Synthesis and structure-activity relationship studies of 2-(1,3,4-oxadiazole-2(3*H*)-thione)-3-amino-5-aryl-thieno[2,3-*b*]pyridines as inhibitors of DRAK2

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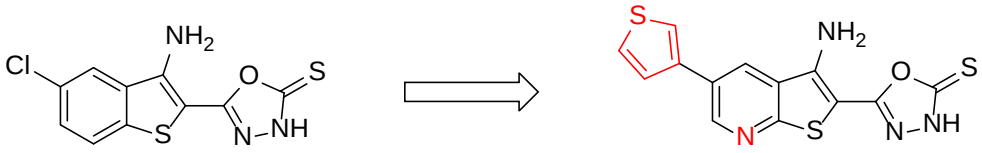
Graphical Table of Contents Entry



DRAK2 K_d=0.25 μM

DRAK2 K_d=0.008 μM
DRAK2 IC_{50}=0.029 μM

Improving the potency of a benzothiophene analogue for its DRAK2 inhibitory activity afforded a series of 2-(1,3,4-oxadiazole-2(3*H*)-thione)-3-amino-5-aryl-thieno[2,3-*b*]pyridines with high affinity for DRAK2. Morover, these compounds are endowed with functional, inhibitory DRAK2 activity.

Abstract

In recent years, DRAK2 emerged as a promising target for the treatment of a variety of auto-immune 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 are not described in literature. Screening of a proprietary compound library led to the discovery of a benzothiophene analogue, displaying 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-aryl-thieno[2,3-*b*]pyridines with strong binding affinity (K_d=0.008 μM for the most potent representative). In addition, these compounds also show promising activity in a functional, biochemical DRAK2 enzymatic assay, displaying 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.

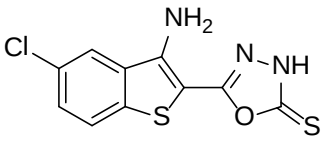
Keywords : DRAK2, Heterocycles, Medicinal Chemistry, 1,3,4-oxadiazoles

Introduction

Currently, clinical treatments to avoid allograft rejection include life-time administration of immunosuppressive drugs.[1] Cyclosporine and tacrolimus are the two immunosuppressants that are most frequently used by transplant patients. Their immunosuppressive activity is linked to inhibition of calcineurine, 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 the current treatments resulting very often 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 after organ transplantation and for the treatment of a variety of auto-immune disorders (such as diabetes, multiple sclerosis and rheumatoid arthritis). A number of potent DRAK2 ligands is known in literature. 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, for these compounds only binding data (i.e. K_d values) are available, 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 to use these as chemical tool compounds or they can be used 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. Then, the experiment is repeated in presence of a "free test" compound. The more test compounds bind to the kinase the fewer the DNA-tagged-protein molecules will 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).



1

MW	283.76
PSA	59.64
*c*log*P*	3.16
Ligand efficiency (kcal·mol^{-1})	0.53
K_d (μM)	0.25

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

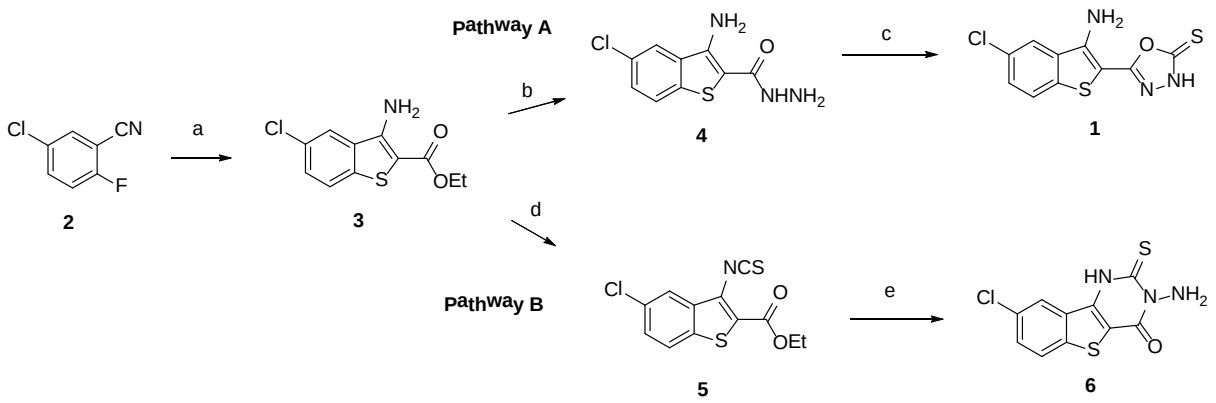
A low molecular weight (MW 283.76), in combination with potent activity (K_d=0.25 μM) furnishes a Ligand Efficiency (LE) of 0.53. It is generally accepted that a LE of at least 0.3 is necessary to have a good hit or lead compound.[8] Calculation of the *c*Log*P* value revealed the compound to have acceptable lipophilicity (*c*Log*P*=3.16). Overall, these characteristics makes hit **1** a good starting point for an optimization program.

