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# Discovery of Dual Death-Associated Protein Related Apoptosis Inducing Protein Kinase 1 and 2 Inhibitors by a Scaffold Hopping Approach

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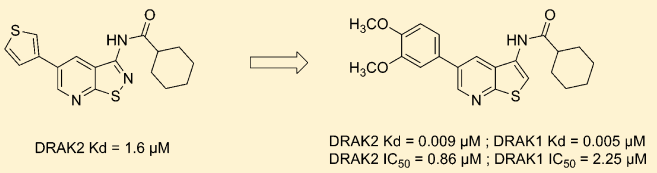
**ABSTRACT:** DRAK2 emerged as a promising drug target for the treatment of autoimmune diseases and to prevent graft rejection after organ transplantation. Screening of a compound library in a DRAK2 binding assay led to the identification of an isothiazolo[5,4-*b*]pyridine derivative as a novel ligand for DRAK2, displaying a $K_d$ value of 1.6 $\mu$M. Subsequent medicinal chemistry work led to the discovery of a thieno[2,3-*b*]pyridine derivative with strong DRAK2 binding affinity ($K_d$ = 9 nM). Moreover, this compound also behaves as a functional inhibitor of DRAK2 enzymatic activity, displaying an $IC_{50}$ value of 0.82 $\mu$M, although lacking selectivity, when tested against DRAK1. This paper describes for the first time functionally active dual DRAK1 and DRAK2 inhibitors that can be used as starting point for the synthesis of chemical tool compounds to study DRAK1 and DRAK2 biology, or they can be considered as hit compounds for hit-to-lead optimization campaigns in drug discovery programs.




## INTRODUCTION

Although the human kinome comprises more than 500 protein kinases,[1] only a small fraction of the kinome is currently being targeted by reasonably selective and potent inhibitors. However, small-molecule cell-permeable inhibitors of protein kinases are of high value. They are useful tool compounds to investigate the physiological role of protein kinases, because they can be used to block the endogenous kinase activity in cells, and, in addition, these compounds can be used as starting points in hit-to-lead optimization campaigns in drug discovery programs if the kinase is sufficiently validated as drug target. Protein kinases are the second most exploited group of drug targets, after the G-protein-coupled receptors (GPCR's). Hence, much research has been devoted to the identification of small molecule kinase inhibitors and it has been stated that currently 20−30% of the drug discovery programs in the pharmaceutical industry are focused on kinases as drug targets.[2]

The vast majority of the kinase targets are being investigated for the treatment of cancer, which has led to the FDA approval of a number of small molecules (Figure 1).[3] Imatinib was the first kinase inhibitor drug to be developed targeting Abelson tyrosine kinase and is used in the treatment of chronic myeloid leukemia (Figure 1). Since its approval, many more compounds have undergone clinical trials in different oncology indications and a number of novel kinase inhibitors were licensed for anticancer use. Examples include ruxolitinib (JAK1/2-inhibitor approved for the treatment of myelofibrosis), dasatinib (targeting Scr, ABL1, KIT, PDGFR, and Eph) and gefitinib (EFGR inhibitor licensed for the treatment of nonsmall cell lung cancer).

Although other therapeutic areas are lagging behind, there is increasing interest in the development of kinase target therapeutics for nononcology indications. Kinases have been proposed as therapeutic targets for heart failure,[4] CNS disorders,[5] and inflammatory diseases.[6]

In this paper, DAPK related apoptosis inducing protein kinase 2 (DRAK2) was selected as a kinase target. DRAK2, also known as STK17B, is a serine/threonine protein kinase which belongs to the DAPK (death-associated protein kinase) family.[7] The remaining four members are DAPK1, DAPK2, DAPK3, and DRAK1.[8] All members of the DAPK family have been shown to induce apoptosis upon ectopic expression in various cell types. Although DRAK2 is broadly expressed at low levels, DRAK2 expression is highly enriched in T and B cells, whereas it is not expressed at significant levels in NK cells, macrophages, or dendritic cells.[9] Due to the lymphoid specific nature of DRAK2, its potential as a therapeutic target in immunological diseases has been investigated. Genetic deletion of DRAK2 afforded mice that are resistant to autoimmune diseases, such as EAE (experimental autoimmune encephalomyelitis, which is an animal model of multiple sclerosis) and type 1 diabetes.[10,11] The absence of DRAK2 in T cells in these animal models leads to an increased death of T-cells and did not affect the survival of T cells expanding during infections. It suggests that DRAK2

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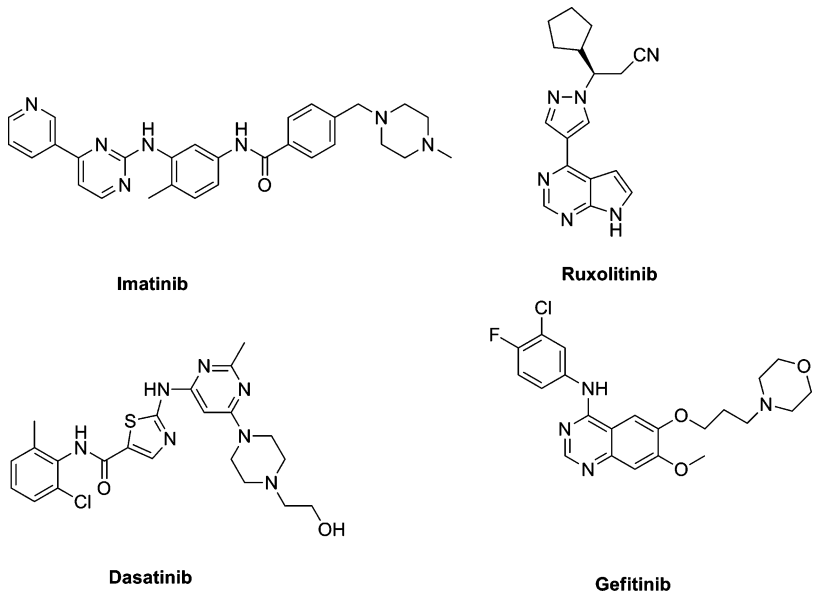


**Figure 1.** Clinically used kinase inhibitors.

is essential for the survival of T cells in chronic, autoimmune stimulation. Recent studies demonstrated that DRAK2 signaling is required for efficient allorejections.[12] It supports the notion that DRAK2 inhibition may lead to long-term allograft maintenance and that DRAK2 inhibition is a therapeutic option to prevent graft rejection after organ transplantation.

