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Discovery of thienopyrimidine-based inhibitors of the human farnesyl pyrophosphate synthase—Parallel synthesis of analogs via a trimethylsilyl ylidene intermediate

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ABSTRACT

Thienopyrimidine-based bisphosphonates were identified as a new class of nitrogen-containing bisphosphonate (N-BP) inhibitors of the human farnesyl pyrophosphate synthase (hFPPS). Analogs were prepared via cyclization of 2-(1-(trimethylsilyl)ethylidene)malononitrile to 2-amino-4-(trimethylsilyl) thiophene-3-carbonitrile in the presence of elemental sulfur. Direct *ipso*-iododesilylation of this intermediate led to selective iodination at C_β of the sulfur atom in high efficiency. The synthetic protocols developed were used in the parallel synthesis of structurally diverse thieno[2,3-*d*]pyrimidin-4-amine-based bisphosphonate inhibitors of hFPPS.

1. Introduction

The thienopyrimidine core is an integral part of numerous biologically active compounds, as evidenced by the plethora of literature and industrial patents disclosing the use of this heterocycle in agrochemicals[1–3] and human therapeutics (e.g., antifungal[4] and antiviral[5] agents). Thienopyrimidines are often used as bioisosteres of purine nucleobases (e.g., adenine),[5] and have been employed extensively in the design of various kinase inhibitors, including Aurora kinase,[6] tyrosine kinases,[7,8] cyclin-dependent kinase,[9] c-Jun N-terminal kinases,[10–12] PERK,[13] phosphoinositide 3-kinase α (PI3Kα),[14,15] and EGFR/ErbB-2.[16] Many of these inhibitors are currently under pre-clinical or clinical investigations for the treatment of cancer, inflammatory, autoimmune and neurodegenerative diseases. Thienopyrimidine-based compounds have also been reported as inhibitors of γ-secretase[17] and antagonists of the adenosine A_{2A} receptor,[18–20] and implicated in the potential treatment or prevention of Alzheimer's disease.

In our own investigations, we focus on the identification of structurally diverse molecules that selectively inhibit mammalian prenyl synthase enzymes. Inspired by the privileged biopharmaceutical properties of the thienopyrimidine scaffold (**1**; Fig. 1), we decided to explore thienopyrimidine-based bisphosphonates (**2**; Fig. 1), as potential inhibitors of the human farnesyl pyrophosphate synthase (hFPPS) and/or geranylgeranyl pyrophosphate synthase (hGGPPS); these prenyl synthase enzymes are functionally closely related.

The development of synthetic methodologies amenable to parallel synthesis of structurally diverse libraries of thienopyrimi-

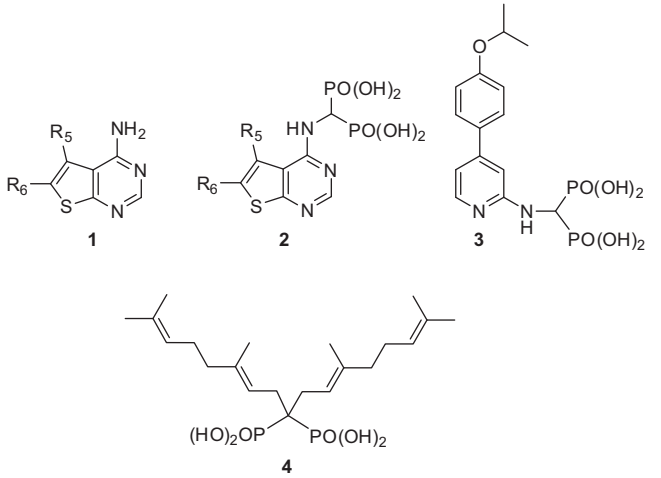

Figure 1. Structures of thieno[2,3-*d*]pyrimid-4-amines (general structure **1**), thienopyrimidine-based bisphosphonate inhibitors of hFPPS/hGGPPS (general structure **2**), hFPPS inhibitor **3** and hGGPPS inhibitor **4**.

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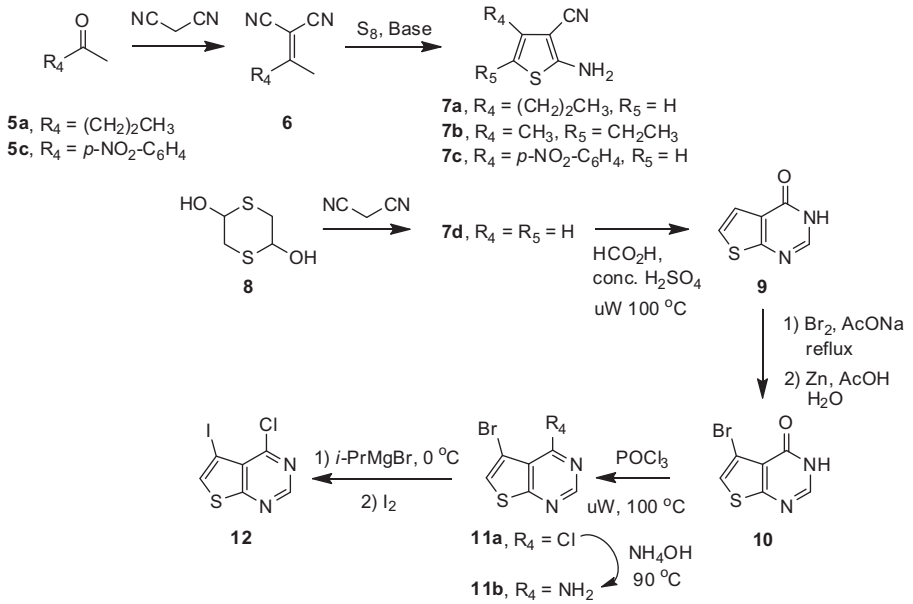


Scheme 1. Gewald synthesis of 2-aminothiophene-3-carbonitriles (**7**) and halo-substituted thienopyrimidines.

dine bisphosphonates (**2**) were required to support our medicinal chemistry efforts. We anticipated that the final compounds could be made from highly substituted thieno[2,3-*d*]pyrimidin-4-amine intermediates (**1**), after coupling of the exocyclic amine (at C-4) with the bisphosphonate moiety; the latter step could be easily achieved using previously developed procedures for the preparation of 2-aminopyridine bisphosphonates (e.g., hFPPS inhibitor **3**; Fig. 1).[21,22]

Traditionally, thieno[2,3-*d*]pyrimid-4-amines (**1**) have been synthesized via a multistep process from 2-aminothiophenes (**7**; Scheme 1). However, preparation of highly substituted thienopyrimidine libraries is usually hampered by limitations in selectively functionalizing the C_α and/or C_β positions of the thiophene core (**7**). Similarly, direct and selective substitution at those positions in the corresponding thieno[2,3-*d*]pyrimidine is very challenging (i.e., the C-5 and C-6 of **1**).

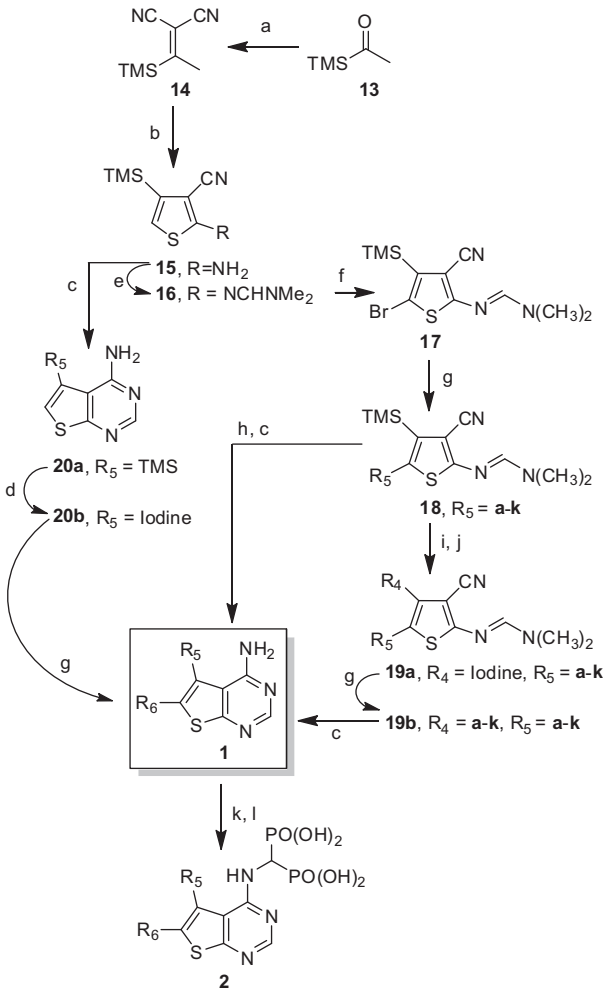
The 2-aminothiophene core (**7**) can be easily prepared by the classical Gewald method (Scheme 1).[23] One-step and two-step procedures, under either thermal or microwave conditions, have been reported with various optimizations for the condensation of the Knoevenagel ylidene **6** with elemental sulfur to give the thiophene **7**. The drawbacks to this elegant protocol can be regioselectivity in the cyclization step, when non-symmetrical ketones (**5**) are used with an alkyl or a benzylic substituent, and the chemical stability of ylidene **6**. For example, the one-pot condensation of pentane-2-one (**5a**) with malononitrile, sulfur and catalytic amounts of imidazole, was recently reported to produce a mixture of thiophenes **7a** and **7b** in 1:3.4 ratio and in modest yield (Scheme 1).[24] Furthermore, acetophenones bearing electron withdrawing substituents on the phenyl ring (e.g., **5c**) often produce very low yields of the desired 2-aminothiophene (**7c**) under standard Gewald reaction conditions.[25] The low yields have been attributed, in part, to decomposition and dimerization of ylidene **6**, under high temperatures and basic conditions.[25]

Alternatively, the unsubstituted 2-amino-thiophene-3-carbonitrile core (**7d**) has also been prepared from 2,5-dihydroxy-1,4-dithiane (**8**) and subsequently elaborated to substituted thieno[2,3-*d*]pyrimidines.[26,27] Chlorination or bromination at C_α of the thiophene sulfur can be easily achieved with NCS or NBS, respectively. However, direct and regiospecific halogenation at the β-carbon, or the equivalent carbon on any thiophene-containing bicyclic heterocycle, is unknown. Recent reports on the preparation of 3-bromothiophenes usually describe the generation of the α-lithium-β-bromothiophene from the 2-bromothiophene using the (so-called) base-catalyzed halogen dance (BCHD) reaction.[28–32] This approach has also been used in the synthesis of 4-chloro-5-iodothieno[2,3-*d*]pyrimidine (**12**) from the LDA-mediated BCHD rearrangement of the 6-bromo-4-chlorothieno [2,3-*d*]pyrimidine first to the 5-bromo analog **11a**, followed by treatment with a Grignard reagent and quenching of the anion with iodine (Scheme 1).[1,3] Several alternative approaches have been reported, most commonly involving 2,3-dibromination of **9**, followed by reductive dehalogenation at the more reactive C_α position of the thiophene with Zn in acetic acid to give intermediate **10** (Scheme 1).[10,11] Two additional synthetic steps are required to convert **10**–**11b**, which can then be used to prepare C-5 monosubstituted thienopyrimidines. Herein, we report an alternative protocol that allows selective disubstitution at either C_α or C_β of the thiophene core and is amenable to modular parallel synthesis of structurally diverse libraries of thieno[2,3-*d*]pyrimidin-4-amines (**1**). We also provide preliminary biological data which indicates that the thienopyrimidine-based bisphosphonates described herein are inhibitors of the human FPPS and new leads for drug discovery. Up-regulation of hFPPS has been linked to numerous human disorders,[33,34] including cancer[35] and neurodegenerative diseases, thus inhibitors of this enzyme have the potential of becoming valuable human therapeutics.

