Practical Synthesis and Application of Halogen-Doped Pyrrole Building Blocks

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ABSTRACT: A practical access to four new halogen-substituted pyrrole building blocks was realized in two to five synthetic steps from commercially available starting materials. The target compounds were prepared on a 50 mg to 1 g scale, and their conversion to nanomolar inhibitors of bacterial DNA gyrase B was demonstrated for three of the prepared building blocks to showcase the usefulness of such chemical motifs in medicinal chemistry.

1. INTRODUCTION

Halogen-substituted pyrrole-2-carboxamide is an integral molecular fragment of bioactive marine natural products as well as natural and synthetic anti-infectives (Figure 1). In particular, mono- and dibromopyrrole-2-carboxamide are found in oroidin and hymenidin, which are postulated precursors for structurally diverse mono- and oligomeric secondary metabolites involved in the chemical defense of Agelas marine sponges. A representative compound ageliferin features a complex multichiral scaffold. Furthermore, 3,4-dichloro-5-methyl-1H-pyrrole-2-carboxamide is a molecular fragment of natural and synthetic antibacterials, crucial for binding to the active site of bacterial topoisomerases, and the 3-fluoro-1H-pyrrole-2-carboxamide moiety is found in promising preclinical candidates, active against hepatitis B virus (Figure 1).

During our ongoing research in the field of dual bacterial DNA gyrase/topoisomerase IV inhibitors, a promising hit compound 1 (Figure 2) was identified, displaying low nanomolar inhibition of the target enzymes and broad-spectrum activity against gram-positive bacterial strains. Due to the compound’s high lipophilicity, its more polar analogues 2–5 were designed by varying the 3,4-dichloro-5-methyl-1H-pyrrole moiety, envisioning improved physical properties (clogP was calculated by ChemDraw) of the analogues while retaining the on-target activity (Figure 2).

With no preceding literature on the synthesis of the required pyrrole building blocks for the preparation of compounds 2–5, we report herein our synthetic endeavors, where the main goal was...
was the timely delivery of at least 100 mg of the sample to be built into the bioactive molecules. The amide bond of the target compounds can be formed using pyrrole-2-carbonyl chloride or 2-trichloroacetylpyrrole; therefore, either would be an acceptable option.

2. RESULTS AND DISCUSSION

The literature procedure for the synthesis of 4-chloro-5-methyl-1H-pyrrole-2-carboxylic acid involves chlorination of ethyl 5-methyl-1H-pyrrole-2-carboxylate using N-chlorosuccinimide at 0 °C and required in our hands laborious chromatographic separation of two barely resolved products.17 The practical synthesis of an alternative acylating agent 8 for the introduction of the same structural fragment was thus developed (Scheme 1). Trichloroacetylpyrrole 7 was prepared from pyrrole-2-carbaldehyde 6 employing the Wolff–Kishner reduction and Friedel-Crafts acylation. It was then directly monochlorinated using N-chlorosuccinimide at r.t. and the pure product 8 was obtained on the gram scale in 61% isolated yield after convenient crystallization from dichlormethane. Its structure was unambiguously determined by two-dimensional (2D) nuclear magnetic resonance (NMR) experiments (Supporting Information), showing that the electrophilic chlorination was selective for the position next to the electron-donating methyl substituent.

