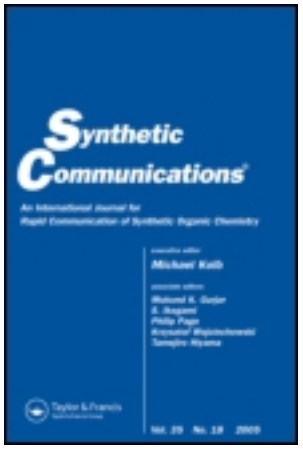

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Synthesis, Crystal Structures, and in Silico Toxicity Prediction of Thienopyridine Phosphoramidates

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SYNTHESIS, CRYSTAL STRUCTURES, AND IN SILICO TOXICITY PREDICTION OF THIENOPYRIDINE PHOSPHORAMIDATES

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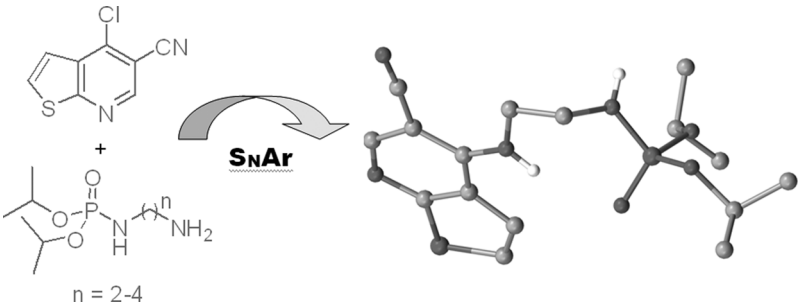
GRAPHICAL ABSTRACT



Abstract *New thieno[2,3-*b*]pyridine phosphoramidates compounds were synthesized and characterized by infrared; ^{1}H, ^{13}C, and ^{31}P NMR spectroscopy; and high-resolution mass spectrometry. The products were obtained in good yields (64–82%) under mild conditions by nucleophilic aromatic substitution reaction of aminoalkylphosphoramidates over 4-chlorothieno[2,3-*b*]pyridine-5-carbonitrile. The crystal structures of two compounds were solved by x-ray diffraction and showed a network of intermolecular interactions involving phosphoramidate groups. Druglike properties and toxicity of the new compounds were studied with the help of the software Molinspiration, Osiris, and Toxtree, and were compared with the standard drugs amphotericin B, miltefosine, benznidazole, and nifurtimox.*

[Supplementary materials are available for this article. Go to the publisher's online edition of Synthetic Communications® *for the following free supplemental resource(s): Full experimental and spectral details.]*

Keywords Crystal structures; druglike properties; phosphoramidates; thienopyridine

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INTRODUCTION

Fused heterocycles containing nitrogen and sulfur have been associated with several biological and medicinal activities.[1] In particular, thienopyridine derivatives play important roles because of their extensive spectrum of pharmacological activities. They can be used as sedatives;[2] analgesics and anti-inflammatories;[3–5] antiparasitic,[6] antibacterial,[7–10] and antitumor agents;[11–13] and antiviral drugs[14–16] and in the treatment of diabetes mellitus,[17,18] antiplatelet aggregation,[19–21] and myeloproliferative disorders.[22] Several 4-substituted thienopyridines have been obtained by our group by nucleophilic substitution of the 4-chloro precursor with variable nucleophiles.