2. Results

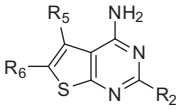
2.1. Chemistry

As part of our continued interest in developing efficient, robust methodologies that are amenable to parallel synthesis of structurally diverse libraries of biologically active compounds,[21,22] we modified the classical Knoevenagel/Gewald approach by incorporating the trimethylsilyl ylidine **14** as the precursor to the 2-amino-thiophene-3-carbonitrile scaffold (**15**; Scheme 2). The ylidine 2-(1-(trimethylsilyl)ethylidene)malononitrile (**14**) was prepared in nearly quantitative yield via condensation of acetyltrimethylsilane (**13**) with malononitrile under acidic conditions (Scheme 2). Ylidine **14** was found to be stable at −20 °C for several months



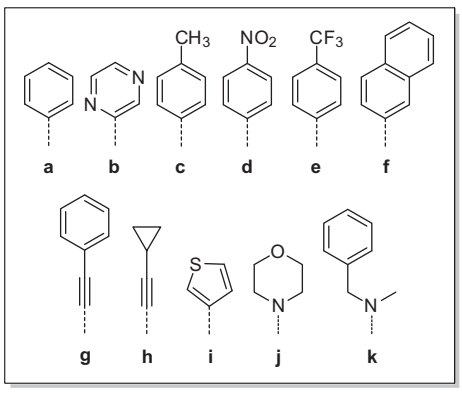
Scheme 2. Modular synthesis of highly substituted thieno[2,3-*d*]pyrimidin-4-amines; the stuctures of substituent **a**–**k** are shown in Table 1. Reagents and conditions: (a) $CH_2(CN)_2$, NH_4OAc, AcOH, C_6H_6, Dean–Stark trap, 95 °C, 24 h (90%); (b) S_8, Et_2NH, pyridine, rt, 18 h (85%); (c) $HCONH_2$, 130 °C, 48 h (75–85%); (d) ICl, DCM, −10 °C (>98%); (e) $(CH_3O)_2CHN(CH_3)_2$, DMF, rt, 4 h (90%); (f) NBS, DMF, rt, 13 h in the dark (80%); (g) various cross-coupling reactions under standard Suzuki, Buchwald–Hartwig, Sonogashira and Stille conditions, isolated yields varied from 50% to 95%, reactions were not individually optimized (for the conversion of **20b** or **18** to **1**, R_6 = H or R_5 = H, respectively); (h) TBAF, THF, 0 °C to rt, 3 h (>95%); (i) CF_3CO_2Ag, THF, −78 °C, 15 min; (j) I_2, THF, −78 °C, 3 h (>98%); (k) $CHOEt_3$, diethylphosphite, 130 °C, 24 h (50–75%); (l) TMSBr, MeOH, rt, 72 h (>85%).

Table 1
Mini-library of thieno[2,3-*d*]pyrimidin-4-amines (**1**) synthesized



1a, R_2 = **a**, R_5 = R_6 = H
1b, R_2 = R_6 = H, R_5 = **a**
1c, R_2 = R_5 = H, R_6 = **Br**
1d, R_2 = R_5 = H, R_6 = **c**
1e, R_2 = R_5 = H, R_6 = **e**
1f, R_2 = R_5 = H, R_6 = **f**
1g, R_2 = R_5 = H, R_6 = **i**
1h, R_2 = R_6 = H, R_5 = **d**
1i, R_2 = R_6 = H, R_5 = **e**
1j, R_2 = R_6 = H, R_5 = **f**
1k, R_2 = H, R_5 = **g**, R_6 = **a**
1l, R_2 = H, R_5 = **h**, R_6 = **a**
1m, R_2 = H, R_5 = **j**, R_6 = **a**
1n, R_2 = H, R_5 = **k**, R_6 = **a**
1o, R_2 = H, R_5 = **b**, R_6 = **a**

R_2, R_5 and/or R_6 = H or a fragment from **a** to **k**



and at RT for several weeks without any evidence of decomposition. Condensation of **14** with elemental sulfur in pyridine provided the 2-amino-4-(trimethylsilyl)-thiophene-3-carbonitrile (**15**) as a dark orange/brown solid in >85% yield.

In order to explore direct cross-coupling reactions selectively at the C_β of the sulfur in the thiophene core (i.e., where intermediate **1** is mono-substituted at C-5), we initially focused on preparing the iodothiophene core, as a precursor to the 5-iodothienopyrimidine **20b** (Scheme 2). Efforts to iodinate intermediate **15** at C_β, using silver trifluoroacetate and elemental iodine,[36,37] lead to decomposition of the starting material at temperatures ranging from 0 to −78 °C. Previous examples of *ipso*-iododesilylation of thiophenes under such conditions were reported to give poor yields when the TMS was adjacent to substituents with sp character.[37] Cyclization of **15** with formamide produced the 5-(trimethylsilyl)thieno[2,3-*d*]pyrimidin-4-amine (**20a**) in 75–80% yield (Scheme 2; step c). Mindful of the facile decomposition of thiophenes upon exposure to light, this reaction was usually carried out in the dark. Subsequently, we reattempted to perform an *ipso*-iododesilylation reaction with silver trifluoroacetate and elemental iodine; however, this reaction resulted in mostly unreacted starting material. Alternatively, excellent yields (>98%) of **20b** were obtained when **20a** was treated with ICl in DCM at −10 °C and the reaction was quenched at low temperatures (below 0 °C) with H_2O.[38,39] We noted that if the reaction mixture was allowed to warm-up above 0 °C, the isolated yield was significantly lower and mixtures of di-halogenated side products were observed by LC–MS. The iodo product **20b** was found to be a useful building block for various cross-coupling reactions in the synthesis of C-5 mono-substituted thieno[2,3-*d*]pyrimidin-4-amines (e.g., **1b**, **1h**, **1i**, **1j**, Table 1). It is noteworthy that fragment **20b** was obtained from **13** in four steps, with an average of 50–60% overall isolated yield, using our protocol (Scheme 2). This methodology is significantly more efficient in preparing analogs of **1** that are selectively mono-substituted at C-5 as compared to those reported previously (e.g., examples shown in Scheme 1).

In the course of these studies, we noted that thiophene **15** was somewhat chemically unstable. Interestingly, protection of the 2-amino moiety with *N,N*-dimethylformamide-dimethylacetal, provided intermediate **16**, which is chemically stable and easy to prepare in high yield and purity (Scheme 2). Bromination of the protected thiophene **16** was also carried-out in >80% yield, providing intermediate **17** (Scheme 2). Compound **17** is a suitable building block for a variety of cross-coupling reactions. We explored selective cross-coupling with various boronic acids or boronate esters to obtain intermediates of general structure **18**, which upon *ipso*-iododesilylation at C-4 (i.e., leading to **19a**), followed by Suzuki, Buchwald–Hartwig, Sonogashira, Stille or other cross-coupling reactions, gave the disubstituted thiophenes **19b**.

Finally, cyclization of **19b** provided a library of novel and structurally diverse C-5/C-6 di-substituted thieno[2,3-*d*]pyrimi-