In this contribution, 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 describing 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 mercaptoacetate with subsequent ring closure yields the ethyl 3-aminobenzo[*b*]thiophene-2-carboxylate derivative **3**.[10] Literature 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.[10] 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, in order to construct the 1,3,4-oxadiazole moiety, the 3-amino-2-acylhydrazide **4** was treated with CS_2 in pyridine at elevated temperature,[11] furnishing hit compound **1**.

However, it has been reported that refluxing of aromatic 2-aminocarboxylic acid hydrazides with CS_2 can also afford 3-amino-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one derivatives, rather than the 1,3,4-oxadiazole-2-thiones.[12] 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 to an isothiocyanate moiety,[13] followed by treatment with hydrazine yielding the 2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one **6**.[14]



Reagents and conditions: a) ethyl mercaptoacetate, NaOH, DMF; b) hydrazine monohydrate, EtOH, reflux; c) CS_2, pyridine, reflux; d) $CSCl_2$, acetone, RT; e) hydrazine solution (35% in H_2O), toluene, 80 °C.

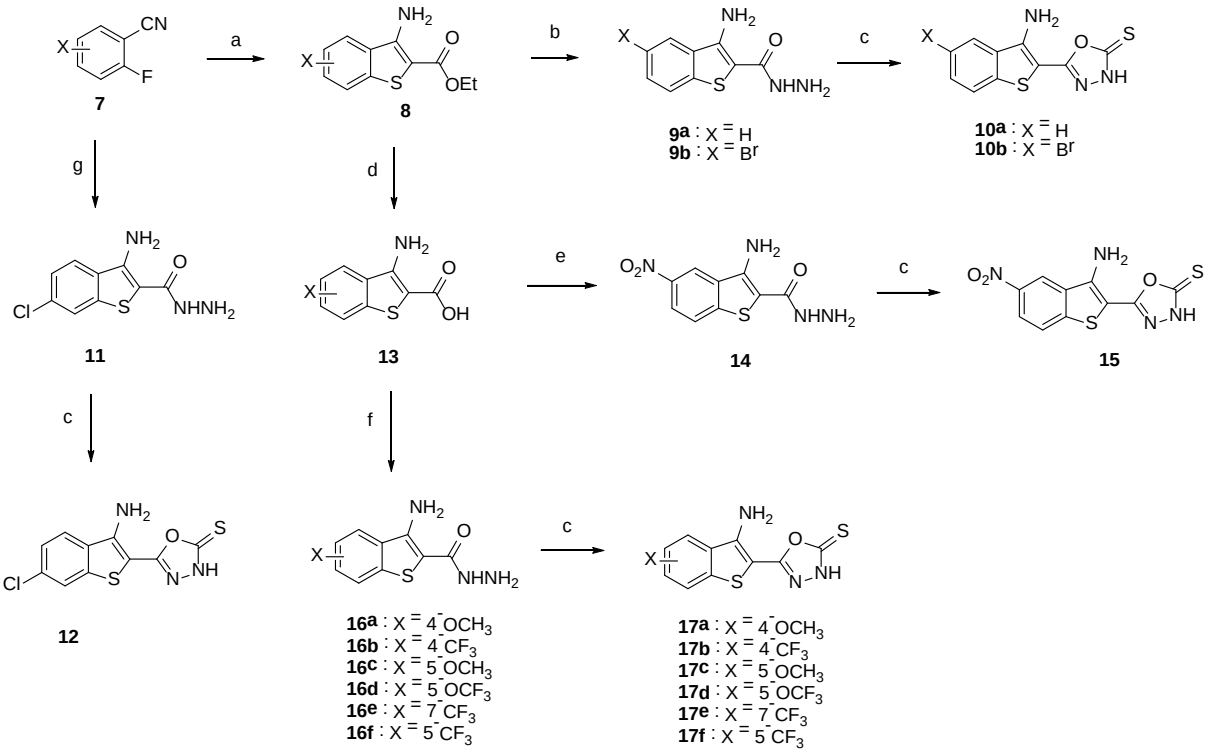
Scheme 1

Compounds **1** and **6** show a very similar polarity (R_f values) on TLC and their proton NMR spectra showed similarity in signal positions.

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 (δ=6.82 ppm) and therefore, the peak at 89.95 ppm can be assigned to C(3). The observation of HMBC correlations (^3J) between the carbon at 132.91 ppm (which can be assigned to C-(3a)) 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 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 mercaptoacetate under basic conditions, afforded ethyl 3-amino-2-carboxylate derivatives **8**.[9] Direct hydrazinolysis of these ethyl ester gave satisfactory results only in case of the unsubstituted and 5-bromo-benzo[*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**. In order to improve the synthesis of the intermediate hydrazide, different approaches were followed, depending on the substitution pattern of the benzonitrile derivative. Reaction of a 2-fluorobenzonitrile derivative with mercaptoacetic acid hydrazide[15] led to the direct formation of the hydrazide moiety, avoiding the need for hydrolysis of the carboxylic acid ethylester.