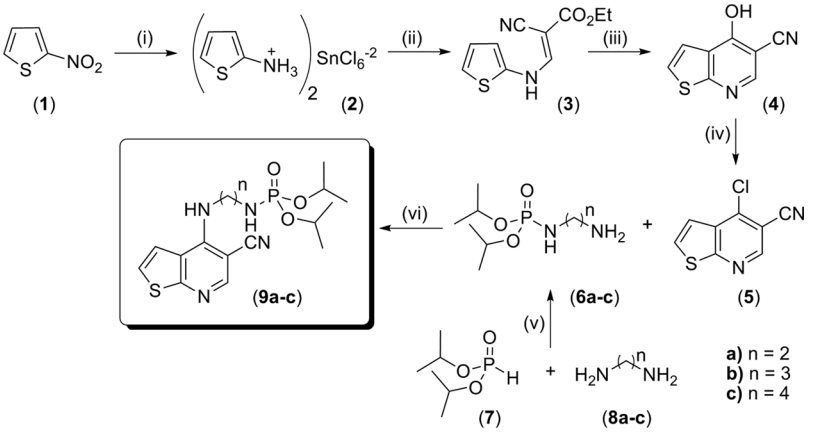
Besides, it is well known that phosphoramidates and their derivatives are an important class of biologically relevant compounds.[23] Introduction of a phosphoramidate group essentially changes the physical and chemical properties of the parent molecule, accentuating the polarization and intermolecular bonding characteristics.[24,25] The P=O group plays a significant role as a strong hydrogen bond acceptor, which is essential for the noncovalent bonding of proteins or other specific ligands to their substrates.[26] The incorporation of a phosphoramidate group into different heterocyclic systems constitutes an interesting synthetic strategy used in the discovery of new drugs.[27] For example, it is widely used in association with neutral lipophilic groups to form a lipophilic-membrane-permeable derivative able to access intracellular target sites.[28] In the past few years, we focused our efforts on the preparation of bioactive heterocycles containing phosphoramidate groups.[29,30] In this work we report the synthesis of three new thieno[2,3-*b*]pyridine phosphoramidate derivatives as well as the crystal structure of two of them. These derivatives were submitted to in silico oral biodisponibility screening, drug score, and druglikeness evaluation to analyze their overall potential to be qualified as drugs, and also compared them to some antiparasitics for leishmaniasis and Chagas disease, such as amphotericin B, miltefosine, benznidazole, and nifurtimox.

RESULTS AND DISCUSSION

Syntheses and Spectroscopic Measurements

The syntheses of the new thieno[2,3-*b*]pyridine phosphoramidates conjugates (**9a–c**) were performed by nucleophilic aromatic substitution of the chlorine atom in 4-substituted thieno[2,3-b]pyridine (**5**) by aminoalkylphosphoramidates **6a–c** (Scheme 1).

The starting 4-chlorothieno[2,3-*b*]pyridine-5-carbonitrile **5** was prepared from 2-nitrothiophene **1**, which was reduced with tin and hydrochloric acid, leading to the bis-(2-thienylammonium)hexachlorostannate **2**. This compound was immediately condensed with ethyl (ethoxymethylene)cyanoacetate in pyridine at 40–50 °C over a period of 24 h to produce ethyl α-cyano-β-(*N*-2-thienylammonium)acrylate **3** in 88% yield. The cyclization of the acrylate was carried out by refluxing in Dowtherm at 250 °C for 40 min, after which 4-hydroxythieno[2,3-*b*]pyridine-5-carbonitrile **4** was isolated in 78% yield, through precipitation from petroleum ether. Compound **4** was easily chlorinated refluxing phosphorus oxychloride at 110 °C over a period of 6 h to afford 4-chlorothieno[2,3-*b*]pyridine-5-carbonitrile **5** in 76% yield.[6,15,31]



Scheme 1. Reagents and conditions: (i) Sn, 35% HCl, 45 °C; (ii) ethyl (ethoxymethylene)cyanoacetate, pyridine, 40–50 °C, 24 h; (iii) Dowtherm, 250 °C, 40 min; (iv) $POCl_3$, 110 °C, 6 h; (v) CCl_4, ethanol, T < 55 °C, 10 min; and (vi) THF, reflux, 9–12 h.

The aminoalkylphosphoramidates **6a–c** were synthesized in 53–58% yield from diisopropylphosphonate **7** and aliphatic diamines **8a–c**.[29,32,33] To guarantee monophosphorylation of the diamines, at least a 2.5-fold excess of diamine in ethanol was used to maintain the (alkaline) pH necessary to catalyze the reaction.

Careful addition of **7** in ethanol and CCl_4 over the diamine solution should not exceed 10 min. At this stage, the temperature must be kept below 55 °C, otherwise bis-phosphorylation will occur preferentially. The nucleophilic aromatic substitution of the chlorine atom in 4-substituted thieno[2,3-*b*]pyridine by amines has been used as a versatile route to new thienopyridine derivatives.[16,34–36] The reaction of 4-chlorothieno[2,3-*b*]pyridine-5-carbonitrile **5** with an excess (2 equiv.) of aminoalkyl phosphoramidates **6a–c** in refluxing tetrahydrofuran (THF) for 9 to 12 h afforded the thieno[2,3-*b*]pyridine phosphoramidate **9a–c** in 64–82% yield. The presence of an electron-withdrawing group (-CN) in the 5-position of the substrate **5** and the excess of the nucleophilic agent **6a–c** facilitates the nucleophilic substitution. The products were fully characterized by infrared; ^1H, ^{13}C, and ^{31}P NMR spectroscopy; and high-resolution mass spectrometry (HRMS).