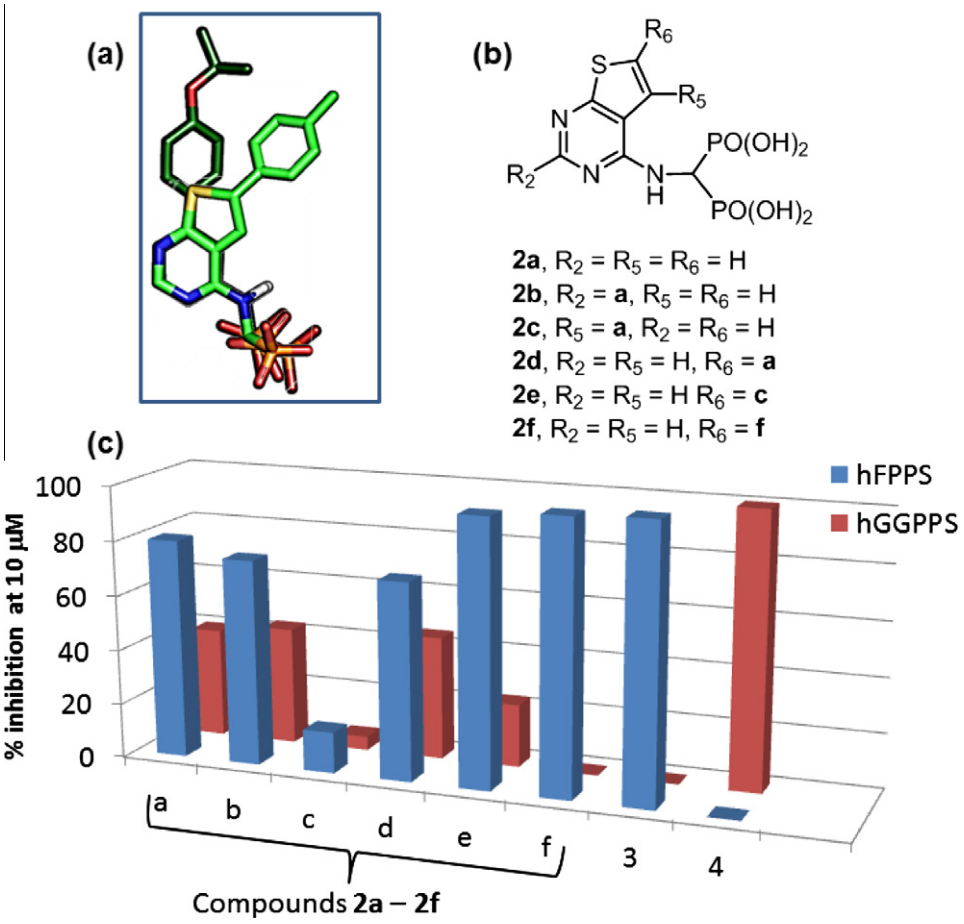


Figure 2. Inhibition data for human FPPS and GGPPS of select thienopyrimide-based bisphosphonates **2a**–**2f** (R_2, R_5 and R_6 substituents are selected from those shown in Table 1). (a) Superposition of analog **2e** with the bioactive conformation of inhibitor **3** (PDB: 4DEM). The carbon skeleton of **2e** and **3** are highlighted in light and dark green colour, respectively; nitrogen, oxygen, sulphur and phosphorus atoms are indicated in blue, red, yellow and orange colour, respectively. (b) Structures of key thienopyrimide-based bisphosphonate compounds. (c) Inhibition data for hFPPS and hGGPPS at 10 μM of inhibitor; the hFPPS and hGGPPS inhibitors **3** and **4**, respectively (Fig. 1) were used as +ve/−ve controls (% inhibition at 10 μM concentration of inhibitor; average values of three determinations; standard deviation ±10%).

din-4-amines (**1k–o**, Table 1) in good overall yield. For example, preparation of analogs **1l** from **15** was achieved in seven steps (including cross coupling under typical Suzuki and Sonogashira conditions at C-5 and C-4, respectively) in an overall isolated yield of 50%. Similarly, analog **1m** (involving Buchwald–Hartwig amination in the conversion of **19a**–**19b**) was prepared from **15** in 30% isolated overall yield. It should be noted that these reactions were not individually optimized during preparation of our library. Analog **1a** (Table 1) was prepared by a different synthetic scheme, involving direct condensation of 2-aminothiophene-3-carbonitrile with benzonitrile under basic condition and at high temperature, as previously reported.[19]

To gain some insight as to the potential for developing favourable structure–activity-relationship (SAR) for thienopyrimidine-based inhibitors of hFPPS and/or hGGPPS, a small cluster of analogs from this library were converted to the corresponding bisphosphonates **2** (Scheme 2). Treatment of fragments **1** with triethyl orthoformate and diethylphosphite, followed by hydrolysis of the tetraethyl bisphosphonate esters with TMSBr/MeOH resulted in the formation of bisphosphonic acids **2** (Scheme 2); details of this two-step protocol were previously described in the synthesis of the hFPPS inhibitor **3**.[21]

2.2. Biological results and discussion

Initially, the bisphosphonic acid analogs **2a–f** (Fig. 2b) were evaluated in our routine hFPPS and hGGPPS inhibition assays at a single concentration of 10 μM (as previously reported),[21] along with inhibitors **3** (IC_{50} = 28 nM in hFPPS; IC_{50} >100 μM in hGGPPS) and **4** (IC_{50} = 410 nM in our own hGGPPS assay;[21] IC_{50} >10 μM in hFPPS[40]) as the positive controls. The inhibition data observed are summarized in Figure 2c (% inhibition of hFPPS and hGGPPS at 10 μM of compound; average values of three determinations; standard deviation of approximately 10%). Significant inhibition of hFPPS was observed with the parent molecule **2a**, as well as the C-2 and C-6 phenyl analogs **2b** and **2d**, respectively (75–80% inhibition at 10 μM). However, analog **2c** exhibited <20% inhibition at 10 μM (Fig. 2b and c). These preliminary data suggest that C-2 and C-6 derivatives of the thienopyrimidine core may be beneficial in developing inhibitors of hFPPS, whereas favourable substitution at C-5 may be limited. In order to gain further insight as to the volume of space within the hFPPS binding pocket occupied by the substituents at C-6, the tolyl and naphthyl moieties (**2e** and **2f**, respectively) were also investigated. These modifications resulted in modest improvement in inhibiting hFPPS (Fig. 2c). The full dose response for hFPPS inhibition was subsequently determined for analogs **2e** and **2f** and their IC_{50} values were calculated to be 390 and 250 nM, respectively. Interestingly, this modest SAR optimization (i.e., compounds **2e** and **2f**) also improved the selectivity in inhibiting hFPPS as compared to hGGPPS (Fig. 2c).

Superposition of the hFPPS-bound conformation of inhibitor **3** (IC_{50} = 28 nM; PDB: 4DEM),[21] with the thienopyrimidine analog **2e** strongly suggests that these compounds cannot adopt the same

binding mode in the active site of hFPPS (Fig. 2a). Therefore, the thienopyrimidine-based bisphosphonates **2e** and **2f** represent novel leads for drug discovery into hFPPS inhibitors. To fully explore the SAR potential of these compounds, synthesis of a larger and structurally diverse library of bisphosphonate analogs is currently in progress. Concurrently, we are pursuing a number of structural investigations in order to determine the mechanism by which these compounds inhibit hFPPS.

3. Conclusion

Currently, nitrogen-containing bisphosphonates (N-BPs) are the only clinically validated drugs that target the human FPPS, thus blocking prenylation.[33,34] Although these drugs are mainly used for the treatment of bone-related diseases, recent clinical data provides evidence that N-BPs are also disease modifying agents that improve the survival of cancer patients (particularly of patients with multiple myeloma[35]) via mechanisms unrelated to their skeletal effects. In an effort to identify compounds with improved biopharmaceutical properties (as compared to those of the current drugs), we designed a new class of bisphosphonates based on the thienopyrimidine scaffold **1**. Thienopyrimidines are usually prepared via the Gewald method. However, this approach is not easily amenable to parallel synthesis of highly substituted and structurally diverse analogs. In addition, structural diversity is typically dictated by the starting ketones **5** (Scheme 1); thus the synthesis is linear and does not allow preparation of permutation libraries. Furthermore, selective mono-substitution at the C_β of the thiophene sulfur is challenging due to the inherent higher reactivity of the C_α carbon. We modified the classical Gewald methodology by employing 2-(1-(trimethylsilyl)-ethylidene)-malononitrile (**14**) as a novel synthon in the preparation of thieno[2,3-*d*]pyrimid-4-amine libraries (**1**; Scheme 2). Preparation of 5-iodothieno[2,3-*d*]pyrimidin-4-amine (**20b**) was achieved in high yields and significantly fewer steps as compared to previous reports. This methodology was then used to prepare a small cluster of thienopyrimidine-based bisphosphonate compounds (**2**) and probe the ability to such N-BPs to inhibit the hFPPS. In depth SAR studies and structural investigations are currently in progress in order to evaluate the potential of these compounds for further optimization into potent and selective inhibitors of hFPPS.

4. Experimental

4.1. General procedures for characterization of compounds

All intermediate compounds were purified by normal phase flash column chromatography on silica gel using a CombiFlash instrument and the solvent gradient indicated. Key thienopyrimidine building blocks, bisphosphonate esters and all final bisphosphonic acid inhibitors were analyzed by reverse-phase HPLC and fully characterized by ^{1}H, ^{13}C and ^{31}P NMR, and MS (HR-MS for key fragments and final inhibitors). Chemical shifts (δ) are reported in ppm relative to the internal deuterated solvent (^{1}H, ^{13}C) or external H_3PO_4 (δ 0.00 ^{31}P), unless indicated otherwise. High-resolution MS spectra were recorded using electrospray ionization (ESI+/−) and Fourier transform ion cyclotron resonance mass analyzer (FTMS). Melting points (when relevant) were determined using a conventional capillary melting point analyzer. The homogeneity of the bisphosphonate tetra esters and the final bisphosphonic acid inhibitors was confirmed by HPLC to >90% (using the conditions indicated below); IC_{50} values were determined only for samples with >95% homogeneity. HPLC analysis was performed using a Waters ALLIANCE® instrument (e2695 with 2489 UV detector and 3100 mass spectrometer).

Method (homogeneity analysis using a Waters Atlantis T3 C18 5 μm column):

Solvent A: H_2O, 0.1% formic acid.
Solvent B: CH_3CN, 0.1% formic acid.
Mobile phase: linear gradient from 95% A and 5% B to 5% A and 95% B in 13 min, then 2 min at 100% B.
Flow rate: 1 mL/min.

4.2. Synthesis of key fragments

4.2.1. 2-(1-(Trimethylsilyl)ethyl)malononitrile (14)

Acetyltrimethylsilane (1.460 g, 12.56 mmol), malononitrile (1.140 g, 12.56 mmol) and ammonium acetate (262.1 mg, 2.386 mmol) were dissolved in acetic acid (0.58 mL, 10.05 mmol) and benzene (30 mL) in a 100 mL round bottom flask attached to a Dean–Stark trap and filled with benzene. The reaction mixture was stirred and heated to 95 °C for 24 h. The resulting orange solution was cooled and diluted with ethyl acetate (20 mL). The organic layer was washed with saturated sodium bicarbonate solution (15 mL), water (45 mL), brine (15 mL) and dried over $MgSO_4$. The product was purified by column chromatography (25% ethyl acetate/hexanes) to give the desired product as clear pale yellow oil in 90% yield (1.973 g).

^{1}H NMR (400 MHz, $CDCl_3$) δ 2.34 (s, 3H), 0.36 (s, 9H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 188.3, 113.2, 111.5, 94.4, 24.3, −2.2.
HRMS (ESI−) Calcd for $C_8H_{11}N_2Si$ m/z $[M-H]^-$: 163.06970, found m/z 163.06875 and HRMS (ESI−) Calcd 327.14667 for $C_{16}H_{23}N_4Si_2$, found m/z 327.14695 $[2M-H]^-$.

4.2.2. 2-Amino-4-(trimethylsilyl)thiophene-3-carbonitrile (15)

2-(1-(Trimethylsilyl)ethyl)malononitrile (1.21 g, 7.365 mmol) and sulfur (248.02 mg, 7.734 mmol) were dissolved in pyridine (25 mL) at room temperature. To this, diethylamine (0.762 mL, 7.365 mmol) was added dropwise. The reaction mixture stirred at room temperature for 18 h. Evaporation of pyridine afforded the crude thiophene, which was dissolved in ethyl acetate (20 mL) and washed with water (45 mL), brine (15 mL), and dried over $MgSO_4$. Purification by column chromatography (25% ethyl acetate/hexanes, R_f = 0.58) afforded the desired product as an orange oil in 85% yield (803.5 mg).