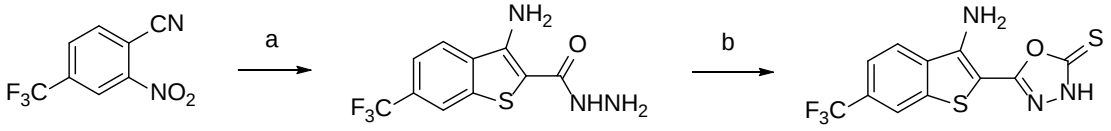
Unfortunately, this method was only successful for the synthesis of the 6-chlorobenzothiophene analogue **11**. Treatment of **11** with carbon disulfide yields the final compound **12**. In an alternative approach, the ethyl ester group was hydrolysed to the corresponding carboxylic acid. Subsequent 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.



Reagents and conditions: a) ethyl mercaptoacetate, 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) mercaptoacetic acid hydrazide, *t*BuOK, DMF, 0 °C.

Scheme 2

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

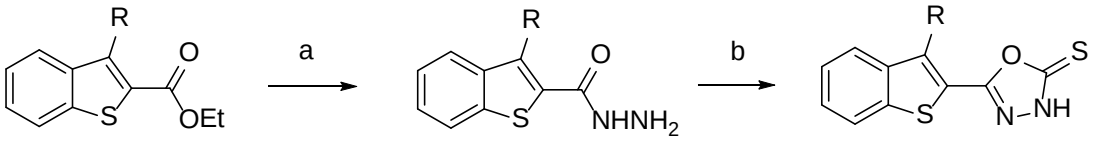


18 **19** **20**

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

Scheme 3

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.



21a : R = H
21b: R = CH_3

22a : R = H
22b : R = CH_3

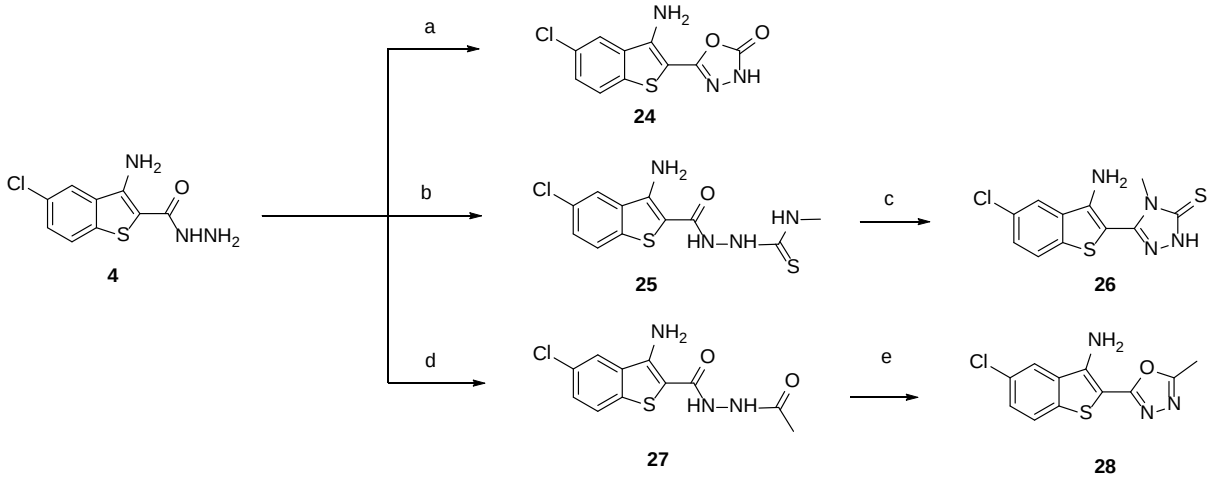
23a : R = H
23b : R = CH_3

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

Scheme 4

In order to prepare analogues with structural modifications of the oxadiazole part, 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 refluxing in an aqueous NaOH solution, 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*'-acetylhydrazide **27**. Subsequent

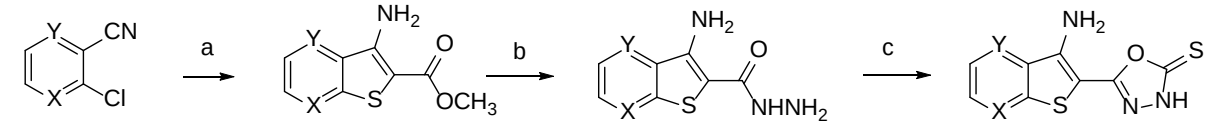
ring closing in a Robinson-Gabriel type reaction using PPh_3/CBr_4 as dehydrating agent afforded 2-methyl-1,3,4-oxadiazole **28** in moderate yield.[16]



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, DCM, 0 °C → rt → reflux.