Next, we targeted the 4-fluoro-substituted building block. The screening of different halogen exchange (“Halex”) conditions involving crown ether 18-C-6 and [2.2.2]cryptand, for the conversion of chloropyrrole 8 or ethyl 4-chloro-5-methylpyrrole-2-carboxylate to the corresponding arylfluorides, returned no hits.19 We thus resorted to electrophilic fluorination of ethyl 5-methyl-1H-pyrrole-2-carboxylate 9 (Scheme 2). Initial 0.5 mmol scale screening of the reaction conditions (Table S1 in the Supporting Information) revealed that Selectfluor-mediated fluorination20 outperformed the N-fluorobenzenesulfonylimide (NFSI)-mediated Lewis acid-catalyzed fluorination,21 as the former resulted in somewhat cleaner conversions. When the fluorination was performed at 0 °C in a mixture of acetonitrile and acetic acid (Table S2, entries 11 and 12), the formation of target compound 10, accompanied by an acetoxo side product 11, was observed. Their structures were confirmed by single-crystal X-ray diffraction analysis (Figures S1 and S2 in the Supporting Information). Aiming for an efficient med–chem synthetic route, the reaction was performed on a 2 g scale (Scheme 2), delivering 10 in a consistent 4.5–6.5% yield after flash chromatography. Esters 10 was hydrolyzed to acid 12, requiring rather forcing conditions, and acyl chloride 13 was finally formed using oxalyl chloride in dichloromethane. It is noteworthy that acyl chloride formation using refluxing sulfonyl chloride or oxalyl chloride with the catalytic quantity of dimethylformamide (DMF) resulted in the formation of a significant amount of unidentified side products.

Ethyl 3-fluoro-1H-pyrrole-2-carboxylate 14 has recently become commercially available at a reasonable price because it is a key building block for a drug candidate against hepatitis B virus.22 This was a good starting point for the synthesis of 3-fluoro-5-methyl-1H-pyrrole-2-carboxylic acid 18 (Scheme 3).

The Vilsmeier–Haack formylation of 14 gave at 68% conversion a 43:57 mixture of 4- and 5-formylated regioisomers 16 and 15, which were separated by flash chromatography. The regioisomers’ identity was assigned by 19F NMR as follows: 4-formyl isomer 16 is a singlet and that of 5-formyl 15 is a doublet, 1JF,H = 4 Hz, confirming the presence of a vicinal proton. Moreover, the 13C NMR peak of the formyl carbon of 15 is a singlet and that of 16 is a doublet, 1JCF = 2.8 Hz.

Based on the literature reports on the reduction of ester-containing formylpyrroles to methylpyrroles,23 we first attempted a BH3·THF-mediated reduction of 15–17, which in this case yielded the intermediate alcohol; no full reduction was observed even after several days of stirring with periodic addition of excess BH3·THF. Other literature reports on aldehyde-to-methyl reduction in the presence of ester include a two-step Mozingo protocol via thioeketone.24 Aiming to secure a

Scheme 1. Synthesis of 2-Trichloroacetyl-4-chloro-5-methyl-1H-pyrrole 8a

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\[ \text{Scheme 1. Synthesis of 2-Trichloroacetyl-4-chloro-5-methyl-1H-pyrrole 8}^a \]
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\[ \begin{align*}
6 & \xrightarrow{a,b} 7 \xrightarrow{c} 8 \\
\text{Reagents and conditions: (a) NH}_{3}\text{NH}_{3}\text{H}_{2}\text{O, ethylene glycol, 90 °C, 1 h, then KOH, 90 °C, 2.5 h (70% yield); (b) CCl}_{3}\text{OCl, Et}_{2}\text{O, r.t., 2 h (60% yield); and (c) N-chlorosuccinimide, dichloromethane, r.t., 4 h (61% yield).} \\
\end{align*} \]
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Scheme 2. Synthesis of 4-Fluoro-5-methyl-1H-pyrrole-2-carboxylic Acid Chloride 13a

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\[ \begin{align*}
9 & \xrightarrow{a} 10 \xrightarrow{b} 12 \\
\text{Reagents and conditions: (a) Selectfluor, MeCN/AcOH 5:1, 0 °C, 2 h (6.5% yield); (b) 10 M NaOH (aq), EtOH, 90 °C, 3 h (76% yield); and (c) oxalyl chloride, dichloromethane, r.t., overnight (quant. yield).} \\
\end{align*} \]
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Scheme 3. Synthesis of 3-Fluoro-5-methyl-1H-pyrrole-2-carboxyl Chloride 19a

