                               | 32                               | 2.1   |

*a* Lowest concentration at which ballooning of *S. aureus* is observed indicating on-target activity. WT: no effect on morphology. *b* Enlarged cells, but not balloons as seen with more potent compounds, were observed at 2048–4096 μg/mL.
MICS against *S. aureus*. Similarly, methyl ester (8i) and 1,2,4-oxadiazol-5-yl (17) substitutions resulted in improved on-target activity.

The design of molecules was largely driven by traditional SAR-directed medicinal chemistry processes; however, the availability of FtsZ crystal structures enabled the use of structure-informed optimization of the ligands.

A cleft between helix 7 and the C-terminal domain of FtsZ was identified as a potential binding site for this series of compounds. The model was not used to design molecules per se, however it was used as a tool to prioritize the synthesis of compounds that docked convincingly into the hydrophobic channel present in the *S. aureus* FtsZ protein. The model was not used to design molecules per se, however it was used as a tool to prioritize the synthesis of compounds that docked well into the binding site.

Because of the hydrophobic nature of the molecules, the protein binding was of concern and the effect of protein on the antibacterial activity was tracked by adding protein (50% v/v serum or 2% w/v bovine serum albumin (BSA)) to the MIC assays. The alkyloxy compounds, such as 1,2,4-oxadiazol-5-yl (>256 μg/mL), were particularly sensitive to the presence of added protein with serum or BSA inducing large fold increases in the MICs. The majority of compounds described herein were less sensitive to added protein, with serum or BSA inducing more modest shifts in the MICs (Tables 1 and 4). Nonetheless, the most active benzothiazole derivatives were still highly bound to protein with plasma protein binding values of >95% being observed using an equilibrium dialysis assay (Table 4).

Because hydrophobic substituents appear to be preferred by the respiratory tract, the effect of protein on the activity was particularly concerning. Because hydrophobic substituents appear to be preferred by the respiratory tract, the effect of protein on the activity was particularly concerning.

Table 2. *S. aureus* MICs and Cell Division Inhibitory Activity for a Set of Substituted Benzothiazoles

| compd | R² group | *S. aureus* MIC (μg/mL) | cell division inhibition (μg/mL) |
|-------|----------|------------------------|-------------------------------|
| 6g    | 4-Cl     | 2                      | 4                             |
| 8j    | 5-Cl     | 0.25                   | 0.25                          |
| 8f    | 5-Me     | 4                      | 8                             |
| 24d   | 6-Me     | 8                      | WT                            |
| 25    | 4-MeO    | 16                     | 64                            |
| 8h    | 5-MeO    | 2                      | 2                             |
| 24b   | 6-MeO    | >256                   | WT                            |
| 22e   | 4-Ph     | >256                   | WT                            |
| 8a    | 5-Ph     | 0.25                   | 0.5                           |
| 24a   | 6-Ph     | >256                   | WT                            |
| 24d   | 7-Ph     | >256                   | WT                            |

*Lower concentration at which ballooning of *S. aureus* is observed indicating on-target activity. WT: No effect on morphology.*

Table 3. *S. aureus* MICs and Cell Division Inhibitory Activity for a Set of 5-Substituted Benzothiazoles

| compd | R³ group | *S. aureus* MIC (μg/mL) | cell division inhibition (μg/mL) |
|-------|----------|------------------------|-------------------------------|
| 6g    | 5-H      | 2                      | 4                             |
| 8j    | 5-Cl     | 0.25                   | 0.25                          |
| 8f    | 5-Me     | 4                      | 8                             |
| 11    | 5-propyl | 0.125                  | 0.125                         |
| 8h    | 5-MeO    | 2                      | 2                             |
| 8b    | 5-EtO    | 0.125                  | 0.125                         |
| 8a    | 5-Ph     | 0.25                   | 0.5                           |
| 8d    | 5-Br     | 0.125                  | 0.25                          |
| 8e    | 5-CF₃    | 0.25                   | 0.5                           |
| 18    | 5-OH     | 32                     | 64                            |
| 9     | 5-NH₂    | >64                    | WT                            |
| 8g    | 5-(dimethylamino) | >64 | WT |
| 16    | 5-CNH₂   | >64                    | WT                            |
| 14    | 5-CO₂H   | >64                    | WT                            |
| 8i    | 5-COOH₂  | >64                    | WT                            |
| 17    | 5-(1,2,4-oxadiazol-5-yl) | 0.5 | 0.5 |
| 13    | 5-(imidazol-2-yl) | >64 | WT |