Scheme 5

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-mercaptoacetate under basic conditions to construct the thiophene ring, affording bicyclic 3-amino-2-carboyxlic acid ethyl ester analogues **30a–b**. The ester functionality was directly transformed into the hydrazide moiety by refluxing 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.



29a : X = N ; Y = CH
29b : X = N ; Y = N

30a : X = N ; Y = CH
30b : X = N ; Y = N

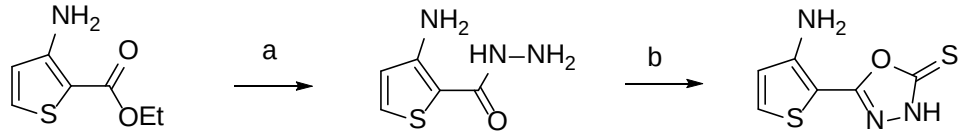
31a : X = N ; Y = CH
31b : X = N ; Y = N

32a : X = N ; Y = CH
32b : X = N ; Y = N

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

Scheme 6

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**.

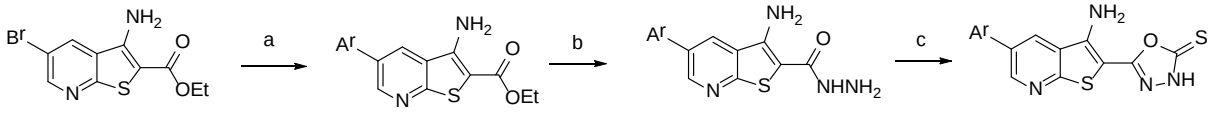


33 **34** **35**

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

Scheme 7

From a library design standpoint of view, the introduction of structural variety in the last step of the synthetic sequence is desirable. Therefore, the 5-bromo derivative **10b** was upscaled as potential building block for a number of Suzuki couplings.[17] Despite using a wide variety of reaction conditions, we were not able to isolate the desired bi-aryl compounds, presumably due to poisoning of the palladium catalyst by the presence of sulfur. Therefore, its synthetic precursor **8** was selected as starting material to be coupled 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). Upon treatment with hydrazine and carbon disulfude, the desired final compounds were obtained (Scheme 8).



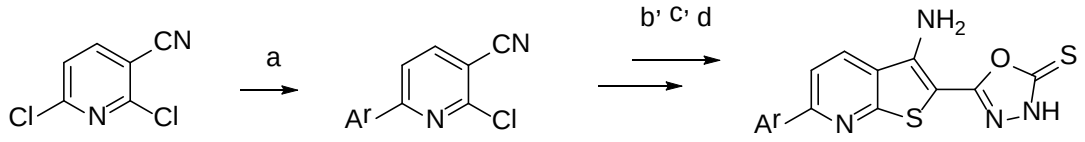
8 **36a-l** **37a-l** **38a-j** (Table 5)

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 8

In order to have access to the regio-isomeric 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 reactions yields selectively the 2-chloro-6-aryl-pyridine-3-carbonitrile analogues **40a–b**. This type of regioselective Suzuki couplings on similar substrates have been reported before in literature.[18] Nucleophilic displacement of the chlorine by ethyl 2-mercaptoacetate with concomitant ringclosure affords the desired 6-aryl-thieno[2,3-*b*]pyridine core structure. Finally, hydrazide and oxadiazole formation yields the final regio-isomeric analogues **41a–b**.



39 **40** **41a-b** (Table 6)

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

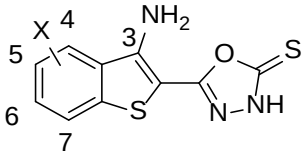
Scheme 9

Biological evaluation and structure-activity relationship studies

DRAK2 binding studies

The SAR study started with probing the optimal substitution pattern on the benzo moiety. A series of compounds with small structural variation was 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 %Ctrl. 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 strongly electron-withdrawing substituents (CF_3 and nitro) at different positions of the phenyl ring, affords benzothiophene analogues which display a decreased affinity for DRAK2 (e.g. compound **17f**) or are completely devoid of DRAK2 affinity (compounds

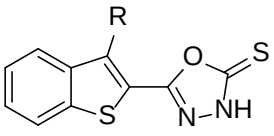
17b, **17e** and **20**). On the other hand, the presence of an electron-donating methoxy group (compounds **17a** and **17c**) affords analogues which are more potent, displaying %Ctrl of less than 10%. The most potent congeners were, however, found among the halogen-substituted (chlorine/bromine) and unsubstituted benzothiophene analogues, with %Ctrl of 5 or less. The 5-bromo (compound **9b**) and the unsubstituted (compound **9a**) analogues were subjected to dose-response curves affording K_d values of 0.3 and 0.61 μM, respectively, which is very comparable with the biological data obtained for the original hit compound **1** (Table 1).



