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Synthetic Strategies towards Imidazopyridinones and 7-Azaoxindoles and their Evaluation as Antibacterial Agents

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Imidazopyridinones and 7-azaoxindoles are two classes of heterocyclic compounds with only limited previous use in organic and medicinal chemistry. Various synthetic methods and routes have been evaluated to identify safe and robust chemistry to advanced imidazopyridinone building blocks and inhibitor structures. Preparation of the 7-azaoxindoles was challenged by instability of the core scaffold. Key to the successful isolation of the target structure was development of a mild Suzuki cross-coupling in non-aqueous media. The imidazopyridinones were potent inhibitors of the *E. coli* thymidylate monophosphate kinase, while the 7-azaoxindole showed low activity. The compounds were inactive in cell-based studies, indicating poor cell wall penetration.

Introduction

The rise of antibiotic resistance has necessitated the development of new antimicrobial agents with novel modes of action.[1] One possible target for new antibiotics is thymidylate monophosphate kinase (TMPK), an essential enzyme of thymidine diphosphate and DNA synthesis.[2] Sufficient sequence differences between human- and bacterial TMPKs allows selective inhibition,[3] and TMPK X-ray co-crystal structures are available from a variety of organisms, making rational drug design possible.[4] Most inhibitors targeting TMPK in prokaryotes are based on modifications of thymine or thymidine.[4–5] The recent years have, however, seen the development of other classes of inhibitors, such as the metal-based complexes of 3-methyl-*N*-hydroxybenzamidine developed by Lautre *et al.*[6] or the imidazopyridinone **1** (Figure 1) developed by Choi *et al.*[7] Besides this work only a few imidazopyridinones have been evaluated for biological activity.[8]

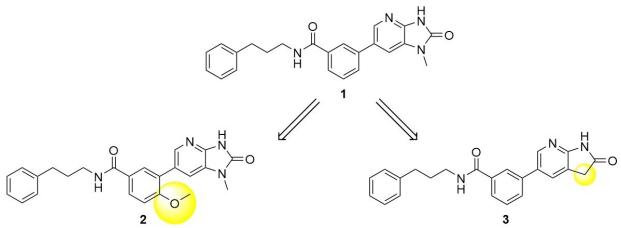

Figure 1. Structures of the known inhibitor **1** and the target molecules **2** and **3**.

We have attempted to identify new design concepts for TMPK inhibitors by using the *E. coli* enzyme as model. Molecular docking based on the structure **1** indicated that increased affinity for TMPK could be obtained by introducing an electron donating substituent at the central aromatic ring (structure **2**). Further, docking could not explain the role of the *N*-methyl group of **1** in binding to TMPK and a possible model compound to investigate this was the 7-azaoxindole **3**, see Figure 1. However, a challenge with 7-azaoxindoles is that the unsubstituted lactam is rather acidic and prone to condensation reactions.[9] Thus, we set out to prepare these model compounds, and herein describe complications and solutions for the chemistry, alongside their preliminary biological evaluation towards *E. coli* TMPK and a panel of human kinases.

Results and Discussion

Docking

Docking of compound **1** was undertaken with two different *E. coli* TMPK structures (PDB: 5TMP and 4TMK) using GLIDE in extra precision (XP) mode with Schrödinger Maestro. This revealed a number of hydrophilic and lipohilic interactions,

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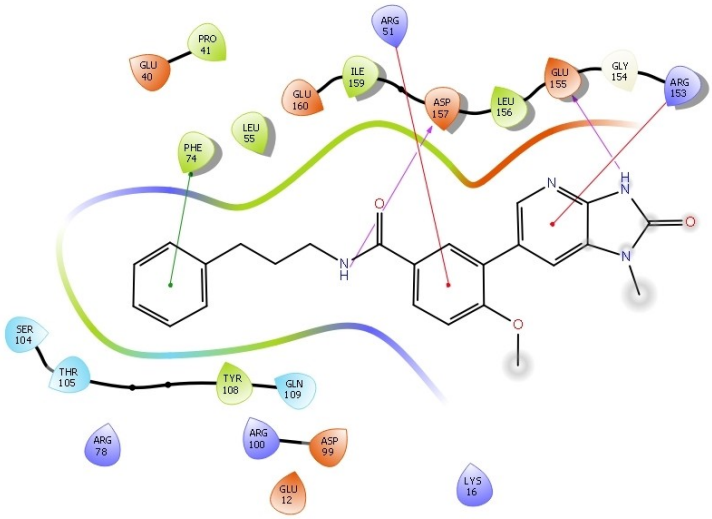


Figure 2. Docking of compound **2** into *E. coli* TMPK (PDB: 5TMP).

among others what appeared as a cation-π interaction between Arg51 and the central carboaromatic ring. Moreover, the role of the *N*-methyl group in binding was unclear, as this group points towards the solvent-exposed entrance with no obvious interactions. Thus, we speculated that the cation-π interaction could be strengthened by adding an electron donating group as in structure **2** (Figure 2) and that the *N*-methyl unit could be replaced by a carbon as seen in the 7-azaoxindole **3**. Indeed, the docking scores (Supplementary information file, Table S2) indicate that compounds **2** and **3** were comparable to structure **1** as inhibitors of TMPK. Figure 3 shows an overlay of the three docked structures **1**–**3** indicating only minimal distortion of the core scaffolds. Compounds **1** and **3** were also subjected to 10 ns dynamics, which indicated very similar flexibility for the two fused 5-membered rings (Supplementary information, Figure S17–S20).

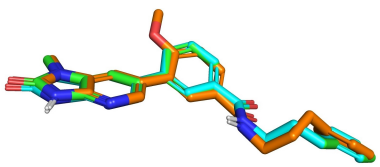
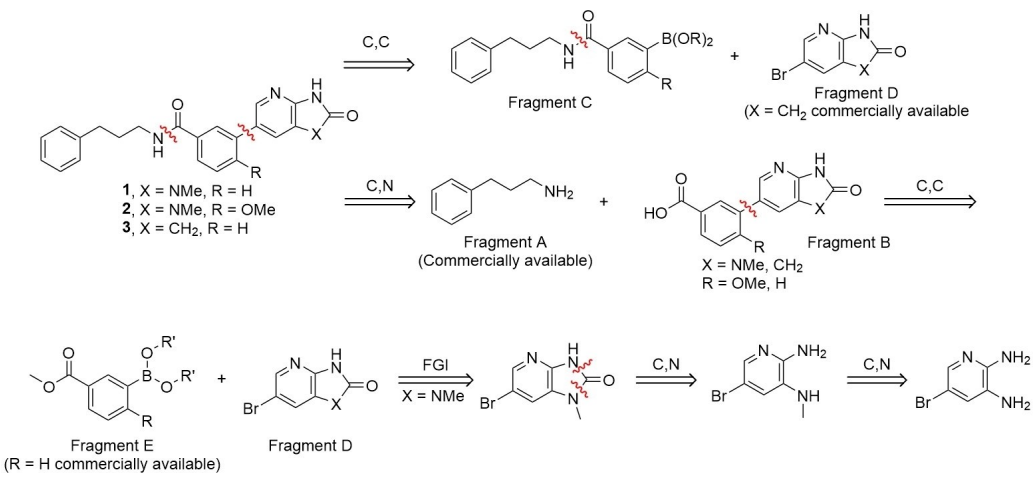


Figure 3. An overlay of the docked structures **1**–**3**.

Retrosynthetic analysis of compounds 1–3

A retrosynthetic analysis of target compounds **1**–**3** is shown in Scheme 1. The linear synthesis used previously[7] to prepare imidazopyridinone **1** involves disconnection to the commercially available 3-phenyl-1-propylamine (fragment A), and an advanced carboxylic imidazopyridinone (fragment B). This in turn can be made from an organoboron reagent (fragment E)



Scheme 1. Retrosynthesis of the target compounds **1**–**3**.

and the heterocyclic building block (fragment D). We also wanted to explore a more convergent synthesis, starting from an advanced organoboron compound (fragment C) and the heterocyclic building block D.

For the synthesis of target molecules **1** and **2**, the heterocyclic fragment D could be prepared from 2,3-diamino-5-bromo-pyridine. Although the synthesis of these building blocks has been reported,[7] we wanted to develop a route with higher yields and without hazardous reagents.

Synthesis of imidazopyridinones 1 and 2

The logical steps from commercially available 2,3-diamino-5-bromopyridine to the key imidazopyridinone fragment D, involves a regioselective methylation and a carbocyclisation. The methylation protocols tested to arrive at 5-bromo-N^3-methylpyridine-2,3-diamine (**5**) are shown in Scheme 2.

Compound **5** was first synthesized via the N^3-Cbz-protected derivative **4**. Initial reactions with benzyl chloroformate (Cbz-Cl) were conducted at 0 °C, but low selectivity towards N^3-protection was observed. The best conditions identified was a reaction time of 30 min at −98 °C, quenching with excess water and then slowly increasing the temperature. However, the high selectivity comes with the price of mediocre conversion and an isolated yield of 46–49% in 3–15 gram scales.

Alternative methylation strategies were also explored. The use of *N*-benzyloxy-carbonyloxy)succinimide (Cbz-OSu) required a much higher reaction temperature and stronger base to proceed and resulted in low selectivity, while methylation with methyl iodide did not lead to product formation under the conditions attempted. Next, reductive cleavage of the Cbz derivative **4** with $LiAlH_4$ gave the methylated aminopyridine **5** in 82% isolated yield. Finally, we tested a two-step one-pot protocol, in which the Cbz derivative **4** was directly reduced by $LiAlH_4$ without intermediate purification. This process performed on par with the original protocol on a small scale but had issues with Cbz-precipitation/mass transfer and aggregates of alumina salts during work-up when scaling to grams. Although simple, more development work is needed to harvest any benefit.

Our synthesis of imidazopyridinone **6** (fragment D) initially made use of triphosgene[7] in yields up to 47% (see Scheme 3). Due to the hazards related to triphosgene, sampling and optimisation was not performed.