All together, these data suggest that DRAK2 inhibitors hold promise for the treatment of a variety of autoimmune diseases, as well as to prevent graft rejection after organ transplantation. To the best of our knowledge, only one example of a small-molecule inhibitor targeting DRAK2 has been reported in the literature.[13] In this paper, a low micromolar inhibitor of DRAK2 was identified, which at a concentration of 1 nM is able to induce neuronal differention. In this study, there seems to be a discrepancy between the DRAK2 target affinity (low $\mu$M) and the cellular activity (low nM). In addition, the exact chemical structure of this compound in not mentioned. In order to evaluate the therapeutic potential of DRAK2 inhibition, we have set up a program aimed at the identification of DRAK2 inhibitors. Conventional kinase screenings rely on assays that measure directly enzymatic activities. These types of assays need to be optimized with respect to the selection of the substrate, the ATP concentration, and the buffer composition. Furthermore, when selectivity data are required against a broad panel of kinases, this classical screening is expensive and time-consuming. Therefore, we opted for a binding, rather than a functional assay, for the identification of novel DRAK2 inhibitors, using the KinomeScan kinase platform. This platform has been extensively used to assess the selectivity of known kinase inhibitors.[14] In contrast, we used this screening platform as a primary tool to discover ligands for the DRAK2 enzyme, and then followed up with the conventional *in vitro* enzymatic assays. Screening of a representative selection of 150 structurally diverse compounds from our proprietary compound library for potential ligands for DRAK2 in the binding assays of DiscoverX, led to the identification of a hit compound based on an isothiazolo[5,4-*b*]pyridine scaffold (Figure 2), displaying a $K_d$ value of 1.6 $\mu$M. Moreover, this compound has no affinity for other kinases when evaluated in a panel of 54 kinases at a concentration of 10 $\mu$M. Other, *in silico* determined

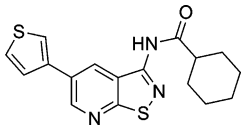

**Figure 2.** Hit compound.

parameters, such as the Polar Surface Area (PSA: 53.82 Å$^2$), partition coefficient (clogP = 4.4), and ligand efficiency (LE = 0.34 kcal/mol/non-H) confirm this compound to be an excellent starting point for medicinal chemistry work.

This manuscript describes our efforts toward the discovery of novel DRAK2 inhibitors, starting from an isothiazolo[5,4-*b*]pyridine derivative, using a combination of classical medicinal chemistry (changing the decoration pattern of the original scaffold) and a scaffold hopping approach.

## CHEMISTRY

**Synthesis of Isothiazolo[5,4-*b*]pyridines 10.** A general synthetic scheme used for the preparation of the isothiazolo-[5,4-*b*]pyridine library is depicted in Scheme 1. Starting from commercially available 2-chloro-3-cyano-pyridine **1**, the corresponding pyridinone derivative **2** was obtained under acidic conditions. The introduction of a bromine atom by treatment with bromine yielded the 2-hydroxy-3-cyano-5-bromo-pyridine derivative **3**. The tautomeric hydroxyl group at position 2 of the pyridine scaffold was converted to its corresponding chlorine, by treatment with $POCl_3$.[15] Nucleophilic displacement of the chlorine by reaction with methyl 3-mercaptopropionate afforded intermediate **5**. Deprotection with sodium hydride yielded the 2-thio-pyridine analogue **6**, which was then *in situ* ring closed with sodium hypochlorite as oxidizing agent, affording the isothiazole ring system **7**.[16,17] This reaction proceeds via a sulfenamide intermediate by the reaction of the 2-thio-pyridine analogue with chloramine (formed *in situ* from sodium hypochlorite and ammonia).[18,19] 3-Amino-5-bromo-isothiazolo[5,4-*b*]pyridine **7** was a key intermediate for library synthesis around the isothiazolo[5,4-*b*]pyridine core. The sequence that was followed depends on the need in the SAR study. Either a palladium-catalyzed Suzuki reaction was


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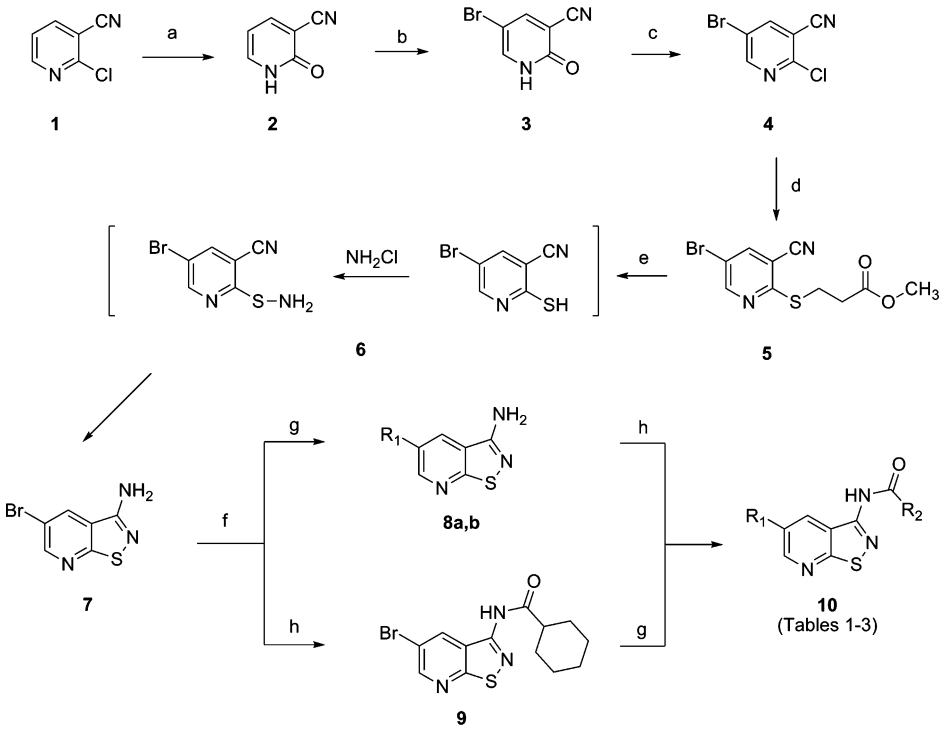
Scheme 1[a]




[a]Reagent and conditions: (a) $CH_3COOH$, reflux; (b) $Br_2$, $CH_3COOH$, rt; (c) $POCl_3$, $PCl_5$, reflux; (d) methyl 3-mercaptopropionate, $NaOCH_3$, DMF, rt; (e) NaH, THF, reflux; (f) $NH_3$, NaOH, NaOCl, $H_2O$, 0 °C; (g) $K_2CO_3$, $Pd(PPh_3)_4$, arylboronic acid, dioxane/water, 90 °C; (h) RC(O)Cl, $CH_2Cl_2$, rt or RCOOH, TBTU, DMF, rt.

performed first, followed by amide formation, or alternatively, the amide moiety was constructed, followed by Suzuki coupling.[20] Suzuki couplings were performed in a mixture of dioxane/water, using potassium carbonate as a base and $(PPh_3)_4Pd$ as catalyst. For the construction of the amide moiety, the coupling with a series of acid chlorides was effected. If the acid chloride was not commercially available, we switched to the coupling of the carboxylic acid using *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU) as coupling reagent. Alternatively, in a number of cases, the carboxylic acid was converted to its corresponding acid chloride, by treatment with thionyl chloride. These synthetic procedures allowed us to prepare a library around the isothiazolo[5,4-*b*]pyridine scaffold (see Tables 1, 2, and 3 for the exact structures that have been synthesized).