Cmpd#	X	%Ctrl (10 μM)[a]	K_d (μM)[a]
1	5-Cl	3.6	0.25
9a	H	0.6	0.61
9b	5-Br	5.2	0.3
12	6-Cl	4.5	ND
15	5-NO_2	16	ND
17a	4-OCH_3	9.6	ND
17b	4-CF_3	35	ND
17c	5-OCH_3	7.6	ND
17d	5-OCF_3	31	ND
17e	7-CF_3	22	ND
17f	5-CF_3	9.1	ND
20	6-CF_3	32	ND

Table 1 : SAR of the benzo-moiety. [a]Values are the average of two independent experiments. ND = not determined.

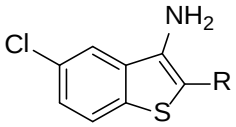
In order to probe into the importance of the amino group for DRAK2 binding, two novel analogues were evaluated. Whereas the 3-desamino congener **23a** shows less potent activity, the 3-methyl analogue **23b** is equipotent with the 3-amino derivative **10a**.



Cmpd#	R	%Ctrl (10 μM)[a]	K_d (μM) [a]
10a	NH_2	0.6	0.61
23b	CH_3	1.1	0.59
23a	H	7	ND

Table 2 : SAR of the amino group. [a]Values are the average of two independent experiments. ND = not determined.

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 an oxo (compound **24**) or methyl (compound **28**) leads to a decreased DRAK2 affinity. In addition, also the oxadiazole ring is essential for DRAK2 binding, as the triazole analogue **26** completely lacks DRAK2 affinity.



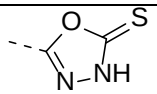
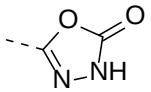
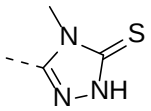
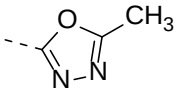
Cmpd#	R	%Ctrl (10 μM)[a]	K_d (μM) [a]
1		3.6	0.25
24		12	ND
26		82	ND
28		39	ND

Table 3 : SAR of the oxadiazole moiety. [a]Values are the average of two independent experiments. ND = not determined.

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 **10a** was chosen as reference. A simplified analogue (compound **35**), in which the benzo part 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 **32a**) or a pyrazine (yielding thieno[2,3-*b*]pyrazine **32b**), gives compounds with a five times stronger binding affinity for DRAK2, when compared to the benzothiophene skeleton **10a**.

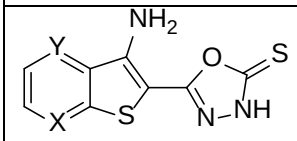
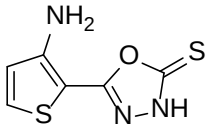
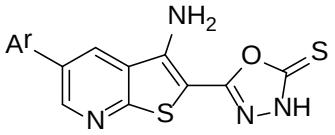
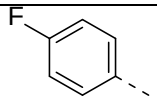
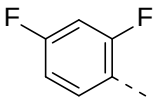
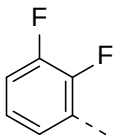
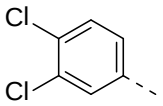
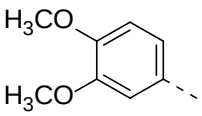
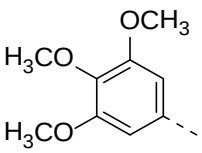
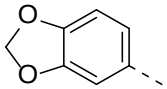
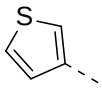
Structure	Cmpd	X	Y	%Ctrl (10 μM)[a]	K_d (μM)[a]
	**10a**	CH	CH	0.6	0.61
	32a	N	CH	0.65	0.12
	32b	N	N	1.5	0.14
	**35**	-	-	33	ND

Table 4 : SAR of the scaffold. [a]Values are the average of two independent experiments. ND = not determined.

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 favourable 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 **9b**) 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 in order 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 bearing structural variation on the 5-aryl part (Table 5). It seems that the presence of an aryl moiety at position 5 leads to strong affinity for the DRAK-2 enzyme and that quite some structural variation is tolerated at that position. Halogens (fluorine, chlorine) on the phenyl moiety afford compounds **38a–d** with K_d values 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 K_d values of 10 nM

and 16 nM, respectively for the 3,4-dimethoxy (compound **38e**) and the 3,4,5-trimethoxy-phenyl (compound **38f**) congener. 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. Especially, five-membered hetero-aromatics such as thienyl (compound **38h**) and furanyl (compound **38i**) give rise to very potent compounds, with K_d values of 8 nM and 9 nM, respectively. A 3-pyridinyl moiety (compound **38j**) leads to a 5-fold drop in DRAK2 affinity.