The ^1H NMR spectra of compounds **9a–c** compounds showed two doublets around 7.58–8.04 and 7.30–7.46 ppm with $^3J(HH) \sim 6.0$ Hz corresponding to the resonances of the thieno ring protons. The same spectra showed a singlet in the range of 8.33–8.45 ppm attributable to the pyridine ring proton. The resonances of the isopropyl protons appeared as two doublets at 1.36–1.42 ppm and a doublet of septets around 4.6 ppm with $^3J(HH) \sim 6.1$ Hz and $^3J(PH) \sim 7.3$ Hz. The NH signal was detected as a broad singlet in the range 8.06–8.84 ppm. On the other hand, NHP protons showed coupling with phosphorus and the neighbor methylene group, giving rise to a doublet of a triplet around 3.20 ppm with $^3J(HH) \sim 7.0$ Hz and $^2J(PH) \sim 10.0$ Hz. In the aliphatic region, the assignment of the signals for methylene protons was based on the correlation spectra (COSY) of these compounds. Typically, the methyne carbon signal in β position to phosphorus appears as a doublet with $^2J(PC) \sim 5.2$ Hz around 71.5 ppm in ^{13}C NMR spectroscopy. In all cases phosphorus and carbon

showed coupling with $^{3}J(PC) \sim 5.2$ Hz, but no coupling $^{2}J(PC)$ was observed. The thieno[2,3-*b*]pyridine phosphoramidates **9a–c** showed in their decoupled ^{31}P NMR spectra one signal in the region of 7.78–9.84 pmm, typical for phosphoramidates.[29,30,37,38] Furthermore, infrared spectra exhibited strong absorptions for the P=O at 1194 cm^{-1}, P-O around 1000–988 cm^{-1}, and weak absorption for the cyano group at 2208 cm^{-1}.

X-Ray Crystallography Investigation

Single crystals of **9a** and **9b** compounds suitable for x-ray diffraction were obtained by slow solvent evaporation at room temperature. The crystal data and structure refinement parameters for thieno[2,3-*b*]pyridine phosphoramidates **9a** and **9b** are provided. The asymmetric units are shown in Fig. 1.

In these compounds, the phosphoramidate groups are linked to an aliphatic skeleton through the C1 atom. The bond lengths and angles are typical of phosphoramidate groups (see Supplementary Information Tables S2 and S3). The 5-cyanothieno[2,3-*b*]pyridin-4-ylamino group is bonded to the alkyl chain through C2 atom in **9a** and C3 atom in **9b**. It is important to highlight that the aromatic substituent group gives rise to weak intermolecular interactions and plays an important role in the crystal packing. In compound **9a**, intramolecular hydrogen bonding involving N2 and O1 atoms lead to a seven-member ring, in which N1 and N2 atoms are in an eclipsed conformation, in relation to the C1–C2 bond axis. A network of hydrogen bonding in the [100] direction is observed due to the interaction between N1 and O1 atoms (Fig. 2).

The bond distances and angles for hydrogen bonding are summarized in Table 1. Besides, the molecular arrangement is also stabilized by weak interactions between the pyridinic and thiophene rings from neighboring molecules.

As a result of these intra- and intermolecular interactions, the compound **9a** presents a more compact crystal packing, leading to a higher calculated density when compared with **9b**. The increase of one carbon atom in the alkyl spacer chain results in a different crystal packing in **9b**. The C1 and N2 atoms are in an eclipsed conformation, viewed through the C2–C3 bond axis. This conformation does not