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\[ \text{Scheme 3. Synthesis of 3-Fluoro-5-methyl-1H-pyrrole-2-carboxyl Chloride 19}^a \]
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\[ \begin{align*}
14 & \xrightarrow{a} 15 \xrightarrow{b} 17 \\
\text{Reagents and conditions: (a) DMF, POC}_{18}\text{H}_{17}, 0 °C, 30 min, then 14, 90 °C, overnight (25% yield); (b) Zn, 4 M HCl/dioxane, r.t., 40 min (20% yield); (c) 10 M NaOH (aq), EtOH, 90 °C, 5 h (82% yield); and (d) oxalyl chloride, dichloromethane, r.t., overnight (quant. yield).} \\
\end{align*} \]
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convenient one-pot procedure, we opted for the modified Clemmensen reduction. A dioxane-soluble [ZnCl₂(dioxane)]₂ complex was prepared by treating zinc dust with anhydrous 4 M HCl in dioxane. This proved to be a very efficient and reasonably selective reduction medium, delivering 17 after 40 min at r.t. in 20% isolated yield. Optimization of the reaction conditions and elucidation of the mechanism is beyond the aim of this study; however, we speculate that a dioxane-soluble Zn(II) species forms a zinc-ylide intermediate more efficiently compared to the classical heterogeneous Clemmensen reduction (Zn/Hg/HCl/H₂O), allowing the reaction to proceed at room temperature. The side products are essentially a result of the zinc-ylide reaction with other present electrophiles (ester, aldehyde, and aryllfluoride). Using the conditions developed for the synthesis of 13, ester 17 was readily transformed to acyl chloride 19.

Ethyl 5-chloromethyl-3,4-dichloro-1H-pyrrole-2-carboxylate 21 was prepared from commercially available 20 according to the literature procedure (Scheme 4). After conversion to azide 22 by KI-mediated nucleophilic substitution, the reduction of 22 to amine 23 was first attempted via Pd/C-catalyzed hydrogenation. This resulted in significant side-product formation, possibly via the nucleophilic attack of amine 23 to the electrophilic methylene moiety of 22, and aryl dehalogenation, as apparent from the ¹H NMR analysis of the crude reaction mixture. Avoiding the coexistence of amine and azide species during the reaction, we resorted to the milder Staudinger reduction, which furnished amine 23 in 78% isolated yield. Saponification to 24, followed by phthalimide protection in neat phthalic anhydride gave 25 with 41% yield over four steps from 21.

To showcase the usefulness of the prepared building blocks in medicinal chemistry, the synthesis of compound 5, the analogue of antibacterial hit compound 1, was tackled (Scheme 5). After the smooth coupling of the acyl chloride, prepared from 25 in neat thionyl chloride, with the 2-amino-benzothiazole building block in refluxing toluene, the deprotection step required some special attention. The formation of stable hydrazinium salt 27 was observed during the phthalimide deprotection, arguably due to the electron-withdrawing character of dichloropyrrole, which increases the acidity of the neighboring amides. It was crucial to first reprotonate the nitrogens of 27 to achieve complete deprotection after refluxing in ethanol overnight. Alkaline hydrolysis of phthalic hydrazide salt 28 yielded phthalate salt 29 and the anion was readily exchanged to the chloride salt of 5 by trituration with methanolic HCl.

Antibacterial hit compound 1 (ε log P = 5.8) inhibited Escherichia coli DNA gyrase with IC₅₀ < 10 nM, and compound 5 (ε log P = 2.0) inhibited the same enzyme with IC₅₀ < 10 nM. Moreover, 5 exhibits activity against Staphylococcus aureus (ATCC29213) with a minimal inhibitory concentration of 1 μg/mL. This confirms the hypothesis that the single-digit nanomolar inhibitory on-target activity coupled to the antibacterial activity can be retained while significantly reducing the lipophilicity by the modification of the pyrrole moiety.