*Lower concentration at which ballooning of *S. aureus* is observed indicating on-target activity. WT: no effect on morphology.*

The thiazolopyridine (28c) where Z² = N was 32-fold less active than the benzothiazole (8j), however where Z² = N (2, 30, and 29), the antibacterial activity was equivalent or only 2–4-fold less potent than the corresponding benzothenes. Furthermore, the plasma protein binding of the thiazolopyridines was lower for the chloro (2) and ethoxy (29) derivatives.

The in vitro ADME properties were determined for molecules where the potency and protein binding were suitable for progression. The upper portion of Table 5 summarizes the results of a set of in vitro screens used to evaluate the drug-like properties of compound 2 with compound 8j shown for comparison. Standard deviations are shown, where available, to illustrate typical assay variability.

Compound 2 did not inhibit any of the cytochrome P450 isozymes tested, nor did it inhibit the hERG ion channel at the concentrations tested. The chemical and plasma stabilities of compound 2 were good, and the clearances in microsomes and hepatocytes were low, suggesting that it should not be rapidly metabolized in vivo. The permeability of compound 2 in Caco-2 cells was good with a low efflux ratio, indicating that compound 2 should not be a substrate of drug transporters such as P-glycoprotein and should be orally bioavailable. Compound 8j had a similar ADME profile to compound 2, with increased plasma protein binding and increased clearance in hepatocytes being the notable exceptions.
contrast, the corresponding benzothiazole (compound 8j) was rapidly cleared both in hepatocytes and in the mouse.

Both compounds 2 and 8j were tested in a murine model of staphylococcal infection (Figure 5). Compound 2 was efficacious following a single administration: intraperitoneal (IP) ED$_{50} <$3 mg/kg, subcutaneous (SC) ED$_{50} =$7 mg/kg, and intravenous (IV) ED$_{50} =$10 mg/kg. While compound 8j was efficacious when administered IP (ED$_{50} =$41 mg/kg), it was not when administered SC (ED$_{50} >$30 mg/kg). Although the bioavailability of compound 2 was good, no efficacy was observed following a single oral administration of 30 mg/kg (data not shown).

Therefore the modest reduction in potency of the thiazolopyridine compared to the benzothiazole is more than offset by reducing protein binding and metabolism, resulting in a drug-like pharmacokinetic profile and efficacy in the murine model of staphylococcal infection.

**Conclusion**

A series of 3-substituted benzamides as inhibitors of FtsZ, which demonstrate potent antistaphylococcal activity by inhibiting cell division, has been synthesized and the SAR has been determined.

Replacement of a 3-alkyloxy substituent with a variety of heteroarylmethoxy substituents resulted in drug-like compounds with on-target antibacterial activity. The most potent of these substitutions was 1,3-benzothiazol-2-ylmethoxy, so the focus was to optimize the benzothiazole by further substitution around the phenyl ring. Substitutions such as chloro, phenyl, or ethyloxy in the 5-position of the benzothiazole resulted in an 8- to 16-fold improvement in the on-target activity, with MICs as low as 0.125 μg/mL against *S. aureus*.

Although these 5-substituted-1,3-benzothiazol-2-ylmethoxy compounds were more drug-like than the 3-alkyloxy derivatives described above, their protein binding, at >95% bound to plasma, was considered too high to progress. Replacing the benzothiazole substituent with a thiazolopyridine reduced the antibacterial activity but also reduced the plasma protein binding. Furthermore, the metabolic stability of the thiazolopyridine was improved compared to the benzothiazole, resulting...
in an approximately 15-fold reduction in clearance following intravenous administration in the mouse.

