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Synthesis and Structure–Activity Relationship Studies of 2-(1,3,4-Oxadiazole-2(3*H*)-thione)-3-amino-5-arylthieno[2,3-*b*]pyridines as Inhibitors of DRAK2

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In recent years, DAPK-related apoptosis-inducing protein kinase 2 (DRAK2) has emerged as a promising target for the treatment of a variety of autoimmune diseases and for the prevention of graft rejection after organ transplantation. However, medicinal chemistry optimization campaigns for the discovery of novel small-molecule inhibitors of DRAK2 have not yet been published. Screening of a proprietary compound library led to the discovery of a benzothiophene analogue that displays an affinity constant (K_d) value of 0.25 μM. Variation of the core scaffold and of the substitution pattern afforded a series of 5-arylthieno[2,3-*b*]pyridines with strong binding affinity (K_d = 0.008 μM for the most potent representative). These compounds also show promising activity in a functional biochemical DRAK2 enzyme assay, with an IC_{50} value of 0.029 μM for the most potent congener. Selectivity profiling of the most potent compounds revealed that they lack selectivity within the DAPK family of kinases. However, one of the less potent analogues is a selective ligand for DRAK2 and can be used as starting point for the synthesis of selective and potent DRAK2 inhibitors.

Introduction

Current clinical treatments to prevent allograft rejection include lifetime administration of immunosuppressive drugs.[1] Cyclosporine and tacrolimus are the two most frequently used immunosuppressants used by transplant patients. Their immunosuppressive activity is linked to the inhibition of calcineurin, a serine/threonine phosphatase that activates intracellular gene-promoting transcription factors involved in IL-2 activation. Sirolimus (rapamycin) also binds to a cytosolic immunophilin called FKBP12. This sirolimus–FKBP12 complex binds proteins downstream of IL-2 in T-cell activation pathways, known as the mammalian target of rapamycin (mTOR) that prevent DNA and protein synthesis in T-cells. Antiproliferative agents, such as azathioprine and mycophenolate mofetil (MMF), while commonly used after transplants, are not ideal due to significant toxic side effects, arising from widespread inhibition of purine biosynthesis in the case of azathioprine and depletion of guanine nucleotides and an inhibition of proliferating lymphocytes in the case of MMF. Corticosteroids have played a central role in the maintenance of immunosuppression as well as the treatment of acute rejection. However, most of these drugs cause toxicity problems. In addition, allogeneic memory T-cells (T_{mem}) cannot be controlled by current treatments, very often resulting in organ failure and chronic rejection. Moreover, most of the targets currently used to specifically block allograft rejection have the characteristic of imposing generalized immunosuppression. Therefore, the search for new therapeutic targets that can be exploited to prevent rejection without compromising the recipient immune response to infections is crucial.

DRAK2 (**D**APK-**r**elated **a**poptosis-inducing protein **k**inase 2) is a serine/threonine kinase belonging to the family of death-associated protein (DAP) kinases (DAPK).[2] Members of this family induce apoptosis in various cell types. It has been demonstrated that DRAK2 mRNA and the DRAK2 protein are mainly expressed in lymphoid organs. DRAK2 expression is highest in B-cells, but is also expressed at high levels in both CD4+ and CD8+ T cells. DRAK2 is not expressed at significant levels in NK cells, macrophages, or dendritic cells.[3] When DRAK2 expression is abolished via genetic deletion, mice develop resistance to the T-cell-mediated autoimmune disease experimental autoimmune encephalomyelitis (EAE, which is a model for multiple sclerosis) and are resistant to type I diabetes.[4] Very recently, it has been demonstrated that DRAK2 signaling is required for productive T-cell alloresponsiveness in two separate allogenic transplant models.[5] These data support the hypothesis that DRAK2 signaling is required for efficient allorejection. Moreover, it has been demonstrated that a loss of DRAK2 does not negatively impact antiviral immunity. Overall, these experimental data strongly suggest that DRAK2 is a promising drug target for the prevention of graft rejection

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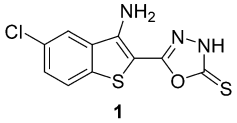
after organ transplantation and for the treatment of a variety of autoimmune disorders (such as diabetes, multiple sclerosis, and rheumatoid arthritis). A number of potent DRAK2 ligands have been reported. However, they are not the result of a DRAK2 drug discovery program, but have been identified as off-target effect in an effort to target other kinases. In addition, only binding data (i.e., K_d values) are available for these compounds, and no functional inhibitory activities (i.e., IC_{50} values) are reported.[6] No specific drug discovery programs focusing on DRAK2 inhibition have been described in the scientific or patent literature. However, the availability of specific and cell-permeable DRAK2 inhibitors would allow the use of these as chemical tool compounds or as lead compounds for drug discovery.

As a primary screening tool for the discovery of novel DRAK2 inhibitors, we opted for the KINOMEscan Profiling Service of DiscoveRx, which is based on a competition binding assay that quantitatively measures the ability of a compound to compete with an immobilized, active-site-directed ligand. In this assay, the binding of DNA-tagged kinase to an immobilized ligand on the solid support is measured by quantitative PCR of the DNA tag. The experiment is then repeated in the presence of a "free test" compound. The more test compounds that bind to the kinase, the fewer DNA-tagged protein molecules bind to the immobilized ligand.[7] This screening campaign led to the discovery of several ligands for DRAK2. One of the most promising hits was 5-(3-amino-5-chlorobenzo[*b*]thiophen-2-yl)-1,3,4-oxadiazole-2(3*H*)-thione **1** (Figure 1). Low molecular weight (M_r = 283.76 Da), in combination with potent activity (K_d = 0.25 μM) furnishes a ligand efficiency (LE) of 0.53. It is generally accepted that an LE of at least 0.3 is necessary to have a good hit or lead compound.[8] Calculation of the log*P* value revealed the compound to have acceptable lipophilicity (clog*P* = 3.16). Overall, these characteristics makes hit **1** a good starting point for an optimization program.



1

M_r:	283.76 Da
PSA:	59.64 Å^2
clog *P*:	3.16
LE:	0.53 kcal mol^{-1}
K_d:	0.25 μM

Figure 1. Hit compound **1** and its properties.

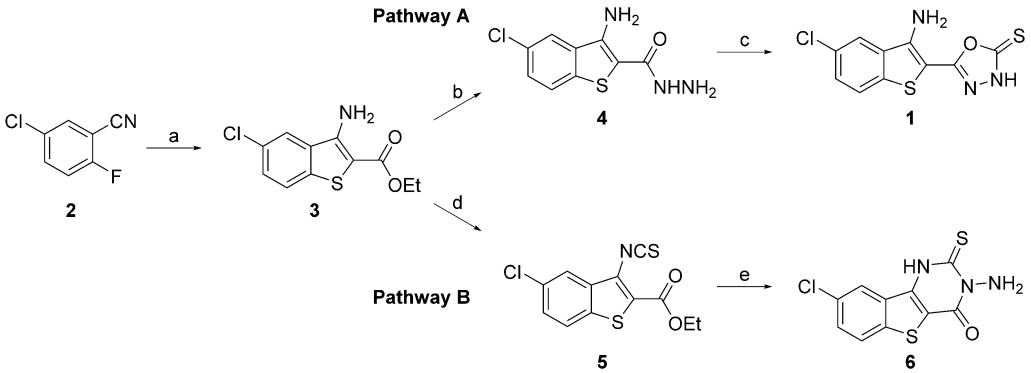
Herein we report our successful efforts to improve the potency of benzo[*b*]thiophene analogue **1** and to identify compounds with improved binding affinity, showing activity in a DRAK2 functional enzymatic assay. This is the first report that describes functionally active DRAK2 inhibitors, which are valuable tool compounds for studying the biological effects of DRAK2 inhibition.

Chemistry

Prior to embarking on an extensive hit-to-lead optimization campaign, hit compound **1** was resynthesized for confirmation of its biological activity. The synthesis starts from a commercially available benzonitrile **2**. Nucleophilic aromatic substitution with ethyl thioacetate with subsequent ring closure yields the ethyl 3-aminobenzo[*b*]thiophene-2-carboxylate derivative **3**.[9] Published reports suggest that the main synthetic route leading to 5-substituted-1,3,4-oxadiazole-2-thione relies on the treatment of a carboxylic acid hydrazide with carbon disulfide in alkaline medium.[9] Therefore, 5-chloro-3-aminobenzo[*b*]thiophene-2-carboxylic acid hydrazide **4** was prepared from the ethyl ester derivative **3** (Scheme 1, Pathway A). Finally, to construct the 1,3,4-oxadiazole moiety, the 3-amino-2-acyl hydrazide **4** was treated with carbon disulfide in pyridine at elevated temperature,[10] furnishing hit compound **1**.

However, it has been reported that holding aromatic 2-aminocarboxylic acid hydrazides with carbon disulfide at reflux can also afford 3-amino-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one derivatives, rather than the 1,3,4-oxadiazole-2-thiones.[11] Both compounds have the same molecular formula, the same molecular weight, and have very similar ^{1}H NMR data, and are therefore hard to distinguish from each other. In order to unambiguously determine the structure of the hit compound **1** as being an oxadiazole derivative, we synthesized fused tricyclic compound **6**, via an independent synthetic route (Scheme 1, Pathway B). The exocyclic amino group was converted into an isothiocyanate moiety,[12] followed by treatment with hydrazine to yield the 2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one **6**.[13]

Compounds **1** and **6** show very similar polarity (R_f values) on TLC and their ^{1}H NMR spectra showed similarity in signal posi-



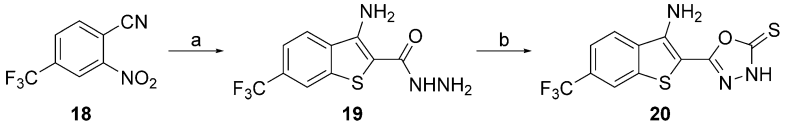
Scheme 1. *Reagents and conditions*: a) ethyl thioacetate, NaOH, DMF, 0 °C, 2 h; b) hydrazine monohydrate, EtOH, reflux, overnight; c) CS_2, pyridine, reflux, 3 h; d) $CSCl_2$, acetone, RT, ~36 h; e) hydrazine solution (35% in H_2O), toluene, 80 °C, 2.5 h.

tions. Differences in chemical shifts in the ^{13}C NMR spectra were observed. The HMBC spectrum of compound **1** showed a clear HMBC correlation (^2J) between the carbon at 89.95 ppm and the NH_2 protons ($\delta = 6.82$ ppm); therefore, the peak at 89.95 ppm can be assigned to C3. The observation of HMBC correlations (^3J) between the carbon at 132.91 ppm (which can be assigned to C3a) and both the proton doublet ($J = 8.7$ Hz) at 7.97 ppm (arising from H-7) and the amino group protons unambiguously confirm the presence of an oxadiazole moiety in hit compound **1**, as these HMBC cross-peaks are not possible in the case of tricyclic compound **6** (Figure S1, Supporting Information).