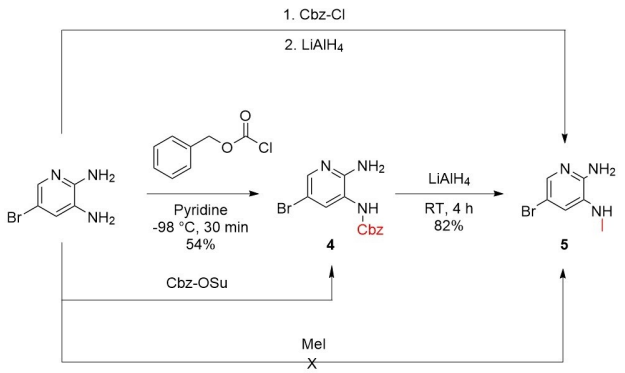
Instead, to make the reaction safer, the carbocyclisation was also tested with urea and carbonyldiimidazole (CDI) as cyclizing agents. The use of urea yielded **6** in 37% yield, owing both to by-product formation and incomplete conversion. However, syntheses with 5 equiv. CDI, employing triethylamine as base at reflux led to full conversion and the isolation of compound **6** in 82% yield. Finally, compound **6** was protected with trityl chloride to form compound **7** in 88% yield.

For the synthesis of target molecule **1**, the more convergent route was used. The assembly of 3-phenyl-1-propylamine (fragment A) and 3-boronobenzoic acid (fragment E) was done through an amide coupling with 1-[bis(dimethylamino) methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluoro-phosphate (HATU) and DIPEA, see Scheme 4.

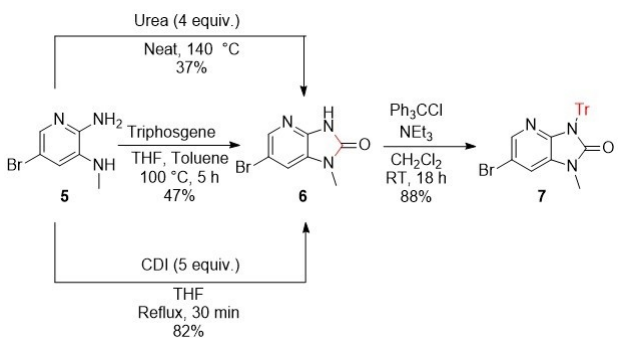
This gave boronic acid **8** in a variable 22–70% yield, depending on the number of purification steps. The subsequent Suzuki coupling with heteroaryl **7** (fragment D) with $Pd(dppf)Cl_2$ gave the protected analogue **9** in 81% yield, and after deprotection with trifluoroacetic acid (TFA) in CH_2Cl_2 compound **1** was obtained in 58% yield.

The aryl boronic ester **10** (see Scheme 5) needed to prepare **2** was made by a previously reported protocol[10] (see Supporting Information). Coupling of **10** to fragment D was first attempted using the unprotected building block **6** (Scheme 5) and a range of palladium catalysts. However, all attempts resulted in low conversion, exhibited low yields of the biaryl **11**, and high amounts of methyl 3-hydroxy-4-methoxybenzoate as the main by-product. Instead the trityl derivative **7** was used as coupling partner, whereupon the Suzuki reaction gave a 78% isolated yield of **12**.

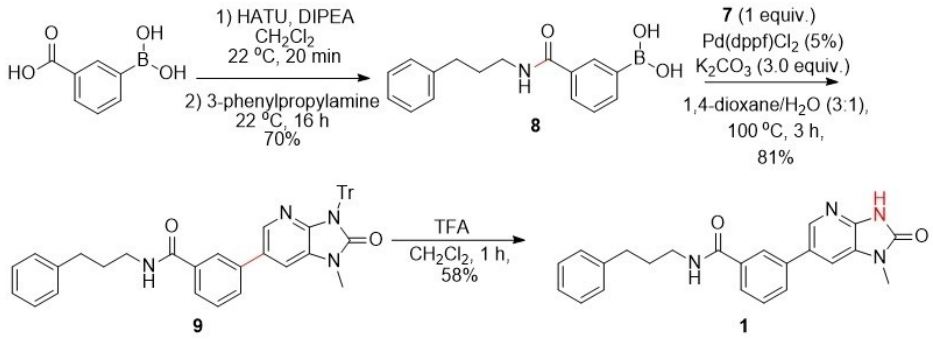
Two strategies were explored for attachment of 3-phenyl-1-propylamine to fragment B, see Scheme 6. The first method employed hydrolysis of **12** with HCl to the free acid **13**. Disappointingly, the following HATU mediated coupling with 3-phenyl-1-propylamine, yielded only 3% of product. Rather than optimizing this amide coupling we instead went for a direct amination of the methyl ester **12**. Traditionally this has been achieved with trimethyl aluminum,[11] but due to its highly



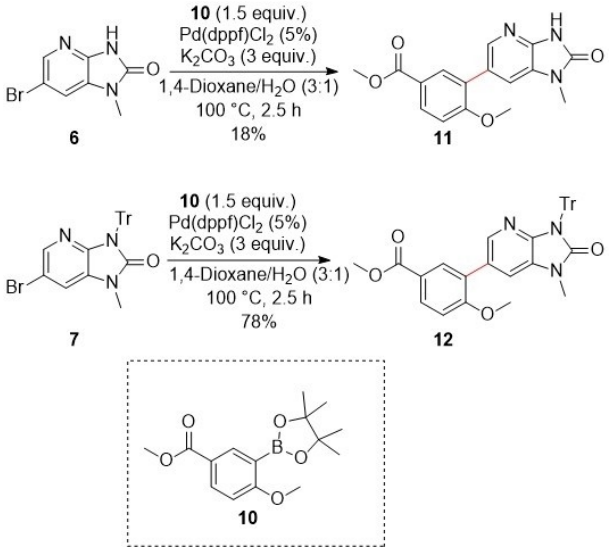
Scheme 2. Preparation of **5** from 2,3-diamino-5-bromopyridine.



Scheme 3. Synthesis of key building block **7** for fragment A.



Scheme 4. Synthesis of target molecule **1**.



Scheme 5. Two strategies for merging fragment D with fragment E.

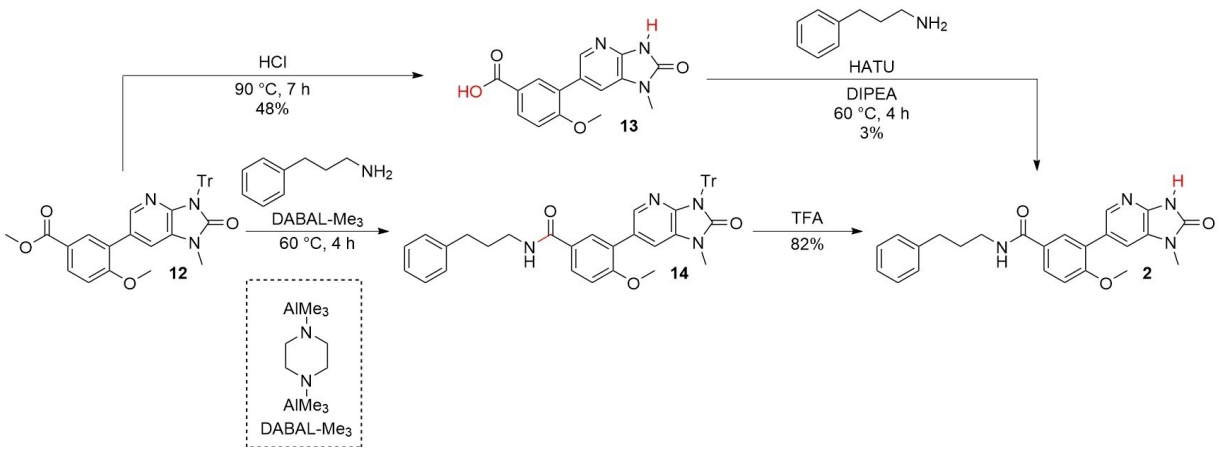
pyrophoric nature, bis(trimethyl-aluminum)-1,4-diazabi-cyclo [2.2.2]octane adduct (DABAL-Me_3), which can release trimethyl aluminum *in situ*, was used.[12] Successful amide formation was achieved in 400 mg scale using 3 equiv. of both coupling agent and amine. The intermediate **14** was not isolated but directly deprotected using TFA, leading to an overall yield of 82%, with the target molecule **2** isolated as its TFA-salt.

Overall, both synthetic routes from precursor **17** to the end products **1** and **2** worked satisfactory, and the choice of method will depend on the task at hand. The three-step approach employing DABAL-Me_3 to form the amide, provided higher yield than the more convergent method employing Suzuki cross-coupling with the boronic acid **8**. It must however be emphasized that these final steps have not been optimised.

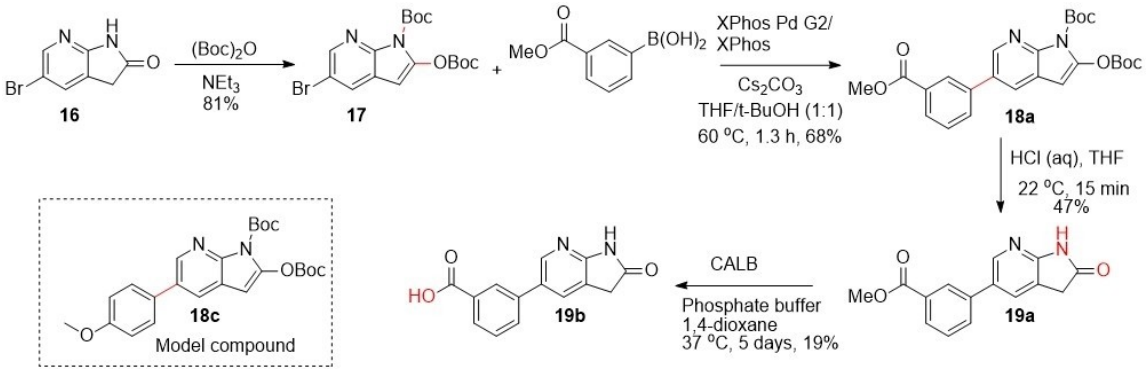
Synthesis of 7-azaoxindole 3

The 7-azaoxindole **3** was first planned prepared as described in Scheme 1.

Although there was some precedence for the cross-coupling between 5-bromo-1,3-dihydro-2*H*-pyrrolo[2,3-*b*]pyridin-2-one (**16**) and aryl boronic acids,[13] the initial Suzuki reactions of **16** were slow, proceeded with low conversions and only trace amounts of product **19a** were formed. The lack of reactivity was assumed to be caused by coordination of the substrate to palladium. To circumvent this issue, a protection strategy was



Scheme 6. The end-game in preparation of the target structure **2**.