**Synthesis of Isothiazolo[5,4-*b*]pyrazine 15 and pyrazolo[3,4-*b*]pyrazine 17.** The synthesis of both fused pyrazine bicycles is shown in Scheme 2 and starts from 6-bromo-3-chloro-pyrazine-2-carbonitrile **11**, which has been synthesized according to literature procedures.[21,22] A Suzuki type of coupling with 3,4-dimethoxyphenylboronic acid yielded pyrazine analogue **12** as a key intermediate. Nucleophilic displacement with methyl 3-mercaptopropionate, followed by alkaline deprotection and oxidative ring closure afforded the 3-amino-isothiazolo[5,4-*b*]pyrazine **14**, which was acylated in a final step affording derivative **15**. Alternatively, by using hydrazine as nucleophile, the 3-pyrazolo[3,4-*b*]pyrazine **16** is accessible. Condensation with cyclohexane carbonyl chloride furnished target compound **17**.

**Synthesis of Benzo[*b*]thiophene 22a, thieno[2,3-*b*]-pyridine 22b and thieno[2,3-*b*]pyrazine 22c.** The synthesis of a series of fused thiophene derivatives is shown in Scheme 3. Nucleophilic aromatic substitution of chloronitriles **11** and **18a**–**b** with ethyl mercaptoacetate upon cooling provides the ethyl carboxylates **19a**–**c**. Hydrolysis of the ethyl carboxylate to the carboxylic acid is followed by acidic decarboxylation[23] affording the 3-amino-thiophene derivatives **20a**–**c**. Subsequently, the cyclohexyl residue and the aryl moiety are introduced by acylation and Suzuki reaction, respectively, to afford the final compounds **22a**–**c**.

**Synthesis of Pyrrolo[2,3-*b*]pyridine 25.** Starting from commercially available 5-bromo-3-nitro-pyrrolo[2,3-*b*]pyridine **23**, the 3,4-dimethoxyphenyl moiety was introduced via standard Suzuki reaction. Catalytic reduction of the nitro group using Raney Nickel as catalyst yielded the 3-amino pyrrolo[2,3-*b*]pyridine derivative.[24] Subsequent coupling with the appropriate acid chloride (Scheme 4) furnished the desired pyrrolo[2,3-*b*]pyridine analogue **25**.

**Synthesis of Benzo[*d*]isothiazole 30 and Benzo[*d*]-isoxazole 33.** The synthesis of both scaffolds starts from ortho-fluoronitrile **26** (Scheme 5). Nucleophilic aromatic displacement of the fluorine with $Na_2S$ followed by treatment with Zn/HCl provides the corresponding mercaptobenzonitrile **27**.[25] Oxidative ring closure with sodium hypochlorite allows buildup of the isothiazole moiety. Acylation of the amino group, followed by Suzuki reaction, affords the desired benzo[*d*]isothiazole **30**. 5-Bromobenzo[d]isoxazol-3-amine **31** is generated via an one-pot reaction of ortho-fluoronitrile **26** and acetohydroxamic acid in basic condition, with concomitant intramolecular cyclization, furnishing the benzo[*d*]isoxazole scaffold **31**.[26] Subsequent amide formation and Suzuki coupling yields the desired benzo[*d*]isoxazole derivative **33**.

**Synthesis of Indazole 36.** In the first step, the endocyclic nitrogen of the indazole building block **34** is protected with a


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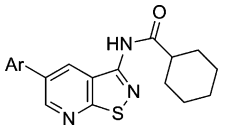
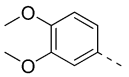
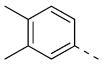
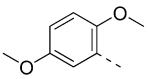
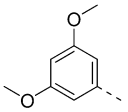
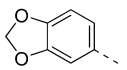
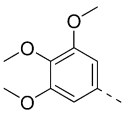
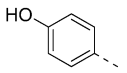
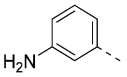
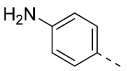
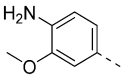
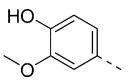
Table 1. SAR of the 5-Aryl Moiety of Isothiazolo[5,4-*b*]pyridines



| Cpmd# | Ar | % Ctrl (10 μM) [a] | Kd (μM) [a] |
|---|---|---|---|
| **10a** |  | 5,9 | 1.6 |
| **10b** |  | 3,9 | 1,9 |
| **10c** |  | 19 | ND |
| **10d** |  | 20 | ND |
| **10e** |  | 5,9 | 1,6 |
| **10f** |  | 22 | ND |
| **10g** |  | 89 | ND |
| **10h** |  | 53 | ND |
| **10i** |  | 76 | ND |
| **10j** |  | 23 | ND |
| **10k** |  | 6,4 | 3,2 |
| **10l** |  | 8,7 | 2,3 |
| **10m** |  | 48 | ND |
| **10n** |  | 3,2 | 0,66 |
| **10o** |  | 11 | 2 |

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

Boc protecting group (Scheme 6). Acylation of the exocyclic amino group is then followed by the standard Suzuki reaction. Finally, acidic deprotection of the Boc protecting group yields the desired indazole **36**.

**Synthesis of Pyrido[2,3-*d*]pyrimidine 40a and pyrido-[3,2-*d*]pyrimidine 40b.** The synthesis starts from suitable amino-nicotinonitrile derivatives **37a**−**b** (Scheme 7). Construction of the pyrimidine moiety was achieved by heating with triethyl orthoformate as one-carbon fragment, yielding the pyrido[2,3-*d*]pyrimidine and pyrido[3,2-*d*]pyrimidine core structures **38a** and **38b**, respectively.[27] The dimethoxyphenyl moiety is introduced via a Suzuki coupling, followed by reaction of the exocyclic amino group with cyclohexane carboxylic acid chloride, affording the desired final compounds **40a**−**b**.

**Synthesis of Quinazoline 45a and Pteridine 45b.** Starting from an appropriately substituted benzoic acid **41a** or pyrazine carboxylate **41b** analogue, formation of the pyrimidin-4(3*H*)-one moiety was achieved by treatment with triethyl


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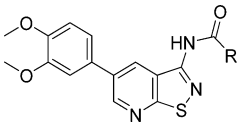
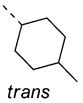
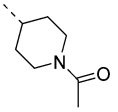
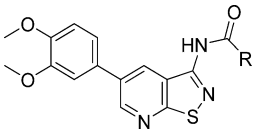
Table 2. SAR of the Cyclohexyl Moiety

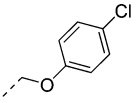


| Cpmd# | Ar | % Ctrl (10 μM) [a] | Kd (μM) [a] |
|---|---|---|---|
| 10p |  | 24 | ND |
| 10q |  | 8,8 | 1,6 |
| 10r |  | 3,8 | 2,2 |
| 10e |  | 5,9 | 1,6 |
| 10s |  | 1,4 | 0,53 |
| 10t |  | 16 | ND |
| 10u |  | 20 | ND |
| 10v |  | 37 | ND |
| 10w |  | 43 | ND |
| 10x |  trans | 20 | ND |
| 10y |  | 17 | ND |
| 10z |  | 8,2 | 7,2 |

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

Table 3. SAR of the Amide Moiety



| Cpmd# | Ar | % Ctrl (10 μM) [a] | Kd (μM) [a] |
|---|---|---|---|
| 10aa |  | 39 | ND |
| 10ab |  | 42 | ND |
| 10ac |  | 74 | ND |
| 10ad |  | 59 | ND |
| 10ae |  | 52 | ND |
| 10af |  | 74 | ND |

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

formate.[28] Suzuki reaction allowed the introduction of the 3,4-dimethoxyphenyl moiety. Chlorination of the lactam group with $POCl_3$ and introduction of the amino group at position 4 was followed by amide formation by reaction with cyclohexanecarboxylic acid chloride, affording the quinazoline and pteridine derivatives **45a** and **45b**, respectively.