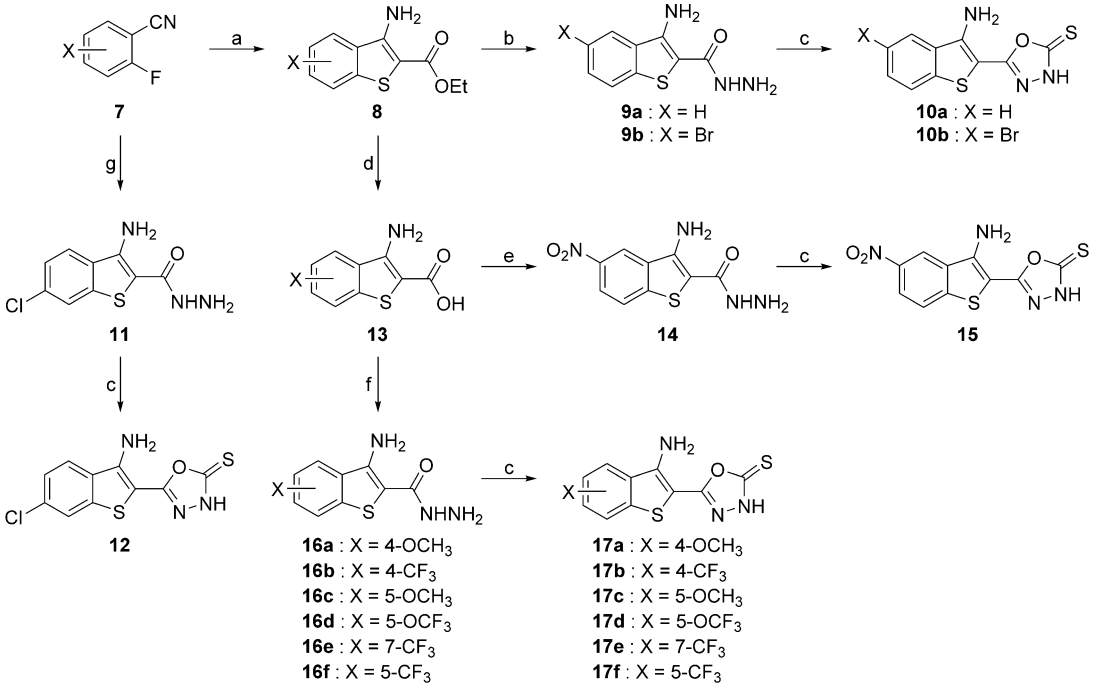
The chemistry started with the synthesis of a series of analogues with subtle structural variations in the phenyl moiety (Scheme 2). Appropriate 2-fluorobenzonitriles **7** were used as a starting material which, upon treatment with ethyl thioacetate under basic conditions, afforded ethyl 3-amino-2-carboxylate derivatives **8**.[14] Direct hydrazinolysis of these ethyl esters gave satisfactory results only in case of the unsubstituted and 5-bromobenzo[*b*]thiophene derivatives **9a**–**b**. Construction of the oxadiazole moiety was achieved by heating **9a**–**b** with carbon disulfide in pyridine, furnishing target compounds **10a**–**b**. To improve the synthesis of the intermediate hydrazide, various approaches were followed, depending on the substitution pattern of the benzonitrile derivative. Reaction of a 2-fluorobenzonitrile derivative with thioacetic acid hydrazide[15] led to the direct formation of the hydrazide moiety, avoiding the need for hydrolysis of the carboxylic acid ethyl ester. Unfortunately, this method was only successful for the synthesis of the 6-chlorobenzothiophene analogue **11**. Treatment of **11** with carbon disulfide yields the final compound **12**. In an alternative approach, the ethyl ester group was hydrolyzed to the corresponding carboxylic acid. Subsequent transformation of the acid into the acid chloride and hydrazinolysis afforded the 5-nitrobenzothiophene analogue **14**. Although this method works fine for the 5-nitro derivative, for the majority of the compounds, a procedure for amide formation using HOBt and EDCI as coupling agents was applied. Treatment of hydrazides **16a**–**f** with carbon disulfide in the presence of pyridine under reflux afforded 2-(1,3,4-oxadiazole-2(3*H*)-thione)-3-aminobenzo[*b*]thiophenes **17a**–**f** in moderate to good yields.

Alternatively, a 2-nitrobenzonitrile derivative **18** was used as starting material (Scheme 3). Nucleophilic displacement of the nitro group with thioacetic acid hydrazide,[15] followed by oxadiazole formation affords the desired analogue **20**.

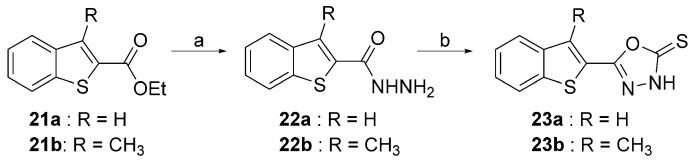
Compounds with structural modification of the amino group were easily accessible from commercially available benzothiophene analogues (Scheme 4), using the standard procedures of the aforementioned schemes.



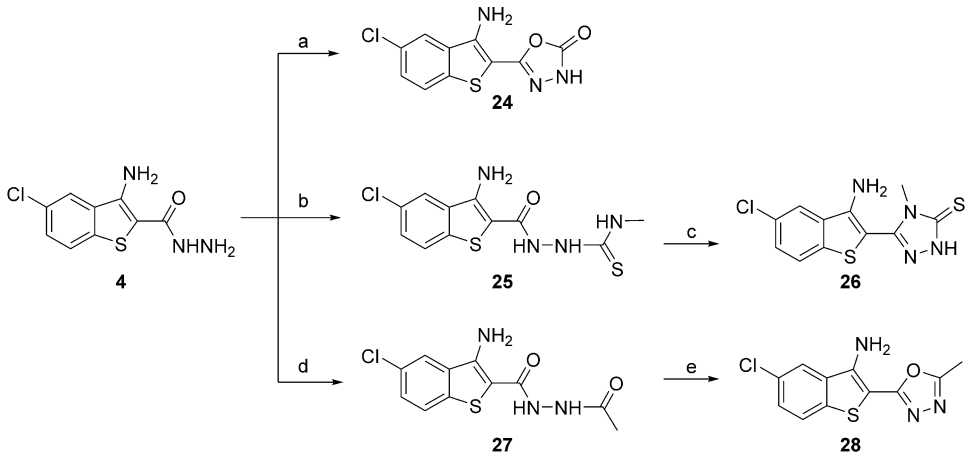
Scheme 3. *Reagents and conditions*: a) thioacetic acid hydrazide, *t*BuOK, DMF, 0 °C; b) CS_2, pyridine, 90 °C.



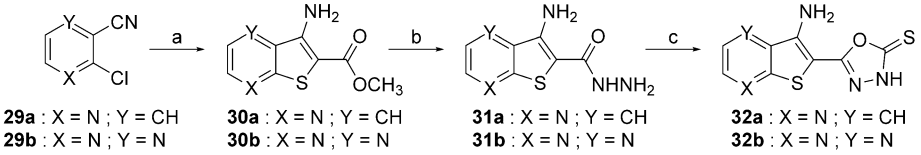
Scheme 2. *Reagents and conditions*: a) ethyl thioacetate, base, DMSO or DMF, 0 °C or RT; b) hydrazine monohydrate, EtOH, reflux; c) CS_2, pyridine, 90 °C; d) 1. 30% NaOH, EtOH, reflux, 2. 1 M HCl; e) 1. $SOCl_2$, THF, reflux, 2. hydrazine solution (1 M in THF), THF, 0 °C→RT; f) 1. HOBt, EDCI, DMF, RT, 2. hydrazine monohydrate, 0 °C→RT; g) thioacetic acid hydrazide, *t*BuOK, DMF, 0 °C.



Scheme 4. *Reagents and conditions*: a) hydrazine monohydrate, EtOH, reflux; b) CS_2, pyridine, 90 °C.



Scheme 5. *Reagents and conditions*: a) CDI, DIPEA, DMF, RT; b) CH_3NCS, 1,4-dioxane, reflux; c) 10% NaOH, reflux; d) Ac_2O, Et_3N, $CHCl_3$, 0 °C→reflux; e) Ph_3P, CBr_4, CH_2Cl_2, 0 °C→RT→reflux.

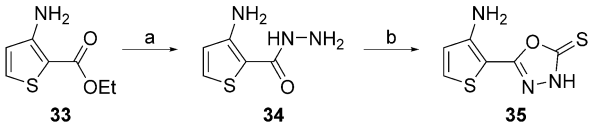


Scheme 6. *Reagents and conditions*: a) methyl thioacetate, Et_3N, MeOH, reflux; b) hydrazine hydrate, EtOH, reflux; c) CS_2, pyridine, reflux.

To prepare analogues with structural modifications of the oxadiazole portion, the synthetic procedures mentioned in Scheme 5 were followed. Starting from key intermediate **4**, three types of modifications were introduced. Reaction of hydrazide **4** with carbonyldiimidazole (CDI) in the presence of a base afforded the 1,3,4-oxadiazole-2-one **24**. Treatment of **4** with methylisothiocyanate led to the *N*-acyl thiosemicarbazide derivative **25**, which, upon holding at reflux in an aqueous solution of sodium hydroxide, yielded 1-methyl-1,3,4-triazole-2-thione **26**. Similarly, heating of **4** with acetic anhydride in the presence of a base resulted in *N*′-acetyl hydrazide **27**. Subsequent ring closing in a Robinson–Gabriel-type reaction using PPh_3/CBr_4 as dehydrating agent afforded 2-methyl-1,3,4-oxadiazole **28** in moderate yield.[16]

The synthesis of a series of analogues with a modified skeleton is shown in Scheme 6. An appropriately substituted 2-chloro-3-cyano-heterocycle **29a**–**b** was condensed with methyl 2-thioacetate under basic conditions to construct the thiophene ring, affording bicyclic 3-amino-2-carboyxlic acid methyl ester analogues **30a**–**b**. The ester functionality was directly transformed into the hydrazide moiety by holding at reflux with an aqueous solution of hydrazine in ethanol. Reaction of the hydrazides with carbon disulfide in pyridine afforded the final 1,3,4-oxadiazole derivatives in good yields.

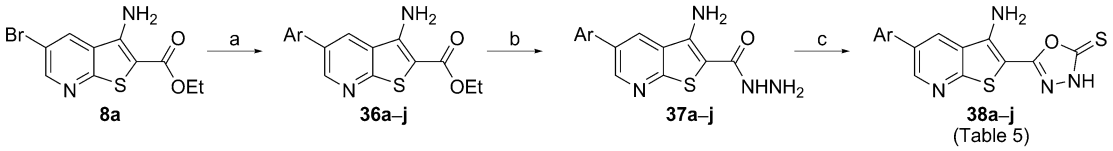
For the synthesis of the monocyclic thiophene analogue **35**, a similar procedure was used starting from ethyl 3-aminothiophene-2-carboxylate **33**. Treatment with hydrazine was followed by oxadiazole formation, yielding the final compound **35** (Scheme 7).



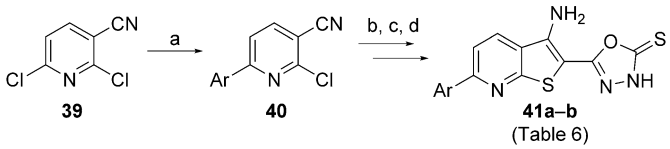
Scheme 7. *Reagents and conditions*: a) hydrazine hydrate, EtOH, reflux; b) CS_2, pyridine, reflux.

For the synthesis of a 5-arylthieno[2,3-*d*]pyridine library, compound **8a** was selected as starting material. Coupling with a wide range of commercially available arylboronic acids under standard Suzuki reactions (K_2CO_3 as a base, $Pd(PPh_3)_4$ as catalyst in a mixture of dioxane and water as solvent) afforded compounds **36a**–**j**.[17] Upon treatment with hydrazine and carbon disulfide, the desired final compounds **38a**–**j** were obtained (Scheme 8).

To gain access to the regioisomeric compounds (i.e., with an aryl group at position 6 of the thieno[2,3-*b*]pyridine scaffold), 2,6-dichloronicotinonitrile **39** was selected as starting material. Reaction with a range of boronic acids under standard Suzuki conditions selectively yields the 2-chloro-6-arylpyridine-3-carbonitrile analogues **40a**–**b**. These types of regioselective Suzuki couplings on similar substrates have been reported before.[18] Nucleophilic displacement of the chlorine by ethyl 2-thioacetate with concomitant ring closure affords the desired 6-arylthieno[2,3-*b*]pyridine core structure. Finally, hydrazide and oxadiazole formation yields the final regioisomeric analogues **41a**–**b** (Scheme 9).



Scheme 8. *Reagents and conditions*: a) $ArB(OH)_2$, K_2CO_3, $Pd(PPh_3)_4$, 1,4-dioxane, H_2O; b) hydrazine hydrate, EtOH, reflux; c) CS_2, pyridine, reflux.



Scheme 9. *Reagents and conditions*: a) $ArB(OH)_2$, K_2CO_3, $Pd(PPh_3)_4$, 1,4-dioxane, H_2O; b) ethyl 2-thioacetate, K_2CO_3, EtOH, reflux; c) hydrazine hydrate, EtOH, reflux; d) CS_2, pyridine, reflux.

Biological evaluation and SAR studies

DRAK2 binding studies

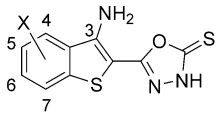
The SAR study started by probing the optimal substitution pattern on the benzene moiety. A series of compounds with small structural variations were tested for their affinity for the DRAK2 enzyme. The choice of substituents was mainly driven by the commercial availability of the necessary starting materials. Compounds were screened at a concentration of 10 μM in the above-mentioned kinase binding assay, and the results are expressed as percent of control. As the assay measures the compound's ability to inhibit binding of a bait ligand, 0% control corresponds to full inhibition, and 100% control to no inhibition. As can be derived from the data in Table 1, the presence of strong electron-withdrawing substituents (CF_3 and NO_2) at different positions of the phenyl ring affords benzothiophene analogues that display decreased affinity for DRAK2 (e.g., compound **17 f**) or are completely devoid of DRAK2 affinity (compounds **17 b**, **17 e**, and **20**). On the other hand, the presence of an electron-donating methoxy group (compounds **17 a** and **17 c**) affords analogues that are more potent, displaying values less than 10% of control. The most potent congeners were, however, found among the halogen-substituted (chlorine/bromine) and unsubstituted benzothiophene analogues, with %Ctrl of 5 or less. The 5-bromo (compound **10 b**) and unsubstituted (compound **10 a**) analogues were subjected to dose–response curves, affording K_d values of 0.3 and 0.61 μM, respectively, which is very similar to the biological data obtained for the original hit compound **1** (Table 1).

To determine the importance of the amino group for DRAK2 binding, two novel analogues were evaluated. Whereas the 3-desamino congener **23 a** shows less potent activity, the 3-methyl analogue **23 b** is equipotent with the 3-amino derivative **10 a** (Table 2).