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.38 (s, 1H), 4.74 (br s, 2H), 0.30 (s, 9H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 164.3, 141.1, 116.8, 116.7, 92.3, −1.3.
HRMS (ESI+) Calcd 197.05632 for $C_8H_{13}N_2SSi$, found m/z 197.05615 $[M+H]^+$.

4.2.3. 5-(Trimethylsilyl)thieno[2,3-*d*]pyrimidin-4-amine (20a)

Fragment **20a** was obtained in two different ways: (a) 2-Amino-4-(trimethylsilyl)thiophene-3-carbonitrile (**15a**, 400 mg, 2.04 mmol, 1 equiv) was added to formamide (8.1 mL, 200 mmol, 100 equiv) in a pressure vessel. The reaction mixture was sealed and stirred at 145 °C in the dark for 16 h. (b) 3-Cyano-4-(trimethylsilyl)thiophen-2-yl)-*N*,*N*-dimethylformimidamide (79.3 mg, 0.315 mmol) was reacted with formamide (anhydrous, 2.5 mL, 63.08 mmol) in a dry 15 mL pressure vessel. The vessel was flushed with argon and the mixture stirred at 130 °C for 45 h. The dark red solution was diluted with ethyl acetate, washed with water (25 mL), brine (10 mL), and dried over Na_2SO_4. The crude mixture

was purified by flash column chromatography (5–30% EtOAc/hexanes, dry loading) to afford the desired product as a pink solid in 80% yield (59.0 mg).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.47 (s, 1H), 7.43 (s, 1H), 5.36 (br s, 2H), 0.46 (s, 9H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 170.9, 158.8, 153.6, 133.2, 131.3, 119.8, 0.4.
HRMS (ESI+) Calcd 224.06722 for $C_8H_{13}N_2SSi$, found *m*/*z* 224.06705 $[M+H]^+$.

4.2.4. 5-Iodothieno[2,3-*d*]pyrimidin-4-amine (20b)

A solution of 5-(trimethylsilyl)thieno[2,3-*d*]pyrimidin-4-amine (220.4 mg, 0.987 mmol, 1 equiv) in dichloromethane (2 mL) was stirred at −10 °C ice slurry for 5 min. 1 M Iodine monochloride in dichloromethane (2.96 mL, 2.96 mmol, 3 equiv) was added to the reaction mixture drop-wise and the reaction mixture was stirred at −10 °C for 30 min. Ice cooled water (30 mL) was directly added to the reaction mixture to quench the reaction. Dichloromethane (2 × 20 mL) at room temperature was added to the mixture and the entire mixture was filtered through a Whatman #5 2.5 μm filter paper. The yellowish solid was washed with dichloromethane (2 × 10 mL) and recrystallized with methanol to give the desired product 5-iodothieno[2,3-*d*]pyrimidin-4-amine as a yellow colored solid (271.9 mg, >98% yield).

^{1}H NMR (300 MHz, CD_3CN): δ 8.33 (1H, s), 7.79 (1H, s), 7.44 (br s, 2H).
^{13}C NMR (126 MHz, acetone-d_6) δ 159.5, 154.7, 128.6, 116.4, 106.1, 70.5.
HRMS (ESI+) Calcd 277.92434 for $C_6H_4IN_3S$, found *m*/*z* 277.92353 $[M+H]^+$.
Melting point: 196 °C (dec).

4.2.5. 3-Cyano-4-(trimethylsilyl)thiophen-2-yl-*N*,*N*-dimethylformimidamide (16)

To a solution of 2-amino-4-(trimethylsilyl)thiophene-3-carbonitrile (**15**, 372.40 mg, 1.90 mmol) in DMF (20 mL) was added DMF–DMA (2.5 mL, 18.97 mmol). After stirring at room temperature for 4 h, the reaction mixture was diluted with ethyl acetate, washed with water (60 mL), brine (20 mL), and dried over $MgSO_4$. Solvent was removed in vacuo to afford the desired product as a brown-yellow solid in 90% yield (440.1 mg).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.72 (s, 1H), 6.63 (s, 1H), 3.10 (s, 3H), 3.09 (s, 3H), 0.32 (s, 9H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 168.9, 154.8, 141.6, 120.7, 117.5, 101.2, 40.7, 35.2, −1.3.
^{29}Si NMR (99 MHz, $CDCl_3$) δ −6.69.
HRMS (ESI+) Calcd 252.09852 for $C_{11}H_{18}N_3SSi$, found *m*/*z* 252.09781 $[M+H]^+$.

4.2.6. 5-Bromo-3-cyano-4-(trimethylsilyl)thiophen-2-yl-*N*,*N*-dimethylformimidamide (17)

N-Bromosuccinimide (121.2 mg, 0.68 mmol) was added to a solution of 3-cyano-4-(trimethylsilyl)thiophen-2-yl-*N*,*N*-dimethylformimidamide (163.0 mg, 0.648 mmol) in DMF (7 mL). The yellow solution was stirred in the absence of light at room temperature for 13 h. The mixture was diluted with ethyl acetate, washed with water (25 mL), brine (10 mL), and dried over $MgSO_4$. Solvent was removed in vacuo to afford the desired product as a brown-orange solid in 80% yield (177.1 mg).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.63 (s, 1H), 3.09 (s, 6H), 0.44 (s, 9H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 168.4, 154.6, 138.9, 116.7, 106.2, 101.9, 40.8, 35.3, 0.2.
HRMS (ESI+) Calcd 330.00903 for $C_{11}H_{17}N_3BrSSi$, found *m*/*z* 330.00926 $[M+H]^+$.

4.2.7. 5-Bromo-3-cyanothiophen-2-yl-*N*,*N*-dimethylformimidamide

A 1 M solution of TBAF (5.13 mL) in THF was added dropwise to a solution of 5-bromo-3-cyano-4-(trimethylsilyl)thiophen-2-yl-*N*,*N*-dimethylformimidamide (**17**, 1.61 g, 4.89 mmol) in THF (100 mL) cooled to 0 °C. The reaction mixture was warmed to room temperature and stirred in the dark for 3 h. The volume of THF was reduced in vacuo and EtOAc was added, washed with water (3 × 50 mL), brine (25 mL), and dried over Na_2SO_4. The desired crude product was obtained as a red oil in 95% yield (1.20 g) and used as such for the synthesis of analogs **1**, where R_5 = H without any further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (s, 1H), 6.86 (s, 1H), 3.11 (s, 6H).
^{13}C NMR (300 MHz, $CDCl_3$) δ 167.0, 154.2, 128.3, 115.1, 99.4, 96.5, 40.8, 35.2.
MS (ESI+) *m*/*z*: 258.06 $[M+H]^+$.

4.2.8. *N*′-(3-Cyano-4-iodo-5-phenylthiophen-2-yl)-*N*,*N*-dimethylformimidamide (19a, R_5 = a)

Silver trifluoroacetate (93.2 mg, 0.42 mmol) was added to a solution of *N*′-(3-cyano-5-phenyl-4-(trimethylsilyl)thiophen-2-yl)-*N*,*N*-dimethylformimidamide (69.1 mg, 0.21 mmol) in THF (20 mL) cooled to −78 °C and stirred under argon for 15 min. Iodine (214.2 mg, 0.84 mmol) dissolved in THF (10 mL) was added dropwise to the cold mixture and stirred in the dark at −78 °C for 4 h. Ethyl acetate was added and the mixture was filtered through Celite. The filtrate was washed with 2 M sodium thiosulfate, brine, and dried over Na_2SO_4. The crude mixture was purified by flash column chromatography (5–30% EtOAc/hexanes, solid loading) to afford the desired product as an orange solid in >98% yield (80.0 mg).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (s, 1H), 7.58–7.51 (m, 2H), 7.40 (m, 3H), 3.13 (s, 6H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 167.2, 154.7, 134.1, 130.7, 129.5, 128.7, 128.6, 116.8, 105.7, 78.1, 40.9, 35.3.
HRMS (ESI+) Calcd 381.98694 for $C_{14}H_{13}N_3IS$, found *m*/*z* 381.98667 $[M+H]^+$.

4.3. Synthesis of thieno[2,3-*d*]pyrimidin-4-amines—general protocols

4.3.1. Suzuki coupling reactions using fragment 20b

Fragment **20b** is not very soluble in non-polar solvents; consequently all reactions were carried out in MeOH. To an argon flushed microwave reactor vial, fragment **20b**, the boronic acid or boronate ester (1.4 equiv) and $Pd(PPh_3)_4$ (0.1 equiv) and KF (2.5 equiv) were added and flushed again with argon. Argon flushed methanol was added to the reaction mixture and the reaction was stirred at 120 °C for 20 min (120 W). The crude mixture was filtered through celite and concentrated to dryness under vacuum. The crude product was purified by normal phase flash column chromatography on silica gel (silica gel was pre-washed

with hexanes) using a CombiFlash instrument and a solvent gradient from 100% hexanes to 100% EtOAc.

4.3.2. General protocol for the Suzuki coupling reactions using fragment 17

In order to probe the versatility and stability of fragment **17**, Suzuki coupling reactions were carried out using two different reaction conditions: (a) The boronic acid or boronate ester (1.5 equiv), $Pd(PPh_3)_4$ (0.1 equiv) and fragment **17** were dissolved in toluene/ethanol (3:1) (approximate concentration with respect to **17** of 0.1 M). The mixture was degassed and flushed with Argon. Aqueous 2 M Na_2CO_3 (2.5 equiv) was added and the mixture was again degassed and flushed with Argon. The reaction mixture was stirred at 85 °C overnight. The crude was filtered through a plug of celite, rinsed with 10 mL of solvent and concentrated under vacuum. The residue was purified on silica gel using a CombiFlash instrument to give the desired products (the common solvent gradient was from 2% EtOAc in hexanes to 100% EtOAc, unless otherwise indicated). (b) The protocol described for the Suzuki reactions using fragment **20b** was also used successfully; presumably the TMS group survives these conditions due to high solvation of the fluoride ion in methanol.