The balance of antibacterial activity, plasma protein binding, and metabolic stability of compound 2 resulted in the compound being tested in the mouse model of staphylococcal infection. Compound 2 was efficacious in an in vivo model of infection, protecting mice infected with a lethal dose of S. aureus. The data validate FtsZ as a target for antibacterial intervention and demonstrate that the series is suitable for optimization into a new antistaphylococcal therapy.

Experimental Section

Antimicrobial Testing. S. aureus ATCC 29213 was obtained from LGC Promochem (Teddington, UK) and was propagated according to standard microbiological practice. MICs were determined by the broth microdilution method according to the recommendations of the Clinical and Laboratory Standards Institute. Cell division phenotype assays were performed as described previously.12

ADME Testing. The cytochrome P450 assay measured the metabolism of fluorogenic substrates by human recombinant enzymes supplied by Invitrogen. The hERG assay, performed at Cyprotex Ltd. (Macclesfield, UK), was a single cell planar patch clamp assay. Plasma protein binding was assessed by equilibrium dialysis of a 10 μM solution using the Pierce RED devices. Plasma stability of a 10 μM solution was determined by LC/MS after 24 h at 37 °C. Microsomal stability of a 10 μM solution was determined over a time course by measuring the remaining parent compound using LC/MS. Permeability assays, performed at Cyprotex Ltd., used LC/MS/MS to determine bi-directional transport across Caco-2 monolayers. Chemical stability of a 10 μM solution in phosphate buffered saline was determined by LC/MS after 24 h at 37 °C. Hepatocyte stability of a 10 μM solution, performed at Cyprotex Ltd., was determined over a time course by measuring the remaining parent compound using LC/MS/MS.

Pharmacokinetics. Parameters were calculated using WinNonlin from the plasma concentrations determined by LC/MS over an 8 h time course following intravenous or oral administration of the compounds in mice.

Efficacy. Mice were inoculated IP with an LD90–100 of S. aureus (Smith) (5 × 10^5 CFU/mouse for SC study and 4 × 10^3 CFU/mouse for IP study) in 0.5 mL of brain heart infusion broth containing 5% mucin. Vehicle and test substances were administered SC or IP 1 h after bacterial inoculation. Mortality was monitored for 7 days. The minimal effective dose (ED50) was determined by nonlinear regression using Graph Pad Prism.

Ligand Docking. Docking of ligands to the putative binding site on Bacillus subtilis FtsZ described previously13 was completed using Benchmark 3D Explorer software (Tripos L.P.).

Table 5. ADME Properties and PK Parameters for Compounds 2 and 8j

| assay                              | units   | 8j  | 2     |
|------------------------------------|---------|-----|-------|
| CYP1A2 inhibition                  | IC50 (μg/mL) | >12 | >12   |
| CYP2C19 inhibition                 | IC50 (μg/mL) | >12 | >12   |
| CYP2C9 inhibition                  | IC50 (μg/mL) | >12 | >12   |
| CYP2D6 inhibition                  | IC50 (μg/mL) | >12 | >12   |
| CYP3A4 inhibition                  | IC50 (μg/mL) | >12 | >12   |
| chemical stability                 | % remaining after 24 h | 99 ± 5.4 | 100 ± 10.3 |
| human microsomal stability         | CL (μL/min/mg) | nd | 7.2 ± 4.4 |
| human plasma stability             | % remaining after 24 h | nd | 100 ± 5.2 |
| mouse hepatocyte stability          | CL (μL/min/10^6 cells) | 31.2 ± 2.7 | 9.51 ± 1.6 |
| mouse microsomal stability         | CL (μL/min/mg) | 14.0 ± 4.8 | 14.5 ± 10.3 |
| mouse plasma stability             | % remaining after 24 h | 99 ± 11.2 | 94 ± 10.8 |
| mouse plasma binding               | fraction unbound | 0.03 ± 0.02 | 0.15 ± 0.06 |
| hERG inhibition                    | IC50 (μM) | nd | >10   |
| Caco-2 permeability                | efflux ratio | nd | 0.645 |
| Caco-2 permeability                | P_{app}AB (cm/s) | nd | 48.7 |
| Caco-2 permeability                | P_{app}BA (cm/s) | nd | 31.4 |
| mouse intravenous PK parameters    | dose (mg/kg) | 2 | 3 [3] |
| [mouse oral PK parameters]         | AUC (μg·h/mL) | 0.1 | 2.42 [1.39] |
|                                   | C0 [Cmax] (μg/mL) | 0.5 | 3.7 [0.7] |
|                                   | CL (mL/min/kg) | 315 | 20.6 |
|                                   | T1/2 [Tmax] (min) | 13 | 37 [24] |
|                                   | Vf (L/kg) | 5.8 | 1.06 |