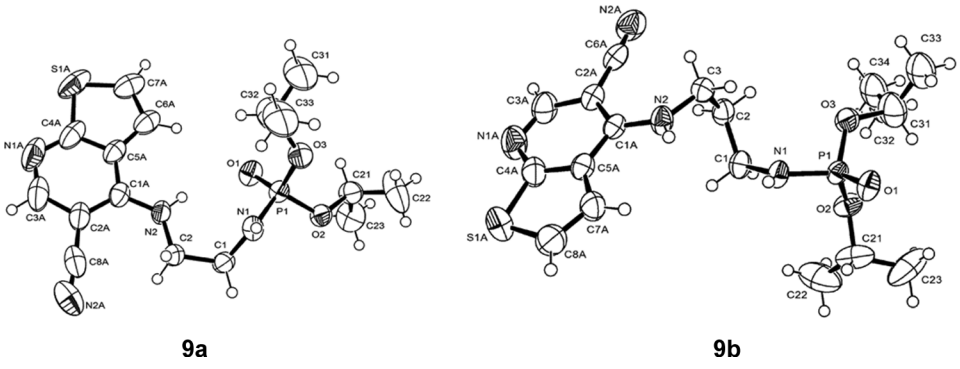


Figure 1. X-ray crystal structure of compounds **9a** and **9b**. Ellipsoids at 50% of probability.

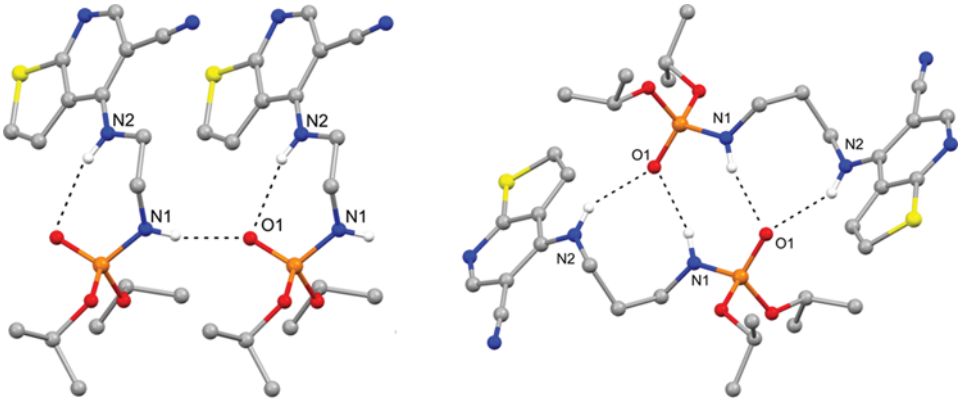


Figure 2. Hydrogen bonding in compounds **9a** and **9b**. (Figure is provided in color online.)

allow intramolecular hydrogen bonding between N2 and O1 atoms, as observed in **9a**. Bifurcated intermolecular hydrogen bonding among O1, N1, and N2 are able to form dimers of molecules (Fig. 2). Moreover, short contact C8-H8A $\cdots$ N2A in the [010] direction and $\pi \cdots \pi$ stacking between the 5-cyanothieno[2,3-*b*]pyridine groups stabilize the crystal packing. A search in the CCDC database shows a tendency of phosphoramidates compounds to form dimers in the solid state, because 47% of the compounds present in this base display this molecular arrangement.

This characteristic is associated to the torsion angle O1–P1–N1–C1, which is close to 180°. In the compound **9a** the intramolecular hydrogen bonding does not allow the dimer formation, while in the **9b** the longer alkyl chain permits a better arrangement of the $\pi \cdots \pi$ stacking together with hydrogen bonds, leading to a supramolecular network.

Druglike Properties and Toxicity Prediction

In silico techniques for the druglike properties and prediction of toxicological endpoints are extremely appealing because of their expeditious return of results and their low cost. Moreover, these techniques can be used in a very early phase of drug discovery even before the molecule is synthesized.[39] Free web-based programs for Lipinski's rule of five and toxicity prediction are available, including the Molinspiration, Osiris, and Toxtree.

Table 1. Hydrogen-bond geometry (Å, °)

Compound	*D*—H $\cdots$ *A*	*D*—H	H $\cdots$ *A*	*D* $\cdots$ *A*	*D*—H $\cdots$ *A*
9a	N1—H1 $\cdots$ O1^a	0.86	2.04	2.879 (3)	165
	N2—H2 $\cdots$ O1	0.86	2.31	3.131 (3)	160
9b	N1—H1 $\cdots$ O1^b	0.86	2.13	2.963 (3)	163
	N2—H2 $\cdots$ O1^b	0.86	2.18	2.953 (3)	149

$^a x-1, y, z$.
$^b -x+1, -y+1, -z+2$.