To explore the reactivity and bioactivity of the fluorinated pyrroles, two additional analogues of 1 were prepared (Scheme 6) and evaluated for their on-target and antibacterial activities. Thus, compounds 31 and 33 inhibited E. coli DNA gyrase with IC₅₀ values of 32 and 150 nM, respectively, and possessed weak activity against S. aureus (ATCC29213) (31: MIC = 64 μg/mL; 33: MIC > 64 μg/mL).

3. CONCLUSIONS
In summary, practical synthetic routes to four new halogen-doped pyrrole building blocks were developed, delivering the target compounds in sufficient quantities for further elaboration. Moreover, the transformation of the building blocks to potent DNA gyrase B inhibitors was demonstrated. Such building blocks are polar alternatives to molecular fragments found in naturally occurring or natural-product-inspired bioactive compounds and are useful in hit-to-lead optimization.

4. EXPERIMENTAL SECTION
4.1. General. Reactions were conducted under an inert atmosphere using anhydrous solvents when required. Analytical thin-layer chromatography (TLC) was performed on silica gel 60 F₂₅₄ plates. Flash column chromatography was
performed using silica gel 60 (40–63 μm). Melting points were determined on a Kofler apparatus and are uncorrected. \(^1\)H NMR (400 MHz, internal Me\(_4\)Si), \(^13\)C NMR (101 MHz, internal CDCl\(_3\)) or DMSO-d\(_6\)), and \(^{19}\)F NMR (376 MHz, external CCl\(_3\)) spectra were recorded on a Bruker AVANCE III 400 spectrometer (Bruker Corporation, Billerica, MA) in a DMSO-d\(_6\) or CDCl\(_3\) solution. HRMS analysis was performed on a VG Analytical Autospec Q mass spectrometer (Fisons, VG Analytical, Manchester, U.K.).

4.2. Synthetic Procedures. 4.2.1. 2-Trichloroacetyl-4-(1-phenylethoxy)benzol[d]thiazole-6-carboxylate (8). A mixture of 2-trichloroacetyl-5-methyl-1H-pyrrole (2.14 g, 9.44 mmol), and dichloromethane (9.0 mL) at 0\(^\circ\)C for 2 h (full conversion by \(^1\)H NMR). The reaction mixture was partitioned between EtOAc (50 mL) and water (50 mL), and the organic layer was washed with water and brine, dried (Na\(_2\)SO\(_4\)), and concentrated. The residue was recrystallized from dichloromethane to get title compound as white crystals (1.51 g, 61% yield). Mp 140\(^\circ\)C (DCM).

4.2.2. Ethyl 4-Fluoro-5-methyl-1H-pyrrole-2-carboxylate (15) and Ethyl 3-Fluoro-4-formyl-1H-pyrrole-2-carboxylate (16). To DMF (8.9 mL, 115 mmol) at 0\(^\circ\)C under Ar was added POC\(_3\) (1.95 mL, 21.0 mmol). After stirring at 0 \(^\circ\)C for 30 min, a solution of ethyl 3-fluoro-1H-pyrrole-2-carboxylate 14 (3.00 g, 19.1 mmol) in DMF (29 mL) was added, and the resulting solution was stirred at 90 \(^\circ\)C overnight. The reaction mixture was cooled to room temperature and poured onto ice (100 mL). The pH was adjusted to 7 by adding 2 M NaOH (aq) and the product was extracted to EtOAc (3 \(\times\) 200 mL). The combined organic layers were washed with brine, dried (Na\(_2\)SO\(_4\)), and concentrated to get the crude product. Crude products from two 2 g runs were combined and purified by flash chromatography, eluent hexane/EtOAc 4:1, to get the title compound (first eluting) as a colorless amorphous solid (288 mg, 6.5% yield). \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 8.92 (bs, 1H), 6.54 (d, 1H, J = 4.0 Hz), 4.30 (q, 2H, J = 7.1 Hz), 2.24 (s, 3H), 1.34 (t, 3H, J = 7.1 Hz). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)): \(\delta\) 161.3 (d, J = 3.3 Hz), 148.9 (d, J = 238.7 Hz), 117.6, 116.2 (d, J = 7.4 Hz), 102.3 (d, J = 15.6 Hz), 60.6, 14.6, 9.4 (d, J = 2.1 Hz). \(^{19}\)F NMR (376 MHz, CDCl\(_3\)): \(\delta\) −166.4. HRMS calcld for C\(_8\)H\(_7\)FNO\(_2\) [M + H]\(^+\) 172.0768, found 172.0769.