*nd: not determined. ± standard deviations.
were obtained from commercial sources and were used without further purification. 2,6-Difluoro-3-methoxybenzamide (3) was purchased from JRD Fluorochemicals Ltd. Yields refer to purified products and are not optimized. Analytical TLC was performed on Merck silica gel 60 F254 aluminum-backed plates. Compounds were visualized by UV light and/or stained with either I2 or potassium permanganate solution followed by heating. Flash column chromatography was performed on silica gel. 1H NMR spectra were recorded on either a JEOL GSX-400 MHz or on a Bruker Avance 400 MHz spectrometer with a Broad Band Observe (BBO) and Broad Band Fluorine Observe (BBFO) probe. Chemical shifts (δ) are expressed in parts per million (ppm) downfield by reference to tetramethylsilane as the internal standard. Splitting patterns are designated as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br s (broad singlet), app (apparent). Coupling constants (J) are given in hertz (Hz). LC-MS analyses were performed on either an Acquity C-18 column (2.10 mm × 100 mm, 1.70 μm) using the electrospray ionization (ESI) technique or on a Gemini C-18 column (4.6 mm × 30 mm, 5 μm) using the atmospheric pressure chemical ionization (APCI) technique. The purity of all tested compounds was determined by analytical HPLC and HPLC-MS analyses and was greater than 95%, unless otherwise stated. Purity assessment for final compounds was based on the following 5 HPLC methods. Method 1 consisted of the following: Discovery HSC-18 column 4.60 mm × 250 mm, 5 μm. Mobile phase: A, acetonitrile with 0.10% formic acid; B, H2O with 0.10% formic acid; gradient, 10% A to 90% A in 6 min with 10 min run time and a flow rate of 1 mL/min. Method 2 consisted of the following: Purospher Star C-18 column 4.60 mm × 250 mm, 5 μm. Mobile phase: A, acetonitrile with 0.10% formic acid; B, H2O with 0.10% formic acid; gradient, 10% A to 90% A in 15 min with 30 min run time and a flow rate of 1 mL/min. Method 3 consisted of the following: Xbridge C-18 column 4.60 mm × 250 mm, 5 μm. Mobile phase: A, acetonitrile with 0.10% formic acid; B, CH3CN with 0.10% formic acid; gradient, 10% A to 90% A in 50 min, 5 μm. Mobile phase: 20% CH3CN:10 mM aqueous ammonium acetate, over 4 min, isocratic for 1 min, 25% CH3CN:10 mM aqueous ammonium acetate for 2 min, flow rate = 1 mL/min at 40 °C. Melting points were measured using a Stuart Scientific SMP10 or a Buchi B545 apparatus and are uncorrected. Experimental and spectroscopic details of compounds not described herein and synthetic schemes and experimental details of the intermediates used to make all compounds are presented in the Supporting Information.