4.3.3. General protocol for Stille cross-coupling reactions using fragment 19a

Palladium acetate (1 equiv) and XPhos (2 equiv) in DME (2 mL) were heated under argon to 85 °C in a 2-dram vial for 10 min. To this mixture cesium carbonate (2 equiv), and fragment **19a** (1 equiv 0.3 mmol) were added and stannane (1.77 equiv). The vial was flushed with argon and the reaction mixture was stirred at 80 °C for 15 h. The reaction mixture was diluted with EtOAc (10 mL), washed with water (3 × 10 mL) and brine (10 mL), dried over Na_2SO_4, and concentrated under vacuum. The crude residue was purified by column chromatography on silica gel using a CombiFlash instrument and a solvent gradient from% EtOAc in hexanes to 100% EtOAc, unless otherwise indicated.

4.3.4. General protocol for Sonogashira cross-coupling reactions using fragment 19a

Triethylamine (0.5 mL) was added to a vial of the iodide **19a** (0.081 mmol), acetylene (0.018 mL, 0.16 mmol), copper(II) bromide (2.7 mg, 0.012 mmol), and $Pd(PPh_3)_4$ (9.4 mg, 0.008 mmol). The vial was purged with argon, capped, and heated to 90 °C for 13 h. Extracted with EtOAc (3 × 10 mL) and washed with saturated sodium bicarbonate (5 mL), water (3 × 5 mL), brine (5 mL), and dried over Na_2SO_4. The crude mixture was purified by flash column chromatography on silica gel (solid loading) using a solvent gradient from 2% EtOAc in hexanes to 100% EtOAc (unless otherwise indicated) to afford the desired product.

4.3.5. General protocol for Buchwald–Hartwig amination reactions using fragment 19a

The amine (5 equiv) was added to a degassed solution of fragment **19a** (usually on a 0.03 mmol scale), $Pd_2(dba)_3$ (5 mol %), XantPhos (11 mol %), and cesium carbonate (1.7 equiv) in toluene (1 mL). The vial was purged with argon and the reaction mixture stirred at 100 °C for 18 h. A second portion of $Pd_2(dba)_3$ (5 mol %) and XantPhos (11 mol %) were added and the reaction mixture was stirred at 100 °C for an additional 18 h. The reaction mixture was diluted with EtOAc (10 mL), washed with water (3 × 10 mL) and brine (10 mL), dried over Na_2SO_4, and concentrated under vacuum. The crude residue was purified by column chromatography on silica gel using a CombiFlash instrument and a solvent gradient from 2% EtOAc in hexanes to 100% EtOAc (unless otherwise indicated) to afford the desired product.

4.3.6. General protocol for the cyclization of C-4 and/or C-5 substituted fragments 18 or 19b to thieno[2,3-*d*]pyrimidin-4-amines 1

The mono-substituted or di-substituted fragments **18** or **19b**, respectively, (0.04 mmol) and dry formamide (excess, >200 equiv) were added to a dry 15 mL pressure vessel. The vessel was flushed with argon and the mixture stirred at 130 °C for 48 h. The dark red solution was diluted with EtOAc, washed with water (25 mL), brine (10 mL), and dried over Na_2SO_4. The crude mixture was purified by flash column chromatography (5–100% EtOAc/hexanes, solid loading) to afford the desired product.

4.4. Spectral data of key synthetic intermediates 18

The R5 groups indicated are taken from the fragments shown in Table 1.

4.4.1. *N*′-(3-Cyano-5-phenyl-4-(trimethylsilyl)thiophen-2-yl)-*N*,*N*-dimethylformimidamide (18, R_5 = a)

Isolated as a beige solid in 90% yield (179 mg).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (s, 1H), 7.36–7.33 (m, 5H), 3.12 (s, 3H), 3.08 (s, 3H), 0.12 (s, 9H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 167.8, 154.6, 139.1, 136.3, 135.9, 130.6, 128.5, 128.1, 118.0, 102.7, 40.7, 35.2, 0.4.
HRMS (ESI+) Calcd 328.12982 for $C_{17}H_{22}N_3SSi$, found *m*/*z* 328.12920 $[M+H]^+$.

4.4.2. *N*′-(3-Cyano-5-(*p*-tolyl)-4-(trimethylsilyl)thiophen-2-yl)-*N*,*N*-dimethylformimidamide (18, R_5 = c)

Isolated as a pale yellow solid in 75% yield (115 mg).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (s, 1H), 7.21 (d, J = 8 Hz, 2H), 7.15 (d, J = 8 Hz, 2H), 3.32 (s, 3H), 3.20 (s, 3H), 2.38 (s, 3H), 0.14 (s, 9H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 167.6, 154.5, 139.3, 138.3, 135.9, 132.8, 130.4, 128.7, 117.9, 102.6, 40.6, 35.0, 21.4, 0.5.
HRMS (ESI+) Calcd 342.14547 for $C_{18}H_{24}N_3SSi$, found *m*/*z* 342.14458 $[M+H]^+$.

4.4.3. *N*′-(3-Cyano-5-(4-(trifluoromethyl)phenyl)-4-(trimethylsilyl)thiophen-2-yl)-*N*,*N*-dimethylformimidamide (18, R_5 = e)

Isolated as a pale brown solid in 71% yield (49.6 mg).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.72 (s, 1H), 7.61 (d, J = 8 Hz, 2H), 7.46 (d, J = 8 Hz, 2H), 3.12 (d, J = 12 Hz, 6H), 0.14 (s, 9H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 168.3, 154.7, 139.7, 137.4, 136.8, 130.5 (q, J_{CF} = 33 Hz), 125.0 (q, J_{CF} = 4 Hz), 124.1 (q, J_{CF} = 272 Hz), 117.7, 103.1, 40.8, 35.2, 0.5.
HRMS (ESI+) Calcd 396.11775 for $C_{18}H_{21}F_3N_3SSi$, found *m*/*z* 396.11519 $[M+H]^+$.

4.4.4. *N*′-(3-Cyano-5-(naphthalen-2-yl)-4-(trimethylsilyl)thiophen-2-yl)-*N*,*N*-dimethylformimidamide (18, R_5 = f)

Isolated as an orange solid in 63% yield (72.1 mg).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.87–7.81 (m, 5H), 7.74 (s, 1H), 7.53–7.51 (m, 2H), 7.46 (m, 1H), 3.15 (s, 3H), 3.10 (s, 3H) 0.13 (9H, s).
^{13}C NMR (75 MHz, $CDCl_3$): δ 167.9, 154.6, 139.1, 136.6, 133.3, 133.0, 132.8, 129.6, 128.4, 128.1, 127.9, 127.7, 126.7, 126.6, 40.7, 35.2, 0.6.
HRMS (ESI+) Calcd 378.14547 for $C_{21}H_{24}N_3SSi$, found *m*/*z* 378.14412 $[M+H]^+$.

4.4.5. *N*′-(4-Cyano-3-(trimethylsilyl)-[2,3′-bithiophen]-5-yl)-*N*,*N*-dimethylformimidamide(18, R_5 = i)

Isolated as light brown solid in 75% yield (53.1 mg).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.70 (s, 1H), 7.31–7.29 (m, 1H), 7.23 (m, 1H), 3.12 (3H, s), 3.09 (3H, s), 0.15 (s, 9H).
^{13}C NMR (75 MHz, $CDCl_3$): δ 167.6, 154.7, 154.6, 137.3, 135.7, 132.9, 130.0, 125.5, 125.3, 117.8, 102.5, 40.7, 35.2, 0.2.
HRMS (ESI+) Calcd 334.08624 for $C_{15}H_{20}N_3S_2Si$, found *m*/*z* 334.08530 $[M+H]^+$.

4.4.6. *N*′-(3-Cyano-4-(trimethylsilyl)-5-vinylthiophen-2-yl)-*N*,*N*-dimethylformimidamide (18, R_5 = h)

Isolated as beige solid in 75% yield (63 mg) and 95% purity (as determined by LC–MS). The impurity was determined to be the dehalogenated thiophene, however, this compound was eliminated from the library for the subsequent steps.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (s, 1H), 6.86 (dd, *J* = 16.9, 10.8 Hz, 1H), 5.36 (d, *J* = 17.0 Hz, 1H), 5.10 (d, *J* = 10.8 Hz, 1H), 3.12 (s, 3H), 3.10 (s, 3H), 0.41 (s, 9H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 166.3, 154.8, 138.1, 137.5, 130.7, 117.5, 113.6, 103.0, 40.7, 35.1, 0.8.
HRMS (ESI+) Calcd 278.11417 for $C_{13}H_{20}N_3SSi$, found *m*/*z* 278.11400 $[M+H]^+$.

4.5. Spectral data of key synthetic intermediates 19

The R_4 and/or R_5 groups indicated are taken from the fragments shown in Table 1.

4.5.1. *N*′-(3-Cyano-5-phenyl-4-(pyrazin-2-yl)thiophen-2-yl)-*N*,*N*-dimethylformimidamide (19b, R_4 = b, R_5 = a)

Isolated as beige solid in 41% yield (8.0 mg) and 95% purity (as determined by LC–MS).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.67 (dd, *J* = 2.6, 1.6 Hz, 1H), 8.46 (d, *J* = 2.6 Hz, 1H), 8.36 (d, *J* = 1.5 Hz, 1H), 7.85 (s, 1H), 7.28–7.25 (m, 3H), 7.17–7.14 (m, 2H), 3.15 (s, 3H), 3.15 (s, 3H).
^{13}C NMR (75 MHz, $CDCl_3$): δ 166.9, 154.6, 149.3, 145.8, 144.5, 143.1, 132.7, 132.2, 131.1, 129.3, 129.0, 128.4, 115.9, 99.2, 40.9, 35.3.
HRMS (ESI+) Calcd 334.11209 for $C_{18}H_{16}N_5S$, found *m*/*z* 334.11144 $[M+H]^+$.

4.5.2. *N*′-(3-Cyano-5-phenyl-4-(phenylethynyl)thiophen-2-yl)-*N*,*N*-dimethylformimidamide (19b, R_4 = g, R_5 = a)

Isolated as a red-orange solid in 95% yield (28.0 mg).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86–7.75 (d, *J* = 7.4 Hz, 3H), 7.83 (s, 1H), 7.57–7.51 (m, 2H), 7.42 (t, *J* = 7.5 Hz, 2H), 7.37–7.30 (m, 4H), 3.15 (s, 3H), 3.15 (s, 3H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 164.2, 154.6, 134.0, 133.3, 131.9, 128.8, 128.7, 128.5, 128.2, 127.5, 122.9, 116.6, 115.5, 100.9, 94.7, 83.3, 40.9, 35.3.
HRMS (ESI+) Calcd 356.12159 for $C_{22}H_{18}N_3S$, found *m*/*z* 356.12098 $[M+H]^+$.