To assess the SAR around the oxadiazole moiety, three novel analogues were assessed for DRAK2 binding (Table 3). The thioxo group is important for DRAK2 affinity, as its replacement by oxo (compound **24**) or methyl (compound **28**) leads to decreased DRAK2 affinity. The oxadiazole ring is also essential for DRAK2 binding, as the triazole analogue **26** completely lacks DRAK2 affinity.

Besides the benzothiophene scaffold of the original hit compound **1**, a number of other core structures was also evaluated (Table 4). For synthetic feasibility reasons, the unsubstituted benzo-analogue **10 a** was chosen as reference. A simplified analogue (compound **35**), in which the fused benzene ring is completely removed, was also evaluated. A fused bicycle seems to be essential for DRAK2 binding, as the monocyclic thiophene analogue **35** is completely devoid of any DRAK2 affinity. On the other hand, the replacement of the phenyl ring by a pyridine (affording thieno[2,3-*b*]pyridine **32 a**) or a pyrazine (yielding thieno[2,3-*b*]pyrazine **32 b**), gives compounds with a fi-

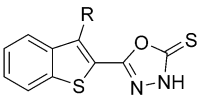
Table 1. SAR of the benzene moiety.



Compd	X	%Ctrl @ 10 μM[a]	K_d [μM][a]
1	5-Cl	3.6	0.25
10 a	H	0.6	0.61
10 b	5-Br	5.2	0.3
12	6-Cl	4.5	ND
15	5-NO_2	16	ND
17 a	4-OCH_3	9.6	ND
17 b	4-CF_3	35	ND
17 c	5-OCH_3	7.6	ND
17 d	5-OCF_3	31	ND
17 e	7-CF_3	22	ND
17 f	5-CF_3	9.1	ND
20	6-CF_3	32	ND

[a] Values are the average of two independent experiments; ND: not determined.

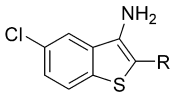
Table 2. SAR of the amino group.

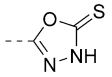
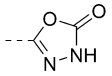
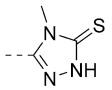
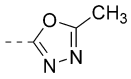


Compd	R	%Ctrl @ 10 μM[a]	K_d [μM][a]
10 a	NH_2	0.6	0.61
23 b	CH_3	1.1	0.59
23 a	H	7	ND

[a] Values are the average of two independent experiments; ND: not determined.

Table 3. SAR of the oxadiazole moiety.



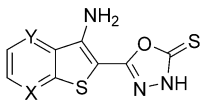
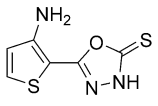
Compd	R	% Ctrl @ 10 μM[a]	K_d [μM][a]
1		3.6	0.25
24		12	ND
26		82	ND
28		39	ND

[a] Values are the average of two independent experiments; ND: not determined.

vefold stronger binding affinity for DRAK2 relative to the benzothiophene skeleton **10a**.

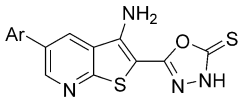
As the pyridine ring imparts better DRAK2 binding affinity, the thieno[2,3-*b*]pyridine scaffold was selected for further structural variation, in combination with other favorable substituents (i.e., the amino group at position 3 and the oxadiazole moiety at position 2). As the presence of a halogen (chlorine/bromine) at position 5 of the benzothiophene scaffold (compounds **1** and **10b**) affords compounds with potent activity, it seems that structural variety is tolerated at that position. In order to increase DRAK2 affinity, we envisioned that it was necessary to introduce larger substituents to exploit additional interactions with the DRAK2 enzyme. Given the low molecular weight of the lead compounds, adding additional groups was still justified. Therefore, a series of compounds was made with structural variation on the 5-aryl portion (Table 5). It seems that the presence of an aryl moiety at position 5 leads to high affinity for DRAK2 and that a rather high degree of structural variation is tolerated at that position. Halogens (fluorine/chlorine) on the phenyl moiety afford compounds **38a**–**d** with K_d values

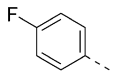
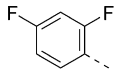
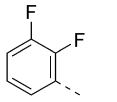
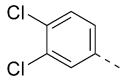
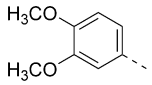
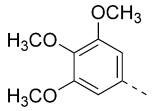
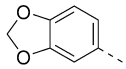
Table 4. SAR of the scaffold.

Structure	Compd	X	Y	% Ctrl @ 10 μM[a]	K_d [μM][a]
	**10a**	CH	CH	0.6	0.61
	32a	N	CH	0.65	0.12
	32b	N	N	1.5	0.14
	**35**	–	–	33	ND

[a] Values are the average of two independent experiments; ND: not determined.

Table 5. SAR of the aryl moiety.



Compd	Ar	% Ctrl @ 10 μM[a]	K_d [μM][a]
38a		0	0.038
38b		3.6	0.110
38c		3.1	0.390
38d		2.5	0.270
38e		3.6	0.010
38f		2	0.016
38g		0.65	0.093
38h		6.6	0.008
38i		7.7	0.009
38j		2.2	0.043

[a] Values are the average of two independent experiments.

in the range of 110–380 nM. The 4-fluorophenyl analogue **38a** is the most potent congener, displaying a K_d value of 38 nM. The presence of electron-donating substituents gives rise to very strong binding affinity, with respective K_d values of 10 and 16 nM for the 3,4-dimethoxy (compound **38e**) and the 3,4,5-trimethoxyphenyl (compound **38f**) congeners. Similarly, the benzodioxolane analogue **38g** does show good binding affinity (K_d = 93 nM). The most potent compounds within this series was found among the 5-heteroaryl derivatives. In particular, five-membered heteroaromatics such as thienyl (compound **38h**) and furanyl (compound **38i**) give rise to very potent compounds, with K_d values of 8 and 9 nM, respectively. A 3-pyridinyl moiety (compound **38j**) leads to a fivefold drop in DRAK2 affinity.

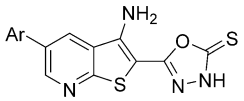
It is clear from the data in Table 5 that the presence of an aryl group at position 5 imparts high

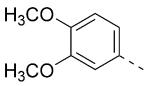
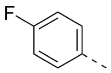
DRAK2 binding affinity. To determine the exact position necessary for optimal activity, the corresponding 6-aryl regioisomers **41a–b** were evaluated as potential DRAK2 ligands (Table 6). Both compounds show strong affinity for DRAK2, but are, however, sevenfold less potent than the 5-substituted congeners.

Functional DRAK2 inhibition

Up to now, compounds were evaluated in assays without the use of ATP or substrate. As a result, these assays measure binding rather than activity. Therefore, compounds with the highest affinity for DRAK2 were also evaluated for functional DRAK2 inhibition in a classical in vitro enzymatic activity assay with a radioactive read-out (SignalChem). As can be derived from Table 7, the compounds behave as potent inhibitors of DRAK2, with IC_{50} values in the range of 30–60 nM.

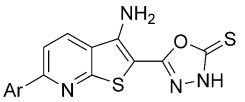
Table 7. Profile of selected lead compounds.

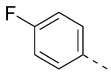


Compd	Ar	K_d [μM][a]	IC_{50} [μM][a]	*clog P*	LE [kcal mol^{-1}]
38a		0.010	0.058	3.17	0.42
38b		0.038	0.057	3.59	0.44
38d		0.008	0.029	3.12	0.53
38e		0.009	0.033	2.62	0.52

[a] Values are the average of two independent experiments.

Table 6. SAR of 6-arylthieno[2,3-*b*]pyridines.



Compd	Ar	%Ctrl @ 10 μM[a]	K_d [μM][a]
41a		0.3	0.270
41b		0.1	0.061

[a] Values are the average of two independent experiments.

The calculation of LE values is useful to determine the impact of the addition of more molecular bulk to the original compounds. Although in the optimization campaign, the molecular weight increases (by the insertion of aryl groups affording compounds **38a–j**), these optimized compounds are still highly efficient, displaying LE values of at least 0.4 kcal mol^{-1} per non-hydrogen atom. Moreover, the optimization campaign proceeded without any gain in lipophilicity, as most compounds display *clog P* values between 2.6 and 3.6.

Selectivity profile

The human kinome comprises more than 500 protein kinases. The vast majority of the compounds target the kinase ATP site, and as all kinases have an ATP binding site, there is great potential for cross-reactivity. In addition, cell-permeable kinase inhibitors are often used in cell biology as specific pathway modifiers, and therefore selectivity is an important issue.

DRAK2 is a serine/threonine kinase belonging to the DAPK family. This family consists of DAPK1, DAPK2 (also called DRP-1) and DAPK3 (also known as ZIPK, Zipper-interacting protein kinase). They share approximately 80% identity in their kinase domains. DRAK1 and DRAK2 are two other members of this family, but they are more distantly related, as they share only approximately 50% identity with DAPK1. To get an initial idea about the selectivity of these compounds, some representatives were screened for affinity for the different kinases of the DAPK family at a concentration of 10 μM (Table 8). It is clear that most of the DRAK2 inhibitors also have a strong binding affinity for DRAK1, DAPK1, and DAPK2, whereas the affinity for DAPK3 is generally less. As a representative example, dose–response curves were generated for compound **38h**, in order to determine K_d values for DRAK1, DAPK1, and DAPK2. It revealed that only a sevenfold level of selectivity was present between DRAK2 and DAPK1. However, compound **38d** (bearing a 5-(3,4-dichlorophenyl) moiety), although being less active as a DRAK2 binder ($K_d = 0.27$ μM) shows an excellent selectivity profile, lacking any affinity for the other members of DAPK family.

Table 8. Selectivity profile of selected lead compounds.

Compd	K_d [μM][a]	%Ctrl @ 10 μM[a]			
	DRAK2	DRAK1	DAPK1	DAPK2	DAPK3
38a	0.038	0.35	0.55	0.9	6
38d	0.270	23	46	30	36
38e	0.010	0.35 0.16[b]	0.4	0.55	5
38h	0.008	0.35 0.099[b]	0.35 0.054[b]	0.35 0.647[b]	1.8
38i	0.009	0.4	1	1.4	6

[a] Values are the average of two independent experiments. [b] K_d value [μM].

Conclusions

This manuscript describes the synthesis and biological evaluation of DRAK2 inhibitors. Starting from a hit with moderate binding affinity for the DRAK2 enzyme, a novel series of compounds was discovered displaying strong affinity for the DRAK2 enzyme. In addition, these compounds also behave as strong, functionally active inhibitors. Moreover, based on determined in silico parameters (such as *clog P* and LE), these compounds possess the desired physicochemical parameters to be used as leads in drug discovery programs.

The most promising compounds were also tested in a panel of kinases, closely related to DRAK2. Whereas most of the analogues lack selectivity, one compound has been discovered that does show selective DRAK2 affinity, although being less potent. This analogue might serve as a starting point for the discovery of novel and specific DRAK2 inhibitors.

Experimental Section

Chemistry

General: For all reactions, analytical-grade solvents were used. All moisture-sensitive reactions were carried out in oven-dried glassware (135 °C). ^{1}H and ^{13}C NMR spectra: Bruker Avance 300 (^{1}H NMR: 300 MHz, ^{13}C NMR: 75 MHz), Bruker Avance 500 (^{1}H NMR: 500 MHz, ^{13}C NMR: 125 MHz, ^{19}F NMR: 470 MHz) or Bruker Avance 600 (^{1}H NMR: 600 MHz, ^{13}C NMR: 150 MHz), using tetramethylsilane as internal standard for ^{1}H NMR spectra, residual solvent peak for [D$_6$]DMSO (39.52 ppm) or $CDCl_3$ (77.16 ppm) for ^{13}C NMR spectra and trifluoroacetic acid (−77.0 ppm) for ^{19}F NMR. Abbreviations used are: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad signal. Coupling constants are expressed in Hz. Mass spectra are obtained with a Finnigan LCQ Advantage Max (ion trap) mass spectrophotometer from Thermo Finnigan, San Jose, CA, USA. High-resolution mass spectrometry spectra were acquired on a quadrupole orthogonal acceleration time-of-flight mass spectrometer (Synapt G2 HDMS, Waters, Milford, MA). Samples were infused at 3 μL min^{-1} and spectra were obtained in positive (or negative) ionization mode with a resolution of 15000 (FWHM) using leucine enkephalin as lock mass. Pre-coated aluminum sheets (Fluka Silica gel/TLC-cards, 254 nm) were used for TLC. Column chromatography (CC) was performed on ICN silica gel 63–200 mesh, 60 Å.