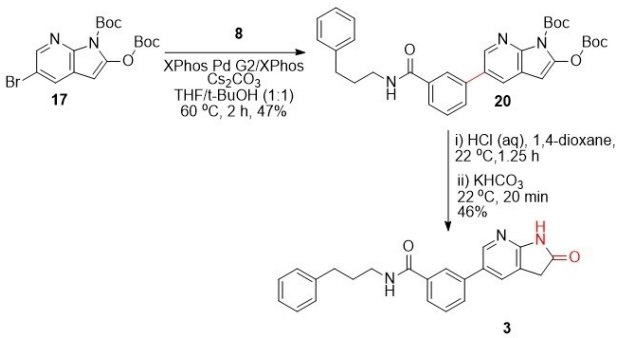


Scheme 7. First route to the target structure **3** and the structure of the model compound **18c**.

sought. Insertion of SEM-, trityl- and acetyl *N*-protections were unsuccessful in our hands (multiple products, data not shown), while reaction of **16** with Boc_2O gave the double *N*,*O*-diBoc derivative **17** in 81% yield (Scheme 7).

The Boc-protecting group is usually stable in basic aqueous media, but the initially tested Suzuki cross-couplings with **17** and 3-methoxycarbonylphenylboronic acid, catalyzed by $Pd_2(dba)_3$ and PCy_3 in 1,4-dioxane/H_2O (1:1) at 100 °C, revealed extensive degradation of both substrate and product. However, it was found that decomposition was far less pronounced in THF/*t*-BuOH. After tuning of the reaction conditions on a model reaction giving **18c**, the use of XPhos Pd G2 and XPhos at 60 °C appeared as the best solution (see Supporting Information). It should be noted that the model compound **18c** (Scheme 8) was unstable during silica-gel column chromatography, but purification by reversed phase silica (RP) gave 75% yield. With a decent outcome for the model reaction, we proceeded with more relevant boronic acids. Ideally, coupling with 3-carboxyphenyl boronic acid would lead to a shorter overall route, but the reactivity was too low compared to the rate of degradation. However, coupling with 3-methoxycarbonylphenyl boronic acid, lead to complete conversion in two hours. Purification of the biaryl **18a** by silica-gel column chromatography resulted in 68% yield (Scheme 7).

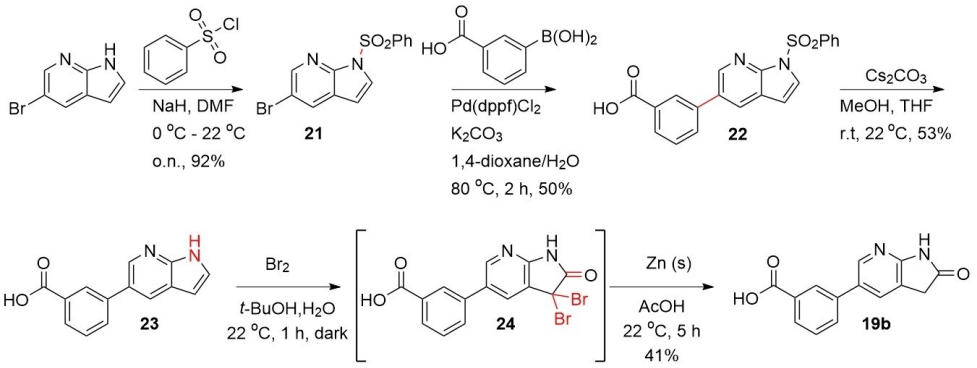
Boc-deprotection of 7-azaoxindole **18a** yielded **19a** in 47% yield. In the subsequent ester hydrolysis, conventional basic or acidic conditions were too harsh giving by-products assumed originating from lactam ring opening and side reactions on the α-carbon. In the search for milder methods, we found that lipase B from *Candida antarctica* mediated the hydrolysis, but at a sluggish pace, giving the acid **19b** in 19% yield after 5 days. Unfortunately, coupling of the carboxylic acid **19b** with 3-phenyl-1-propylamine using HATU failed to give the target structure **3** and instead formed a complex mixture of components (not shown). Fortunately, we knew that the 7-azaoxindole scaffold tolerated mild Suzuki cross-coupling conditions and we instead performed a reaction between **17** and **8**, which gave precursor **20** in 47% yield (Scheme 8). Finally, acid hydrolysis enabled the isolation of the target **3** in 46% yield.



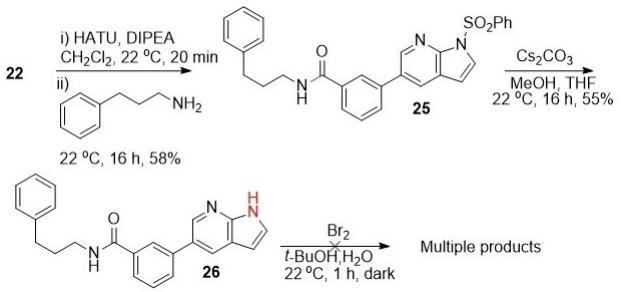
Scheme 8. Final steps towards the target **3**.

Although compound **3** was successfully prepared, carrying the unstable azaoxoindole through three steps is challenging. Thus, in an alternative approach, the plan was to introduce the labile γ-lactam at the end. As our major concern was the oxidation step, we first evaluated the chemistry from **23** to **19b**. The synthesis is outlined in Scheme 9.

Oxidation of 7-azaindols has previously been described using a two-step procedure containing double-bromination at C-3 followed by zinc reduction.[9a] In our hands the aqueous bromination was carried out at room temperature, and after one hour full conversion was observed by ^{1}H NMR spectroscopy. However, quenching of the reaction mixture with either $Na_2S_2O_5$, $Na_2S_2O_3$ or Na_2SO_3 led to decomposition, and we therefore proceeded with a simple extraction as work up. Attempts at purifying the dibrominated intermediate **24** also led to severe decomposition on both NP and RP columns. Instead, the following reduction with Zn powder in AcOH was performed on the crude material which gave the acid **19b** in 41% yield over two steps The process described above was then tested in the oxidation of the more advanced derivative **26**. To prepare the 7-azaindole **26**, the protected intermediate **22** was first coupled with 3-phenyl-1-propylamine using HATU giving the amide **25** in 58% yield (Scheme 10). This was followed by deprotection with Cs_2CO_3 in MeOH/THF giving the precursor **26** in 55% yield. However, the final oxidation failed at the bromination stage. ^{1}H NMR analysis indicated that multiple sites were brominated and treatment with Zn powder did not



Scheme 9. Synthesis of **19b** with oxidation of the 7-azaindole **23**.



Scheme 10. Preparation of the advanced intermediate **26** and attempted oxidation.

Table 1. Evaluation of compound **1**–**3** as *E.coli* TMPK inhibitors in enzymatic and in antimicrobial assays.

Compound	Enzymatic assay TMPK *E. coli* Inhibition [%][a]	IC_{50} [μM][b]	Antimicrobial activity, MIC [μg/Ml] *E. coli*[c]	*S. aureus*[d]
1	61	1.4	>256	>256
2	69	5.6	>256	>256
3	9	83	>256	>256

[a] Measured at 8.3 μM. [b] See Supporting Information for IC_{50} curves. [c] *E. coli*–MG1655. [d] *S. aureus* – ATCC29213.

reduce the complexity of the mixture. Although the strategy might be applicable for other more electron deficient aromatic amines, we decided to abandon this route for the preparation of **3**.

Overall synthesis of the 7-azaoxindole **3** proved challenging mainly due to the instability of the core structure. Among others, the typically mild HATU amidation protocol could not be applied. However, the 7-azaoxindole in protected form tolerated Suzuki cross-coupling to yield advanced intermediates, providing a route for future design of new 7-azaoxindoles. The second route employing a late stage oxidation/reduction sequence is highly applicable if the molecule in question is not prone to over- bromination.

Biological evaluation of compounds 1–3

Compound **1** has previously been found active towards TMPK from *Pseudomonas aeruginosa*.[7] We initially confirmed that compound **1** was also an active *E. coli* TMPK inhibitor. Although the sequence similarity as analysed by BLAST[14] was only 44%, the two enzymes have very similar folding.[4] The potency of compounds **1**–**3** towards *E. coli* TMPK was determined in an enzymatic fluorescence assay. Single-point inhibition measurements at 8.3 μM revealed the imidazopyridinones **1** and **2** to be equipotent, while the 7-azaoxindole **3** showed low inhibition (Table 1). Further, IC_{50} measurements confirmed that **1** and **2** are potent inhibitors towards the *E. coli* TMPK variant (IC_{50} curves are shown in Supporting Information). In contrast to that indicated by molecular docking, efficient binding was not seen for compound **3**. Obviously, our docking procedures on this rather flexible protein must be improved. We have attempted to grow co-crystal structures of *E. coli* TMPK and compounds **1**–**3**, but these efforts have not yielded crystals of sufficient quality.

Antimicrobial activity testing in culture revealed all three compounds to have no activity towards both *E. coli* and *S. aureus*, likely due to poor cell wall penetration. Although certain design concepts can be applied to improve cell wall penetration,[15] these are difficult to apply on inhibitors for TMPK without proper structural information of binding mode.

To reveal if the imidazopyridinone **2** and the 7-azaoxindole **3** could have any off-targets or alternative applications, they were assayed towards a panel of 60 human kinases at 500 nM test concentration. Overall, these two model compounds possessed low ability to inhibit these selected kinases, with the highest activity seen towards the colony simulated factor 1 receptor (CSF1R) with 17 and 18% inhibition, respectively. Thus, further development as CSF1R inhibitors is a potential application of both compound classes. Interestingly, in contrast to that seen in the TMPK assay, compounds **2** and **3** have rather similar potency towards the human kinases. Figure 4 shows the 15 most inhibited kinases ranked by the inhibition towards compound **2**. All data can be found in the Supporting Information file.

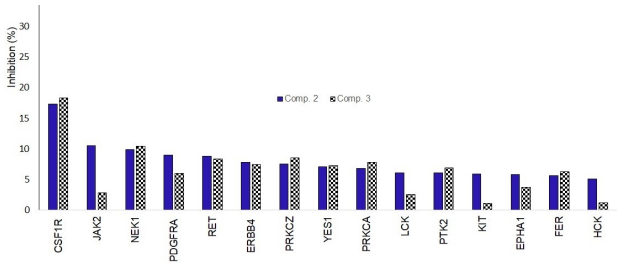


Figure 4. Evaluation of compound **2** and **3** towards a panel of kinases.