Cmpd	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

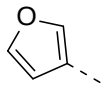
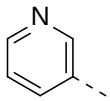
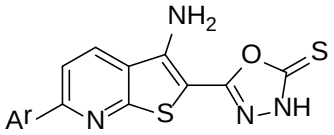
38i		7.7	0.009
38j		2.2	0.043

Table 5 : SAR of the aryl moiety. [a]Values are the average of two independent experiments. ND = not determined.

It is clear from the data in Table 5 that the presence of an aryl group at position 5, imparts high DRAK2 binding affinity. In order to determine the exact position, which is necessary for optimal activity, the corresponding 6-aryl regio-isomers **41a–b** were evaluated as potential DRAK2 ligands (Table 6). Both compounds show strong affinity for DRAK2, but are, however, 7-fold less potent when compared to the 5-substituted congeners.



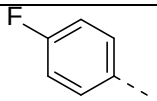
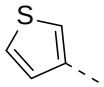
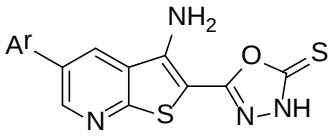
Cmpd	Ar	%Ctrl (10 μM) [a]	K_d (μM) [a]
41a		0.3	0.270
41b		0.1	0.061

Table 6 : SAR of 6-aryl-thieno[2,3-*b*]pyridines [a]Values are the average of two independent experiments.

Functional DRAK2 inhibition

Up to now, compounds were evaluated in assays, without using 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 as potent inhibitors of DRAK2 with IC_{50} values in the range of 30-60 nM.

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



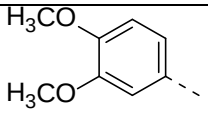
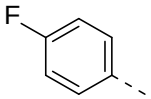
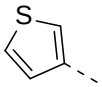
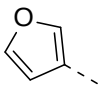
Cmpd#	Ar	K_d (μM)[a]	IC_{50} (μM)[a]	*c*Log*P*	LE (kcal·mol^{-1})
38a		0.010	0.058	3.17	0.42
38b		0.038	0.057	3.59	0.44
38d		0.008	0.029	3.12	0.53
38e		0.009	0.033	2.62	0.52

Table 7 : Profile of selected lead compounds. [a]Values are the average of two independent experiments

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. In order to have a first 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 have also a strong binding affinity for DRAK1, DAPK1 and DAPK2, whereas the affinity for DAPK3 is in general 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 7-fold level of selectivity was present between DRAK2 and DAPK1. However, compound **38d** (bearing a 5-(3,4-dichlorophenyl) moiety), although being less active as DRAK2 binder (K_d=0.27 μM) shows an excellent selectivity profile, lacking any affinity for the other members of DAPK family.

Cmpd#	DRAK2 K_d (μM)	DRAK1	DAPK1	DAPK2	DAPK3
38a	0.038	%Ctrl=0.35	%Ctrl=0.55	%Ctrl=0.9	%Ctrl=6
38d	0.270	%Ctrl=23	%Ctrl=46	%Ctrl=30	%Ctrl=36
38e	0.010	%Ctrl=0.35 K_d=0.16 μM	% Ctrl=0.4	%Ctrl=0.55	%Ctrl=5
38h	0.008	%Ctrl=0.35 K_d=0.099 μM	%Ctrl=0.35 K_d =0.054 μM	%Ctrl=0.35 K_d=0.647 μM	%Ctrl=1.8
38i	0.009	%Ctrl=0.4	%Ctrl=1	%Ctrl=1.4	%Ctrl=6

Table 8 : Selectivity profile of selected lead compounds
All values are the average of two independent experiments.

Conclusion

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 behave also as strong, functionally active inhibitors. Moreover, based on determined in silico parameters (such as *c*log*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

General

For all reactions, analytical grade solvents were used. All moisture-sensitive reactions were carried out in oven-dried glass-ware (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. Precoated 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 thiophosgene (0.026 mL, 0.340 mmol) in acetone (0.35 mL) was added dropwise a solution of aminoester **3** in acetone (1.15 mL) and the mixture was stirred overnight at room temperature. Then, an additional amount of thiophosgene (0.020 mL) was added and stirring was continued for 24 h. Water was added, the precipitate was filtered off, washed with water until neutral pH of filtrate 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 down to room temperature. The resulting precipitate was filtered off, washed with toluene, hexane and dried. The crude product was purified by column chromatography (DCM/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 mercaptoacetate (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 mercaptoacetate (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 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 mercaptoacetate (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, [D6]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 mercaptoacetate (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 mercaptoacetate (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

yellowish 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 mercaptoacetate (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 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 mercaptoacetate (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 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 mercaptoacetate (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 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 mercaptoacetate (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, quantit.): ^{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*≈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 refluxed 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 yellowish 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 yellowish 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 yellowish 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 mercaptoacetic 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)benoznitrile (**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):