2,6-Difluoro-3-hydroxybenzamide (4). Boron tribromide solution (1 M in CH2Cl2, 8.55 mL, 8.55 mmol, 2 equiv) was added dropwise to a stirred suspension of 2,6-difluoro-3-methoxybenzamide (3) (800 mg, 4.3 mmol) in CH2Cl2 (20 mL) at room temperature, under N2 atmosphere. The reaction mixture was stirred at room temperature for 48 h. The solvent was removed under reduced pressure, and the residue was taken up in water (50 mL) and extracted with EtOAc (30 mL). The combined organic extracts were washed with water (2 × 40 mL), dried (Na2SO4), and filtered through a pad of silica. The filtrate was evaporated to dryness under reduced pressure, to give 4 as a light-brown solid (580 mg, 78%). 1H NMR (DMSO-d6): δ 6.90–6.95 (m, 2H), 7.73 (br s, 1H), 8.03 (br s, 1H), 9.89 (br s, 1H).

2-(Benzyloxy)-N-(2,5-dichloropyridin-3-yl)acetamide (32). To an ice-cold solution of 2,5-dichloropyridin-3-amine (31) (2.50 g, 15.33 mmol) in CH2Cl2 (50 mL) was added triethylamine (3.17 mL, 23 mmol, 1.5 equiv) followed by the addition of a solution of 2-(benzyloxy)acetyl chloride (4.20 g, 23 mmol, 1.5 equiv) in CH2Cl2 (50 mL). The resulting mixture was stirred at room temperature for 2 h. The solvent was removed under reduced pressure, and the residue was taken up in water (100 mL) and extracted with EtOAc (3 × 100 mL). The combined organic extracts were washed with brine, dried (Na2SO4), filtered, and
concentrated under reduced pressure. The residue was purified over silica eluting with 5% EtOAc–hexane to obtain the title compound 32 (2.20 g, 46%). LCMS (ESI) 311.04 [M + H]+, 84%. 2-(Benzoxylmethyl)-6-chloropyrido[3,2-d][1,3]thiazole (33). To a solution of 32 (2.20 g, 7.07 mmol) in toluene (50 mL) was added Lawesson’s reagent (2.84 g, 7.07 mmol, 1 equiv), and the resulting reaction mixture was refluxed for 4–5 h. The solvent was evaporated and the residue was purified over silica eluting with 3% EtOAc–hexane to obtain the title compound 33 (1.75 g, 85%). LCMS (ESI) 291.18 [M + H]+, 87.50%.

Scheme 7. Synthesis of the Alkyl Halide Intermediate 26

To a solution of 32 (2.20 g, 7.07 mmol) in toluene (50 mL) was added Lawesson’s reagent (2.84 g, 7.07 mmol, 1 equiv), and the resulting reaction mixture was refluxed for 4–5 h. The solvent was evaporated and the residue was purified over silica eluting with 3% EtOAc–hexane to obtain the title compound 33 (1.75 g, 85%). LCMS (ESI) 291.18 [M + H]+, 87.50%.

2-(Bromomethyl)-6-chloropyrido[3,2-d][1,3]thiazole (26, Scheme 7). A solution of 33 (2 g, 6.87 mmol) in CHCl3 (100 mL) was cooled to −78 °C followed by dropwise addition of BBr3 (3.30 mL, 34.39 mmol, 5 equiv). The mixture was stirred at room temperature for 3 h then cooled again to −78 °C and quenched by dropwise addition of water followed by extraction with EtOAc (3 × 100 mL). The combined organic extracts were washed with saturated NaHCO3 solution, dried (Na2SO4), filtered, and concentrated under reduced pressure. The residue was purified over silica eluting with 5% EtOAc–hexane to obtain the title compound 26 (1.50 g, 88%). 1H NMR (DMSO-d6): δ 5.16 (s, 2H), 7.12 (m, 1H), 7.86 (br s, 1H), 8.14 (br s, 1H), 8.67 (d, J = 2.4 Hz, 1H). MS (ESI) m/z: 262.90 [M + H]+.