Conclusion

Synthetic routes for preparing three potential bacterial thymidylate monophosphate kinase inhibitors have been evaluated. The key building block for the imidazopyridinone based inhibitors were made in 4 steps. Three of the transformations were carefully evaluated and the yield and safety were improved by substituting triphosgene with 1,1′-carbonyldiimidazole in the key carbo-cyclisation step. A modular approach employing bis(trimethylaluminum)-1,4-diazabicyclo-[2.2.2] octane in the amide forming reaction proved highly efficient. Synthesis of the 7-azaoxindole was challenged by instability of the core scaffold. This was solved by proper selection of protection group and development of a mild Suzuki cross-coupling in non-aqueous media, allowing for the isolation of target **3** by a 3-step route. An alternative strategy employing late stage oxidation with molecular bromine was successfully executed on model compounds but failed when performed on a more electron rich substrate. The imidazopyridinones were potent inhibitors of the *E. coli* TMPK in enzymatic assays. However, in contrast to that indicated by molecular docking, the 7-azaoxindole possessed low activity. Obviously, the docking procedures on these flexible proteins must be developed. Unfortunately, none of the compounds showed activity towards cultures of *E. coli* or *S. aureus*, probably due to poor cell membrane permeability.

Experimental Section

Reagents and general methods

All reagents and solvents used were purchased from Merch, VWR or Alpha Aesar and used without further purification. Reactions sensitive to moisture or oxygen were conducted under an N_2 atmosphere using oven-dried glassware and solvents dried over molecular sieves for 24 hours, or collected from an MBraun SPS-800 solvent purifier. HPLC was performed on an Agilent 1100 series instrument with an Agilent Poroshell C18 (4.6×100 mm) column with 2.7 μm pore size. Agilent Chemstation was used as software. Purity analysis and reaction monitoring was done starting with a 5-minute isocratic elution with H_2O/MeCN (9:1) followed by a 30-minute linear gradient ending at H_2O/MeCN (0:100) at 0.8 mL/min flow. Hydrolytic reactions were analyzed with a 2-minute isocratic elution with H_2O/MeCN (9:1) followed by a 13-minute linear gradient ending at H_2O/MeCN (0:100) at 0.5 mL/min flow. Accurate mass determination in positive and negative mode was performed on a "Synapt G2-S" Q-TOF instrument from WatersTM. Samples were ionized using an ASAP probe (APCI). No chromatographic separation was done prior to the mass analysis. Calculated exact mass and spectra processing was done by WatersTM Software (Masslynx V4.1 SCN871). NMR spectra were recorded using the Bruker DPX 400 MHz and 600 MHz Avance III HD NMR spectrometers. Chemical shifts (δ) are recorded in parts per million relative to TMS (δH = 0.00, δC = 0.0) or [D_6]DMSO (δH = 2.50, δC = 39.5), and coupling constants (J) are measured in hertz (Hz).

Synthesis of 4[16]

A solution of 2,3-diamino-5-bromopyridine (15.0 g, 79.9 mmol), dry THF (190 mL) and dry pyridine (22.5 mL) was stirred at −98 °C for 10 minutes before the dropwise addition of benzyl chloroformate (14.8 mL, 102 mmol). The reaction was then stirred for a further 30 minutes, quenched with H_2O (10 mL) and stirred for an additional 15 minutes at −98 °C. The reaction mixture was subsequently concentrated to approx. 50% of the original volume *in vacuo*, and EtOAc (200 mL) and brine (100 mL) were added. The resulting emulsion was split in two, and the bottom phase extracted with EtOAc (100 mL) and brine (50 mL). The resulting organic phase was added to the top phase, which was then extracted with EtOAc (100 mL) and brine (50 mL). This resulted in phase separation. The combined water-phases were extracted with EtOAc (6×100 mL) and the combined organic phases washed with brine (100 mL), dried over anhydrous Na_2SO_4, filtered, concentrated *in vacuo* and washed with toluene (5×2 mL), yielding a dark brown solid. The crude material was then immobilized on Celite, split in three and purified by flash chromatography on three columns (R_f = 0.27, EtOAc/*n*-pentane 1:1). Impure product fractions were combined and recrystallized from MeCN (15 mL), resulting in a total of 11.7 g (36.4 mmol, 46%) of a brown solid, mp. 152–153 °C (lit. [16] 157–158 °C). ^{1}H NMR (400 MHz, [D_6]DMSO) δ = 8.98 (s, 1H), 7.87 (br s, 1H), 7.79 (d, J = 2.3 Hz, 1H), 7.32–7.45 (m, 5H), 6.09 (s, 2H), 5.16 (s, 2H). ^{1}H NMR and ^{13}C NMR were in accordance with the literature.[7,16]

Synthesis of 5[16]

$LiAlH_4$ (1.17 g, 30.8 mmol) was added to a solution of benzyl-(2-amino-5-bromopyridin-3-yl)-carbamate (**4**) (2.50 g, 7.77 mmol) in diethyl ether (150 mL) cooled at 0 °C under an N_2-atmosphere. Following stirring at 0 °C for 15 minutes and at RT for 5 hours, the mixture was cooled to 0 °C and H_2O (1.6 mL), aqueous NaOH (10%, 1.6 mL) and more H_2O (3.6 mL) were added sequentially. The solution was then filtered to remove $LiAlH_4$ residues and the filtrate concentrated *in vacuo*. The resulting brown solid was immobilized on Celite and purified by flash chromatography (R_f = 0.30, EtOAc/*n*-pentane 3:1), yielding 1.28 g (6.36 mmol, 82%) of the desired product as a white solid, mp. 136–137 °C (lit. [16] 137–138 °C). ^{1}H NMR (400 MHz, [D_6]DMSO) δ = 7.28 (d, J = 1.9 Hz, 1H), 6.55–6.56 (m, 1H), 5.65 (s, 2H), 5.22 (m, 1H), 2.69 (d, J = 4.9 Hz, 3H), ^{1}H NMR and ^{13}C NMR were in accordance with the literature.[7,16]

Synthesis of 6[7]

5-Bromo-N^3-methylpyridine-2,3-diamine (**5**) (503 mg, 2.50 mmol) and carbonyldiimidazole (2.00 g, 12.4 mmol) were flushed with N_2 three times. THF (10 mL) and NEt_3 (1.7 mL) were added, and then refluxed for 30 min. The solvent was removed *in vacuo*, EtOAc (50 mL) and H_2O (25 mL) were added and the pH in the aqueous phase adjusted to 3 with HCl (2 M) and $NaHCO_3$ soln. (sat.). The phases were separated, and the aqueous phase extracted with

EtOAc (2×50 mL). The combined organic layers were washed with brine (25 mL), dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The resulting crude was immobilized on Celite and purified by flash chromatography (R_f = $CHCl_3$/acetone 5:1), yielding 463 mg (2.03 mmol, 82%) of the desired product as a yellow solid. ^{1}H NMR (400 MHz, [D_6]DMSO) δ = 11.72 (s, 1H), 7.99 (d, *J* = 2.0 Hz, 1H), 7.73 (d, *J* = 2.0 Hz, 1H), 3.27 (s, 3H). ^{1}H NMR and ^{13}C NMR were in accordance with literature.[7]

Synthesis of 7[7]

Trityl chloride (1.45 g, 5.20 mmol) was added to a solution of 6-bromo-1-methyl-1,3-dihydro-2*H*-imidazo[4,5-*b*]pyridin-2-one (**6**) (1.04 g, 4.57 mmol), NEt_3 (2 mL) in CH_2Cl_2 (30 mL) and stirred overnight. The solvent was removed *in vacuo*, CH_2Cl_2 (50 mL) and H_2O (25 mL) was added and the phases separated. The aqueous phase was extracted with CH_2Cl_2 (3×50 mL) and H_2O (25 mL). The combined organic layers were washed with brine (25 mL) before drying with anhydrous Na_2SO_4, filtration and concentration *in vacuo* yielded the crude product as a yellow solid. The solid was purified by flash chromatography (EtOAc/*n*-pentane 2:9–1:1), resulting in 1.93 g (4.02 mmol, 88%) of a yellow solid, mp. 219–220 °C. ^{1}H NMR (600 MHz, [D_6]DMSO) δ = 8.01–8.03 (m, 1H), 7.96 (d, *J* = 2.2 Hz, 1H), 7.92 (d, *J* = 1.9 Hz, 1H), 7.52–7.54 (m, 6H), 7.23–7.27 (m, 7H), 7.17–7.19 (m, 3H), 6.98 (d, *J* = 8.7 Hz, 1H), 3.89 (s, 3H), 3.87 (s, 3H), 3.34 (s, 3H); ^{13}C NMR (150 MHz, [D_6]DMSO) δ = 152.90, 142.68, 142.02 (3C), 138.40, 128.47 (6C), 127.28 (6C), 126.34, 126.29 (3C), 116.08, 112.31, 73.84, 26.90; IR (neat, cm^{-1}) ν = 705 (s), 1136 (m), 1367 (s), 1467 (m), 1737 (s); HRMS (TOF ASAP+): m/z calcd. for $C_{26}H_{21}N_3O^{79}Br$: 470.0868 $[M+H]^+$, found: 470.0863. ^{1}H NMR, ^{13}C NMR and MS spectra were in accordance with literature.[7]

Synthesis of 8

To a solution of 3-boronobenzoic acid (504 mg, 3.04 mmol) and HATU (1.37 g, 3.61 mmol) in dry CH_2Cl_2 (40 mL), DIPEA (0.67 mL, 3.85 mmol) was added. The solution was stirred at ambient temperature for 30 minutes before 3-phenylpropan-1-amine (435 µL, 3.06 mmol) was added, and the reaction was stirred for 20 hours. Full conversion was observed by TLC (R_f = 0.41, CH_2Cl_2/MeOH 1:9), and water (50 mL) was added. The mixture was extracted with CH_2Cl_2 (3×25 mL) before the combined organic layer was washed with brine (25 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography (silica, R_f = 0.41, CH_2Cl_2/MeOH gradient 0:100 to 1:9) before the obtained oil was added CH_2Cl_2 (6 mL) and *n*-pentane (20 mL). The formed solids were filtered off and a portion of the solids (84%) were purified by flash chromatography (C-18 silica, R_f = 0.24, H_2O/MeOH gradient 1:1 to 1:2) before the obtained solids were dissolved in CH_2Cl_2 (6 mL) and were dropwise added Et_2O (4 mL). The formed solids were filtered off and were stirred in refluxing H_2O (150 mL) for 30 min, before the mixture was cooled to ambient temperature and the solids were filtered off. This afforded 212 mg (0.749 mmol) of (3-((3-phenylpropyl)carbamoyl)phenyl)boronic acid (**8**) as a white powder, which corresponds to a yield of 29%. ^{1}H NMR (600 MHz, [D_6]DMSO) δ = 8.42 (t, *J* = 5.5 Hz, 1H), 8.24 (br s, 1H), 8.15 (s, 2H), 7.90–7.89 (m, 1H), 7.84–7.83 (m, 1H), 7.41 (t, *J* = 7.5 Hz, 1H), 7.30–7.27 (m, 2H), 7.24–7.23 (m, 2H), 7.19–7.16 (m, 1H), 3.29–3.26 (m, 2H), 2.65–2.62 (m, 2H), 1.86–1.81 (m, 2H); ^{13}C NMR (150 MHz, [D_6]DMSO) δ = 166.8, 141.8, 136.5, 134.0, 133.0, 128.6, 128.29, 128.26, 127.2, 125.7, 38.9, 32.6, 30.9.