## BIOLOGICAL EVALUATION AND STRUCTURE–ACTIVITY RELATIONSHIP STUDIES

As mentioned in the Introduction, we opted for a binding assay to detect DRAK2 ligands. In a first round of screening, compounds were tested at a concentration of 10 $\mu$M. Compounds that bind to the kinase ATP site displace the immobilized ligand from the kinase/phage, which is then detected using quantitative PCR. The results are reported as the percentage of kinase/phage remaining bound to the ligands/beads, relative to a control. High affinity compounds have %Ctrl = 0, while weaker binders have higher % control values. For the most promising compounds, dose–response curves were generated to determine $K_d$ values.

In a first round of optimization, the isothiazolo[5,4-*b*]pyridine scaffold, as well as the amide moiety were fixed, and structural variation was introduced by the synthesis of a number of substituted aryl and heteroaryl groups (Table 1). Replacement of the 3-thienyl group of the hit compound **10a** by a 2-thienyl afforded compound **10b** with similar DRAK2 affinity. In contrast, the presence of a 3-furanyl moiety furnished analogue **10c** with greatly reduced DRAK2 affinity. Similarly, changing the thienyl into a phenyl (which is considered as a bioisoster of the thiophene) led to compound **10d**, displaying a strongly reduced DRAK2 affinity. The presence of a 3,4-dimethoxyphenyl moiety affords an analogue with potent DRAK2 binding affinity, displaying a $K_d$ value of 1.6 $\mu$M. A small series of analogues was prepared around this 3,4-dimethoxyphenyl moiety. Dimethyl derivative **10f** (carrying two electron-donating groups as in the dimethoxyphenyl substructure) was synthesized, but has no activity as DRAK2 ligand. Different regio-isomeric dimethoxyphenyl derivatives (compounds **10g–h**) were also prepared and were found to be completely devoid of any DRAK2 affinity, indicating the importance of the exact positioning of both methoxy groups. The dioxolane analogue **10i** (to be considered as the ring closed derivative of the 3,4-dimethoxyphenyl analogue) is completely devoid of DRAK2 affinity. The insertion of an additional methoxy group affords the 3,4,5-trimethoxy analogue **10j**, which lacks DRAK2 affinity.

Electron-donating substituents, such as a hydroxyl function (compound **10k**) and an amino group (compound **10l**) furnishes compounds with affinity for the DRAK2 enzyme, displaying $K_d$ values in the low $\mu$M range. It seems although that the exact position of the amino group is important, as the 4-amino analogue **10m** does not show any affinity for the DRAK2 enzyme.

Finally, structural features endowed with promising DRAK2 affinity (i.e., the dimethoxyphenyl moiety and the amino/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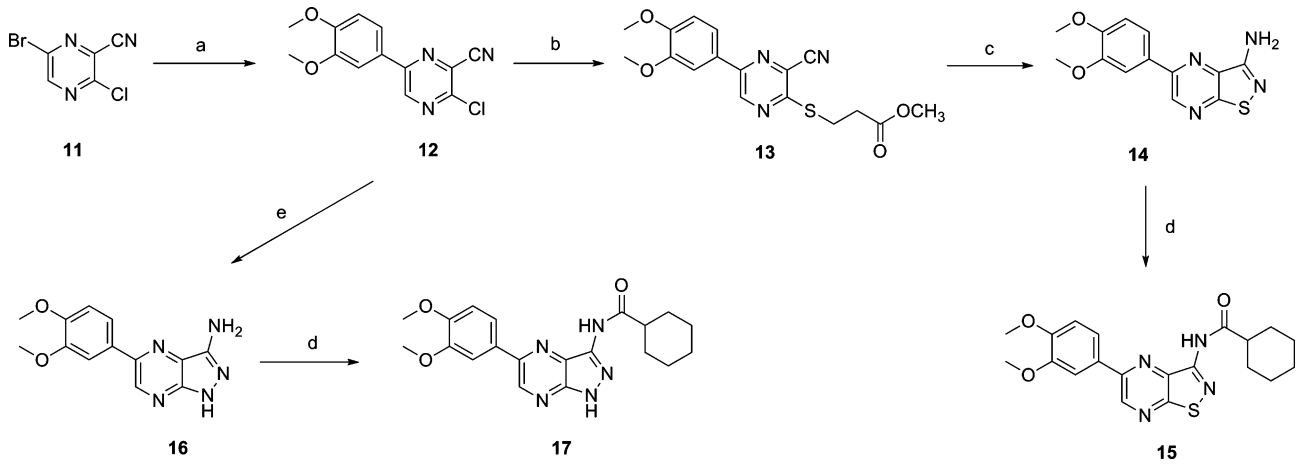
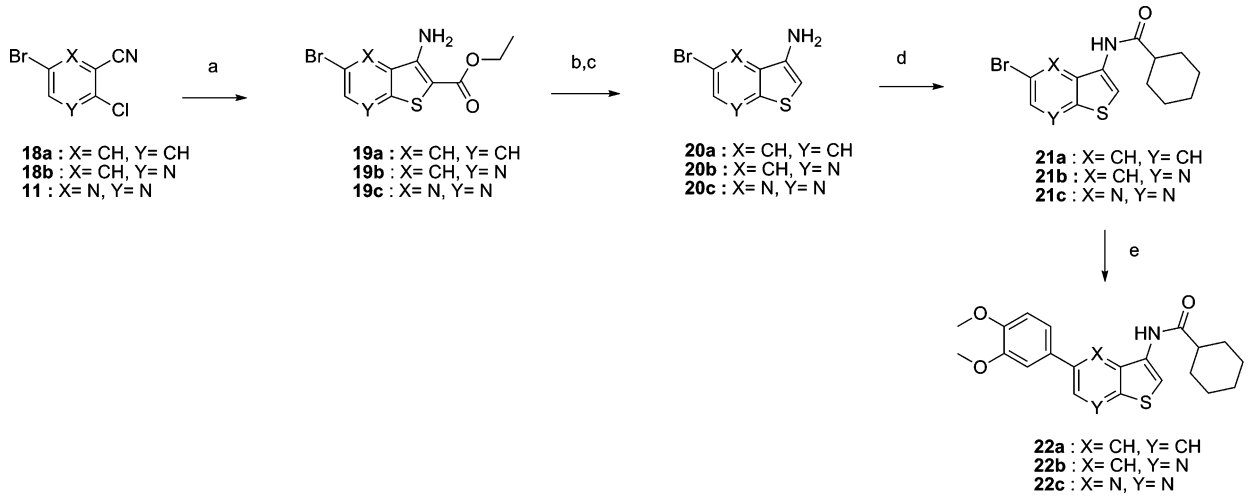
**Scheme 2**[a]