Ethyl 5-chloro-3-isothiocyanatobenzo[*b*]thiophene-2-carboxylate (5): To a solution of $CSCl_2$ (0.026 mL, 0.340 mmol) in acetone (0.35 mL) was added dropwise a solution of amino ester **3** in acetone (1.15 mL) and the mixture was stirred overnight at room temperature. Then, an additional amount of $CSCl_2$ (0.020 mL) was added and stirring was continued for 24 h. Water was added, the precipitate was filtered off, washed with water until the filtrate reached neutral pH, and dried affording compound **5** as a white solid (85 mg, 91%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 1.45 (t, J = 7.2 Hz, 3H), 4.46 (q, J = 7.2 Hz, 2H), 7.48 (dd, J = 8.7 Hz, J = 2.1 Hz, 1H), 7.72 (dd, J = 8.7 Hz, J = 0.3 Hz, 1H), 7.88 ppm (dd, J = 2.1 Hz, J = 0.3 Hz, 1H).

3-Amino-8-chloro-2-thioxobenzothieno[3,2-*d*]pyrimidin-4(1*H*)-one (6): A mixture of isothiocyanate **5** (84 mg, 0.282 mmol) and pyridine (0.045 mL, 0.564 mmol) in toluene (3.0 mL) was treated with a hydrazine solution (35% in H_2O, 0.055 mL). The resulting mixture was stirred at 80 °C for 2.5 h. The mixture was cooled to room temperature. The resulting precipitate was filtered off, washed with toluene, hexane and dried. The crude product was purified by column chromatography (CH_2Cl_2/MeOH 98:2→9:1) affording compound **6** as a white solid (57 mg, 71%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 6.52 (br s, 2H), 7.70 (dd, J = 8.7 Hz, J = 1.8 Hz, 1H), 8.18 (d, J = 8.7 Hz, 1H), 8.75 (d, J = 1.8 Hz, 1H), 14.07 ppm (br s, 1H); ^{13}C NMR (300 MHz, [D$_6$]DMSO): δ = 114.61, 123.36, 125.70, 129.20, 130.71, 138.54, 138.62, 152.93, 168.71 ppm; HRMS (ESI): m/z $[M-H]^-$ calcd for $C_{10}H_6ClN_3OS_2$: 281.9568, found 281.9561.

Ethyl 3-amino-5-chlorobenzo[*b*]thiophene-2-carboxylate (3): A solution of 5-chloro-2-fluorobenzonitrile (**2**) (1.50 g, 9.64 mmol) in DMF (8.9 mL) was cooled in an ice-water bath and ethyl thioacetate (1.11 mL, 10.12 mmol) was added dropwise. After stirring for 30 min at 0 °C, an aqueous solution of NaOH (5 M, 2.9 mL, 14.5 mmol) was added. Stirring was continued for another 2 h. Then, water was added to the mixture and the resulting precipitate was filtered off, washed with water and dried in a vacuum desiccator affording compound **3** as a white solid (2.17 g, 88%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 1.29 (t, J = 7.2 Hz, 3H), 4.27 (q, J = 7.2 Hz, 2H), 7.16 (br s, 2H), 7.54 (dd, J = 8.7 Hz, J = 1.8 Hz, 1H), 7.88 (d, J = 8.7 Hz, 1H), 8.30 ppm (d, J = 1.8 Hz, 1H).

Ethyl 3-amino-5-bromobenzo[*b*]thiophene-2-carboxylate (8; X = 5-Br): The title compound was synthesized from 5-bromo-2-fluorobenzonitrile (**7**) (100 mg; 0.50 mmol) and ethyl thioacetate (0.058 mL, 0.68 mmol) using procedure described for the synthesis of compound **3.** The crude product was purified by flash chromatography on silica gel (hexane/EtOAc 91:9) affording compound **8** as a pale-yellow solid (90 mg, 60%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 1.29 (t, J = 7.2 Hz, 3H), 4.27 (q, J = 7.2 Hz, 2H), 7.16 (br s, 2H), 7.64 (dd, J = 8.7 Hz, J = 1.8 Hz, 1H), 7.82 (d, J = 8.7 Hz, 1H), 8.44 ppm (d, J = 1.8 Hz, 1H).

Ethyl 3-amino-5-nitrobenzo[*b*]thiophene-2-carboxylate (8; X = 5-NO_2): To a solution of 5-nitro-2-fluorobenzonitrile (**7**) (1.0 g, 6.02 mmol) and Et_3N (1.68 mL, 12.04 mmol) in DMSO (5.45 mL) was added ethyl thioacetate (0.660 mL, 6.02 mmol) dropwise. After stirring for 3 h at room temperature, the mixture was poured into ice-water, stirred for 10 min and the resulting precipitate was filtered off, washed with water and dried in vacuum desiccator affording compound **8** as an orange solid (1.45 g, 90%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 1.31 (t, J = 7.2 Hz, 3H), 4.29 (q, J = 7.2 Hz, 2H), 7.44 (br s, 2H), 8.10 (d, J = 8.9 Hz, 1H), 8.28 (d, J = 8.9 Hz, 1H), 9.22 ppm (s, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 14.53, 60.33, 97.29, 119.56, 122.21, 124.55, 131.58, 144.70, 145.06, 149.83, 164.14 ppm.

Ethyl 3-amino-4-methoxybenzo[*b*]thiophene-2-carboxylate (8; X = 4-OCH_3): To a solution of 2-fluoro-6-methoxybenzonitrile (**7**) (300 mg; 1.985 mmol) and ethyl thioacetate (0.435 mL; 3.970 mmol) in DMF (1.4 mL) was added potassium *tert*-butoxide (445 mg; 3.970 mmol) portionwise at 0 °C. The cooling bath was removed and the mixture was stirred at room temperature for 1.5 h. The reaction mixture was poured into ice-water and stirred vigorously for 5 min. The resulting precipitate was filtered off, washed with water, dried and crystallized from MeOH, affording 169 mg of white solid. The filtrate was concentrated and purified by silica gel flash chromatography, the mobile phase being a mixture of cyclohexane and EtOAc (in a ratio of 3:1) affording an additional 114 mg of compound **8** as white powder (total yield: 283 mg, 57%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 1.28 (t, J = 7.2 Hz, 3H), 3.93 (s, 3H), 4.23 (q, J = 7.2 Hz, 2H), 6.88 (d, J = 7.5 Hz, 1H), 6.98 (br s, 2H), 7.34 (d, J = 7.5 Hz, 1H), 7.43 ppm (t, J = 7.5 Hz, 1H).

Ethyl 3-amino-4-(trifluoromethyl)benzo[*b*]thiophene-2-carboxylate (8; X = 4-CF_3): The title compound was synthesized from 2-fluoro-6-(trifluoromethyl)benzonitrile (**7**) (300 mg, 1.586 mmol) and ethyl thioacetate (0.348 mL, 3.172 mmol) using the procedure described for the synthesis of **8 (X = 4-OCH_3)**. The crude product was filtered off, washed with water and dried affording target compound **8 (X = 4-CF_3)** as a pale-yellow powder (456 mg, 99%): ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 1.32 (t, J = 7.2 Hz, 3H), 4.32 (q, J = 7.2 Hz, 2H), 6.56 (br s, 2H), 7.69 (t, J = 7.8 Hz, 1H), 7.86 (d, J = 7.2 Hz, 1H), 8.26 ppm (d, J = 8.1 Hz, 1H).

Ethyl 3-amino-5-(trifluoromethyl)benzo[*b*]thiophene-2-carboxylate (8; X = 5-CF_3): The title compound was synthesized from 2-fluoro-5-(trifluoromethyl)benzonitrile (**7**) (300 mg, 1.586 mmol) and ethyl thioacetate (0.348 mL, 3.172 mmol) using the procedure described for the synthesis of **8 (X = 4-OCH_3)**. The crude product was filtered off, washed with water and dried affording compound **8 (X = 5-CF_3)** as a pale-yellow powder (439 mg, 96%): ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 1.31 (t, J = 7.2 Hz, 3H), 4.29 (q, J = 7.2 Hz, 2H), 7.32 (br s, 2H), 7.80 (d, J = 8.7 Hz, 1H), 8.09 (d, J = 8.7 Hz, 1H), 8.66 ppm (s, 1H).

Ethyl 3-amino-5-methoxybenzo[*b*]thiophene-2-carboxylate (8; X = 5-OCH_3): The title compound was synthesized from 2-fluoro 5-methoxybenzonitrile (**7**) (300 mg, 1.985 mmol) and ethyl thioacetate (0.443 mL, 3.970 mmol) using the procedure described for the synthesis of **8 (X = 4-OCH_3)**. The crude product was purified by flash chromatography on silica gel (using CH_2Cl_2 as mobile phase) affording compound **8 (X = 5-OCH_3)** as a pale-yellow powder (141 mg, 28%): ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 1.29 (t, J = 7.2 Hz, 3H), 3.82 (s, 3H), 4.25 (q, J = 7.2 Hz, 2H), 7.08 (br s, 2H), 7.14 (dd, J = 9.0 Hz, J = 2.4 Hz, 1H), 7.69 (d, J = 9 Hz, 1H), 7.72 ppm (d, J = 2.4 Hz, 1H).

Ethyl 3-amino-5-(trifluoromethoxy)benzo[*b*]thiophene-2-carboxylate (8; X = 5-OCF_3): The title compound was synthesized from 2-fluoro 5-(trifluoromethoxy)benzonitrile (**7**) (300 mg, 1.463 mmol) and ethyl thioacetate (0.327 mL, 2.926 mmol) using the procedure described for the synthesis of **8 (X = 4-OCH_3)**. The crude product was filtered off, washed with water and dried affording compound **8 (X = 5-OCF_3)** as a pale-yellow powder (415 mg, 93%): ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 1.30 (t, J = 7.2 Hz, 3H), 4.28 (q, J = 7.2 Hz, 2H), 7.22 (br s, 2H), 7.53 (dd, J = 9 Hz, J = 0.9 Hz, 1H), 7.98 (d, J = 9 Hz, 1H), 8.24 ppm (d, J = 0.9 Hz, 1H).

Ethyl 3-amino-7-(trifluoromethyl)benzo[*b*]thiophene-2-carboxylate (8; X = 7-CF_3): The title compound was synthesized from 2-fluoro-3-(trifluoromethyl)benzonitrile (**7**) (300 mg, 1.586 mmol) and ethyl thioacetate (0.348 mL, 3.172 mmol) using the procedure described for the synthesis of **8 (X = 4-OCH_3)**. The crude product was filtered-off, washed with water and dried affording compound **8 (X = 7-CF_3)** as a pale-beige powder (458 mg, quant.): ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 1.31 (t, J = 7.2 Hz, 3H), 4.29 (q, J = 7.2 Hz, 2H), 7.30 (br s, 2H), 7.63 (br t, $J \approx$ 7.8 Hz, 1H), 7.94 (d, J = 7.2 Hz, 1H), 8.46 ppm (d, J = 8.1 Hz, 1H).

3-Amino-5-chlorobenzo[*b*]thiophene-2-carbohydrazide (4): To a suspension of **3** (40 mg, 0.157 mmol) in EtOH (1 mL) was added hydrazine monohydrate (0.174 mL) and the mixture was held at reflux overnight. The volatiles were removed under reduced pressure. The solid residue was washed with water and dried in vacuum desiccator affording compound **4** as a pale-yellow solid (22 mg, 58%): ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 4.44 (br s, 2H), 7.06 (br s, 2H), 7.43 (d, J = 7.5 Hz, 1H), 8.00–8.05 (m, 2H), 9.00 ppm (br s, 1H).

3-Aminobenzo[*b*]thiophene-2-carbohydrazide (9a): Ethyl carboxylate **8 (X = H)** (930 mg, 4.5 mmol) was treated according to the procedure described for the synthesis of hydrazide **3** affording compound **9a** as a pale-yellow solid (930 mg, > 99%): ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 8.92 (br s, 1H), 8.02 (d, J = 7.7 Hz, 1H), 7.81 (d, J = 7.9 Hz, 1H), 7.41 (m, 2H), 7.01 (br s, 2H), 4.39 ppm (br s, 2H); MS: m/z (%): 208.0 ([M + H]$^+$, 100).

3-Amino-5-bromobenzo[*b*]thiophene-2-carbohydrazide (9b): Ethyl carboxylate **8 (X = 5-Br)** (80 mg, 0.268 mmol) was treated according to the procedure described for the synthesis of hydrazide **4** affording compound **9b** as a pale-yellow solid (52 mg, 68%): ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 4.33 (br s, 2H), 7.03 (br s, 2H), 7.58 (d, J = 8.4 Hz, 1H), 7.81 (d, J = 8.4 Hz, 1H), 8.32 (s, 1H), 8.95 ppm (br s, 1H).

3-Amino-6-chlorobenzo[*b*]thiophene-2-carbohydrazide (11): To a solution of 4-chloro-2-nitrobenzoznitrile (**7**) (100 mg, 0.643 mmol) in DMF (0.460 mL) was added thioacetic acid hydrazide[15] (102 mg, 0.964 mmol) and the mixture was cooled to 0 °C. Potassium *tert*-butoxide (144 mg, 1.286 mmol) was added portionwise and the mixture was stirred at room temperature for 1 h. The reaction mixture was poured into ice-water. The precipitate was filtered off, washed with H_2O and dried affording compound **11** as a red solid (96 mg, 62%). The crude product was not characterized and used in the next step without further purification.