Molinspiration software was utilized to screen the compounds **9a–c** based on Lipinski's rule of five and oral bioavailability and compared with standard drugs against leishmaniasis and Chagas disease currently available (i.e., amphotericin B, miltefosine, benznidazole, and nifurtimox). The prediction results are in Table 2.

According to stated procedures,[40–42] the parameters of Lipinski's rule of five are as follows: the molecular weight must be less than 500 Da, logP less than 5, the amount of hydrogen donors (nOHNH) must be less than 5, and the amount of acceptor hydrogen (nON) must be less than 10. miLogP (octanol/water partition coefficient) is calculated by the methodology developed by Molinspiration as a sum of fragment-based contributions and correction factors. The method is very robust and is able to process practically all organic and most organometallic molecules.

For all the compounds the calculated logP values were around 2.058–2.6, below the limit for the drugs to be able to penetrate through biomembranes according to Lipinski's rule. Thus, compounds **9a–c** are expected to exhibit good bioavailability. It is worth mentioning that all the compounds showed zero violation of the rule of five, having successfully passed Lipinski's rules. Two or more violations suggest the probability of problems in bioavailability.

The oral bioavailability of drugs could be measured by the molecular weight, number of rotatable bonds (nrotb), number of hydrogen bonds (nON and nOHNH), and total polar surface area (TPSA). This set of criteria is called Veber's rule.[43]

The oral bioavailability was marked by small molecular weight (less than 500); also, the number of rotatable bond must be less than 10, the number of hydrogen bond donors and acceptors must be less than 12, and TPSA values less than 140 Å. TPSA has been shown to be a very good descriptor characterizing drug absorption, including intestinal absorption, bioavailability, and blood–brain barrier penetration. Lipophilicity and polar surface area values are two important properties for the prediction of oral bioavailability of drug molecules.[44] Molecules with TPSA values around 160 Å or more are expected to exhibit poor intestinal absorption. Table 2 shows that **9a–c** are within this limit and in accordance with Veber's rule.

Table 2. Molinspiration calculation of properties for Lipinski's rule[a]

	Acceptable range							
	miLog P	TPSA	natoms	MW	nON	nOHNH	nrotb	nviolations
Compound	*<5*			*<500*	*<10*	*<5*		
9a	2.058	96.277	25.0	382.426	7	2	9	0
9b	2.329	96.277	26.0	396.453	7	2	10	0
9c	2.6	96.277	27.0	410.48	7	2	11	0
Amphotericin B	0.365	299.381	65.0	922.119	17	12	3	3
Miltefosine	1.303	58.596	30.0	449.657	5	0	23	0
Benznidazole	0.778	92.748	19.0	260.253	7	1	5	0
Nifurtimox	0.709	108.708	19.0	287.297	8	0	3	0

[a]miLog P, Molinspiration-predicted log P; TPSA, total polar surface area; natoms, number of atoms; MW, molecular weight; nON, number of hydrogen-bond acceptors; nOHNH, number of hydrogen-bond donors; nrotb, number of rotatable bonds; and nviol, number of violations.

It has to be kept in mind that log P and TPSA values are the most important two features, although not sufficient criteria for predicting oral absorption of a drug.

Toxicity risks (mutagenicity, tumorogenicity, irritation, reproduction) and physicochemical properties (drug likeness and drug score) of compounds **9a–c** were calculated by the methodology developed by Osiris (Table 3).

The toxicity prediction using Osiris Property Explorer was shown in color codes. Green color shows the low toxicity tendency, yellow shows the moderate tendency, and red shows high tendency. The result of toxicity analysis of compounds **9a–c** showed low toxicity tendency.

Drug likeness may be defined as a complex balance of various molecular properties and structure features that determine whether particular molecule is similar to the known drugs. The drug-likeness value of standard and modified ligand shows the fragment content of the drugs. If the drug-likeness values are increasing, then it has the same fragment content with existing drugs.[45,46] Table 3 shows that the drug-likeness values of **9a–c** were lower than the standard drugs, with the exception of amphotericin B. This result tells us that our thienopyridine phosphoramidates have few fragments content of drugs.