4.2.3. Ethyl 4-Acetoxy-5-methyl-1H-pyrrole-2-carboxylate (11). Ethyl 4-acetoxy-5-methyl-1H-pyrrole-2-carboxylate was isolated as a second eluting product (see purification of 10 above), a white amorphous solid. \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 10.10 (s, 1H), 6.72 (d, J = 2.8 Hz, 1H), 4.29 (g, J = 7.1 Hz, 1H), 2.24 (s, 1H), 2.16 (s, 1H), 1.32 (t, J = 7.1 Hz, 3H). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)): \(\delta\) 169.23, 161.46, 134.49, 123.33, 117.80, 108.22, 60.46, 20.85, 14.55, 10.16. A monocrystal suitable for single-crystal X-ray diffraction analysis was grown from dichloromethane/hexane.

ACS Omega 2021, 6, 9723−9730
8.32 (br s, 1H), 6.58 = 7.1 Hz, 2H), 3.99 (s, 2H), 1.38 (t, δ = 7.1 Hz, 3H). 13C NMR (101 MHz, CDCl3) δ 160.08, 126.48, 117.72, 113.26, 61.66, 45.22, 14.45. HRMS calcd for C8H7O2N2Cl2 [M − H] − 260.99515, found 260.99529.

4.2.11. Ethyl 5-(aminomethyl)-3,4-dichloro-1H-pyrrole-2-carboxylate (23). To a solution of the above azide (1.70 g, 6.46 mmol) in THF/H2O 10:1 (35 mL) was added PPh3 (3.39 g, 12.9 mmol) at r.t. The resulting amber solution was stirred at r.t. (caution: gas evolution) for 2.5 h, and then it was concentrated under reduced pressure. The oily residue was partitioned between EtOAc (200 mL) and 0.5 M HCl (300 mL). The water layer was brought to pH = 11 by adding 2 M NaOH. The precipitate was collected, washed with water, and air-dried to yield the title compound as a white amorphous powder (1.3 g, 78%). 1H NMR (400 MHz, CDCl3) δ 9.87 (s, 1H), 4.36 (q, J = 7.1 Hz, 2H), 3.99 (s, 2H), 1.63 (s, 2H), 1.38 (t, J = 7.1 Hz, 3H). 13C NMR (101 MHz, CDCl3) δ 160.16, 133.16, 117.50, 116.76, 110.24, 61.02, 36.85, 14.49. HRMS calcd for C8H6O2N2Cl [M − H] − 235.00466, found 235.00459.

4.2.12. 5-(Aminomethyl)-3,4-dichloro-1H-pyrrole-2-carboxylic Acid (24). A mixture of the above ester (1.42 g, 6.00 mmol), abs. EtOH (60 mL), and 10 M NaOH (10.8 mL) was stirred at 90 °C under argon for 3 h. The reaction mixture was concentrated under reduced pressure, dissolved in water (20 mL), filtered through cotton, and cooled to 0 °C. Conc. HCl(aq) (7.5 mL) was added, followed by 2 M HCl to adjust the pH to 8. The precipitate was collected, washed with water, and air-dried to yield the title compound as a white amorphous powder (1.3 g, quant. yield). 1H NMR (400 MHz, DMSO-d6) δ 3.91 (s, 2H). HRMS calcd for C8H6O2N2Cl [M − H] − 206.97336, found 206.97342.