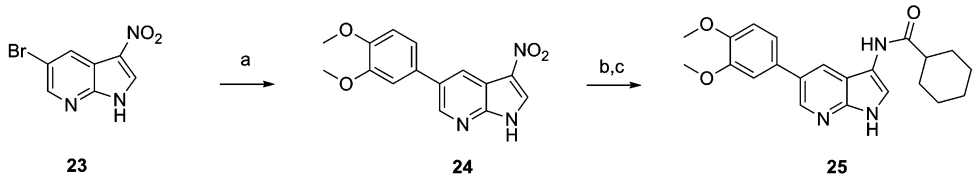

[a]Reagents and conditions: (a) $K_2CO_3$, $Pd(PPh_3)_4$, 3,4-dimethoxyphenylboronic acid, dioxane/water, 90 °C; (b) methyl 3-mercaptopropionate, $NaOCH_3$, DMF, rt; (c) (i) NaH, THF, reflux ; (ii) $NH_3$, NaOH, NaOCl, $H_2O$, 0 °C; (d) cyclohexanecarboxylic acid chloride, DCM; (e) $N_2H_4$, EtOH, reflux.

**Scheme 3**[a]




[a]Reagents and conditions: (a) ethyl 2-mercaptoacetate, *t*-BuOK, DMF, 0 °C; (b) NaOH, EtOH, reflux; (c) $H_3PO_4$; (d) cyclohexanecarboxylic acid chloride, DCM; (e) $K_2CO_3$, $Pd(PPh_3)_4$, 3,4-dimethoxyphenylboronic acid, DME, 90 °C.

**Scheme 4**[a]




[a]Reagents and conditions: (a) $K_2CO_3$, $Pd(PPh_3)_4$, 3,4-dimethoxyphenylboronic acid, dioxane/water, 90 °C; (b) Raney Ni/$H_2$, methanol, rt; (c) cyclohexanecarboxylic acid chloride, DIPEA, dichloromethane, rt.

hydroxyl substituent) were combined into one molecule. It afforded the 4-hydroxy-3-methoxyphenyl derivative (compound **10o**) and the 4-amino-3-methoxyphenyl congener **10n**, displaying $K_d$ values of 2 and 0.66 $\mu$M, respectively.

As structural modifications of the aryl part did not lead to the desired drastic improvement in DRAK2 affinity, a series of analogues was prepared in which the 3,4-dimethoxyphenyl group and the isothiazolo[5,4-*b*]pyridine core were kept intact and the exocyclic amino group was used to introduce structural variety by the synthesis of a series of amides. The study started with the synthesis of a series of cyclo-aliphatic amides, ranging from 3- to 7-membered rings (Table 2). A cycloheptyl ring seems to optimal for activity, displaying a $K_d$ value of 0.53 $\mu$M (compound **10s**). A cyclopentyl (compound **10r**) and a cyclohexyl derivative (compound **10e**) only lose 3- to 5-fold activity when compared to analogue **10s**. The introduction of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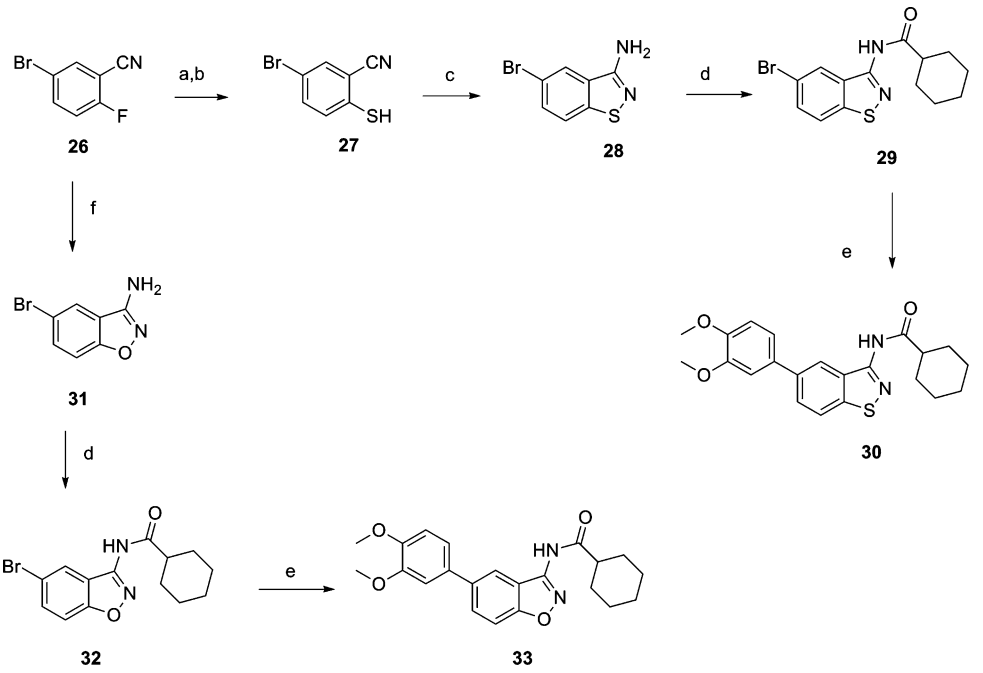
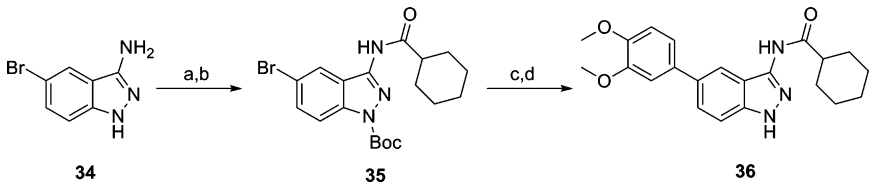
**Scheme 5**[a]



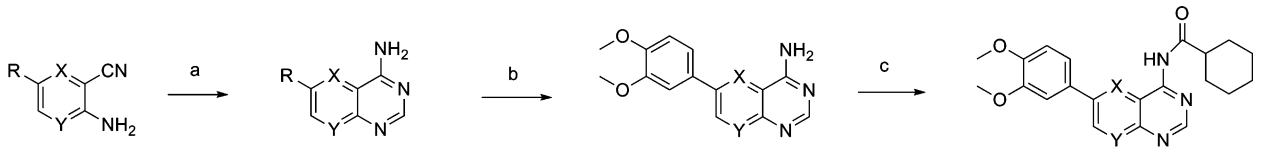

[a]Reagents and conditions: (a) $Na_2S$, DMF; (b) Zn powder, 10% HCl; (c) NaClO, 3% NaOH, $NH_4OH$; (d) cyclohexanecarboxylic acid chloride, DIPEA, DCM; (e) $K_2CO_3$, $Pd(PPh_3)_4$, 3,4-dimethoxyphenylboronic acid, DME, 90 °C; (f) acetohydroxamic acid, *t*-BuOK, DMF.