The drug score values are the combination of solubility, molecular weight, log P, drug likeness, and toxicity risk within one useful practical value. It could be used for evaluating the potential of the drug candidate.[47] When the drug score is better, then the compound has a better chance to be a drug candidate. The compounds **9a–c** showed better drug score than all standard drugs tested (Table 3).

Estimation of toxicity could be verified by using Toxtree v2.5.0, and the result could be seen at Table 4. Toxtree v2.5.0 determines the toxicity level of compounds based on Benigni and Bossa rules.[48] It stipulated that certain functional groups, which have mutagenic or carcinogenic properties, could influence the toxicity. Table 4 shows that compounds **9a–c** have no tendency to be mutagenic and carcinogenic, while benznidazole and nifurtimox have potential to be carcinogenic. Thus, when Toxtree software result was compared with quantitative structure–activity relationship (QSAR) analysis, the thieno[2,3-*b*]pyridine phosphoramidates presented no mutagenic and carcinogenic potential.

In conclusion, the methodology for the nucleophilic aromatic substitution of the chlorine atom in 4-substituted thieno[2,3-*b*]pyridine was successfully applied to aminoalkylphosphoramidates as the nucleophile. Greater yields were obtained when there

Table 3. Toxicity of thieno[2,3-*b*]pyridine phosphoramidates (**9a–c**) and standard drugs based on Osiris Property Explorer

Compound	Mutagenic	Tumorigenic	Irritant	Reproductive effective	Drug likeness	Drug score
9a	Green	Green	Green	Green	−22.47	0.35
9b	Green	Green	Green	Green	−22.48	0.33
9c	Green	Green	Green	Green	−23.70	0.30
Amphotericin B	Green	Green	Green	Green	−0.14	0.26
Miltefosine	Green	Green	Green	Green	−54.74	0.30
Benznidazole	Red	Green	Green	Red	−3.32	0.18
Nifurtimox	Red	Red	Green	Red	0.65	0.16

Table 4. Toxicity of thieno[2,3-*b*]pyridine phosphoramidates (**9a–c**) and standard drugs based on Toxtree v2.5.0

Compound	Negative for genotoxic carcinogenity	Negative for nongenotoxic carcinogenity	Potential *S. Typhiurium* TA 100 mutagen based on QSAR	Potential carcinogen based on QSAR
9a	Yes	Yes	No	No
9b	Yes	Yes	No	No
9c	Yes	Yes	No	No
Amphotericin B	Yes	Yes	No	No
Miltefosine	Yes	Yes	No	No
Benznidazole	No	Yes	No	No
Nifurtimox	No	Yes	No	No

is a greater distance between the amino and phosphoramidates groups, suggesting that the nucleophilic attack is somewhat dependent on the steric effect caused by the phosphoramidate group. This assumption is based on the crystal structures of compounds **9a** and **9b** in which hydrogen bonding contributed to approximate phosphoramidate and the amino groups. In the case of compound **9a**, the short alkyl chain between phosphoramidate and thieno[2,3-*b*]pyridine groups leads to intramolecular and intermolecular hydrogen bonding P—O1—H—N2 atoms, increasing the steric hindrance.

The compounds **9a–c** showed suitable druglike properties and are expected to present good bioavailability profiles. The theoretical study of these molecules also showed that they fulfilled the Lipinski rule of five and present drug scores better than some standard drugs used as antiparasitics. The results encouraged us to invest in the synthesis of new thienopyridine phosphoramidates and perform antiparasitic tests in vitro and in vivo.

EXPERIMENTAL

Analytical-grade reagents and solvents were purchased from commercial sources and used without further purification. Uncorrected melting points were obtained with a Fisher-Johns apparatus. ^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded on a Varian UP-300 spectrometer at 299.95, 75.42, and 121.42 MHz, respectively, with tetramethylsilane (TMS) as internal standard or 85% H_3PO_4 as external standard. The chemical shifts (δ) are reported in parts per million (ppm) and the coupling constants (*J*) are in hertz. Thin-layer chromatography (TLC) was carried out using silica-gel F-254 glass plates (20 × 20 cm). Infrared spectra were recorded on a Perkin-Elmer Spectrum One Fourier transform–infrared (FT-IR) spectrometer. High-resolution mass spectra (EI-70 eV) were performed on a Varian MAT CH7 8500 direct inlet instrument. The 4-chlorothieno[2,3-*b*]pyridine-5-carbonitrile (**5**)[6,15,31] and aminoalkylphosphoramidates (**6a–c**) compounds[32,33] were prepared as previously reported.