4.2.13. 5-(Phthalimidomethyl)-3,4-dichloro-1H-pyrrole-2-carboxylic Acid (25). A homogeneous mixture of the above amino acid 24 (300 mg, 1.44 mmol) and powdered phthalic anhydride (213 mg, 1.44 mmol) in a 25 mL round-bottom flask was heated on an oil bath under a stream of argon from 150 to 180 °C for 15 min while stirring with a magnetic stirrer at 100 rpm and agitating the flask manually. The temperature was kept at 180 °C for 30 min during which the reaction mixture cooled. After cooling to r.t., the crude product was triturated successively with dichloromethane, EtOAc, and 1 M HCl, washed with water, and air-dried to get the title compound as a light gray amorphous solid (270 mg, 55% yield). 1H NMR (400 MHz, DMSO-d6) δ 13.04 (s, 1H), 12.57 (s, 1H), 8.01−7.76 (m, 4H), 4.79 (s, 2H). 13C NMR (101 MHz, DMSO-d6) δ 167.31, 160.04, 134.42, 131.83, 127.18, 123.14, 117.38, 114.98, 109.12, 33.00. HRMS calcd for C8H6O2N2Cl [M − H] − 336.97884, found 336.97929.

4.2.14. Methyl 4-(benzoxyl)-2-(3,4-dichloro-5-((1,3-dioxoisoadinol-2-yl) methyl)-1H-pyrrole-2-carboxamido)benzo[d]thiazole-6-carboxylate (26). A suspension of the above carboxylic acid 25 (110 mg, 0.324 mmol) in SOCl2 (1 mL) was refluxed for 1 h and then concentrated under reduced pressure. To the solid residue were added methyl 2-amino-4-(benzoxyl)benzo[d]thiazole-6-carboxylate (102 mg, 0.324 mg) and normal grade toluene (6.5 mL), and the resulting suspension was refluxed overnight. After cooling to r.t., the
precipitate was collected, washed with toluene, and air-dried to get the title compound as a gray amorphous solid (165 mg, 81% yield).1H NMR (400 MHz, DMSO-d$_6$) δ 12.66 (s, 1H), 12.51 (s, 1H), 11.64 (s, 1H), 8.42–8.28 (m, 4H), 8.19–7.98 (m, 2H), 7.89 (dd, J = 5.9, 3.3 Hz, 2H), 7.64 (d, J = 1.5 Hz, 1H), 7.58–7.49 (m, 2H), 7.48–7.41 (m, 2H), 7.41–7.35 (m, 1H), 5.34 (s, 2H), 4.06 (q, J = 5.2 Hz, 2H), 3.89 (s, 3H). HRMS calcd for C$_{22}$H$_{19}$O$_3$N$_3$Cl$_2$S [M − H]$^-$ 438.0938; found 438.0933.

A mixture of the above ester (58 mg, 0.36 mmol) and methyl 2-(3-isopropoxybenzo[d]thiazole-6-carboxylate (93 mg, 0.35 mmol) was suspended in MeOH (7 mL) was stirred at 80 °C for 30 min, and then the reaction mixture was concentrated and triturated with acetone to give the title compound as a beige amorphous solid (55 mg, 81% yield).1H NMR (400 MHz, DMSO-d$_6$) δ 12.82 (s, 1H), 12.20 (s, 1H), 11.37 (s, 1H), 8.13 (s, 1H), 7.52–7.40 (m, 2H), 7.40–7.31 (m, 3H), 7.31–7.20 (m, 2H), 6.91 (app t, J = 4.2 Hz, 1H), 1.99 (s, 3H), 1.64 (d, J = 6.3 Hz, 3H). 13C NMR (376 MHz, DMSO-d$_6$) δ −153.97 (d, J = 4 Hz). HRMS calcd for C$_{25}$H$_{19}$O$_3$N$_3$FS 438.0929; found 438.0923.