4.5.3. *N*′-(3-Cyano-4-(cyclopropylethynyl)-5-phenylthiophen-2-yl)-*N*,*N*-dimethylformimidamide (19b, R_4 = h, R_5 = a)

Isolated beige solid in 90% yield (15 mg, based on the recovery of some starting material).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78–7.75 (m, 3H), 7.38–7.35 (m, 2H), 7.30–7.26 (m, 1H), 3.10 (s, 6H), 1.52–1.46 (m, 1H), 0.91–0.87 (m, 2H), 0.87–0.83 (m, 2H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 163.8, 154.5, 133.30, 132.9, 128.6, 127.9, 127.1, 117.2, 115.6, 101.4, 99.6, 69.4, 40.9, 35.2, 9.1, 0.7.
HRMS (ESI+) Calcd 320.12159 for $C_{19}H_{18}N_3S$, found *m*/*z* 320.12139 $[M+H]^+$.

4.5.4. *N*′-(3-Cyano-4-morpholino-5-phenylthiophen-2-yl)-*N*,*N*-dimethylformimidamide (19b, R_4 = j, R_5 = a)

Isolated as a beige solid in 90% yield (9.5 mg).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (s, 1H), 7.50 (d, *J* = 7.3 Hz, 2H), 7.35 (t, *J* = 7.5 Hz, 2H), 7.29 (t, *J* = 7.3 Hz, 1H), 3.75–3.71 (m, 4H), 3.12 (s, 3H), 3.10 (s, 3H), 3.10–3.07 (m, 4H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 164.4, 153.9, 143.8, 133.6, 129.3, 128.4, 127.6, 118.4, 116.3, 96.6, 67.5, 51.7, 40.9, 35.2.
HRMS (ESI+) Calcd 341.14306 for $C_{18}H_{21}ON_4S$, found *m*/*z* 341.14218 $[M+H]^+$.

4.5.5. *N*′-(4-(Benzyl(methyl)amino)-3-cyano-5-phenylthiophen-2-yl)-*N*,*N*-dimethylformimidamide (19b, R_4 = k, R_5 = a)

The crude residue was purified by column chromatography on a CombiFlash instrument using a solvent gradient from 3% EtOAc/hexanes to 100% EtOAc with 0.1% triethylamine. The desired product was obtained in 45% yield (19 mg), 70% purity (as determined by ^{1}H NMR). The impurity was determined to be the dehalogenated thiophene and was eliminated in the following step.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (s, 1H), 7.50–7.44 (m, 1H), 7.38–7.27 (m, 5H), 7.25–7.21 (m, 4H), 4.16 (s, 2H), 3.14–3.07 (m, 8H), 2.74 (s, 3H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 164.2, 153.9, 144.6, 138.4, 133.7, 129.1, 128.9, 128.3, 128.2, 127.6, 127.3, 127.1, 125.3, 121.9, 120.1, 116.6, 96.9, 60.1, 41.1, 35.2.
HRMS (ESI+) Calcd 375.16379 for $C_{22}H_{23}N_4S$, found *m*/*z* 375.16296 $[M+H]^+$.

4.6. Spectral data of key thieno[2,3-*d*]pyrimidin-4-amines (1)

The yields indicated are for the isolated pure fragments after the cyclization step of **19b**–**1** from the library synthesis. For the key building blocks 5-(trimethylsilyl)thieno[2,3-*d*]pyrimidin-4-amine (**20a**) and 5-iodothieno[2,3-*d*]pyrimidin-4-amine (**20b**), refer to Sections 4.2.3 and 4.2.4, respectively.

4.6.1. 2-Phenylthieno[2,3-*d*]pyrimidin-4-amine (1a)

Synthesis of derivative **20c** was achieved via direct condensation of 2-aminothiophene-3-carbonitrile with benzonitrile, as previously reported.[12b] To an argon flushed pressure vessel, 2-

aminothiophene-3-carbonitrile (150 mg, 1.21 mmol, 1 equiv), benzonitrile (174.4 mg, 1.69 mmol, 1.4 equiv) and potassium *tert*-butoxide (135.3 mg, 1.21 mmol, 1 equiv) were added and the mixture was flushed again with argon. Anhydrous dioxane (1 mL) was added and the reaction mixture was stirred at 150 °C for 14 h. The crude mixture was concentrated to dryness under vacuum and was purified by flash column chromatography (0–50% EtOAc/hexanes, solid loading) to afford the final product in 27% yield (37.7 mg).

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.45–8.42 (m, 2H), 7.48–7.45 (m, 3H), 7.26 (d, J = 6.0 Hz, 1H), 7.14 (d, J = 6.0 Hz, 1H), 5.55 (s, 2H).
^{13}C NMR (126 MHz, $CDCl_3$): δ 168.9, 160.3, 157.8, 137.9, 130.1, 128.4, 128.2, 123.3, 117.4, 114.3.
MS (ESI+) m/z: 228.1 $[M+H]^+$.

4.6.2. 5-Phenylthieno[2,3-*d*]pyrimidin-4-amine (1b)

Isolated as pale yellow solid in 22% yield (27 mg).

^{1}H NMR (500 MHz, CD_3OD): δ 8.29 (s, 1H), 7.51–7.46 (m, 5H), 7.31 (s, 1H).
^{13}C NMR (126 MHz, CD_3OD) δ 168.3, 160.0, 154.6, 137.2, 136.8, 130.2, 130.1, 129.8, 122.1, 114.9.
MS (ESI+) m/z: 228.4 $[M+H]^+$.

4.6.3. 6-Bromothieno[2,3-*d*]pyrimidin-4-amine (1c)

Isolated as a pale yellow solid in 35% yield (321 mg).

^{1}H NMR (300 MHz, DMSO-d_6): δ 8.22 (1H, s), 7.72 (1H, s), 7.56 (2H, s).
^{13}C NMR (300 MHz, DMSO-d_6): δ 167.5, 157.7, 154.8, 123.3, 116.7, 109.7.
MS (ESI+) m/z: 229.9 and 232.0 $[M+H]^+$.

4.6.4. 6-(*p*-Tolyl)thieno[2,3-*d*]pyrimidin-4-amine (1d)

Isolated as a beige solid in 65% yield (49.6 mg).

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.28 (s, 1H), 8.18 (s, 1H), 8.15 (s, 1H), 8.06–8.01 (m, 2H), 7.96–7.94 (m, 1H), 7.84–7.83 (m, 1H), 7.62–7.53 (m, 4H).
^{13}C NMR (75 MHz, DMSO-d_6) δ 165.5, 162.9, 158.2, 154.0, 138.2, 130.5, 129.9, 125.5, 117.0, 114.9, 20.8.
HRMS (ESI+) Calcd for $C_{13}H_{12}N_3S$ m/z $[M+H]^+$: 242.07464, found m/z 242.07383.

4.6.5. 6-(4-(Trifluoromethyl)phenyl)thieno[2,3-*d*]pyrimidin-4-amine (1e)

Isolated as a pale yellow solid in 56% yield (18.6 mg).

^{1}H NMR (500 MHz, DMSO-d_6): δ 8.29 (s, 1H), 8.14 (s, 1H), 7.86 (s, 4H), 7.66 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.3, 158.5, 154.6, 137.1, 135.7, 128.31 (q, J = 32.0 Hz), 126.3 (q, J = 3.7 Hz), 124.09 (q, J = 272.0 Hz), 117.8, 116.9.
HRMS (ESI+) Calcd 296.04638 for $C_{13}H_9F_3N_3S$, found m/z 296.04552 $[M+H]^+$.

4.6.6. 6-(Naphthalen-2-yl)thieno[2,3-*d*]pyrimidin-4-amine (1f)

Isolated as a brown solid in 35% yield (15.4 mg).

^{1}H NMR (500 MHz, DMSO-d_6): δ 8.28 (s, 1H), 8.18 (s, 1H), 8.15 (s, 1H), 8.06–8.01 (m, 2H), 7.96–7.94 (m, 1H), 7.84–7.83 (m, 1H), 7.62–7.53 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 165.9, 158.3, 154.3, 133.0, 132.6, 130.7, 129.0, 128.1, 127.7, 127.0, 126.7, 124.4, 123.4, 117.1, 116.3, 104.6.
HRMS (ESI+) Calcd 278.07464 for $C_{16}H_{11}N_3S$, found m/z 278.07371 $[M+H]^+$.

4.6.7. 6-(Thiophen-3-yl)thieno[2,3-*d*]pyrimidin-4-amine (1g)

Isolated as a brown solid in 28% yield (9.6 mg).

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.24 (s, 1H), 7.78 (m, 2H), 7.73 (dd, J = 5.0, 2.9 Hz, 1H), 7.50 (br s, 2H), 7.41 (dd, J = 5.0, 1.4 Hz, 1H).
^{13}C NMR (126 MHz, DMSO-d_6) δ 165.3, 158.1, 154.0, 134.6, 133.1, 128.3, 125.4, 121.9, 116.6, 115.3.
HRMS (ESI+) Calcd 234.01542 for $C_{10}H_7N_3S_2$, found m/z 234.01433 $[M+H]^+$.

4.6.8. 5-(4-Nitrophenyl)thieno[2,3-*d*]pyrimidin-4-amine (1h)

Isolated as orange solid in 40% yield (14.2 mg); all NMR data was consistent with previous reports.[24]

^{1}H NMR (500 MHz, DMSO-d_6): δ 8.37 (s, 1H), 8.34 (d, J = 8.2 Hz, 2H), 7.73 (d, J = 8.2 Hz, 2H), 7.69 (s, 1H), 7.06 (s, 2H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 167.7, 158.3, 154.0, 147.1, 142.0, 133.2, 130.1, 123.9, 122.9, 112.2.
MS (ESI+) m/z: 273.1 $[M+H]^+$.

4.6.9. 5-(4-(Trifluoromethyl)phenyl)thieno[2,3-*d*]pyrimidin-4-amine (1i)

Isolated as a pale yellow solid in 57% yield (28.2 mg).

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.36 (s, 1H), 7.87 (d, J = 8.0 Hz), 7.70 (d, J = 8.0 Hz), 7.62 (s, 1H).
^{13}C NMR (126 MHz, DMSO-d_6) δ 167.5, 158.3, 153.9, 139.6, 133.6, 129.1, 128.5 (q, J = 32.1 Hz), 125.7 (q, J = 3.8 Hz), 124.3 (q, J = 272.1 Hz), 122.2, 112.4.
HRMS (ESI+) Calcd 296.04638 for $C_{13}H_8F_3N_3S$, found m/z 294.04552 $[M+H]^+$.