General Procedure for Synthesis of the Thieno[2,3-*b*]pyridine Phosphoramidates (9a–c)

4-Chlorothieno[2,3-*b*]pyridine-5-carbonitrile (**5**) (2.2 mmol) and the aminoalkylphosphoramidate (**6a–c**) (4.4 mmol) were dissolved in THF (10 mL), and the reaction

mixture was heated at reflux until the disappearance of the starting **5** (9–12 h, monitored by TLC). The mixture was poured onto ice, and the resulting solid (except **9c**) was filtered off, washed with distilled water, and dried. Compounds **9a** and **b** were recrystallized from ethanol/water (1:3). Compound **9c** was diluted with chloroform and washed with water (3×10 mL). The solvent was evaporated under reduced pressure, giving an oily product.

Diisopropyl 2-(5-cyanothieno[2,3-b]pyridin-4-ylamino)ethyl phosphoramidate (9a). Yield 82%; pale brown solid; mp 130–131 °C; IR (KBr, cm^{-1}): 3342 (m, νN-H), 3213 (m), 2982 (m), 2933 (m), 2208 (w, νCN), 1592 (s, δN-H), 1544(s), 1521 (m), 1450 (m), 1384 (w), 1358 (m), 1235 (m), 1194 (m, νP=O), 1137 (m), 1099 (m), 1000 (s, νP-O), 842 (w), 775 (w), 707 (w); ^{1}H NMR ($CDCl_3$): δ = 1.31 and 1.33 [2d, 12H, ^3J(HH) = 6.4], 2.08 (br s, 1H), 3.43 (m, 2H), 4.05 (m, 2H), 4.59 [dsep, 2H, ^3J(HH) = 6.3 and ^3J(PH) = 7.5], 7.46 [d, 1H, ^3J(HH) = 6.0], 8.04 [d, 1H, ^3J(HH) = 6.0], 8.45 (s, 1H), 8.86 (br s, 1H); ^{13}C NMR ($CDCl_3$): δ = 23.66 and 23.59 [2d, ^3J(PC) = 5.3 and 4.7], 40.30 (s), 47.04 (s), 71.95 [d, ^2J(PC) = 5.9], 119.36 (s), 119.56 (s), 124.54 (s), 150.93 (s); ^{31}P NMR ($CDCl_3$): δ = 9.84 (s); HRMS (EI): m/z [M + 1] calcd. for $C_{16}H_{23}N_4O_3PS$: 382.12285. Found: 382.12280.

Diisopropyl 3-(5-cyanothieno[2,3-b]pyridin-4-ylamino)propyl phosphoramidate (9b). Yield 73%; pale brown solid; mp 125 °C; IR (KBr, cm^{-1}): 3332 (m, νN-H), 3271 (m), 2978 (m), 2932 (m), 2210 (w, νCN), 1591 (s, δN-H), 1546 (s), 1437 (w), 1374 (w), 1220 (s), 1194 (m, νP=O), 1130 (m), 1082 (w), 1018 (s), 988 (s, νP-O), 899 (w), 838 (w), 755 (w), 713 (m); ^{1}H NMR ($CDCl_3$): δ = 1.26 and 1.28 [2d, 12H, ^3J(HH) = 6.1], 1.85 [quin, 2H, ^3J(HH) = 5.4], 3.07 (m, 3H), 3.98 [dt, 2H, ^3J(HH) = 6.5], 4.55 [dsep, 2H, ^3J(HH) = 6.1 and ^3J(PH) = 7.3], 7.30 [d, 1H, ^3J(HH) = 6.0], 7.71 [d, 1H, ^3J(HH) = 6.0], 7.97 (br s, 1H), 8.33 (s, 1H); ^{13}C NMR ($CDCl_3$): δ = 23.73 and 23.79 [2d, ^3J(PC) = 4.8], 31.50 [d, ^3J(PC) = 3.6], 37.30 (s), 39.52 (s), 71.47 [d, ^2J(PC) = 6.0], 119.31 (s), 119.38 (s), 124.02 (s), 150.48 (s), 151.45 (s); ^{31}P NMR ($CDCl_3$): δ = 9.20 (s); HRMS (EI): m/z [M + 1] calcd. for $C_{17}H_{25}N_4O_3PS$: 396.13850. Found: 396.13850.