Synthesis of 9

A mixture of 6-bromo-1-methyl-3-trityl-1,3-dihydro-2H-imidazo[4,5-b]pyridin-2-one (**7**) (201 mg, 0.428 mmol), (3-((3-phenylpropyl) carbamoyl)phenyl)boronic acid (**8**) (156 mg, 0.552 mmol), $PdCl_2$(dppf) (16 mg, 0.022 mmol) and K_2CO_3 (176 mg, 1.273 mmol) under N_2 were added 1,4-dioxane (1.5 mL) and water (0.5 mL) and was agitated at 100 °C for 3 hours. The mixture was cooled to ambient temperature and was added water (25 mL) and EtOAc (25 mL), before the aqueous layer was extracted with EtOAc (3× 15 mL). The combined organic layer was dried over Na_2SO_4, filtered through a celite pad and concentrated *in vacuo*. The crude product was purified by flash chromatography (Silica, R_f = 0.33, gradient 100% CH_2Cl_2 to 1:9 EtOAc/CH_2Cl_2) to afford **9** in 81% yield (217 mg, 0.345 mmol) as a white waxy solid; ^{1}H NMR (600 MHz, [D_6]DMSO) δ = 8.53 (t, *J* = 5.6 Hz, 1H), 8.16 (d, *J* = 2.1 Hz, 1H), 8.10 (t, *J* = 1.9 Hz, 1H), 7.83–7.79 (m, 3H), 7.54–7.50 (m, 7H), 7.29–7.22 (m, 10H), 7.19–7.14 (m, 4H), 3.33 (s, 3H), 3.31–3.28 (m, 2H), 2.65–2.63 (m, 2H), 1.87–1.82 (m, 2H). ^{13}C NMR (150 MHz, [D_6]DMSO) δ = 165.9, 153.3, 143.0 (3C), 142.9, 141.7, 137.4, 137.0, 135.3, 129.1, 129.01, 128.98, 128.5 (6C), 128.30 (2C), 128.28 (2C), 127.3 (6C), 126.5, 126.3 (3C), 125.7, 125.4, 124.9, 111.8, 73.8, 39.0, 32.7, 30.9, 26.9;

Synthesis of 1[7]

To a stirring solution of 3-(1-methyl-2-oxo-3-trityl-2,3-dihydro-1H-imidazo[4,5-b]pyridin-6-yl)-N-(3-phenylpropyl)benzamide (**9**) (172 mg, 0.274 mmol) in CH_2Cl_2 (5 mL) TFA (1 mL) was added. After 1 hour, the solution was concentrated under reduced pressure, and residual TFA was removed by co-evaporation with MeOH (3× 15 mL). A portion (56%, 103 mg) of the crude material was added brine (25 mL) and EtOAc (25 mL) before the aqueous phase was extracted with additional EtOAc (3×25 mL) and the combined organic phase was dried over Na_2SO_4, filtered and dried *in vacuo*. The crude material was purified by flash chromatography (silica, R_f = 0.16, gradient: 100% EtOAc to 1:9 MeOH/EtOAc) to afford **1** in 58% yield (35 mg, 0.091 mmol) as a white solid; mp 183.0–184.1 °C; ^{1}H NMR (600 MHz, [D_6]DMSO) δ = 11.65 (s, 1H), 8.60 (t, *J* = 5.6 Hz, 1H), 8.30 (d, *J* = 2.0 Hz, 1H), 8.14 (t, *J* = 1.8 Hz, 1H), 7.87–7.83 (m, 2H), 7.81 (d, *J* = 2.0 Hz, 1H), 7.57 (t, *J* = 7.7 Hz, 1H), 7.31–7.24 (m, 4H), 7.20–7.17 (m, 1H), 3.38 (s, 3H), 3.34–3.31 (m, 2H), 2.66 (t, *J* = 7.7 Hz, 2H), 1.89–1.84 (m, 2H); ^{13}C NMR (150 MHz, [D_6]DMSO) δ = 166.0, 154.1, 143.3, 141.8, 138.3, 138.1, 135.4, 129.2, 128.98, 128.96, 128.30 (2C), 128.27 (2C), 126.2, 125.7, 125.5, 125.2, 112.2, 39.0, 32.7, 30.9, 26.5; HRMS (ASAP-TOF) *m/z* calcd. for $C_{23}H_{23}N_4O_2$: 387.1821, $[M+H]^+$, found 387.1822. ^{1}H NMR and ^{13}C NMR were in accordance with literature.[7]

Synthesis of 12

6-Bromo-1-methyl-3-trityl-1,3-dihydro-2*H*-imidazo[4,5-*b*]pyridin-2-one (**7**) (196 mg, 0.417 mmol), methyl 4-methoxy-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate (**10**) (185 mg, 0.634 mmol), K_2CO_3 (173 mg, 1.25 mmol) and Pd(dppf)Cl_2 (17.9 mg, 24.4 µmol) were dissolved in degassed 1,4-dioxane (1.5 mL) and H_2O (0.5 mL) under an N_2 atmosphere, heated to 100 °C and stirred for 2.5 hours. The mixture was then cooled, filtered through a Celite pad and concentrated *in vacuo*. The resulting brown solid was distributed between EtOAc (10 mL) and H_2O (10 mL). The aqueous phase was extracted with EtOAc (6×10 mL) and the combined organic layers washed with H_2O (10 mL) and brine (10 mL), dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to yield the crude product as a brown oil. Immobilisation on Celite and purification by flash chromatography (R_f = 0.19, EtOAc/toluene 1:10) yielded 213 mg (0.324 mmol, 78%) of a yellow solid, decomp. point: 215 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ = 8.01 (dd, $^3J_{H-H}$ = 8.7 Hz,

$^4J_{H-H}$ = 2.3 Hz, 1H), 7.96 (d, J = 2.2 Hz, 1H), 7.92 (d, J = 1.8 Hz, 1H), 7.53–7.54 (m, 6H), 7.23–7.26 (m, 10H), 7.17–7.19 (m, 3H), 6.98 (d, J = 8.5 Hz, 1H), 3.89 (s, 3H), 3.86 (s, 3H), 3.33 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ = 165.7, 160.1, 154.2, 143.1, 142.9 (3C), 140.1, 132.3, 131.0, 129.2 (6C), 127.4, 127.3 (6C), 126.5 (3C), 126.6, 124.4, 122.9, 113.5, 111.6, 74.71, 55.8, 52.0, 26.9; IR (neat, cm^{-1}) ν = 739 (s), 1141 (m), 1238 (m), 1263 (m), 1367 (s), 1450 (m), 1460 (m), 1707 (s), 1723 (s); HRMS (TOF ASAP +): m/z calcd. for $C_{35}H_{30}N_3O_4$: 556.2236 $[M+H]^+$, found: 556.2238.

Synthesis of 2

3-Phenyl-1-propylamine (310 μL, 2.18 mmol) was added to a solution of DABAL-Me_3 (557 mg, 2.17 mmol) in dry THF (8 mL) and stirred at 40 °C under an N_2 atmosphere for 1 hour. Methyl 4-methoxy-3-(1-methyl-2-oxo-3-trityl-2,3-dihydro-1H-imidazo[4,5-b] pyridin-6-yl)-benzoate (**12**) (385 mg, 0.693 mmol) in dry THF (8 mL) was added, and the reaction refluxed for a further 4 hours. The reaction was then concentrated *in vacuo*, re-dissolved in CH_2Cl_2 (10 mL) and TFA (2 mL) and stirred for 1.5 hours at 22 °C to remove the protective group. Concentration *in vacuo* and co-evaporation with CH_2Cl_2 (2×10 mL) yielded the crude product as a dark yellow solid. Immobilisation on Celite and gradient flash chromatography (EtOAc/MeOH 100:0–9:1) yielded 366 mg (0.567 mmol, 98% purity, 82%) of the TFA-salt of the desired product as a light brown solid, mp. 119–122 °C (decomp. on melting). ^{1}H NMR (600 MHz, [D_6] DMSO) δ = 11.58 (s, 1H), 8.43 (t, J = 5.6 Hz, 1H), 8.03 (d, J = 1.8 Hz, 1H), 7.89 (dd, $^3J_{H,H}$ = 8.5 Hz, $^4J_{H,H}$ = 2.3 Hz, 1H), 7.85 (d, J = 2.2 Hz, 1H), 7.55 (d, J = 1.8 Hz, 1H), 7.15–7.28 (m, 6H), 3.83 (s, 3H), 1.83 (quintet, J = 7.3 Hz, 2H), 2.62 (t, J = 7.7 Hz, 2H), 3.32 (s, 3H), 3.28 (q, J = 6.8 Hz, 2H); ^{13}C NMR (150 MHz, [D_6]DMSO) δ = 165.5, 158.4, 154.1, 142.7, 141.8, 140.2, 129.6, 128.5, 128.27–128.30 (4C), 127.1, 126.75, 125.7, 126.67, 124.7, 114.7, 111.2, 55.9, 38.9, 32.7, 31.0, 26.4; IR (neat, cm^{-1}) ν = 657 (m), 699 (m), 1019 (s), 1047 (s), 1604 (m), 1633 (m), 1686 (br s), 2936 (br s); HRMS (TOF ASAP +): m/z calcd. for $C_{24}H_{25}N_4O_3$: 417.1927 $[M+H]^+$, found: 417.1921.