4.6.10. 5-(Naphthalen-2-yl)thieno[2,3-*d*]pyrimidin-4-amine (1j)

Isolated as a pale yellow solid in 54% yield (27.0 mg).

^{1}H NMR (500 MHz, DMSO-d_6): δ 8.37 (s, 1H), 8.08–8.00 (m, 4H), 7.62–7.59 (m, 4H).
^{13}C NMR (75 MHz, DMSO-d_6) δ 167.3, 158.4, 153.8, 134.9, 133.0, 132.8, 132.4, 128.5, 128.0, 127.8, 127.7, 126.8, 126.7, 126.6, 121.1, 113.0.
HRMS (ESI+) Calcd 278.07464 for $C_{16}H_{11}N_3S$, found m/z 278.07371 $[M+H]^+$.

4.6.11. 6-Phenyl-5-(phenylethynyl)thieno[2,3-*d*]pyrimidin-4-amine (1k)

Isolated as a brown solid in 85% yield (12 mg).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.45 (s, 1H), 7.96–7.94 (m, 1H), 7.94–7.93 (m, 1H), 7.51–7.47 (m, 4H), 7.45–7.37 (m, 4H), ~6.30 (br s, 2H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 165.3, 158.4, 154.6, 143.9, 133.0, 131.4, 129.4, 129.3, 129.0, 128.9, 128.6, 122.0, 116.5, 108.8, 94.8, 85.2.
HRMS (ESI+) Calcd 328.09029 for $C_{20}H_{14}N_3S$, found m/z 328.09015 $[M+H]^+$.

4.6.12. 5-(Cyclopropylethynyl)-6-phenylthieno[2,3-*d*]pyrimidin-4-amine (1l)

Isolated as a beige solid in 85% yield (11 mg).

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.39 (s, 1H), 7.88–7.81 (m, 2H), 7.49–7.35 (m, 3H), 1.52 (m, 1H), 0.98–0.92 (m, 2H), 0.84 (m, 2H).
^{13}C NMR (126 MHz, $CDCl_3$): δ 164.9, 158.4, 154.4, 143.0, 133.1, 129.1, 128.8, 128.4, 116.7, 109.3, 99.5, 71.6, 8.8, 0.55.
HRMS (ESI+) Calcd 292.09029 for $C_{17}H_{14}N_3S$, found m/z 292.09030 $[M+H]^+$.

4.6.13. 5-Morpholino-6-phenylthieno[2,3-*d*]pyrimidin-4-amine (1m)

The crude mixture was purified by flash column chromatography (5–100% EtOAc/hexanes with 0.1% Et_3N, solid loading) to afford the desired product as a beige solid in 50% yield (20.5 mg).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 (s, 1H), 7.49–7.41 (m, 5H), 3.84 (d, J = 10.6 Hz, 2H), 3.60 (td, J = 11.5, 2.3 Hz, 2H), 3.03 (td, J = 11.6, 2.8 Hz, 2H), 2.95 (d, J = 11.8 Hz, 2H), 1.60 (s, 1H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 164.5, 158.8, 154.3, 138.3, 133.4, 131.2, 131.0, 129.2, 128.6, 113.9, 67.8, 53.2.
HRMS (ESI+) Calcd 313.11176 for $C_{16}H_{17}ON_4S$, found m/z 313.11125 $[M+H]^+$.

4.6.14. N^5-Benzyl-N^5-methyl-6-phenylthieno[2,3-*d*]pyrimidine-4,5-diamine (1n)

The crude mixture was purified by flash column chromatography (5–100% EtOAc/hexanes with 0.1% Et_3N, solid loading) to afford the desired product as a pale orange solid in 53% yield (9.0 mg).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.41 (s, 1H), 7.47–7.36 (m, 5H), 7.29–7.21 (m, 3H), 7.12–7.06 (m, 2H), 3.99 (d, J = 13.3 Hz, 1H), 3.66 (d, J = 13.3 Hz, 1H), 2.77 (s, 3H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 164.4, 158.6, 154.3, 139.3, 138.1, 133.6, 130.8, 130.6, 128.9, 128.9, 128.7, 128.46, 127.7, 114.2, 60.7, 43.4.
HRMS (ESI+) Calcd 347.13249 for $C_{20}H_{19}N_4S$, found m/z 347.13188 $[M+H]^+$.

4.6.15. 6-Phenyl-5-(pyrazin-2-yl)thieno[2,3-*d*]pyrimidin-4-amine (1o)

Isolated as a beige solid in 95% yield (7.0 mg).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.68 (dd, J = 2.6, 1.5 Hz, 1H), 8.50 (d, J = 2.6 Hz, 1H), 8.48 (s, 1H), 8.29 (d, J = 1.4 Hz, 1H), 7.36–7.30 (m, 3H), 7.22–7.19 (m, 2H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 167.3, 159.1, 154.1, 150.8, 149.0, 143.1, 143.0, 141.9, 132.9, 130.2, ~129.4 (3 × C), 126.1, 115.9.
HRMS: Calcd 306.08079 for $C_{16}H_{12}N_5S$, found m/z 306.08165 $[M+H]^+$.

4.7. Synthesis of bisphosphonic acids 2

4.7.1. Standard 2-step procedure for the conversion of thienopyrimidin-4-amines 1 to inhibitors 2

The R_2, R_5 and R_6 groups indicated are taken from the fragments shown in Table 1.

Step (a): A solution of the thienopyrimidin-4-amine **1** (0.2 mmol) in anhydrous toluene (10 mL) was flushed with argon. Triethyl orthoformate (1.5 equiv) and diethylphosphite (7 equiv) was added to the reaction mixture via syringe and the reaction mixture was flushed again with argon, sealed, and stirred for 130 °C in the dark for 48 h. The crude mixture was concentrated to dryness under vacuum and purified by flash column chromatography (20–100% EtOAc/hexanes to 0–20% MeOH/EtOAc, solid loading) to afford the desired tetraethyl bisphosphonate ester.

Step (b): A solution of the tetraethyl bisphosphonate ester (1 equiv) in CH_2Cl_2 was cooled to 0 °C and trimethylsilyl bromide (15 equiv) was added via syringe. The reaction mixture was stirred at room temperature for 3–5 days; completion of conversion was monitored by ^{31}P NMR. The mixture was then concentrated under vacuum, diluted with HPLC grade MeOH (~5 mL), and the solvent was evaporated to dryness; this step was repeated four times. The organic solvents were evaporated under vacuum, the residue was suspended in 0.5 mL MeOH, excess water (~5 mL Milli-Q grade) was added to induce full precipitation of the final bisphosphonic acid. The amorphous powder was collected by filtration, washed with de-ionized water (2×), with HPLC-grade CH_3CN (2×), with distilled Et_2O or toluene (2×) and dried under vacuum to give the final compound as a white solid.

4.7.2. (Thieno[2,3-*d*]pyrimidin-4-ylamino)methylene bisphosphonic acid (2a)

The tetraethyl ((5-(trimethylsilyl)thieno[2,3-*d*]pyrimidin-4-yl)amino)methylene bisphosphonate was prepared from intermediate **20a**; isolated in 60% yield (36.7 mg).

^{1}H NMR (400 MHz, CD_3OD): δ 8.50 (s, 1H), 7.45 (s, 1H), 5.98 (td, J = 22.0, 9.9 Hz, 1H), 5.78 (d, J = 10.3 Hz, 1H), 4.28–4.12 (m, 8H), 1.26 (td, J = 7.1, 1.7 Hz, 12H), 0.52 (s, 9H).
^{13}C NMR (126 MHz, CD_3OD): δ 170.1, 156.2, 152.7, 133.0, 131.3, 120.3, 63.4 (d, J_{CP} = 122.5 Hz), 44.2 (t, J_{CP} = 583 Hz), 16.3, 0.09.
^{31}P NMR (81 MHz, CD_3OD): δ 14.81.
MS (ESI) m/z 532.15 $[M+Na]^+$.

A solution of the bisphosphonate tetraester (47.4 mg, 0.093 mmol) in THF (2 mL) was then cooled to 0 °C and 0.1 mL (23.6 mg of a 1 M solution, 0.1 mmol) of TBAF in THF was added. After stirring for 20 min at 0 °C, the mixture was warmed to ambient temperature and stirred for and additional 4 h in the dark. THF was removed under vacuum and the crude mixture was dissolved in 10 mL of EtOAc, washed with water (1 × 5 mL) and brine (1 × 5 mL), and dried over anhydrous sodium sulfate. The desilylated product was obtained quantitatively as a pale yellow solid (41.2 mg).

^{1}H NMR (500 MHz, CD_3OD): δ 8.47 (s, 1H), 7.72 (d, J = 6.0 Hz, 1H), 7.57 (d, J = 6.0 Hz 1H), 6.00 (t, J = 23.7 Hz, 1H), 4.20 (m, 8H), 1.26 (m, 12H).
^{13}C NMR (126 MHz, CD_3OD): δ 167.6, 157.6, 154.1, 125.1, 119.8, 118.5, 65.16, 45.6 (t, J_{CP} = 150.1 Hz), 16.6.
^{31}P NMR (81 MHz, CD_3OD): δ 18.52.
HRMS (ESI+) Calcd 460.08370 for $C_{15}H_{25}N_3NaO_6P_2S$, found 461.08320 $[M+Na]^+$.

The final inhibitor, thieno[2,3-*d*]pyrimidin-4-ylamino methylene bisphosphonic acid (**2a**) was isolated as a white solid in 70% yield (20.8 mg).

^{1}H NMR (400 MHz, D_2O, 10% ND_4OD, 10% DMSO internal standard): δ 8.284 (s, 1H), 7.549 (d, J = 5.9 Hz, 1H), 7.427 (d, J = 6.1 Hz, 1H), 4.594 (t, J = 19.1 Hz, 1H).
^{13}C NMR (126 MHz, D_2O, 20% ND_4OD, 10% DMSO internal standard): δ 164.3, 157.3, 154.2, 123.4, 119.8, 117.9, 51.7 (t, J_{CP} = 125.6 Hz).
^{31}P NMR (81 MHz, D_2O, 20% ND_4OD): δ 13.308.
HRMS (ESI−) Calcd 323.96873 for $C_7H_8SN_3O_6P_2$, found 323.96125 $[M-H]^-$.