3-Amino-6-(trifluoromethyl)benzo[*b*]thiophene-2-carbohydrazide (19): 2-Nitro-4-(trifluoromethyl)benzonitrile (**18**) (100 mg, 0.463 mmol) was treated according to the procedure described for the synthesis of hydrazide **11** affording compound **19** as a yellow solid (102 mg, 80%). The crude product was not characterized and used in the next step without further purification.

Synthesis of 3-aminobenzo[*b*]thiophene-2-carboxylic acids (13): A mixture of ethyl carboxylate **8** (0.563 mmol) and 30% aqueous NaOH (0.282 mL, 2.815 mmol) in EtOH (10 mL) was held at reflux for 1.5–5 h, during which a precipitate was formed. The progress of the reaction was monitored by TLC. After disappearance of starting material, the volatiles were removed under reduced pressure. The solid residue was re-dissolved in H_2O and the resulting solution was brought to pH ~ 2 using a 1 M HCl solution. The resulting precipitate was filtered off, washed with water and dried (purified by flash chromatography when needed).

3-Amino-4-methoxybenzo[*b*]thiophene-2-carboxylic acid (13; X = 4-OCH_3): Ethyl carboxylate **8 (X = 4-OCH_3)** (100 mg, 0.398 mmol) was treated according to the general procedure given above affording compound **13 (X = 4-OCH_3)** as a pale-yellow powder (69 mg, 78%): ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 3.94 (s, 3H), 6.87 (d, J = 7.5 Hz, 1H), 7.32–7.49 ppm (m, 2H).

3-Amino-4-(trifluoromethyl)benzo[*b*]thiophene-2-carboxylic acid (13; X = 4-CF_3): Ethyl carboxylate **8 (X = 4-CF_3)** (400 mg, 1.384 mmol) was treated according to the general procedure given above. The crude product was purified by column chromatography (CH_2Cl_2) affording compound **13 (X = 4-CF_3)** as a pale-yellow powder (127 mg, 35%): ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 7.66 (t, J = 7.8 Hz, 1H), 7.85 (d, J = 7.2 Hz, 1H), 8.25 ppm (d, J = 8.1 Hz, 1H).

3-Amino-5-nitrobenzo[*b*]thiophene-2-carboxylic acid (13; X = 5-NO_2): Ethyl carboxylate **8 (X = 5-NO_2)** (157 mg, 0.563 mmol) was treated according to the general procedure given above affording compound **13 (X = 5-NO_2)** as a dark brown solid (103 mg, 77%). The product was not characterized and used in the next step without further purification.

3-Amino-5-methoxybenzo[*b*]thiophene-2-carboxylic acid (13; X = 5-OCH_3): Ethyl carboxylate **8** (X = 5-OCH_3) (135 mg, 0.537 mmol) was treated according to the general procedure given above affording compound **13** (X = 5-OCH_3) as a pale-beige powder (107 mg, 89%): ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 3.83 (s, 3H), 7.12 (d, J = 9.0 Hz, 1H), 7.67–7.70 ppm (m, 2H).

3-Amino-5-(trifluoromethoxy)benzo[*b*]thiophene-2-carboxylic acid (13; X = 5-OCF_3): Ethyl carboxylate **8** (X = 5-OCF_3) (250 mg, 0.819 mmol) was treated according to the general procedure given above affording compound **13** (X = 5-OCF_3) as a pale-beige powder (197 mg, 87%): ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 7.49 (d, J = 8.7 Hz, 1H), 7.97 (d, J = 8.7 Hz, 1H), 8.22 ppm (s, 1H).

3-Amino-5-(trifluoromethyl)benzo[*b*]thiophene-2-carboxylic acid (13; X = 5-CF_3): Ethyl carboxylate **8** (X = 5-CF_3) (300 mg, 1.038 mmol) was treated according to the general procedure given above. The crude product was extracted from the water layer with EtOAc, dried using $MgSO_4$, filtered and concentrated in vacuo affording compound **13** (X = 5-CF_3) as a pale-yellow powder (232 mg, 86%). The product was not characterized and used in the next step without further purification.

3-Amino-7-(trifluoromethyl)benzo[*b*]thiophene-2-carboxylic acid (13; X = 7-CF_3): Ethyl carboxylate **8** (X = 7-CF_3) (200 mg,0.692 mmol) was treated according to the general procedure given above affording compound **13** (X = 7-CF_3) as a white powder (153 mg, 85%): ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 7.62 (t, J = 7.8 Hz, 1H), 7.93 (d, J = 7.6 Hz, 1H), 8.43 ppm (d, J = 8.1 Hz, 1H).

3-Amino-5-nitrobenzo[*b*]thiophene-2-carbohydrazide (14): A mixture of carboxylic acid **13** (X = 5-NO_2) (100 mg, 0.419 mol) and $SOCl_2$ (0.032 mL, 0.504 mmol) in THF (5 mL) was held at reflux for 2 h. Then additional amount of $SOCl_2$ (0.020 mL, 0.275 mmol) was added and reflux was continued overnight. Volatiles were removed under reduced pressure; the solid residue was dissolved in THF (5 mL) and added dropwise to the cold (ice-water bath) mixture of hydrazine (1 M in THF, 1.263 mL) and Et_3N (0.071 mL, 0.505 mmol). Cooling bath was removed and the mixture was stirred at RT for 1.5 h. Next, the mixture was concentrated under reduced pressure; the residue was washed with H_2O and dried affording compound **14** as deep red solid (102 mg, 96%) which was used in the next step without further purification and spectral analysis.

Synthesis of hydrazides (16a–16f): To a mixture of carboxylic acid **13** (1 mmol) and HOBt monohydrate (1 mmol) in DMF (3.75 mL), was added EDCI hydrochloride (1.2 mmol) and the mixture was stirred at room temperature for 1–2.5 h (TLC analysis). Next, it was cooled in ice-water bath and hydrazine monohydrate (0.736 mL, 15 mol. equiv) was added. The mixture was stirred at room temperature and the progress of the reaction was monitored by TLC. Water was added and resulted precipitate was filtered off, washed with H_2O and dried. The crude product was purified by column chromatography.

3-Amino-4-methoxybenzo[*b*]thiophene-2-carbohydrazide (16a): Carboxylic acid **13** (X = 4-OCH_3) (65 mg, 0.291 mmol) was treated according to the general procedure given above. The crude product was purified by column chromatography (CH_2Cl_2/MeOH 99:1) affording compound **16a** as a slightly purple solid (36 mg, 52%): ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 3.92 (s, 3H), 4.35 (br s, 2H), 6.84 (dd, J = 6.9 Hz, J = 1.5 Hz, 1H), 6.97 (br s, 2H), 7.32–7.39 (m, 2H), 8.79 ppm (br s, 1H).

3-Amino-4-(trifluoromethyl)benzo[*b*]thiophene-2-carbohydrazide (16b): Carboxylic acid **13** (X = 4-CF_3) (122 mg, 0.467 mmol) was treated according to the general procedure given above. The crude product was purified by column chromatography (CH_2Cl_2/MeOH 99:1) affording compound **16b** as a pale-yellow solid (69 mg, 54%). The crude product was not characterized and used in the next step without further purification.

3-Amino-5-methoxybenzo[*b*]thiophene-2-carbohydrazide (16c): Carboxylic acid **13** (X = 5-OCH_3) (103 mg, 0.461 mmol) was treated according to the general procedure given above. The crude product was purified by column chromatography (CH_2Cl_2/MeOH 100:0→96:4) affording compound **16c** as a solid (88 mg, 75%): ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 3.82 (s, 3H), 4.37 (br s, 2H), 6.95 (br s, 2H), 7.08 (dd, J = 8.7 Hz, J = 2.1 Hz, 1H), 7.61 (d, J = 2.1 Hz, 1H), 7.68 (d, J = 8.7 Hz, 1H), 8.68 ppm (br s, 1H).

3-Amino-5-(trifluoromethoxy)benzo[*b*]thiophene-2-carbohydrazide (16d): Carboxylic acid **13** (X = 5-OCF_3) (191 mg, 0.689 mmol) was treated according to the general procedure given above. The crude product was purified by column chromatography (CH_2Cl_2/MeOH 100:0→96:4) affording compound **16d** as a pale-yellow solid (151 mg, 71%): ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 4.42 (br s, 2H), 7.08 (br s, 2H), 7.44 (d, J = 8.4 Hz, 1H), 7.96 (d, J = 8.4 Hz, 1H), 8.12 (s, 1H), 9.07 ppm (br s, 1H).

3-Amino-7-(trifluoromethyl)benzo[*b*]thiophene-2-carbohydrazide (16e): Carboxylic acid **13** (X = 7-CF_3) (148 mg, 0.567 mmol) was treated according to the general procedure given above. Crude product was purified by column chromatography (CH_2Cl_2/MeOH 98:2) affording compound **16e** as a pale-yellow solid (120 mg, 77%): ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 4.42 (br s, 2H), 7.14 (br s, 2H), 7.59 (br t, J = 7.5 Hz, 1H), 7.87 (d, J = 7.2 Hz, 1H), 8.35 (d, J = 8.1 Hz, 1H), 9.16 ppm (br s, 1H).

3-Amino-5-(trifluoromethyl)benzo[*b*]thiophene-2-carbohydrazide (16f): Carboxylic acid **13** (X = 5-CF_3) (200 mg, 0.766 mmol) was treated according to the general procedure given above. Crude product was purified by column chromatography (CH_2Cl_2/MeOH 98:2) affording compound **16f** as a pale-yellow solid (92 mg, 44%): ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 4.43 (br s, 2H), 7.19 (br s, 2H), 7.73 (d, J = 8.4 Hz, 1H), 8.07 (d, J = 8.4 Hz, 1H), 8.53 (s, 1H), 9.13 ppm (br s, 1H).

Synthesis of 5-(3-aminobenzo[*b*]thiophen-2-yl)-1,3,4-oxadiazole-2-thiones (1, 10a, 10b, 12, 15, 17a–17f, 20): Acyl hydrazide **4, 9b, 11, 14, 16a–16f** or **19** (0.100 mmol) was treated with CS_2 (0.250 mL) in pyridine (0.750 mL) under reflux for 3 h. Volatiles were removed under reduced pressure and the crude product was purified by flash chromatography.

5-(3-Amino-5-chlorobenzo[*b*]thiophen-2-yl)-1,3,4-oxadiazole-2(3*H*)-thione (1): Acyl hydrazide **4** (20 mg, 0.083 mmol) was treated according to the general procedure given above. The crude product was purified by column chromatography (CH_2Cl_2/EtOAc 10:1→ 4:1) affording compound **1** as a yellow solid (11 mg, 50%): ^{1}H NMR (500 MHz, [D_6]DMSO): δ = 6.82 (br s, 2H), 7.52 (dd, J = 8.5 Hz, J = 2.0 Hz, 1H), 7.97 (d, J = 8.7 Hz, 1H), 8.31 ppm (d, J = 2.0 Hz, 1H); ^{13}C NMR (125 MHz, [D_6]DMSO): δ = 89.95, 122.32, 125.10, 127.66, 129.70, 132.91, 136.56, 143.71, 158.68 ppm; HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{10}H_6ClN_3OS_2$: 283.9714, found: 283.9722.

5-(3-Aminobenzo[*b*]thiophen-2-yl)-1,3,4-oxadiazole-2(3*H*)-thione (10a): Acyl hydrazide **9a** (415 mg, 2.0 mmol) was treated according to the general procedure given above. The crude product was purified by column chromatography (CH_2Cl_2/EtOAc 4:1) affording compound **10a** as a brown solid (400 mg, 80%): ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 6.83 (br s, 2H), 7.48 (m, 2H), 7.92 (d, J = 7.9 Hz, 1H), 8.17 ppm (d, J = 7.6 Hz, 1H); ^{13}C NMR (150 MHz, [D_6]DMSO): δ =

87.61, 122.89, 123.36, 124.57, 128.01, 131.56, 138.18, 145.19, 158.91, 175.31 ppm; HRMS (ESI): *m/z* $[M-H]^-$ calcd for $C_{10}H_7N_3OS_2$: 250.0103, found: 250.0102.

5-(3-Amino-5-bromobenzo[*b*]thiophen-2-yl)-1,3,4-oxadiazole-2(3*H*)-thione (10b): Acyl hydrazide **9b** (52 mg, 0.186 mmol) was treated according to the general procedure given above. The crude product was purified by column chromatography (CH_2Cl_2/EtOAc 4:1) affording compound **10b** as a dark-yellow solid (17 mg, 29%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=6.85$ (br s, 2H), 7.65 (d, $J=8.7$ Hz, 1H), 7.91 (d, $J=8.7$ Hz, 1H), 8.47 ppm (s, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=89.24$, 117.70, 125.29, 125.35, 130.36, 133.16, 137.00, 143.95, 158.46, 175.25 ppm; HRMS (ESI): *m/z* $[M-H]^-$ calcd for $C_{10}H_6BrN_3OS_2$: 325.9063, found: 325.9061.