**Scheme 6**[a]




[a]Reagents and conditions: (a) $Boc_2O$, DMAP, DCM; (b) cyclohexanecarboxylic acid chloride, DIPEA, DCM; (c) $K_2CO_3$, $Pd(PPh_3)_4$, 3,4-dimethoxyphenylboronic acid, DME, 90 °C; (d) 20% TFA/DCM.

**Scheme 7**[a]




[a]Reagents and conditions: (a) $CH(OEt)_3$, $NH_4OAc$, reflux; (b) $K_2CO_3$, $Pd(PPh_3)_4$, 3,4-dimethoxyphenylboronic acid, dioxane/water, 90 °C; (c) cyclohexanecarboxylic acid chloride, DIPEA, DCM, rt.

unsaturation (compound **10t**) and the insertion of a methylene linker between the carbonyl and the cyclohexyl moiety (compound **10u**) give rise to less potent compounds. Similarly, when the cyclohexyl ring is part of a bicyclic adamantyl ring system (compounds **10v–w**), the compounds have a much lower affinity for the DRAK2 enzyme. Substitution at position 4 of the cyclohexyl moiety with a methyl group gives rise to a strongly diminished DRAK2 binding. Replacement of the cyclohexane ring by a piperidine ring afforded compound **10z** that retains reasonable DRAK2 affinity ($K_d$ = 7.2 $\mu$M). This compound offers the advantage of reduced lipophilicity and increased aqueous solubility.

Besides cyclo-aliphatic amides, a number of aliphatic and aromatic amides were prepared (Table 3). All these analogues are completely devoid of DRAK2 affinity, pointing toward an essential role of a cyclo-alkyl moiety in DRAK2 binding.

At this stage of the project, it was decided to evaluate the most potent DRAK2 binders for their ability to functionally inhibit the enzyme in a traditional, enzymatic assay, based on a radioactive read-out. As can be derived from the data in Table 4, compounds **10s** and **10n** are completely devoid of functional inhibitory activity.

Therefore, we felt it was necessary to further improve the binding affinity, to identify compounds that could also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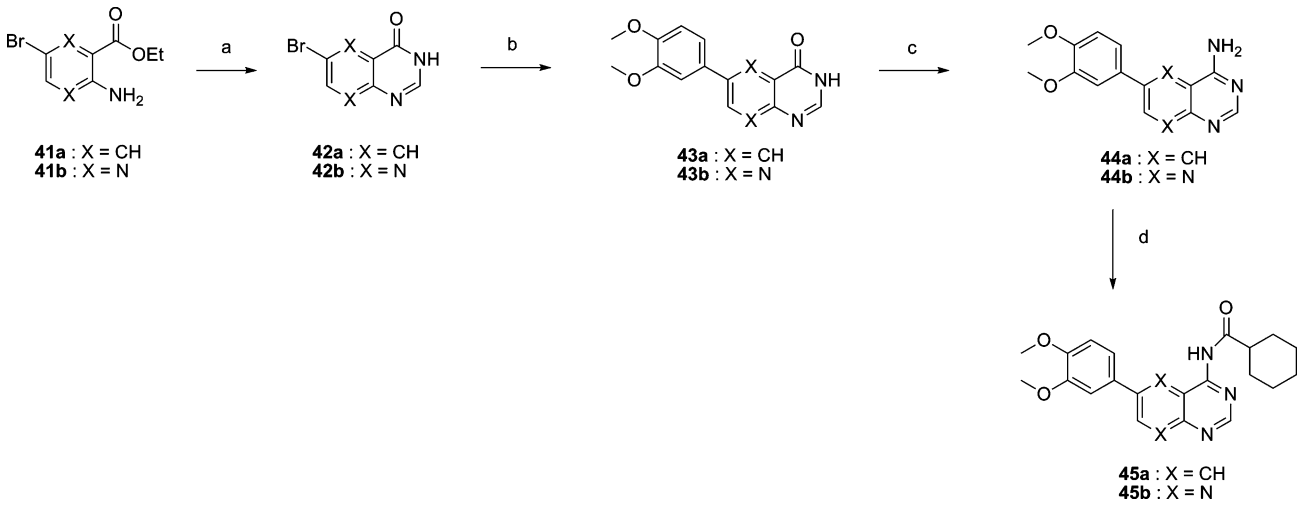
**Scheme 8**[a]




[a]Reagents and conditions: (a) $CH(OEt)_3$, $NH_4OAc$, reflux; (b) $K_2CO_3$, $Pd(PPh_3)_4$, 3,4-dimethoxyphenylboronic acid, dioxane/water, 90 °C; (c) (i) $POCl_3$ (ii) $NH_3$/Methanol; (d) cyclohexanecarboxylic acid chloride, DIPEA, DCM, rt.

**Table 4. Functional Inhibition of DRAK2 Activity**

| Cmpd | $K_d$ ($\mu$M)[a] | % inh @ 10 $\mu$M[a] |
|---|---|---|
| **10s** | 0.53 $\mu$M | 10% |
| **10n** | 0.66 $\mu$M | 0% |

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

functionally inhibit the DRAK2 enzymatic activity. It turns out that by changing the decoration pattern of the isothiazolopyridine scaffold, we were not able to improve DRAK2 affinity substantially and only compounds were obtained with mediocre binding affinity, lacking any functional, inhibitory activity. Therefore, we switched the strategy toward a scaffold hopping approach. Scaffold hopping is a useful approach to discover structurally novel compounds starting from known active molecules by changing the molecular core of the hit, yielding a number of skeletons, keeping the substitution pattern intact.

Two types of heteroaromatic bicyclic scaffolds, i.e., the 6−5 and the 6−6 bicycles, were prepared (Table 5). Most of the 6−5 bicycles do show some affinity for the DRAK2 enzyme, with the exception of the isothiazolo[5,4-*b*]pyrazine derivative **15** and the indazole **36**. The benzo[*b*]thiophene **22a**, the benzo[*d*]isothiazole **30**, and the benzo[*d*]isoxazole **33** are endowed with very similar DRAK2 affinity, with $K_d$ values ranging from 0.51 to 2.7 $\mu$M, which does not represent any major step forward when compared to the DRAK2 affinity data of the original hit compound. However, the thieno[2,3-*b*]pyridine **22b**, thieno[2,3-*b*]pyrazine **22c**, and the pyrrolo-[2,3-*b*]pyridine **25** display strong DRAK2 afffinity with $K_d$ values of 9 nM, 27 nM, and 33 nM, respectively. Among the 6−6 type of bicycles, the pyrido[2,3-*d*]pyrimidine scaffold **40a** as well as the quinazoline scaffold **45a** are completely devoid of DRAK2 affinity. The pyrido[3,2-*d*]pyrimidine scaffold **40b** shows marginal activity, whereas the pteridine scaffold **45b** is a potent ligand for the DRAK2 enzyme, with a $K_d$ value of 0.18 $\mu$M.