Synthesis of 17

5-Bromo-1,3-dihydro-2*H*-pyrrolo[2,3-*b*]pyridine-2-one (**16**) (1.00 g, 4.72 mmol), di-*tert*-butyl decarbonate (5.14 g, 23.55 mmol) and NEt_3 (4.00 mL, 28.70 mmol) was dissolved in THF (11 mL) and heated to reflux. After 24 h, the mixture was concentrated under reduced pressure, before EtOAc (50 mL) was added. The organic phase was washed with water (50 mL), and the aqueous phase was extracted with EtOAc (2×50 mL) before the combined organic layer was washed with brine (50 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash chromatography (silica, R_f = 0.53, Et_2O/*n*-pentane, 5:95,). This gave **17** in 81% yield (1.57 g, 3.80 mmol) as a viscous, colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.42 (d, J = 2.2 Hz, 1H), 7.87 (d, J = 2.2 Hz, 1H), 6.14 (s, 1H), 1.62 (s, 9H), 1.53 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 149.8, 147.1, 144.9, 143.7, 143.2, 130.5, 121.5, 115.0, 92.6, 85.5, 85.2 28.0 (3C), 27.6 (3C); IR (neat, cm^{-1}) ν = 697, 734, 1144, 1264, 1378, 1450, 1460, 1537, 1633, 1717, 2859, 2934, 3026, 3056, 3085, 3326; HRMS (TOF ASAP +): *m/z* calcd. for $C_{17}H_{22}N_2O_5{}^{79}Br$: 413.0712 $[M+H]^+$, found 413.0704.

Synthesis of 18a

A mixture of (3-(methoxycarbonyl)phenyl)boronic acid (122, mg, 0.680 mmol), XPhos Pd G2 (14.8 mg, 0.019 mmol), XPhos (8.6 mg, 0.018 mmol) and Cs_2CO_3 (340 mg, 1.05 mmol) was added *t*-BuOH (2 mL) and a solution of *tert*-butyl 5-bromo-2-((*tert*-butoxycarbonyl) oxy)-1*H*-pyrrolo[2,3-*b*]pyridine-1-carboxylate (**17**) (140 mg, 0,340 mmol) in THF (2 mL). After 2 h the solution was filtered through celite and concentrated under reduced pressure before EtOAc (25 mL) and brine (25 mL) was added. The aqueous layer was extracted with EtOAc (2×25 mL), before the combined organic layer was washed with brine (2×25 mL), dried over Na_2SO_4, and dried under reduced pressure. The crude product was purified by flash chromatography (silica, R_f = 0.20, Et_2O/*n*-pentane, 3:7). This gave **18a** in 68% yield (109 mg, 0.233 mmol) as a light-brown oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.67 (d, J = 2.2 Hz, 1H), 8.28–8.27 (m, 1H), 8.06–8.04 (m, 1H), 8.00 (d, J = 2.2 Hz, 1H), 7.80–7.78 (m, 1H), 7.54 (t, J = 7.7 Hz, 1H), 6.27 (s, 1H), 1.68 (s, 9H), 1.58 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.0, 150.0, 147.5, 145.2, 143.5, 143.1, 139.1, 131.8, 131.7, 131.1, 129.2, 128.7, 128.6, 126.9, 120.1, 93.6, 85.5, 85.1, 52.4, 28.2, 27.8; HRMS (ASAP-TOF +) *m/z* calcd. for $C_{25}H_{29}N_2O_7$ 469.1975 $[M+H]^+$, found 469.1977.

Preparation of 19a

To a mixture of *tert*-butyl 2-((*tert*-butoxycarbonyl)oxy)-5-(3-(methoxycarbonyl)phenyl)-1*H*-pyrrolo[2,3-*b*]pyridine-1-carboxylate (**18a**) in THF (0.10 M, 1 mL, 0.10 mmol), HCl (37%, 1 mL) was added. The reaction was left for 15 min before $NaHCO_3$ (sat., 20 mL) and EtOAc (10 mL) was added. The aqueous layer was extracted with EtOAc (2×10 mL), and the combined organic layers were washed with brine (10 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The crude product was adsorbed on celite and purified by flash chromatography (C18 silica, R_f = 0.18, EtOAc/*n*-hexane 3:2). This gave **19a** in 47% yield (12.5 mg, 0.047 mmol); mp 239.8–242.4 °C (decomp.); ^{1}H NMR (400 MHz, [D_6]DMSO) δ = 11.13 (s, 1H), 8.39 (d, J = 2.0 Hz, 1H), 8.153–8.145 (m, 1H), 7.95–7.91 (m, 3H), 7.62 (t, J = 7.8 Hz, 1H), 3.89 (s, 3H), 3.63 (s, 2H); ^{13}C NMR (100 MHz, [D_6] DMSO) δ = 175.9, 166.1, 158.2, 144.4, 138.2, 131.1, 130.44, 130.40, 129.6, 128.8, 127.9, 129.8, 120.9, 52.3, 35.4; IR (neat, cm^{-1}): 3092, 1714, 1284, 1242, 1228, 763, 571; HRMS (ASAP-TOF +) *m/z* calcd. for $C_{15}H_{13}N_2O_3$, 269.0926 $[M+H]^+$, found 269.0927.

Enzymatic hydrolysis of methyl ester 19a to 19b

To a mixture of lipase B from *Candida antarctica* (39.6 mg, 807 U/g) in phosphate buffer (0.100 M, 9.1 mL, pH 7.0), a solution of methyl 3-(2-oxo-2,3-dihydro-1*H*-pyrrolo[2,3-*b*]pyridin-5-yl)benzoate (**19a**) (20.6 mg, 0.077 mmol) in 1,4-dioxane (0.9 mL) was added. The mixture was placed in an orbital shaker at 37 °C and 200 rpm for 5 days, and incomplete conversion was observed by TLC (R_f = 0.13, CH_2Cl_2/MeOH 9:1). The reaction mixture was saturated with NH_4SO_4 and extracted with EtOAc (3×10 mL) before the combined organic layer was washed with $(NH_4)_2SO_4$-solution (sat., 10 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was immobilized on celite and purified by flash chromatography (silica, grad. EtOAc 100%– EtOAc/MeOH 9:1). This gave **19b** in 19% yield (3.8 mg, 0.015 mmol) as a light pink solid; ^{1}H NMR (400 MHz, [D_6]DMSO) δ = 11.11 (s, 1H), 8.38 (d, J = 2.1 Hz, 1H), 8.14 (br t, J = 1.5 Hz, 1H), 7.93–7.88 (m, 3H), 7.59 (t, J = 7.7 Hz, 1H), 3.62 (s, 1H); ^{13}C NMR (100 MHz, [D_6]DMSO) δ = 175.9, 167.2, 158.1, 144.4, 138.0, 131.7, 130.7, 130.4, 129.4, 129.0, 128.1, 127.0, 120.9, 35.4; HRMS (TOF ASAP$^-$) *m/z* calcd. for $C_{14}H_9N_2O_3$: 253.0613 $[M-H]^-$, found 253.0608.

Synthesis of 20

To a mixture of XPhos Pd G2 (27.3 mg, 0.035 mmol), XPhos (17.1 mg, 0.036 mmol) and Cs_2CO_3 (657 mg, 2.016 mmol) under N_2 atmosphere, a solution of (3-((3-phenylpropyl)carbamoyl)phenyl) boronic acid (**8**) (483 mg, 1.705 mmol) and tert-butyl 5-bromo-2-((tert-butoxycarbonyl)oxy)-1H-pyrrolo[2,3-b]pyridine-1-carboxylate

(**17**) (273 mg, 0.661 mmol) in THF (2 mL) and *t*-BuOH (2 mL) was added. After 1 hour and 40 minutes, full conversion was observed by ^{1}H NMR and CH_2Cl_2 (25 mL), water (50 mL) and brine (5 ml) was added. The aqueous phase was extracted with CH_2Cl_2 (2×25 mL) and the combined organic layer was washed with brine (50 mL), dried over Na_2SO_4 and filtered before it was concentrated under reduced pressure. The crude product was purified by reverse phase flash chromatography (C18-silica, R_f = 0.36, gradient H_2O/MeOH 1:4 to 1:9). This gave **20** in 47% yield (179 mg, 0.313 mmol) as a pale-yellow solid; mp 105.8–107.2 °C (decomp.); ^{1}H NMR (600 MHz, [D_6] DMSO) δ = 8.76 (d, *J* = 2.2 Hz, 1H), 8.63 (br t, *J* = 5.6, 5.4 Hz, 1H), 8.33 (d, *J* = 2.1 Hz, 1H), 8.21 (br s, 1H), 7.91 (br d, *J* = 7.8 Hz, 1H), 7.88 (br d, *J* = 7.8 Hz, 1H), 7.59 (t, *J* = 7.7, 7.8 Hz, 1H), 7.30–7.27 (m, 2H), 7.25–7.23 (m, 2H), 7.19–7.16 (m, 1H), 6.56 (s, 1H), 3.34–3.30 (m, 2H), 2.66 (br t, *J* = 7.6, 7.7 Hz, 2H), 1.87 (br quint., *J* = 7.3, 7.4 Hz, 2H), 1.61 (s, 9H), 1.52 (s, 9H); ^{13}C NMR (150 MHz, [D_6]DMSO) δ = 165.9, 149.6, 146.9, 144.1, 142.9 142.3, 141.8, 137.6, 135.4, 131.2, 129.5, 129.2, 128.31 (2C), 128.27 (2C), 126.9, 126.7, 125.7, 125.5, 119.3, 93.8, 85.3, 84.5, 39.0, 32.7, 30.9, 27.6, 27.1; IR (neat, cm^{-1}) ν = 698, 1106, 1141, 1235, 1255, 1368, 1742, 1771, 3061, 3320; HRMS (ES-TOF): *m/z* calcd. for $C_{33}H_{38}N_3O_6$: 572.2761, $[M+H]^+$, found 572.2764.