General Procedure for the Alkylation of 4 Using Alkyl or Arylmethyl Halides. K2CO3 (1.5–3.5 equiv) and NaI (0.2 equiv) were added to a solution of 4 in DMF (3–8 mL per mmol). The resulting suspension was stirred for 5 min at room temperature before the corresponding halide (1.0 equiv) was added. The reaction mixture was stirred at 20–60 °C for 3–18 h under N2 atmosphere. After cooling to room temperature, one of two workup procedures was followed. In some cases, the reaction mixture was poured into water (15 mL per mmol), and the precipitant solid was filtered, washed with water, and dried to give the crude product. In other cases, the reaction mixture was partitioned between EtOAc (30 mL per mmol) and water (20 mL per mmol), the organic phase was separated, washed with water (2 × 15 mL), dried (MgSO4), filtered, and concentrated in vacuo to give the crude solid.

3-(6-Chloropyrido[3,2-d][1,3]thiazol-2-yl) methoxy]-2,6-difluorobenzamide (2). Compound 2 was prepared from 4 (590 mg, 3.41 mmol) and 26 (900 mg, 3.41 mmol, 1 equiv) using 3.5 equiv of K2CO3, according to the general procedure (20 °C, 3 h, EtOAc/H2O workup). The residue was purified over silica eluting with 60% EtOAc–hexane to obtain the title compound 2 (940 mg, 78%), mp 218 °C. 1H NMR (DMSO-d6): δ 5.72 (s, 2H), 7.12 (m, 1H), 7.40 (m, 1H), 7.89 (br s, 1H), 8.17 (br s, 1H), 8.55 (d, J = 2.4 Hz, 1H), 8.73 (d, J = 2.4 Hz, 1H). MS (ESI) m/z: 356.27 [M + H]+. HPLC (method 3) Rf = 15.40 min.

3-(Cyanomethoxy)-2,6-difluorobenzamide (19). Compound 19 was prepared from 4 (1 g, 5.8 mmol) and chlorosuccinonitrile (0.40 mL, 6.4 mmol, 1.1 equiv) using 1.5 equiv K2CO3, according to the general procedure (60 °C, overnight, EtOAc/H2O workup). The crude product was triturated with diethyl ether (30 mL), filtered, and dried in vacuo to give 19 as a gray solid (1.06 g, 86%); mp 122–123 °C. 1H NMR (DMSO-d6): δ 5.26 (s, 2H), 7.18 (app dt, J = 9.0, 1.8 Hz, 1H), 7.40 (m, 1H), 7.86 (br s, 1H), 8.14 (br s, 1H). HPLC-MS (APCI) m/z: 213 [M + H]+, (method 5) Rf = 1.97 min.

General Procedure for the synthesis of substituted benzothiazole derivatives from 3-cyanomethoxy-2,6-difluoro-benzamide (19) and substituted 1,3-benzothiazol-2-amines. A solution of KOH (20 equiv) in water (25 mL) was added to a solution of the substituted 1,3-benzothiazol-2-amine in 2-methoxy-ethanol (25 mL), and the reaction mixture was heated to reflux overnight. After cooling at room temperature, the mixture was diluted with water (200 mL), acidified with 5N HCl solution to pH 4, and extracted with CH2Cl2 (3 × 150 mL). The combined organic extracts were washed with brine (100 mL), dried (Na2SO4), and concentrated under reduced pressure to dryness, to give crude substituted 2-aminobenzethiol. A portion of this material (1.5 equiv) were mixed with 19 (150 mg, 0.7 mmol), and the mixture was stirred at 120 °C. In a preheated oil bath, under N2, for 2 h. EtOH (2 mL) was added and the reaction mixture was heated at 120 °C for a further 2 h. The products either precipitated from the reaction mixture on cooling or the reaction mixtures were evaporated and the crude products purified by trituration of the residue. See the Supporting Information for exact details of each example.

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Supporting Information Available: Synthetic schemes and experimental details of the intermediates used to make the compounds described herein. This material is available free of charge via the Internet at http://pubs.acs.org.

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