2.4.19. 2-(3-Fluoro-5-methyl-1H-pyrrole-2-carboxamido)-4-(1-phenylethoxy)benz[d]thiazole-6-carboxylic Acid (31). A solution of the above ester 30 (70 mg, 0.154 mmol) in MeOH (3.0 mL) and 2 M NaOH (0.40 mL) was stirred at 40 °C overnight. NaOH (2 M, 0.40 mL) was added and stirred another night, and then the reaction mixture was concentrated. The residue was suspended in water (2 mL), the pH was adjusted to 2 by adding 4 M HCl, and the precipitate was collected, washed with water, air-dried, and triturated with MeOH to get the title compound as a fine yellow amorphous solid (31 mg, 49% yield).1H NMR (400 MHz, DMSO-d$_6$) δ 13.05 (s, 1H), 12.64 (s, 1H), 11.85 (s, 1H), 8.13 (d, J = 1 Hz, 1H), 7.44 (d, J = 1 Hz, 1H), 7.26 (d, J = 2.3 Hz, 1H), 4.95–4.84 (m, 1H), 2.20 (s, 3H), 1.36 (d, J = 6 Hz, 6H). MS 432.2 [M − H]$^-$.

2.4.20. tert-Butyl 2-(4-fluoro-5-methyl-1H-pyrrole-2-carboxamido)-4-isoproxybenz[d]thiazole-6-carboxylate (32). A suspension of 13 (58 mg, 0.36 mmol) and methyl 2-aminooxy-5-methyl-1H-pyrrole-2-carboxamide (93 mg, 0.30 mmol) in toluene (7 mL) was stirred at 130 °C overnight. The gray amorphous precipitate was collected and washed with toluene. Yield: 79% (104 mg).1H NMR (400 MHz, DMSO-d$_6$) δ 12.66 (s, 1H), 11.85 (s, 1H), 8.13 (d, J = 1 Hz, 1H), 7.44 (d, J = 1 Hz, 1H), 7.26 (d, J = 2.3 Hz, 1H), 4.95–4.84 (m, 1H), 2.20 (s, 3H), 1.36 (d, J = 6 Hz, 6H). MS 432.2 [M − H]$^-$.
4.3. Biological Assays. 4.3.1. Determination of Inhibitory Activity on E. coli DNA Gyrase. The supercoiling assay for the determination of IC50 values was performed according to previously reported procedures.30

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c00331.

Additional experimental data; copies of 1H and 13C and 2D NMR spectra; and single-crystal X-ray diffraction analysis (PDF)

Compound 10 (CIF)

Compound 11 (CIF)

checkCIF/PLATON report (PDF)

Accession Codes
CCDC 2022198 and 2022199 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ, UK; fax: +44 1223 336033.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

Some of the research leading to these results was conducted as part of the ND4BB ENABLE Consortium and has received support from the Innovative Medicines Initiative Joint Undertaking under Grant No. 115583, resources of which comprise financial contributions from the European Union’s seventh framework program (FP7/2007-2013) and EFPIA companies’ in-kind contribution. We gratefully acknowledge the Slovenian Research Agency (Grant No. P1-0208), COST Action CA15135 MuTaLiG, the French Agence Nationale pour la Recherche (ANR) (grant number ANR-17-CE07-0008-01, DEFIS), CNRS, and Université de Strasbourg for financial support. The French Fluorine Network (GIS Fluor) is also acknowledged. T.G. is much grateful to the French Ministry of Education and Research for funding. The authors thank L. Karmazin and C. Bailly from the Service de radiocrystallographie de la Fédération de Chimie Le Bel FR 2010 for the SCXRD analyses. Maja Frelih is acknowledged for the acquisition of HRMS spectra and Dr. Žiga Skok for performing the enzyme inhibition assay.

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