5-(3-Amino-6-chlorobenzo[*b*]thiophen-2-yl)-1,3,4-oxadiazole-2(3*H*)-thione (12): Acyl hydrazide **11** (95 mg, 0.393 mmol) was treated according to the general procedure given above. The crude product was purified by flash chromatography (cyclohexane/EtOAc 2:1) affording compound **12** as a pale-brown solid (25 mg, 22%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=6.87$ (br s, 2H), 7.50 (dd, $J=8.7$ Hz, $J=1.5$ Hz, 1H), 8.12 (br s, 1H), 8.17 ppm (d, $J=8.7$ Hz, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=88.30$, 122.81, 124.15, 125.01, 130.25, 132.72, 139.41, 144.42, 158.57, 175.10 ppm; HRMS (ESI): *m/z* $[M-H]^-$ calcd for $C_{10}H_6ClN_3OS_2$: 281.9568, found: 281.9570.

5-(3-Amino-5-nitrobenzo[*b*]thiophen-2-yl)-1,3,4-oxadiazole-2(3*H*)-thione (15): Acyl hydrazide **14** (98 mg, 0.388 mmol) was treated according to the general procedure given above. The crude product was purified by column chromatography (CH_2Cl_2/MeOH 100:0→96:4) affording compound **15** as a brown-red solid (60 mg, 53%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=7.11$ (br s, 2H), 8.20 (d, $J=9.0$ Hz, 1H), 8.28 (dd, $J=9.0$ Hz, $J=2.1$ Hz, 1H), 9.24 ppm (d, $J=2.4$ Hz, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=$ 90.33, 119.08, 121.58, 124.69, 131.58, 144.25, 145.10, 145.17, 158.20, 175.59 ppm; HRMS (ESI): *m/z* $[M-H]^-$ calcd for $C_{10}H_6N_4O_3S_2$: 292.9808, found 292.9808.

5-(3-Amino-4-methoxybenzo[*b*]thiophen-2-yl)-1,3,4-oxadiazole-2(3*H*)-thione (17a): Acyl hydrazide **16a** (33 mg, 0.139 mmol) was treated according to the general procedure given above. The crude product was purified by column chromatography (CH_2Cl_2/MeOH 99.5:0.5→99:1) affording compound **17a** as a red-orange solid (25 mg, 64%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=3.97$ (s, 3H), 6.60 (br s, 2H), 6.93 (dd, $J=6.3$ Hz, $J=2.4$ Hz, 1H), 7.40–7.46 ppm (m, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=55.81$, 85.68, 105.61, 115.55, 120.19, 129.32, 140.08, 145.58, 157.16, 159.20, 173.68 ppm; HRMS (ESI): *m/z* $[M-H]^-$ calcd for $C_{11}H_9N_3O_2S_2$: 278.0063, found 278.0056.

5-(3-Amino-4-(trifluoromethyl)benzo[*b*]thiophen-2-yl)-1,3,4-oxadiazole-2(3*H*)-thione (17b): Acyl hydrazide **16b** (69 mg, 0.251 mmol) was treated according to the general procedure given above. The crude product was purified by column chromatography ($CHCl_3$/MeOH 100:0→99:1) affording compound **17b** as a yellow solid (72 mg, 90%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=6.10$ (br s, 2H), 7.68 (t, $J=7.8$ Hz, 1H), 7.90 (d, $J=7.5$ Hz, 1H), 8.36 ppm (d, $J=8.1$ Hz, 1H); ^{13}C NMR (150 MHz, [D$_6$]DMSO): $\delta=93.17$, 122.97 (q, $^2J_{C\text{-}F}=32$ Hz), 123.81 (q, $^3J_{C\text{-}F}=6$ Hz), 123.92 (q, $^1J_{C\text{-}F}=273$ Hz), 125.64, 126.52, 128.84, 141.04, 142.51, 158.17, 175.42 ppm; ^{19}F NMR (150 MHz, [D$_6$]DMSO): $\delta=-54.85$ ppm; HRMS (ESI): *m/z* $[M-H]^-$ calcd for $C_{11}H_6F_3N_3OS_2$: 315.9832, found 315.9839.

5-(3-Amino-5-methoxybenzo[*b*]thiophen-2-yl)-1,3,4-oxadiazole-2(3*H*)-thione (17c): Acyl hydrazide **16c** (80 mg, 0.316 mmol) was treated according to the general procedure given above. The crude product was purified by column chromatography (CH_2Cl_2/EtOAc 5:1) affording compound **17c** as an orange solid (11 mg, 12.5%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=3.84$ (s, 3H), 6.79 (br s, 2H), 7.14 (dd, $J=9.0$ Hz, $J=2.4$ Hz, 1H), 7.77–7.80 ppm (m, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=55.53$, 88.60, 105.09, 117.77, 118.04, 124.04, 130.19, 132.46, 144.86, 157.32, 159.00 ppm; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{11}H_9N_3O_2S_2$: 280.0209, found 280.0207.

5-(3-Amino-5-(trifluoromethoxy)benzo[*b*]thiophen-2-yl)-1,3,4-oxadiazole-2(3*H*)-thione (17d): Acyl hydrazide **16d** (144 mg, 0.469 mmol) was treated according to the general procedure given above. The crude product was purified by column chromatography (CH_2Cl_2/MeOH 100:2) affording compound **17d** as a yellow solid (43 mg, 27%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=6.90$ (br s, 2H), 7.51 (d, $J=8.7$ Hz, 1H), 8.07 (d, $J=8.7$ Hz, 1H), 8.28 ppm (s, 1H); ^{13}C NMR (150 MHz, [D$_6$]DMSO): $\delta=89.99$, 115.18, 120.18 (q, $^1J_{C\text{-}F}=$ 256 Hz), 121.24, 125.24, 132.35, 136.82, 144.29, 145.85, 158.50, 175.18 ppm; ^{19}F NMR (470 MHz, [D$_6$]DMSO): $\delta=-56.98$ ppm; HRMS (ESI): *m/z* $[M-H]^-$ calcd for $C_{11}H_6F_3N_3O_2S_2$: 331.9781, found 331.9778.

5-(3-Amino-7-(trifluoromethyl)benzo[*b*]thiophen-2-yl)-1,3,4-oxadiazole-2(3*H*)-thione (17e): Acyl hydrazide **16e** (104 mg, 0.378 mmol) was treated according to the general procedure given above. The crude product was purified by column chromatography (CH_2Cl_2/EtOAc 4:1) affording compound **17e** as a yellow solid (26 mg, 22%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=6.99$ (br s, 2H), 7.68 (t, $J=7.8$ Hz, 1H), 7.93 (d, $J=7.5$ Hz, 1H), 8.49 ppm (d, $J=8.4$ Hz, 1H); ^{13}C NMR (150 MHz, [D$_6$]DMSO): $\delta=88.47$, 123.39 (q, $^2J_{C\text{-}F}=33$ Hz), 123.92 (q, $^1J_{C\text{-}F}=273$ Hz), 124.96, 125.74 (q, $^3J_{C\text{-}F}=3.6$ Hz), 127.11, 133.43, 133.94, 144.39, 158.35, 174.67 ppm; ^{19}F NMR (470 MHz, [D$_6$]DMSO): $\delta=-61.75$ ppm; HRMS (ESI): *m/z* $[M-H]^-$ calcd for $C_{11}H_6F_3N_3OS_2$: 315.9832, found 315.9825.

5-(3-Amino-5-(trifluoromethyl)benzo[*b*]thiophen-2-yl)-1,3,4-oxadiazole-2(3*H*)-thione (17f): Acyl hydrazide **16f** (110 mg, 0.400 mmol) was treated according to the general procedure given above. The crude product was purified by column chromatography (CH_2Cl_2/MeOH 100:0→99:1) affording compound **17f** as a yellow solid (39 mg, 31%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=6.99$ (br s., 2H), 7.79 (d, $J=8.4$ Hz, 1H), 8.19 (d, $J=8.4$ Hz, 1H), 8.68 ppm (s, 1H); ^{13}C NMR (150 MHz, [D$_6$]DMSO): $\delta=89.65$, 120.29, 123.40, 124.52, 124.53 (q, $^1J_{C\text{-}F}=272$ Hz), 125.41 (q, $^2J_{C\text{-}F}=32$ Hz), 131.34, 141.67, 144.47, 158.41, 175.37 ppm; ^{19}F NMR (470 MHz, [D$_6$]DMSO): $\delta=-60.11$ ppm; HRMS (ESI): *m/z* $[M-H]^-$ calcd for $C_{11}H_6F_3N_3OS_2$: 315.9832, found 315.9842.

5-(3-Amino-6-(trifluoromethyl)benzo[*b*]thiophen-2-yl)-1,3,4-oxadiazole-2(3*H*)-thione (20): Acyl hydrazide **19** (92 mg, 0.334 mmol) was treated according to the general procedure given above. The crude product was purified by column chromatography (CH_2Cl_2/EtOAc 4:1) affording compound **20** as a yellow solid (62 mg, 58%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=6.94$ (br s, 2H), 7.77 (dd, $J=8.4$ Hz, $J=1.2$ Hz, 1H), 8.38 (d, $J=8.4$ Hz, 1H), 8.48 ppm (br s, 1H); ^{13}C NMR (150 MHz, [D$_6$]DMSO): $\delta=90.75$, 120.87, 121.10 (q, $^3J_{C\text{-}F}=$ 3.3 Hz), 123.74, 124.26 (q, $^1J_{C\text{-}F}=272$ Hz), 127.51 (q, $^2J_{C\text{-}F}=32$ Hz), 134.13, 138.14, 144.12, 158.36, 175.37 ppm; ^{19}F NMR (470 MHz, [D$_6$]DMSO): $\delta=-60.38$ ppm; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{11}H_6F_3N_3OS_2$: 317.9977, found: 317.9977.

Benzo[*b*]thiophene-2-carbohydrazide (22a): This compound was obtained from ethyl benzo[*b*]thiophene-2- carboxylate (**21a**), using the procedure described for the synthesis of compound **4**, yielding the title compound as a brown solid (90%).

3-Methylbenzo[*b*]thiophene-2-carbohydrazide (22b): This compound was obtained from ethyl 3-methylbenzo[*b*]thiophene-2-carboxylate (**21b**), using the procedure described for the synthesis of compound **4**, yielding the title compound as a brown solid (96%).

5-(Benzo[*b*]thiophen-2-yl)-1,3,4-oxadiazole-2(3*H*)-thione (23a): This compound was obtained from benzo[*b*]thiophene-2-carbohydrazide (**22a**), using the procedure described for the synthesis of compound **1**, affording the title compound as a white solid (98%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=8.18$ (s, 1H), 8.12 (d, $J=7.4$ Hz, 1H), 8.03 (d, $J=7.7$ Hz, 1H), 7.52 ppm (m, 2H); ^{13}C NMR (150 MHz, [D$_6$]DMSO): $\delta=123.03$, 123.27, 125.50, 125.62, 127.24, 127.66, 138.75, 139.92, 156.99, 177.34 ppm; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{10}H_6N_2OS_2$: 234.9994, found: 235.0007.

5-(3-Methylbenzo[*b*]thiophen-2-yl)-1,3,4-oxadiazole-2(3*H*)-thione (23b): This compound was obtained from 3-methylbenzo[*b*]thiophene-2-carbohydrazide (**22b**), using the procedure described for the synthesis of compound **1**, affording the title compound as a white solid (96%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=8.07$ (d, $J=6.9$ Hz, 1H), 7.98 (d, $J=6.9$ Hz, 1H), 7.54 (m, 2H), 2.70 ppm (s, 3H); ^{13}C NMR (150 MHz, [D$_6$]DMSO): $\delta=13.10$, 117.69, 122.98, 123.70, 125.33, 127.33, 136.95, 138.89, 139.53, 157.65, 176.71 ppm; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{11}H_8N_2OS_2$: 249.0151, found: 249.0156.

5-(3-Amino-5-chlorobenzo[*b*]thiophen-2-yl)-1,3,4-oxadiazol-2(3*H*)-one (24): To a mixture of acyl hydrazide **4** (83 mg, 0.344 mmol) and DIPEA (0.090 mL, 0.516 mmol) in DMF (3.2 mL) was added carbonyldiimidazole (84 mg, 0.516 mmol). Stirring was continued for 18 h at room temperature. Then, water was added and the volatiles were removed under reduced pressure. Crystallization from EtOH afforded compound **24** as a grey solid (53 mg, 58%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=6.64$ (brs; 2H), 7.50 (d, $J=8.4$ Hz, 1H), 7.94 (d, $J=8.4$ Hz, 1H), 8.27 (s, 1H), 12.51 ppm (brs, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=91.34$, 121.95, 124.88, 127.15, 129.50, 133.05, 136.04, 142.55, 152.18, 153.46 ppm; HRMS (ESI): *m/z* $[M-H]^-$ calcd for $C_{10}H_6ClN_3O_2S$: 265.9796, found: 265.9804.