Selectivity is an important issue in kinase research. Selective small-molecule kinase inhibitors are important when useful conclusions regarding the physiological role of a certain kinase need to be made. Moreover, when compounds are considered as leads in drug discovery programs, specificity is important to avoid unwanted side effects, caused by inhibition of off-targets. As DRAK2 belongs to the superfamily of DAPK, consisting of DAPK1, DAPK2, DAPK3, DRAK1, and DRAK2, the most promising compounds were profiled against this kinase panel in the binding assay at a concentration of 10 $\mu$M (Table 6). The data clearly indicate that the synthesized DRAK2 inhibitors completely lack affinity for the different members of the DAPK family (% Ctrl between 70 and 100), whereas they bind to the DRAK1 enzyme (% Ctrl of 0.55 and 0.65, respectively). As representative examples, the $K_d$ values of compounds **22b** and **22c** for DRAK1 were experimentally determined to be 4.9 nM and 13 nM, respectively. It can be concluded that these compounds behave as dual DRAK1/DRAK2 ligands.

With compounds with strong DRAK1 and DRAK2 binding affinity in hand, they were tested in a classical, biochemical assay, to determine if they are functionally active DRAK1 and DRAK2 inhibitors (Table 7). The two most potent DRAK1 and DRAK2 binders **22b** and **22c** do behave as inhibitors in functional DRAK1 and DRAK2 assays. However, there seem to be a discrepancy between the binding data (low nM affinity) and the $IC_{50}$ values (low $\mu$M values). This contradictory results are quite surprising, given the fact that binding affinity data usually correlate very well with the data obtained in functional enzyme assays.[29] Compound **25**, although having strong affinity for DRAK2, lacks completely functional inhibitory activity.

A possible reason for the greatly diminished activity could be the fact that the compounds do not target the ATP binding site. Therefore, enzyme kinetic experiments were performed, using different concentrations of compound **22b** (as representative example) and ATP (Table 8). Increasing the concentration of ATP from 1 $\mu$M to 500 $\mu$M leads to a 10-fold drop in activity of compound **22b** against DRAK1 and DRAK2, suggesting that this class of compounds acts as ATP competitive inhibitors.

## CONCLUSION

Starting from an isothiazolo[5,4-*b*]pyridine based hit compound with weak affinity for the DRAK2 enzyme, classical medicinal chemistry with systematic variation of the substituents afforded novel compounds with binding affinities of approximately 0.5 $\mu$M, but lacking any functional inhibitory activity. In a second round of optimization, a scaffold hopping


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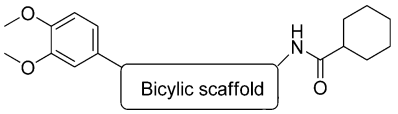
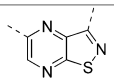
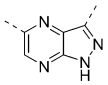
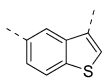
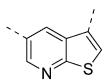
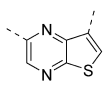
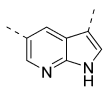
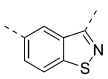
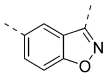
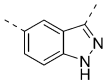
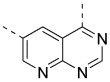
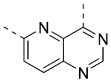
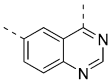
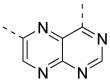
**Table 5. SAR of the Isothiazolopyridine Scaffold**




| Cmpd# | Name of scaffold | Structure | % Ctrl (10 μM)[a] | Kd (μM)[a] |
|---|---|---|---|---|
| **15** | Isothiazolo[5,4-b]pyrazine |  | 30 | ND |
| **17** | Pyrazolo[3,4-b]pyrazine |  | 18 | ND |
| **22a** | Benzo[b]thiophene |  | 6,5 | 0,51 |
| **22b** | Thieno[2,3-b]pyridine |  | 7,2 | 0,009 |
| **22c** | Thieno[2,3-b]pyrazine |  | 5,4 | 0,027 |
| **25** | Pyrrolo[2,3-b]pyridine |  | ND | 0,033 |
| **30** | Benzo[d]isothiazole |  | ND | 2,7 |
| **33** | Benzo[d]isoxazole |  | 5,3 | 0,96 |
| **36** | Indazole |  | 63 | ND |
| **40a** | Pyrido[2,3-d]pyrimidine |  | 100 | ND |
| **40b** | Pyrido[3,2-d]pyrimidine |  | 56 | ND |
| **45a** | Quinazoline |  | 100 | ND |
| **45b** | Pteridine |  | 3,3 | 0,18 |

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

**Table 6. Selectivity Profile of the Lead Compounds**

| Cmpd | DAPK1[a] | DAPK2[a] | DAPK3[a] | DRAK1[a] | DRAK2[a] |
|---|---|---|---|---|---|
| **22b** | % Ctrl = 83 | % Ctrl = 100 | % Ctrl = 72 | % Ctrl = 0,55 $K_d$ = 4.9 nM | % Ctrl = 7.2 $K_d$ = 9 nM |
| **22c** | % Ctrl = 77 | % Ctrl = 98 | % Ctrl = 84 | % Ctrl = 0,65 $K_d$ = 13 nM | % Ctrl = 5.4 $K_d$ = 27 nM |

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

strategy was applied. It led to the discovery of a thieno[2,3-*b*]pyridine and a thieno[2,3-*b*]pyrazine derivative as potent ligands for the DRAK2 enzyme. In addition, these compounds show promising activity as DRAK2 inhibitors. A preliminary selectivity profile demonstrates that the compounds do not bind to DAPK1, 2, and 3, but, however, do not discriminate between DRAK1 and DRAK2 and therefore need to be considered as dual DRAK1 and DRAK2 inhibitors.

**Table 7. Functional DRAK1 and DRAK2 Inhibition**

| Cmpd | DRAK1 $K_d$[a] (nM) | DRAK1 $IC_{50}$[a] (μM) | DRAK2 $K_d$[a] (nM) | DRAK2 $IC_{50}$[a] (μM) |
|---|---|---|---|---|
| **22b** | 4.9 | 2.25 | 9 | 0.86 |
| **22c** | 13 | 4.94 | 27 | 1.78 |
| **25** | ND | ND | 33 | 38% inh @ 10 μM |

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

**Table 8. $IC_{50}$ Determination of Compound 22b against DRAK1 and DRAK2 at Different ATP Concentrations**

| ATP conc (μM) | DRAK1 $IC_{50}$[a] (μM) | DRAK2 $IC_{50}$[a] (μM) |
|---|---|---|
| 1 | 0.56 | 0.37 |
| 5 | 0.63 | 0.51 |
| 10 | 0.71 | 0.53 |
| 25 | 0.94 | 0.65 |
| 50 | 1.33 | 0.72 |
| 100 | 2.11 | 0.99 |
| 250 | 2.85 | 1.47 |
| 500 | 5.91 | 2.22 |

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

## EXPERIMENTAL SECTION

**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 MHz ($^1$H NMR: 300 MHz, $^{13}$C NMR: 75 MHz), 500 MHz ($^1$H NMR: 500 MHz, $^{13}$C NMR: 125 MHz) and 600 MHz ($^1$H NMR: 600 MHz, $^{13}$C NMR: 150 MHz) using tetramethylsilane as internal standard for $^1$H NMR spectra and ($D_6$)-DMSO (39.5 ppm) or $CDCl_3$ (77.2 ppm) and $CD_3OD$ (49.0 ppm) for $^{13}$C NMR spectra. Abbreviations used are s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. 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 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, 60 Å. All final compounds possess a purity of at least 95% (with the exception of compound **10c** which shows a purity of 93%), as determined by analytical RP-HPLC analysis on an XBridge column (C-18, 5 μm, 4.6 × 150 mm) in combination with a Waters 600 HPLC system, a Waters 717 plus Autosampler, and a Waters 2996 Photodiode Array Detector. The mobile phase consisted of a mixture of solvent A (water with 0.1% trifluoroacetic acid) and solvent B (acetonitrile with 0.1% trifluoroacetic acid). A gradient was used starting with 0% solvent B, gradually increasing to 100% of solvent B, over 30 min.