Synthesis of target structure 3

A solution of *tert*-butyl 2-((*tert*-butoxycarbonyl)oxy)-5-(3-((3-phenylpropyl)carbamoyl)phenyl)-1*H*-pyrrolo[2,3-*b*]pyridine-1-carboxylate (**20**) (100 mg, 0.175 mmol) in 1,4-dioxane (4 mL) was added HCl (37%, 2 mL), and was stirred at ambient temperature. The reaction was monitored by reverse phase TLC (C18-silica, R_f = 0.57, MeCN/H_2O 7:3), and full conversion was observed after 1 hour. A solution of $KHCO_3$ (sat., 20 mL) was added to the reaction which was further stirred for 20 minutes. The mixture was then extracted with CH_2Cl_2 (7×25 mL) before the combined organic layer was washed with brine (25 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was recrystallized from a minimal amount of EtOAc, this gave **3** in 46% yield (30 mg, 0.081 mmol) as a white solid; mp 190.5–196.3 °C (decomp.); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 10.03 (s, 1H), 8.32 (s, 1H), 7.88 (s, 1H), 7.67 (s, 1H), 7.62 (s, 1H), 7.68–7.58 (m, 3H), 7.45 (t, *J* = 7.7 Hz, 1H), 7.30–7.15 (m, 5H), 6.54 (m, 1H), 3.58 (s, 2H), 3.56–3.51 (m, 2H), 2.76–2.72 (m, 2H), 2.04–1.97 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 175.6, 167.3, 157.2, 144.9, 141.6, 138.2, 135.7, 131.4, 131.1, 129.6, 129.4, 128.7 (2C), 128.5 (2C), 126.2, 126.1, 125.6, 120.4, 40.2, 35.7, 33.7, 31.2; IR (neat, cm^{-1}) ν = 569, 696, 745, 761, 809, 1228, 1600, 1628, 1650, 1705, 3061, 3312, 3439; HRMS (ASAP-TOF) *m/z* calcd. for $C_{23}H_{22}N_3O_2$: 372.1712, $[M+H]^+$, found 372.1712.

5-Bromo-1-(phenylsulfonyl)-1*H*-pyrrolo[2,3-*b*]pyridine (21)[17]

5-Bromo-1*H*-pyrrolo[2,3-*b*]pyridine (2.13 g, 10.8 mmol) was dissolved in DMF (20 mL) and cooled to 0 °C, before NaH (283 mg, 10.8 mmol) was added in two portions over 5 minutes. After addition, the mixture was heated to ambient temperature, and $PhSO_2Cl$ (1.65 mL, 12.93 mmol) was added dropwise over 5 minutes. After 1.5 hours full conversion was observed by TLC (R_f = 0.48, EtOAc/*n*-pentane 1:3), and water (60 mL) was added. After stirring for an additional 20 minutes, the solids were filtered and washed with water (2×15 mL), before they were air-dried for 30 minutes. This gave **21** in 92% yield (3.35 g, 9.93 mmol) as a white solid; mp 148.6–150.3 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ = 8.43 (d, *J* = 2.1 Hz 1H), 8.16 (d, *J* = 7.6 Hz, 2H), 7.95 (d, *J* = 2.1 Hz, 1H), 7.73 (d, *J* = 4.0 Hz, 1H), 7.59–7.67 (m, 1H), 7.50–7.47 (m, 2H), 6.53 (d, *J* = 4.0 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ = 145.59, 145.55, 138.1, 134.4, 131.9 (2C), 129.2 (2C), 128.1, 128.0, 124.5, 115.4, 104.8; IR (neat, cm^{-1}) ν = 590, 728, 1155, 1173, 1190, 1271, 1376, 1443; HRMS (TOF ASAP+) *m/z* calcd. for $C_{13}H_{10}N_2O_2S^{79}Br$, 336.9646 $[M+H]^+$, found 336.9650.

3-(1-(Phenylsulfonyl)-1*H*-pyrrolo[2,3-*b*]pyridin-5-yl)benzoic acid (22)

5-Bromo-1-(phenylsulfonyl)-1H-pyrrolo[2,3-*b*]pyridine (**21**) (3.01 g, 8.93 mmol), 3-boronobenzoic acid (2.21 g, 13.5 mmol), Pd(dppf)Cl_2 (328 mg, 0,448 mmol) and K_2CO_3 (3.71 g, 26.9 mmol) were mixed with 1,4-dioxane (30.0 mL) and water (10.0 mL) under a N_2 atmosphere. The mixture was stirred at 80 °C for 2 hours, and full conversion was observed by TLC (R_f = 0.95, MeOH/CH_2Cl_2 5:95). HCl (4 M) was added to the mixture until pH = 4, before it was saturated with NaCl and extracted with EtOAc (6×20 mL). The combined organic layers were washed with brine (20 mL), dried over Na_2SO_4 and filtered before the solvents were removed under reduced pressure. The solids were re-dissolved in a minimal amount of MeOH, and the product was precipitated by slow addition of water. This gave **22** in 50% yield (1.68 g, 4.44 mmol) as an off-white solid; mp 185.4–189.4 °C (decomp.) ; ^{1}H NMR (400 MHz, [D_6]DMSO) δ = 8.69 (d, *J* = 2.1, 1H), 8.37 (d, *J* = 2.1 Hz, 1H), 8.20–8.19 (m, 1H), 8.15–8.13 (m, 2H), 7.98–7.96 (m, 3H), 7.75–7.71 (m, 1H), 7.66–7.60 (m, 3H), 6.89 (d, *J* = 3.9 Hz, 1H), the CO_2H proton was not seen; ^{13}C NMR (100 MHz, [D_6]DMSO) δ = 167.1, 146.3, 143.4, 137.8, 138.5, 134.8, 131.68, 131.60, 131.1, 129.7 (2C), 129.5 (2C), 128.56, 128.53, 127.8, 127.5, 122.7, 106.4; IR (neat, cm^{-1}) ν = 580, 596, 725, 1150, 1169, 1188, 1264, 1376, 1692, 3405; HRMS (TOF ASAP+) *m/z* calcd. for $C_{20}H_{15}N_2O_4S$, 379.0753 $[M+H]^+$, found 379.0755.

3-(1*H*-Pyrrolo[2,3-*b*]pyridin-5-yl)benzoic acid (23)

To a stirring mixture of 3-(1-(phenylsulfonyl)-1H-pyrrolo[2,3-b] pyridin-5-yl)benzoic acid (**22**) (821 mg, 2.17 mmol) in MeOH (9 mL) and THF (9 mL), Cs_2CO_3 (2.12 g, 6.51 mmol) was added. The reaction was left at ambient temperature overnight, before full conversion was observed by TLC (R_f = 0.17, MeOH/EtOAc 1:9). The mixture was added EtOAc (10 mL) under stirring before it was added HCl (0.1 M) until pH = 4 and was extracted with a mixture of MeOH and EtOAc (1:9, 3×50 mL). The combined organic layer was washed with $(NH_4)_2SO_4$ (30%, 30 mL), dried over Na_2SO_4 and filtered before the solvents were evaporated under reduced pressure. The crude product was stirred in *i*-PrOH (10 mL) for 15 minutes and filtered. This gave **23** in 53% yield (273 mg, 1.15 mmol) as a light brown solid; mp 256.2–257.5 °C (decomp.); ^{1}H NMR (400 MHz, [D_6]DMSO) δ = 11.75 (s, 1H), 8.53 (d, *J* = 2.1 Hz, 1H), 8.26 (d, *J* = 2.0 Hz, 1H), 8.22–8.21 (m, 1H), 7.98–7.96 (m, 1H), 7.94–7.92 (m, 1H), 7.61 (t, *J* = 7.8 Hz, 1H), 7.54–7.52 (br t, *J* = 3.1, 2.7 Hz, 1 H), 6.53–6.52 (dd, *J* = 1.8, 1.6 Hz, 1H), the CO_2H proton was not seen; ^{13}C NMR (100 MHz, [D_6]DMSO) δ = 167.4, 148.2, 141.4, 139.5, 131.7, 131.3, 129.4, 127.7, 127.5, 127.3, 127.2, 126.3, 119.8, 100.3; IR (neat, cm^{-1}) ν: 534, 726, 754, 1263, 1281, 1304, 1670, 3318; HRMS (TOF ASAP-) *m/z* calcd. $C_{14}H_9N_2O_2$, 237.0664, $[M-H]^-$, found 237.0660.

3-(3,3-Dibromo-2-oxo-2,3-dihydro-1*H*-pyrrolo[2,3-*b*] pyridin-5-yl)benzoic acid (24)

To a stirring solution of 3-(1*H*-pyrrolo[2,3-*b*]pyridin-5-yl)benzoic acid (**23**) (214 mg, 0.898 mmol) in *t*-BuOH (3.2 mL) and water (3.2 mL), Br_2 (115 μL, 2.25 mmol) was added. The reaction was placed in the dark and let stir for 1 hour, after which full conversion was observed by ^{1}H NMR. The reaction mixture was added water (15 mL) and MeOH (3 mL), before it was extracted with EtOAc (4×20 mL). The combined organic layer was washed with brine (40 mL), dried over Na_2SO_4, filtered, and the solvents were evaporated under reduced pressure. This gave crude **24** in 44% yield (169 mg, 0.396 mmol) as

a red solid; analysis of the crude material ^{1}H NMR (400 MHz, [D_6] DMSO) δ = 13.15 (br s, 1H). 12.14 (s, 1H), 8.58 (d, J = 2.2 Hz, 1H), 8.40 (d, J = 2.2 Hz, 1H), 8.21 (br t, J = 1.6, 1.5 Hz, 1H), 7.99 (m, 2H), 7.63 (t, J = 7.8 Hz, 1H); ^{13}C NMR (100 MHz, [D_6]DMSO) δ = 170.2, 167.1, 151.9, 148.7, 136.7, 132.0, 131.7, 131.3, 131.2, 129.5, 128.8, 127.4, 126.1, 44.3; IR (neat, cm^{-1}) ν = 614, 735, 760, 1277, 1290, 1602, 1666, 1750, 2853, 2923, 3017, 3078 ;HRMS (TOF ASAP+) *m/z* calcd. $C_{14}H_9N_2O_3{}^{79}Br_2$, 410.8980 $[M+H]^+$, found 410.8976.