2-(3-Amino-5-chlorobenzo[*b*]thiophene-2-carbonyl)-*N*-methylhydrazinecarbothioamide (25): To a suspension of acyl hydrazide **4** (83 mg, 0.344 mmol) in 1,4-dioxane (2.1 mL) was added methyl isothiocyanate (0.024 mL, 0.344 mmol). The mixture was held at reflux for 2 h. The volatiles were removed under reduced pressure. The solid residue was suspended in toluene, filtrated and the collected solid was crystallized from EtOH affording compound **25** as a pale-yellow solid (70 mg, 65%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=2.87$ (d, $J=3.9$ Hz, 3H), 7.23 (brs, 2H), 7.50 (d, $J=8.4$ Hz, 1H), 7.91 (d, $J=8.4$ Hz, 1H), 7.99 (brs, 2H), 8.24 (s, 1H), 9.26 (brs, 1H), 9.58 ppm (brs, 1H).

3-(3-amino-5-chlorobenzo[*b*]thiophen-2-yl)-4-methyl-1H-1,2,4-triazole-5(4*H*)-thione (26): A solution of *N*-acetyl thiosemicarbazide **25** (64 mg, 0.203 mmol) in 10% aqueous NaOH (1 mL) was held at reflux for 4 h. Then, it was cooled to room temperature, diluted with H_2O and acidified using a 1 N HCl solution. The formed precipitate was filtered off, washed with H_2O and dried affording compound **26** as a beige solid (54 mg, 89%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=3.63$ (s, 3H), 6.56 (brs, 2H), 7.49 (d, $J=7.5$ Hz, 1H), 7.96 (d, $J=7.5$ Hz, 1H), 8.24 (s, 1H), 13.96 ppm (brs, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=31.37$, 92.36, 121.72, 124.51, 126.58, 129.35, 133.09, 136.00, 142.33, 147.36, 166.65 ppm; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{11}H_9ClN_4S_2$: 297.0030, found: 297.0037.

***N*′-Acetyl-3-amino-5-chlorobenzo[*b*]thiophene-2-carbohydrazide (27)**: To a suspension of acyl hydrazide **4** (100 mg, 0.415 mmol) in $CHCl_3$ (10 mL) at 0 °C was added acetic anhydride (0.039 mL, 0.415 mmol) followed by Et_3N (0.058 mL, 0.415 mmol). The resulting mixture was held at reflux for 4 h. Then, it was cooled in an ice-water bath. The precipitate was filtered-off, washed with cold CH_2Cl_2, H_2O and Et_2O and dried affording compound **27** as a white solid (97 mg, 82%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=1.91$ (s, 3H), 7.18 (brs, 2H), 7.51 (dd, $J=8.7$ Hz, $J=1.8$ Hz, 1H), 7.91 (d, $J=8.7$ Hz, 1H), 8.23 (d, $J=1.8$ Hz, 1H), 9.48 (brs, 1H), 9.75 ppm (brs, 1H).

5-Chloro-2-(5-methyl-1,3,4-oxadiazol-2-yl)benzo[*b*]thiophen-3-amine (28): To a suspension of carbohydrazide **27** (45 mg, 0.157 mmol) in CH_2Cl_2 (1.5 mL) at 0 °C Ph_3P (82 mg, 0.314 mmol) followed by CBr_4 (104 mg, 0.314 mmol) was added. The brown mixture was stirred at 0 °C for 45 min, then 1 h 45 min at room temperature and then another 3 h at reflux. The mixture was left overnight at room temperature, then it was concentrated under reduced pressure. The crude product was purified by column chromatography (CH_2Cl_2/EtOAc 100:0→95:5) affording compound **28** as a grey solid (12 mg, 29%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=2.56$ (s, 3H), 6.98 (brs, 2H), 7.51 (dd, $J=8.7$ Hz, $J=1.8$ Hz, 1H), 7.96 (d, $J=8.7$ Hz, 1H), 8.30 ppm (d, $J=1.8$ Hz, 1H); ^{13}C NMR (300 MHz, [D$_6$]DMSO): $\delta=10.42$, 90.36, 122.19, 124.99, 127.38, 129.56, 132.99, 136.51, 143.66, 161.24, 161.53 ppm; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{11}H_8ClN_3OS$: 266.0149, found: 266.0161.

Methyl 3-aminothieno[2,3-*b*]pyridine-2-carboxylate (30a): To a solution of 2-chloronicotinonitrile (**29a**) (2.65 g, 19 mmol) in MeOH (76 mL) was added methyl thioacetate (2.55 mL, 28.5 mmol) and triethylamine (28.5 mmol, 3.97 mL). The reaction mixture was held at reflux overnight. The solvents were evaporated and the residue was purified by flash chromatograph on silica, the mobile phase being a mixture of EtOAc and cyclohexane (in a ratio of 2:8), affording the pure title compound as a white solid (3.23 g, 81%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=1.30$ (t, $J=7.1$ Hz, 3H), 4.28 (q, $J=7.1$ Hz, 2H), 7.30 (brs, 2H), 7.46 (dd, $J=8.1$, 4.6 Hz, 1H), 8.54 (dd, $J=8.1$, 1.5 Hz, 1H), 8.68 ppm (dd, $J=4.6$, 1.5 Hz, 1H).

3-Aminothieno[2,3-*b*]pyridine-2-carbohydrazide (31a): A mixture of ethyl 3-aminothieno[2,3-*b*]pyridine-2-carboxylate (**30a**) (1.11 g, 5.0 mmol) and a 60% hydrazine solution (5 mL) in EtOH (20 mL) was held at reflux till TLC indicated disappearance of the starting material. After removing the solvents under reduced pressure, the residue was suspended in water (10 mL). The precipitate was filtered off, washed with water and dried, yielding the title compound as a pale-yellow solid (1.0 g, 96%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=4.43$ (brs, 2H), 7.14 (brs, 2H), 7.42 (dd, $J=8.1$ Hz, 4.6 Hz, 1H), 8.42 (dd, $J=8.1$ Hz, 1.5 Hz, 1H), 8.62 (dd, $J=4.6$ Hz, 1.5 Hz, 1H), 9.06 ppm (s, 1H); MS *m/z* (%): 209.0 ($[M+H]^+$, 100).

5-(3-Aminothieno[2,3-*b*]pyridin-2-yl)-1,3,4-oxadiazole-2(3*H*)-thione (32a): A solution of 3-aminothieno[2,3-*b*]pyridine-2-carbohydrazide (**31a**) (520 mg, 2.5 mmol) in pyridine (20 mL) and carbon disulfide (5 mL) was held at reflux for 8 h. After removing the solvents under reduced pressure, the residue was suspended in water (10 mL). The precipitate was filtered off, washed with water and dried yielding the title compound as yellow solid (440 mg, 70%); ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=6.95$ (brs, 2H), 7.49 (dd, $J=8.1$ Hz, 4.6 Hz, 1H), 8.55 (dd, $J=8.1$ Hz, 1.5 Hz, 1H), 8.66 ppm (dd, $J=4.6$ Hz, 1.5 Hz, 1H); ^{13}C NMR (150 MHz, [D$_6$]DMSO): $\delta=86.52$, 119.98, 125.64, 131.24, 142.99, 150.02, 158.54, 159.29, 175.46 ppm; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_9H_6N_4OS_2$: 251.0056, found: 251.0060.

Methyl 7-aminothieno[2,3-*b*]pyrazine-6-carboxylate (30b): This compound was synthesized from 3-chloropyrazine-2-carbonitrile **29b** according to procedure for the synthesis of compound **30a**. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 3.84 (s, 3H), 7.12 (br s, 2H), 8.77 ppm (s, 2H).

7-Aminothieno[2,3-*b*]pyrazine-6-carbohydrazide (31b): This compound was prepared according to the procedure for the synthesis of compound **31a**. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 4.48 (br s, 2H), 6.86 (br s, 2H), 8.71 (m, 2H), 9.30 ppm (br s, 1H).

5-(7-Aminothieno[2,3-*b*]pyrazin-6-yl)-1,3,4-oxadiazole-2(3*H*)-thione (32b): This compound was prepared from 7-aminothieno-[2,3-*b*]pyrazine-6-carbohydrazide **31b** according to the procedure for the preparation of compound **32a**. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 6.76 (br s, 2H), 8.76 (d, J = 2.31 Hz, 1H), 8.81 ppm (d, J = 2.31 Hz, 1H).

3-Aminothiophene-2-carbohydrazide (34): This compound was prepared from ethyl 3-aminothiophene-2-carboxylate **33** with hydrazine according to the procedure for the synthesis of compound **4**, yielding the title compound as brown solid (89% yield): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 4.28 (br s, 2H), 6.39 (br s, 2H), 6.57 (d, J = 5.3 Hz, 1H), 7.35 (d, J = 5.3 Hz, 1H), 8.71 ppm (s, 1H); MS m/z (%): 158.0 ([M + H]$^+$, 100).

5-(3-Aminothiophen-2-yl)-1,3,4-oxadiazole-2(3*H*)-thione (35): This compound was synthesized from 3-aminothiophene-2-carbohydrazide **34**, according to the procedure for the synthesis of compound **1**, yielding the title compounds as white solid (61% yield). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 6.26 (br s, 2H), 6.72 (d, J = 5.3 Hz, 1H), 7.63 ppm (d, J = 5.3 Hz, 1H); ^{13}C NMR (150 MHz, [D$_6$]DMSO): δ = 90.29, 120.85, 130.99, 151.15, 158.70, 175.08 ppm; HRMS (ESI): m/z [M + H]$^+$ calcd for $C_6H_5N_3OS_2$: 199.9947, found: 199.9943.

Ethyl 3-amino-5-bromothieno[2,3-*b*]pyridine-2-carboxylate (8a): A mixture of 5-bromo-2-chloronicotinonitrile (2.17 g, 10 mmol), ethyl 2-thioacetate (1.31 mL, 12 mmol) and K_2CO_3 (2.76 g, 20 mmol) in EtOH (30 mL) was held at reflux for 3 h. After cooling to room temperature, H_2O (100 mL) was added. The precipitate was filtered off, washed with water, and dried, yielding the title compound as a pale-yellow solid (2.90 g, 96%); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.87 (d, J = 2.2 Hz, 1H), 8.78 (d, J = 2.2 Hz, 1H), 7.28 (br s, 2H), 4.28 (q, J = 7.1 Hz, 2H), 1.30 ppm (t, J = 7.1 Hz, 3H).

Synthesis of 5-(3-amino-5-arylthieno[2,3-*b*]pyridin-2-yl)-1,3,4-oxadiazole-2(3*H*)-thiones 38a–j; *Step A*: A mixture of ethyl 3-amino-5-bromothieno[2,3-*b*]pyridine-2-carboxylate (**8a**) (301 mg, 1.0 mmol), an appropriate arylboronic acid (1.2 mmol), K_2CO_3 (2.0 mmol) and tetrakis(triphenylphosphine)palladium(0) (0.04 mmol) in 1,4-dioxane (10 mL) and water (3 mL) was held at reflux for 1 h. After cooling to room temperature, the yellow solid was filtered off. This crude product **36a-l** was used in the following step without further purification.

Step B: The crude product **36a–i** was dissolved in EtOH (5 mL), and a 60% aqueous hydrazine solution was added (3 mL). The mixture was heated under reflux till all starting material disappeared, according to TLC analysis. After cooling to room temperature, the yellow solid was filtered off, washed with water and dried, yielding an intermediate **37a–j**, being used as such in the following step.

Step C: A suspension of this crude product **37a–j** in pyridine (5 mL) and CS_2 (2 mL) was heated under reflux till TLC indicated disappearance of the starting material. After concentration under reduced pressure, the residue was purified by flash chromatography on silica, the mobile phase being a mixture of MeOH and CH_2Cl_2 affording the pure title compounds **38a–j**.

5-(3-amino-5-(4-fluorophenyl)thieno[2,3-*b*]pyridin-2-yl)-1,3,4-oxadiazole-2-thiol (38a): This compound was obtained using 4-fluorophenylboronic acid. The crude residue was purified by silica gel flash chromatography, the mobile phase being a mixture of MeOH and CH_2Cl_2 (in a ratio gradually ranging from 1:10→1:5) affording compound **38a** as a pale-yellow solid (59%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 6.99 (s, 2H), 7.40 (t, J = 8.8 Hz, 2H), 7.87 (dd, J = 8.8 Hz, 5.4 Hz, 2H), 8.93 (d, J = 2.1 Hz, 1H), 8.99 ppm (d, J = 2.1 Hz, 1H); ^{13}C NMR (150 MHz, [D$_6$]DMSO): δ = 87.27, 116.19 ($J_{C\text{-}F}$ = 21.36 Hz), 125.81, 128.79, 129.10 ($J_{C\text{-}F}$ = 8.2 Hz), 129.13, 131.14, 133.17, 142.87, 148.29, 157.99, 158.46, 162.42 ($J_{C\text{-}F}$ = 244.2 Hz) 175.53 ppm; HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{15}H_9FN_4OS_2$: 345.0275, found: 345.0276.