General procedure

A mixture of ethyl carboxylate **8** (0.563 mmol) and 30% aqueous NaOH (0.282 mL, 2.815 mmol) in EtOH (10 mL) was refluxed 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 redissolved 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 (DCM) affording compound **13 (X=4-CF_3)** as a yellowish 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 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 yellowish 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 refluxed for 2 h. Then additional amount of $SOCl_2$ (0.020 mL, 0.275 mmol) was added and refluxing 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):
General procedure
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 yellowish 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 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 (DCM/MeOH 98:2) affording compound **16e** as a 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 (DCM/MeOH 98:2) affording compound **16f** as a 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):
General procedure
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 (DCM/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 (DCM/EtOAc 4:1) affording compound **10a** as a brown solid (400 mg, 80%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=3.45 (br s, H), 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 $C_{10}H_7N_3OS_2$: 250.1032, 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 (DCM/EtOAc 4:1) affording compound **10b** as a dark yellow solid (17 mg, 29%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=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): δ=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 brownish solid (25 mg, 22%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=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): δ=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 (DCM/MeOH 100:0→96:4) affording compound **15** as a brown-red solid (60 mg, 53%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=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): δ=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 (DCM/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): δ=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): δ=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): δ=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): δ=93.17, 122.97 (q, $^2J_{C–F}$=32 Hz), 123.81 (q, $^3J_{C–F}$=6 Hz), 123.92 (q, $^1J_{C–F}$=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): δ=–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 (DCM/EtOAc 5:1) affording compound **17c** as an orange solid (11 mg, 12.5%): ^{1}H NMR

(300 MHz, [D_6]DMSO): δ=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): δ=55.53, 88.60, 105.09, 117.77, 118.04, 124.04, 130.19, 132.46, 144.86, 151.53, 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 (DCM/MeOH 100:2) affording compound **17d** as a yellow solid (43 mg, 27%): ^{1}H NMR (300 MHz, [D_6]DMSO): δ=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): δ=89.99, 115.18, 120.18 (q, $^1J_{C-F}$=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): δ=−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 (DCM/EtOAc 4:1) affording compound **17e** as a yellow solid (26 mg, 22%): ^{1}H NMR (300 MHz, [D_6]DMSO): δ=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): δ=88.47, 123.39 (q, $^2J_{C-F}$=33 Hz), 123.92 (q, $^1J_{C-F}$=273 Hz), 124.96, 125.74 (q, $^3J_{C-F}$=3.6 Hz), 127.11, 133.43, 133.94, 144.39, 158.35, 174.67 ppm; ^{19}F NMR (470 MHz, [D_6]DMSO): δ=−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 (DCM/MeOH 100:0→99:1) affording compound **17f** as s yellow solid (39 mg, 31%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=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): δ=89.65, 120.29, 123.40, 124.52, 124.53 (q, $^{1}J_{C-F}$=272 Hz), 125.41 (q, $^{2}J_{C-F}$=32 Hz), 131.34, 141.67, 144.47, 158.41, 175.37 ppm; ^{19}F NMR (470 MHz, [D$_6$]DMSO): δ=−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 (DCM/EtOAc 4:1) affording compound **20** as a yellow solid (62 mg, 58%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=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): δ=90.75, 120.87, 121.10 (q, $^{3}J_{C-F}$=3.3 Hz), 123.74, 124.26 (q, $^{1}J_{C-F}$=272 Hz), 127.51 (q, $^{2}J_{C-F}$=32 Hz), 134.13, 138.14, 144.12, 158.36, 175.37 ppm; ^{19}F NMR (470 MHz, [D$_6$]DMSO): δ=−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): δ=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): δ=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): δ=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): δ=13.10, 117.69, 122.98, 123.70, 125.33, 127.33, 136.95, 138.89, 139.53, 154.11, 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): δ=6.64 (br s; 2H), 7.50 (d, *J*=8.4 Hz, 1H), 7.94 (d, *J*=8.4 Hz, 1H), 8.27 (s, 1H), 12.51 ppm (br s, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ=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 acylhydrazide **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 refluxed 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 yellowish solid (70 mg, 65%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=2.87 (d, *J*=3.9 Hz, 3H), 7.23 (br s, 2H), 7.50 (d, *J*=8.4 Hz, 1H), 7.91 (d, *J*=8.4 Hz, 1H), 7.99 (br s, 2H), 8.24 (s, 1H), 9.26 (br s, 1H), 9.58 ppm (br s, 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 refluxed for 4 h. Then, it was cooled to room temperature, diluted with H$_2$O and acidified using a 1N HCl solution. The formed precipitate was filtered off, washed with H$_2$O and dried affording compound **26** as a solid (54 mg, 89%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=3.63 (s, 1H), 6.56 (br s, 2H), 7.49 (d, *J*=7.5 Hz, 1H), 7.96 (d, *J*=7.5 Hz, 1H), 8.24 (s, 1H), 13.96 ppm (br s, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ=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 acylhydrazide **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 refluxed for 4 h. Then, it was cooled in an ice-water bath. The precipitate was filtered-off, washed with cold DCM, H$_2$O and Et_2O and dried affording compound **27** as a solid (97 mg, 82%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=1.91 (s, 3H), 7.18 (br s, 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 (br s, 1H), 9.75 ppm (br s, 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 DCM (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 (DCM/EtOAc 100:0→95:5) affording compound **28** as a grey solid (12 mg, 29%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=2.56 (s, 3H), 6.98 (br s, 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): δ=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 methanol (76 mL) was added methyl mercaptoacetate (2.55 mL, 28.5 mmol) and triethylamine (28.5 mmol, 3.97 ml). The reaction mixture was refluxed overnight. The solvents were evaporated and the residue was purified by flash chromatograph on silica, the mobile phase being a mixture of ethylacetate 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): δ=1.30 (t, *J*=7.1 Hz, 3H), 4.28 (q, *J*=7.1 Hz, 2H), 7.30 (br s, 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 ethanol (20 ml) was refluxed 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 yellowish solid (1.0 g, 96%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=4.43 (br s, 2H), 7.14 (br s, 2H), 7.42 (dd, *J*=8.1 Hz, 4.6 Hz, 1H), 8.42 (dd, *J*=8.1Hz, 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 refluxed 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): δ=6.95 (br s, 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): δ=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), 8.78 ppm (dd, *J*=15.54 Hz, 2.31 Hz, 2H).