4.7.3. (((2-Phenylthieno[2,3-*d*]pyrimidin-4-yl)amino)methylene)diphosphonic acid (2b)

The tetraethyl (((2-phenylthieno[2,3-*d*]pyrimidin-4-yl)amino)-methylene)bis(phosphonate) was isolated as an orange oil 61% (52.0 mg).

^{1}H NMR (500 MHz, MeOD-d_4): δ 8.43–8.41 (m, 2H), 7.71 (d, J = 6.0 Hz, 1H), 7.51 (d, J = 6.0 Hz, 1H), 7.49–7.47 (m, 4H), 6.21 (t, J = 23.7 Hz, 1H), 4.22–4.19 (m, 8H), 1.25–1.22 (m, 12H).
^{13}C NMR (126 MHz, MeOD-d_4): δ 169.5, 160.5, 157.3, 139.0, 131.5, 129.5, 129.1, 124.8, 119.7, 116.8, 65.1 (m), 45.5 (t, J = 150.1 Hz), 16.7.
^{31}P NMR (81 MHz, MeOD-d_4): δ 17.6.
MS (ESI+) m/z: 514.7 $[M+H]^+$.

The final product (((2-phenylthieno[2,3-*d*]pyrimidin-4-yl)amino)methylene)diphosphonic acid (**2b**) was isolated as a pale yellow solid in 66% yield (22.0 mg).

^{1}H NMR (300 MHz, D_2O): δ 8.15–8.12 (m, 2H), 7.44–7.40 (m, 4H), 7.28 (d, J = 6.0 Hz, 1H), 4.86 (t, J = 19.0 Hz, 1H).
^{13}C NMR (126 MHz, D_2O): δ 166.3, 161.5, 157.8, 138.4, 131.4, 129.6, 129.1, 128.8, 123.6, 119.9, 116.6, 105.9, 50.6 (t, J = 123.6).
^{31}P NMR (81 MHz, D_2O): δ 14.2.
HRMS (ESI−) Calcd 399.9928 for $C_{13}H_{12}N_3P_2O_6S$, found m/z 399.9922 $[M-H]^-$.

4.7.4. (((5-Phenylthieno[2,3-*d*]pyrimidin-4-yl)amino)methylene)diphosphonic acid (2c)

The tetraethyl (((5-phenylthieno[2,3-*d*]pyrimidin-4-yl)amino)-methylene)bis(phosphonate) was isolated as a pale yellow solid 24% yield (61 mg).

^{1}H NMR (500 MHz, MeOD-d_4): δ 8.52 (s, 1H), 7.60–7.55 (m, 3H), 7.52–7.50 (m, 2H), 7.45 (s, 1H), 5.80 (t, J = 22.4 Hz, 1H), 4.11–4.03 (m, 8H), 1.22–1.19 (m, 12H).
^{13}C NMR (75 MHz, MeOD-d_4): δ 166.9, 152.8, 135.4, 134.4, 129.2, 128.9, 128.8, 122.0, 63.7 (m), 43.6 (t, J = 150.1 Hz), 15.2.
^{31}P NMR (81 MHz, MeOD-d_4): δ 16.1.
MS (ESI+) m/z: 515.3 $[M+H]^+$.

The final inhibitor (((5-phenylthieno[2,3-*d*]pyrimidin-4-yl)amino)methylene)diphosphonic acid (**2c**) was isolated as a white solid in 54% yield (5.1 mg)

^{1}H NMR (500 MHz, D_2O): δ 8.17 (s, 1H), 7.68 (s, 1H), 7.54 (d, J = 7.0 Hz, 2H), 7.43 (t, J = 7.6 Hz, 2H), 7.35 (d, J = 7.4 Hz, 1H), 7.12 (s, 1H).
^{13}C NMR (126 MHz, D_2O): δ 165.0, 153.2, 135.3, 129.4, 129.1, 128.3, 120.7, 113.9, 50.6 (t, J = 123.6).
^{31}P NMR (81 MHz, D_2O): δ 13.4.
HRMS (ESI−) Calcd 399.9928 for $C_{13}H_{12}N_3P_2O_6S$, found m/z 399.9918 $[M-H]^-$.

4.7.5. (6-Phenylthieno[2,3-*d*]pyrimidin-4-ylamino)methylene bisphosphonic acid (2d)

The tetraethyl ((6-phenylthieno[2,3-*d*]pyrimidin-4-yl)amino)-methylene bisphosphonate was isolated in 65% yield (38.0 mg).

^{1}H NMR (500 MHz, CD_3OD) δ 8.45 (s, 1H), 8.04 (s, 1H), 7.75 (d, J = 7.3 Hz, 2H), 7.48 (t, J = 7.6 Hz, 2H), 7.41 (d, J = 7.3 Hz, 1H), 6.00 (t, J = 23.6 Hz, 1H), 4.22 (dd, J = 7.7, 3.2 Hz, 8H), 1.45–1.09 (m, 12H).
^{13}C NMR (126 MHz, CD_3OD) δ 167.0, 157.2, 154.1, 142.7, 134.6, 130.4, 130.1, 127.2, 119.8, 115.2, 65.2, 45.6, 16.7.
^{31}P NMR (81 MHz, CD_3OD) δ 17.004.
MS (ESI) m/z 536.15 $[M+Na]^+$.

The final inhibitor (6-phenylthieno[2,3-*d*]pyrimidin-4-ylamino)methylene bisphosphonic acid (**2d**) was isolated as a white solid in 63% yield (18.8 mg).

^{1}H NMR (400 MHz, D_2O, 10% ND_4OD): δ 8.18 (s, 1H), 7.80 (s, 1H), 7.71 (d, J = 7.4 Hz, 2H), 7.42 (t, J = 7.6 Hz, 2H), 7.33 (t, J = 7.4 Hz, 1H).
^{13}C NMR (126 MHz, D_2O, 10% ND_4OD): δ 163.1, 156.2, 153.6, 139.2, 133.2, 129.3, 128.6, 125.9, 118.64, 114.8.
^{31}P NMR (81 MHz, D_2O, ND_4OD) δ 13.29.
HRMS (ESI−) Calcd 399.99166 for $C_{13}H_{12}SN_3O_6P_2$, found 399.99268 $[M-H]^-$.

4.7.6. (((6-(*p*-Tolyl)thieno[2,3-*d*]pyrimidin-4-yl)amino)methylene)diphosphonic acid (2e)

The tetraethyl (((6-(*p*-tolyl)thieno[2,3-*d*]pyrimidin-4-yl)amino)methylene)bis(phosphonate) was isolated as a light brown powder 94% (27.3 mg).

^{1}H NMR (500 MHz, MeOD-d_4): δ 8.43 (s, 1H), 7.97 (s, 1H), 7.60 (d, J = 8.1 Hz, 2H), 7.27 (d, J = 7.8 Hz, 1H), 6.00 (t, J = 23.6 Hz, 1H), 4.24–4.21 (m, 8H), 2.37 (s, 3H), 1.31–1.24 (m, 12H).
^{13}C NMR (126 MHz, MeOD-d_4): δ 166.8, 157.0, 153.9, 142.9, 140.4, 131.8, 130.9, 127.1, 119.8, 114.4, 65.10 (m), 45.6 (t, J = 150 Hz), 21.3, 16.7 (m).
^{31}P NMR (81 MHz, MeOD-d_4): δ 17.4.
MS (ESI+) m/z: 528.2 $[M+H]^+$.

The final inhibitor (((6-(*p*-tolyl)thieno[2,3-*d*]pyrimidin-4-yl)amino)methylene)diphosphonic acid (**2e**) was isolated as a white solid in 94% yield (27.3 mg).

^{1}H NMR (500 MHz, D_2O): δ 8.22 (s, 1H), 7.80 (s, 1H), 7.66 (d, J = 8.2 Hz, 2H), 7.30 (d, J = 8.0 Hz, 2H), 2.33 (s, 3H).

^{13}C NMR (126 MHz, D_2O): δ 164.3, 157.6, 155.0, 140.8, 140.7, 131.9, 127.5, 120.2, 115.7, 21.8.
^{31}P NMR (81 MHz, D_2O): δ 13.72.
HRMS (ESI−) Calcd 414.00840 for $C_{14}H_{14}N_3P_2O_6S$, found m/z 414.00863 $[M-H]^-$.

4.7.7. (((6-(Naphthalen-2-yl)thieno[2,3-*d*]pyrimidin-4-yl)amino)methylene)diphosphonic acid (2f)

The tetraethyl (((6-(naphthalen-2-yl)thieno[2,3-*d*]pyrimidin-4-yl)amino)methylene)bis(phosphonate) was isolated as a white powder 71% (30.8 mg)

^{1}H NMR (500 MHz, MeOD-d_4): δ 8.45 (s, 1H), 8.14 (s, 2H), 7.94–7.83 (m, 4H), 7.53–7.47 (m, 2H), 6.02 (t, J = 23.6 Hz, 1H), 4.24–4.21 (m, 8H), 1.31–1.24 (m, 12H).
^{13}C NMR (126 MHz, MeOD-d_4): δ 167.1, 157.1, 154.2, 142.6, 135.0, 134.8, 131.9, 130.0, 129.3, 128.8, 128.0, 127.9, 126.3, 124.6, 119.9, 115.6, 65.1 (m), 45.7 (t, J = 150 Hz), 16.7 (m).
^{31}P NMR (81 MHz, MeOD-d_4): δ 17.4.
MS (ESI+) m/z: 564.3 $[M+H]^+$.

The final inhibitor (((6-(naphthalen-2-yl)thieno[2,3-*d*]pyrimidin-4-yl)amino)methylene)diphosphonic acid (**2f**) was isolated as a white solid in 86% yield (29.5 mg).

^{1}H NMR (500 MHz, D_2O): δ 8.19 (s, 1H), 8.13 (s, 1H), 7.91–7.83 (m, 5H), 7.50–7.44 (m, 2H).
^{13}C NMR (126 MHz, D_2O): δ 164.8, 157.8, 155.1, 140.7, 134.7, 134.3, 132.2, 130.4, 129.6, 129.3, 128.6, 128.3, 126.2, 125.3, 120.3, 116.8.
^{31}P NMR (81 MHz, D_2O): δ 13.74.
HRMS (ESI−) Calcd 450.0084 for $C_{17}H_{14}N_3P_2O_6S$, found m/z 450.0085 $[M-H]^-$.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2013.02.006.

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