Diisopropyl 4-(5-cyanothieno[2,3-b]pyridin-4-ylamino)butyl phosphoramidate (9c). Yield 64%; yellow oil; IR (CH_2Cl_2, cm^{-1}): 3268 (m, νN-H), 3109 (m), 2977 (s), 2931 (s), 2208 (w, νCN), 1592 (m, δN-H), 1543 (s), 1437 (m), 1373 (m), 1225 (s), 1106 (m, νP=O), 987 (s, νP-O), 896 (m), 836 (w), 775 (m), 736 (m), 702 (w), 665 (w); ^{1}H NMR ($CDCl_3$): δ = 1.26 and 1.28 [2d, 12H, ^3J(HH) = 6.3], 1.49 (br s, 1H), 1.65 (m, 2H), 1.82 [quin, 2H, ^3J(HH) = 6.5], 3.04 (m, 2H), 3.79 [dt, 2H, ^3J(HH) = 6.5 and 6.0], 4.55 [dsep, 2H, ^3J(HH) = 6.3 and ^3J(PH) = 7.5], 6.41 (br s, 1H), 7.30 [d, 1H, ^3J(HH) = 6.0], 7.58 [d, 1H, ^3J(HH) = 6.0], 8.38 (s, 1H); ^{13}C NMR ($CDCl_3$): δ = 23.63 and 23.70 [2d, ^3J(PC) = 4.6], 26.85 (s), 28.43 [d, ^3J(PC) = 5.8], 40.72 (s), 44.14 (s), 70.76 [d, ^2J(PC) = 5.2], 119.26 (s), 118.90 (s), 119.34 (s), 123.75 (s), 150.52 (s), 151.10 (s), 163.95 (s); ^{31}P NMR ($CDCl_3$): δ = 7.79 (s), HRMS (EI): m/z [M + 1] calcd. for $C_{18}H_{27}N_4O_3PS$: 410.15415. Found: 410.15420.

X-Ray Structure Determination

The crystallographic data for compounds **9a** and **9b** were collected on an Enraf Nonius Bruker Kappa CCD diffractometer, using graphite monochromatic MoKα radiation ($\lambda = 0.71069$ Å). Final unit cell parameters were based on the fitting of all reflections positions using DIRAX.[49] Collected reflections were integrated using the EVALCCD program.[50] Empirical multiscan absorption corrections using equivalent reflections were performed with the SADABS program.[51] The structure solutions and full-matrix least-squares refinements based on $F2$ were performed with the SHELXS-97 and SHELXL-97 program packages.[52] All atoms except hydrogen were refined anisotropically. Hydrogen atoms were treated by a mixture of independent and constrained refinement. Details of data collection and structure refinement are summarized in Table S1. Selected distances and angles are given in Tables 1, S2, and S3. CCDC ID 886689 (**9a**) and 886690 (**9b**) contains the supplementary crystallographic data for this article. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk).

Computational Methods Applicable for Lipinski's Rule and Toxicity Prediction

The molecular properties of compounds were predicted in Molinspiration software[53] using the SMILES format. These properties include molecular mass, miLogP, TPSA, number of hydrogen-bond acceptors, number of hydrogen-bond donors, nRotb, and number of Lipinski's rule of five violations. The fragment-based approach used by Molinspiration has proven to be reliable and is employed in relevant scientific publications and chemical databases.

The OSIRIS Property Explorer[54] is an integral part of Actelion's inhouse substance registration system. It lets you draw chemical structures and calculates on-the-fly various drug-relevant properties whenever a structure is valid. Prediction results are valued and color coded. Green color shows the low toxicity tendency, yellow shows the moderate tendency, and red shows high tendency.

Toxicology analysis was performed using software ToxTree v2.5.0[55] based on the Benigni and Bossa rules for mutagenicity and carcinogenicity developed by Romualdo Benigni and Cecilia Bossa from the Instituto Superiore in Sanita, Rome, Italy, and approved by the European Chemical Bureau, Institute for Health and Consumers Protection, European Commission Joint Research Centre (JRC) in 2008.

SUPPORTING INFORMATION

Crystal data and ^{1}H, ^{13}C, and ^{31}P NMR spectra can be found via the Supplementary Content section of this article's Web page.

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