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.

Synthesis of 5-(3-amino-5-aryl-thieno[2,3-*b*]pyridin-2-yl)-1,3,4-oxadiazole-2(3*H*)-thiones 38a–j

General procedure

Step a

A mixture of ethyl 3-amino-5-bromobenzo[*b*]thiophene-2-carboxylate (**8**) (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 refluxed 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 methanol and dichloromethane affording the pure title compounds **38a–j**.

The following compounds were made according to this procedure :

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 silicagel 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 yellowish 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, 5.4Hz, 2H), 8.93 (d, *J*=2.1 Hz, 1H), 8.99 ppm (d, *J*=2.1Hz, 1H); ^{13}C NMR (150 MHz, [D$_6$]DMSO): δ=87.27, 116.19 (J_{C-F}=21.36 Hz), 125.81, 128.79, 129.10 (J_{C-F}=8.2 Hz), 129.13, 131.14, 133.17, 142.87, 148.29, 158.22 (J_{C-F}=70.62 Hz), 161.61, 163.24, 175.53 ppm; HRMS (ESI): *m*/*z* [*M*+H]$^+$ calcd for $C_{14}H_9N_4OS_2$: 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 silicagel 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 silicagel 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 silicagel 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 silicagel 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 $C_{17}H_8N_{14}O_3S_2$: 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 silicagel 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): δ=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): δ=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 silicagel 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): δ=6.11 (s, 2H), 7.10 (d, *J*=8.0 Hz, 1H), 7.32 (d, *J*=6.5 Hz, 1H7.41 (s, 1H), 8.86 (s, 1H), 8.89 (s, 1H), 8.95 ppm (s, 1H); ^{13}C NMR (150 MHz, [D$_6$]DMSO): δ=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 silicagel 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): δ=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): δ=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 silicagel 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 compound **38j** as a yellow solid (49%): ^{1}H NMR (300 MHz, [D_6]DMSO): δ=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 ppm (s, 1H), 9.06 (s, 1H); ^{13}C NMR (150 MHz, [D_6]DMSO): δ=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-aryl-thieno[2,3-*b*]pyridin-2-yl)-1,3,4-oxadiazole-2(3*H*)-thione analogues 41a–41b

General procedure

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 ethanol (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 methanol and dichloromethane (in a ratio of 1/10 to 1/5), yielding the pure title compounds **41a−b** as a white solid.

The following compounds were synthesized according to this procedure :

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 4 steps). ^{1}H NMR (300 MHz, [D_6]DMSO): δ=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): δ=89.25, 115.86 (J_{C-F}=21.33 Hz), 116.44, 124.83, 129.26 (J_{C-F}=8.4 Hz), 131.50, 134.43, 140.53, 154.38, 158.99 (J_{C-F}=77.01 Hz), 162.40, 164.03, 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 4 steps). ^{1}H NMR (300 MHz, [D_6]DMSO): δ=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): δ=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.

DRAK2 binding assay

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

DRAK2 functional enzymatic Assay

DRAK2 functional assays have been performed at SignalChem uses a radioisotope assay format, as described by the provider (www.signalchem.com)

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