3-(2-Oxo-2,3-dihydro-1*H*-pyrrolo[2,3-*b*]pyridin-5-yl)benzoic acid (19b)

To a mixture of 3-(3,3-dibromo-2-oxo-2,3-dihydro-1*H*-pyrrolo[2,3-*b*] pyridin-5-yl)benzoic acid (**24**) (133 mg, 0.323 mmol) in AcOH (3 mL), zinc powder (213 mg, 3.258 mmol) was added. The mixture was stirred overnight, and full conversion was observed by ^{1}H NMR before water (125 mL) was added. The mixture was extracted with EtOAc (3×50 mL) before additional water (25 mL) and EtOAc (25 mL) was added. The combined organic layer was washed with brine (2×25 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. This gave **19b** in 93% yield (76 mg, 0.299 mmol) as a light yellow solid; ; mp 147.9–195.7 °C (decomp.); ^{1}H NMR (400 MHz, [D_6]DMSO) δ = 11.11 (s, 1H), 8.38 (d, J = 2.1 Hz, 1H), 8.14 (br t, J = 1.5 Hz, 1H), 7.93–7.88 (m, 3H), 7.59 (t, J = 7.7 Hz, 1H), 3.62 (s, 2H), the CO_2H proton is not seen; ^{13}C NMR (100 MHz, [D_6]DMSO) δ = 175.9, 167.2, 158.1, 144.4, 138.0, 131.7, 130.7, 130.4, 129.4, 129.0, 128.1, 127.0, 120.9, 35.4; IR (neat, cm^{-1}) ν = 567, 730, 753, 1211, 1242, 1279, 1463, 1684, 1723, 3157; HRMS (TOF ASAP+) *m/z* calcd. $C_{14}H_9N_2O_3$, 253.0613, $[M+H]^+$, found 253.0608.

N-(3-Phenylpropyl)-3-(1-(phenylsulfonyl)-1*H*-pyrrolo[2,3-*b*] pyridin-5-yl)benzamide (25)

To a solution of 3-(1-(phenylsulfonyl)-1H-pyrrolo[2,3-b]pyridin-5-yl) benzoic acid (**22**) (118 mg, 0.312 mmol) and HATU (147 mg, 0.387 mmol) in dry CH_2Cl_2, DIPEA (0.070 mL, 0.402 mmol) was added. The solution was stirred at ambient temperature for 20 minutes before 3-phenylpropan-1-amine (0.060 mL, 0.422 mmol) was added. The solution was left overnight, and full conversion was observed by TLC (R_f = 0.26, EtOAc/toluene 1:4). Phosphate buffer (0.100 M, 20 mL, pH 5.8) was added and the mixture was extracted with CH_2Cl_2 (3×10 mL), before the combined organic layer was washed with brine (20 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was dissolved in a minimal amount of DMSO and was precipitated by dropwise addition of water. This gave **25** in 58% yield (89 mg, 0.180 mmol) as an off-white solid; mp 65–75 °C (decomp.); ^{1}H NMR (400 MHz, [D_6]DMSO) δ = 8.73 (d, J = 2.1 Hz, 1H), 8.58 (t, J = 5.4 Hz, 1H), 8.38 (d, J = 2.1 Hz, 1H), 8.147–8.145 (m, 2H), 8.132–8.131 (m, 1H), 7.98 (d, J = 3.9 Hz, 1H), 7.86 (d, J = 1.6 Hz, 1H), 7.85 (d, J = 1.6, 1H), 7.73 (bt, J = 7.4, 1H), 7.65–7.63 (m, 2H), 7.57 (t, J = 7.4, 1H), 7.29–7.27 (m, 2H), 7.24–7.23 (m, 2H), 7.18–7.16 (m, 1H), 6.90 (d, J = 4.0 Hz, 1H), 3.33–3.30 (m, 2H), 2.65 (t, J = 7.7 Hz, 2H), 1.85 (quint., J = 7.6, 7.3 Hz, 2H); ^{13}C NMR (100 MHz, [D_6]DMSO) δ = 165.8, 146.2, 143.5, 141.7, 137.5, 137.4, 135.4, 134.8, 131.4, 129.6 (2C), 129.1 (2C), 128.4, 128.34, 128.32 (2C), 128.2 (2C), 127.8, 127.5, 126.7, 125.7, 125.6, 122.6, 106.3, 39.0*, 32.6, 30.8; IR (neat, cm^{-1}) ν = 580, 596, 751, 1153, 1188, 1375, 1603, 1639, 1721, 3314; HRMS (TOF ASAP+) *m/z* calcd. $C_{29}H_{26}N_3O_3S$, 496.1695, $[M+H]^+$, found 496.1692.

*Overlaps with DMSO. Detected by HSQC and HMBC.

N-(3-Phenylpropyl)-3-(1*H*-pyrrolo[2,3-*b*]pyridin-5-yl) benzamide (26)

To a stirring solution of *N*-(3-phenylpropyl)-3-(1-(phenylsulfonyl)-1*H*-pyrrolo[2,3-*b*]pyridin-5-yl)benzamide (**25**) (0.53 g, 1.069 mmol) in MeOH (10.0 mL) and THF (10.0 mL), Cs_2CO_3 (1.02 g, 3.13 mmol) was added. The mixture was left overnight, and full conversion was observed by TLC (R_f = 0.49 MeOH/EtOAc 1:9). The solvents were removed under reduced pressure before the solids were dissolved in CH_2Cl_2 (20 mL) and water (40 mL) was added. The mixture was extracted with CH_2Cl_2 (3×20 mL) before the combined organic layer was washed with $KHCO_3$ (sat., 20 mL) and brine (20 mL), and dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified through hot filtration with CH_2Cl_2 (3× 10 mL) which gave **26** as a white solid in 55% yield (207 mg, 0.582 mmol); mp 199.8–204.1 °C (decomp.); ^{1}H NMR (400 MHz, [D_6] DMSO) δ = 11.77 (s, 1H), 8.64–8.60 (m, 2H), 8.29 (d, J = 2.0 Hz, 1H), 8.19 (br s, 1H), 7.89–7.83 (m, 2H), 7.59–7.53 (m, 2H), 7.31–7.23 (m, 4H), 7.20–7.16 (m, 1H), 6.53 (dd, J = 3.2, 1.8 Hz, 1H), 3.34–3.31 (m, 2H (overlaps with H_2O signal)), 2.68–2.64 (m, 2H), 1.91–1.84 (m, 2H); ^{13}C NMR (100 MHz, [D_6]DMSO) δ = 166.1, 148.2, 141.8, 141.6, 139.1, 135.3 129.4, 129.0, 128.33 (2C), 128.29 (2C), 127.6, 127.1, 126.3, 125.9, 125.7, 125.4, 119.7, 100.2, 39.0, 32.7, 30.9; IR (neat, cm^{-1}) ν: 698, 754, 893, 1265, 1579, 1630, 3321; HRMS (TOF ASAP+) *m/z* calcd. $C_{23}H_{22}N_3O$, 356.1763 $[M+H]^+$, found 356.1767.

E. coli TMPK assay

Inhibition of *Ec*TMPK was determined by Profoldin using the *E. coli* Thymidylate Kinase Assay Kit Plus (ProFoldin Catalog No. TMK100KE), where dTMP was phosphorylated by ATP and TMPK and the formation of ADP was measured by adding a fluorescing dye. Single-point inhibitions were measured at 8.3 μM inhibitor concentrations, and an ADP control assay was performed to correct for possible ADP inhibition of the compounds. The IC_{50}-values were determined from a similar assay, where 2-fold dilution series from 10 mM to 0.02 mM were used.

MIC measurements

The MIC of compound **1**–**3** towards *Escherichia coli* (MG1655) and *Staphylococcus aureus* (ATCC29213) was determined following the standards recommended by the Clinical and Laboratory Standards Institute (CLSI) for the broth microdilution method.[18] Briefly, the bacterial suspension were adjusted to 0.5 McFarland standard (~1× 10^8 colony forming units (CFU)/mL) and diluted 1:200 in Cation-Adjusted Mueller-Hinton Broth (CAMHB, 22.5 mg/mL Ca^{2+}, 11 mg/mL Mg^{2+}). The suspension was subsequently added to polypropylene microtiter plates (100 μL/well, ~5×104 CFU/well) already prepared with different concentrations of the thymidylate kinase inhibitors (11 μL/well, twofold serial dilutions). The plates were incubated at 37 °C overnight for 20–24 h before inspection for visible growth and determination of MIC values.

Kinase panel

The compounds were supplied in a 10 mM DMSO solution, and enzymatic kinase inhibition potency was determined by Termo-Fisher (Invitrogen) using their Z′-LYTE® assay technology,[19] at 500 nM in duplicates. ATP concentration used was in most cases equal to K_m. Otherwise the ATP concentration was 100 μM.

Molecular modelling

The X-ray crystal structures of the protein (PDB: 5TMP and 4TMK) were prepared using the protein preparation wizard, which is part of the Maestro software package (Maestro, v11.6.013, release 2018–2; Schrödinger, LLC, New York, NY, USA). Bond orders and formal charges were added for het-groups, and hydrogens were added to all atoms in the system. Water molecules beyond 5 Å from het-groups were removed. To alleviate steric clashes that may exist in the original PDB structures, an all-atom constrained minimization was carried out with the Impact Refinement module (Impref) (Impact, v5.0; Schrödinger, LLC) using the OPLS3 force field. The minimization was terminated when the energy converged or the RMSD reached a maximum cutoff of 0.30 Å. The resulting protein structures were used in the following docking study. Ligands were drawn using ChemBioDraw (ChemBioDraw Ultra 13.0, CambridgeSoft, PerkinElmer) and were prepared using LigPrep2.2 (LigPrep, v2.2; Schrödinger, LLC). For the computational investigation of the receptor-inhibitor structures, the energy minimized structures of 5TMP and ligands were subsequently docked using GLIDE in XP mode and Maestro Schrödinger.[20] The resulting docked poses were analysed using Glide pose viewer tool. For dynamic simulation, the best poses from docking were used as starting points when building the model systems. Dynamic simulations were conducted for 10 ns simulation time using Maestros Desmond suite, the OPLS3e force field and a TIP4P solvent model. Briefly, this was performed by putting the docked protein- ligand complex inside a minimized solvent box and adding ions (Na^+ or Cl^-) in order to have an electrical neutral system. Finally, NaCl was added to a total concentration of 0.15 M, which is approximately the physiological concentration of monovalent ions. This gave normally a system of approximately 39 000 atoms. Molecular dynamics were then calculated on these systems using the isothermal-isobaric (NPT) ensemble at 300 K and 1.01325 bar. Trajectory analysis were performed using Desmond's Simulation Interactions Diagram tool. All the graphical pictures were made using Maestro.

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Conflict of Interest

The authors declare no conflict of interest.

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