5-(3-Amino-5-(2,4-difluorophenyl)thieno[2,3-*b*]pyridin-2-yl)-1,3,4-oxadiazole-2(3*H*)-thione (38b): This compound was obtained using 2,4-difluorophenylboronic acid. The crude residue was purified by silica gel flash chromatography, the mobile phase being a mixture of MeOH and CH_2Cl_2 (in a ratio gradually ranging from 1:10→1:5) affording compound **38b** as a yellow solid (63%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 7.02 (s, 2H), 7.32 (m, 1H), 7.49 (m, 1H), 7.76 (m, 1H), 8.81 ppm (s, 2H); ^{13}C NMR (150 MHz, [D$_6$]DMSO): δ = 87.78, 104.86, 112.49, 112.62, 121.45, 121.54, 125.67, 126.51, 131.03, 131.14, 132.34, 132.37, 132.41, 132.43, 142.35, 149.39, 152.02, 158.38, 158.45, 158.56, 160.13, 160.22, 161.52, 161.60, 163.17, 163.25, 175.70 ppm; HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{15}H_8F_2N_4OS_2$: 363.0180, found: 363.0181.

5-(3-Amino-5-(2,3-difluorophenyl)thieno[2,3-*b*]pyridin-2-yl)-1,3,4-oxadiazole-2(3*H*)-thione (38c): This compound was obtained using 2,3-difluorophenylboronic acid. The crude residue was purified by silica gel flash chromatography, the mobile phase being a mixture of MeOH and CH_2Cl_2 (in a ratio gradually ranging from 1:10→1:5) affording compound **38c** as a yellow solid (56%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 7.04 (s, 2H) 7.50 (m, 3H), 8.88 ppm (s, 2H); ^{13}C NMR (150 MHz, [D$_6$]DMSO): δ = 87.37, 117.54, 117.65, 125.61, 126.24, 127.07, 127.14, 131.29, 142.80, 146.35, 146.44, 148.00, 148.09, 149.39, 149.49, 151.09, 151.17, 152.05, 158.42, 158.84, 161.19, 175.50 ppm; HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{15}H_8N_4OS_2$: 363.0180, found: 363.0182.

5-(3-Amino-5-(3,4-dichlorophenyl)thieno[2,3-*b*]pyridin-2-yl)-1,3,4-oxadiazole-2(3*H*)-thione (38d): This compound was obtained using 3,4–dichlorophenylboronic acid. The crude residue was purified by silica gel flash chromatography, the mobile phase being a mixture of MeOH and CH_2Cl_2 (in a ratio gradually ranging from 1:20→1:5) affording compound **38d** as a yellow solid (85%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 6.99 (s, 2H), 7.81 (m, 2H), 8.09 (s, 1H), 8.98 (s, 1H), 9.03 ppm (s, 1H); ^{13}C NMR (150 MHz, [D$_6$]DMSO): δ = 87.35, 125.76, 126.98, 128.58, 128.99, 129.41, 131.09, 131.33, 132.11, 137.27, 142.85, 148.14, 158.71, 175.40 ppm; HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{15}H_8Cl_2N_4OS_3$: 394.9589, found: 394.9586.

5-(3-Amino-5-(3,4-dimethoxyphenyl)-thieno[2,3-*b*]pyridin-2-yl)-1,3,4-oxadiazole-2(3*H*)-thione (38e): This compound was obtained using 3,4-dimethoxyphenylboronic acid. The crude residue was purified by silica gel flash chromatography, the mobile phase being a mixture of MeOH and CH_2Cl_2 (in a ratio gradually ranging from 1:10→1:5) affording compound **38e** as a yellow solid (66%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 3.83 (s, 3H), 3.90 (s, 3H), 7.00 (s, 1H), 7.12 (d, J = 8.0 Hz, 1H), 7.37 (d, J = 8.0 Hz, 1H), 7.40 (s, 1H), 8.89 (s, 1H), 9.02 ppm (s, 1H); ^{13}C NMR (150 MHz, [D$_6$]DMSO): δ = 55.70, 55.80, 87.05, 110.57, 112.41, 119.32, 125.80, 128.09, 129.10, 132.04, 143.00, 148.32, 149.25, 149.39, 157.37, 158.51, 175.48 ppm;

HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{17}H_{14}N_4O_3S_2$: 387.0580, found: 387.0569.

5-(3-Amino-5-(3,4,5-trimethoxyphenyl)thieno[2,3-*b*]pyridin-2-yl)-1,3,4-oxadiazole-2(3*H*)-thione (38f): This compound was obtained using 3,4,5-trimethoxyphenylboronic acid. The crude residue was purified by silica gel flash chromatography, the mobile phase being a mixture of MeOH and CH_2Cl_2 (in a ratio gradually ranging from 1:10→1:5) affording compound **38f** as a yellow solid (54%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=3.73$ (s, 3H), 3.92 (s, 6H), 7.07 (s, 2H), 7.23 (s, 1H), 7.41 (s, 1H), 8.86 (s, 1H), 8.98 (s, 1H), 9.08 ppm (s, 1H); ^{13}C NMR (150 MHz, [D$_6$]DMSO): $\delta=56.23$, 60.22, 87.08, 104.56, 125.78, 128.64, 132.15, 132.19, 137.87, 142.99, 148.62, 153.59, 157.85, 158.48, 175.52 ppm; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{18}H_{16}N_4O_4S_2$: 417.0686, found: 417.0679.

(5-(3-Amino-5-(benzo[d][1,3]dioxol-5-yl)thieno[2,3-*b*]pyridin-2-yl)-1,3,4-oxadiazole-2(3*H*)-thione (38g): This compound was obtained using 3,4,5-trimethoxyphenylboronic acid. The crude residue was purified by silica gel flash chromatography, the mobile phase being a mixture of MeOH and CH_2Cl_2 (in a ratio gradually ranging from 1:20→1:5) affording compound **38g** as a yellow solid (46%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=6.11$ (s, 2H), 7.10 (d, $J=8.0$ Hz, 1H), 7.32 (d, $J=6.5$ Hz, 1H), 7.41 (s, 1H), 8.86 (s, 1H), 8.89 (s, 1H), 8.95 ppm (s, 1H); ^{13}C NMR (150 MHz, [D$_6$]DMSO): $\delta=87.08$, 101.50, 107.26, 109.06, 120.79, 125.81, 128.41, 130.68, 131.90, 142.97, 147.62, 148.27, 148.36, 157.51, 158.49, 175.48 ppm; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{16}H_{10}N_4O_3S_2$: 371.0267, found: 371.0274.

5-(3-Amino-5-(thiophen-3-yl)thieno[2,3-*b*]pyridin-2-yl)-1,3,4-oxadiazole-2(3*H*)-thione (38h): This compound was obtained using 3-thienylboronic acid. The crude residue was purified by silica gel flash chromatography, the mobile phase being a mixture of MeOH and CH_2Cl_2 (in a ratio gradually ranging from 1:20→1:5) affording compound **38h** as a yellow solid (52%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=6.97$ (s, 2H), 7.68 (d, $J=4.8$ Hz, 1H), 7.76 (d, $J=4.8$ Hz, 1H), 8.07 (s, 1H), 8.97 (s, 1H), 9.10 (s, 1H) ppm; ^{13}C NMR (150 MHz, [D$_6$]DMSO): $\delta=87.14$, 122.15, 125.83, 126.00, 127.45, 127.82, 127.99, 137.83, 142.93, 148.08, 157.39, 158.47, 175.49 ppm; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{13}H_8N_4OS_3$: 332.9933, found: 332.9939.

5-(3-Amino-5-(pyridin-3-yl)thieno[2,3-*b*]pyridin-2-yl)-1,3,4-oxadiazole-2(3*H*)-thione (38j): This compound was obtained using 3-pyridinylboronic acid. The crude residue was purified by silica gel flash chromatography, the mobile phase being a mixture of MeOH and CH_2Cl_2 (in a ratio gradually ranging from 1:10→1:2) affording compound **38j** as a yellow solid (49%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=6.92$ (s, 2H), 7.58 (dd, $J=8.1$ Hz, 3.3 Hz, 1H), 8.23 (d, $J=8.1$ Hz, 1H), 8.65 (d, $J=3.3$ Hz, 1H), 8.97 (s, 1H), 9.05 (s, 1H), 9.06 ppm (s, 1H); ^{13}C NMR (150 MHz, [D$_6$]DMSO): $\delta=87.18$, 108.52, 112.70, 124.54, 125.85, 127.29, 139.96, 142.80, 144.98, 147.68, 157.23, 158.46, 175.51 ppm; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{14}H_9N_5OS_2$: 328.0321, found: 328.0322.

Synthesis of 5-(3-amino-6-arylthieno[2,3-*b*]pyridin-2-yl)-1,3,4-oxadiazole-2(3*H*)-thione analogues 41a–41b; *Step A*: A mixture of 2,4-dichloronicotinonitrile (**39**) (173 mg, 1.0 mmol), an appropriate arylboronic acid (1.2 mmol), K_2CO_3 (2.0 mmol) and tetrakis(triphenylphosphine)palladium(0) (0.03 mmol) in 1,4-dioxane (10 mL) and water (3 mL) was heated at 90 °C for 3 h. After concentration under reduced pressure, the residue was purified by flash chromatography on silica (using a mixture of EtOAc and cyclohexane as mobile phase, in a ratio gradually ranging from 1/15 to 1/5), yielding the pure intermediate **40** as white solid (210 mg, 90%).

Step B: To a solution of this crude product **40** in EtOH (15 mL) was added ethyl 2-mercatoacetate (1.0 mmol) and K_2CO_3 (2.0 mmol). The mixture was heated at 95 °C for 2 h. After cooling to room temperature, the yellow product was filtered off, washed with water and dried. It was used in the next step.

Step C: To a solution of the above crude product in EtOH (5 mL) was added a 60% aqueous hydrazine solution (3 mL). The mixture was heated under reflux for 8 h. After cooling to room temperature, the yellow solid was filtered off, washed with water, and dried yielding a yellow solid which is used as such in the following step.

Step D: A suspension of above product in pyridine (5 mL) and CS_2 (2 mL) was heated at 95 °C for 4 h. After concentration under reduced pressure, the residue was purified by flash chromatography on silica, the mobile phase being a mixture of MeOH and CH_2Cl_2 (in a ratio of 1:10 to 1:5), yielding the pure title compounds **41a–b** as a white solid.

5-(3-amino-6-(4-fluorophenyl)thieno[2,3-*b*]pyridin-2-yl)-1,3,4-oxadiazole-2-thiol (41a): This compound was obtained using 4-fluorophenylboronic acid in a yield of 73% (over four steps). ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=6.82$ (s, 2H), 7.35 (t, $J=8.8$ Hz, 2H), 8.08 (d, $J=8.6$ Hz, 1H), 8.25 (dd, $J=8.8$ Hz, 5.6 Hz, 2H), 8.54 ppm (d, $J=8.6$ Hz, 1H); ^{13}C NMR (150 MHz, [D$_6$]DMSO): $\delta=89.25$, 115.86 ($J_{C\text{-}F}=21.33$ Hz), 116.44, 124.83, 129.26 ($J_{C\text{-}F}=8.4$ Hz), 131.50, 134.43, 140.53, 154.38, 158.93, 159.25, 163.21 ($J_{C\text{-}F}=245.8$ Hz), 176.49 ppm; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{15}H_9FN_4OS_2$: 345.0275, found: 345.0276.

5-(3-amino-6-(thiophen-3-yl)thieno[2,3-*b*]pyridin-2-yl)-1,3,4-oxadiazole-2-thiol (41b): This compound was obtained using 3-thienylboronic acid in a yield of 64% (over four steps). ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=6.94$ (s, 2H), 7.70 (dd, $J=5.0$ Hz, 3.0 Hz, 1H), 7.76 (dd, $J=5.0$ Hz, 1.2 Hz, 1H), 8.03 (d, $J=8.5$ Hz, 1H), 8.37 (dd, $J=3.0$ Hz, 1.2 Hz, 1H), 8.58 ppm (d, $J=8.5$ Hz, 1H); ^{13}C NMR (150 MHz, [D$_6$]DMSO): $\delta=86.32$, 117.06, 123.98, 126.01, 126.67, 127.54, 131.99, 141.02, 143.06, 153.05, 158.66, 159.47, 175.26 ppm; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{13}H_8N_4OS_3$: 332.9933, found: 332.9939.

Bioassays

DRAK2 binding assays: Compounds were screened at a single concentration of 10 μM, using binding assays as previously described.[7,19] For the most potent compounds from this primary screening, binding constants (K_d values) were also determined.

DRAK2 functional enzymatic assays: DRAK2 functional assays were performed at SignalChem through a radioisotope assay format, as described by the provider (http://www.signalchem.com).

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