**2-Hydroxynicotinonitrile (2).** A solution of 2-chloronicotinonitrile (**1**, 10 g, 72.5 mmol) in acetic acid (50 mL) was heated under reflux for 60 h. After cooling to room temperature, the crystals was collected by filtration and washed with acetic acid (10 mL), yielding the title compound as white crystals (6.1 g, 70%). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ = 6.35 (t, $J$ = 6.8 Hz, 1H, arom H), 7.78 (dd, $J$ = 6.8 Hz and $J$ = 2.2 Hz, 1H, arom H), 8.13 (dd, $J$ = 6.9 and 2.2 Hz, 1H, arom H), 12.56 (br s, 1H, OH) ppm.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**5-Bromo-2-hydroxynicotinonitrile (3).** To a stirring suspension of 2-hydroxynicotinonitrile (**2**, 4.8 g, 40 mmol) in acetic acid (40 mL), bromine was added dropwise (3.1 mL, 60 mmol). The resulting mixture was stirred at room temperature for 2 h. Then, water (100 mL) was added and the mixture was extracted with dichloromethane (3 × 50 mL). The combined organic phases were washed with brine and water. After the solvents were evaporated under reduced pressure, the title compound was obtained as white solid (6.0 g, 75%). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ = 8.09 (d, $J$ = 2.8 Hz, 1H, arom H), 8.38 (d, $J$ = 2.8 Hz, 1H, arom H), 12.87 (br s, 1H, OH) ppm.

**5-Bromo-2-chloronicotinonitrile (4).** A mixture of 5-bromo-2-hydroxynicotinonitrile (**3**, 5.0 g, 25 mmol) and phosphorus pentachloride (5.2 g, 25 mmol) in phosphorusoxychloride (50 mL) was heated under reflux for 48 h. After concentration under reduced pressure, ice water was added to the residue. The resulting reaction mixture was stirred at room temperature for 3 h. The precipitate was filtered off, washed with water, and dried to yield the title compound as a gray solid (4.3 g, 79%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 8.14 (d, $J$ = 2.3 Hz, 1H, arom H), 8.68 (d, $J$ = 2.3 Hz, 1H, arom H) ppm.

**Methyl 3-(5-Bromo-3-cyanopyridin-2-ylthio)propanoate (5).** To a mixture of 5-bromo-2-chloronicotinonitrile (**4**, 4.3 g, 19.8 mmol) and $K_2CO_3$ (4.15 g, 30 mmol) in DMF (50 mL) was added methyl 3-mercaptopropionate (3.3 mL, 30 mmol). The mixture was stirred at room temperature for 2 h. After addition of water (150 mL), the precipitate was filtered off, washed with water, and dried, yielding the title compound as a brown solid (4.5 g, 72%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 2.73 (t, $J$ = 7.2 Hz, 2H, $CH_2$), 3.31 (t, $J$ = 7.2 Hz, 2H, $CH_2$), 3.73 (s, 3H, $OCH_3$), 7.93 (d, $J$ = 1.9 Hz, 1H, Ar−H), 8.54 (d, $J$ = 1.9 Hz, 1H, Ar−H) ppm. MS $m/z$ (%): 316.15 ([M + H]$^+$, 100).

**3-Amino-5-bromoisothiazolo[5,4-*b*]pyridine (7).** To a solution of 3-(5-bromo-3-cyanopyridin-2-ylthio)propanoate (**5**, 3.8 g, 12 mmol) in THF (60 mL) was added NaH (60%, 1.42 g, 36 mmol). The mixture was heated under reflux for 2 h. After concentration under reduced pressure, the residue was triturated with water (10 mL). The crude product was collected by filtration and was used in the following reaction without further purification.

To a solution of this crude product in a 25% ammonia in water (40 mL) were added NaOH (0.96 g, 24 mmol) and NaOCl (13% active chlorine, 8.0 mL) at 0 °C. The mixture was stirred at 0 °C for 3 h. The precipitate was collected by filtration, washed with water, and dried, yielding the title compound as brown solid in 87% yield (2.4 g, 10.4 mmol). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ = 7.09 (s, 2H, $NH_2$), 8.80 (s, 2H, arom H) ppm.

**3-Amino-5-aryl-isothiazolo[5,4-*b*]pyridines (8a−b).** *General Procedure A.* To a solution of 3-amino-5-bromo-isothiazolo[5,4-*b*]pyridine (**7**, 230 mg, 1.0 mmol) in a mixture of dioxane (8 mL) and water (2 mL) were added potasium carbonate (2 equiv, 296 mg, 2.0 mmol), $(PPh_3)_4Pd$ (0.05 eq, 55 mg, 0.05 mmol) and an appropriate arylboronic acid (1.2 equiv, 1.2 mmol). The reaction mixture was stirred at 95 °C until the starting material was disappeared on TLC (2−6 h). The solvents were evaporated and the crude residue was purified by silicagel flash chromatography, yielding the pure title compounds. The following compounds were made according to this procedur.

**3-Amino-5-(3-thienyl)-isothiazolo[5,4-*b*]pyridine (8a).** This compound was prepared from compound **7** according to the general procedure A using 3-thiopheneboronic acid (124 mg, 0.98 mmol) and the crude residue was purified on silicagel, using a mixture of cyclohexane and ethyl acetate (in a ratio of 2:1) as mobile phase, yielding the title compound as a white powder (69 mg, 60%). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ = 7.03 (br s, 2H, $NH_2$), 7.65 (dd, $J$ = 0.96 Hz and $J$ = 5.04 Hz, 1H, arom H), 7.77 (m, 1H, arom H), 8.04 (m, 1H, arom H), 8.84 (d, $J$ = 1.98 Hz, 1H, arom H), 9.14 (d, $J$ = 1.92 Hz, 1H, arom H) ppm.

**3-Amino-5-(3,4-dimethoxy-phenyl)-isothiazolo[5,4-*b*]-pyridine (8b).** This compound was prepared from compound **7** according to the general procedure A using 3,4-dimethoxyphenylboronic acid (178 mg, 0.98 mmol) and the crude residue was purified on silica gel, using a mixture of cyclohexane and ethyl acetate (in a ratio of 2:1) as mobile phase, yielding the title compound as a white powder (119 mg, 85%). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ = 3.82 (s, 3H, $OCH_3$), 3.89 (s, 3H, $OCH_3$), 7.04 (br s, 2H, $NH_2$), 7.13 (s, 1H, arom H), 7.36 (m, 2H, arom H), 8.78 (d, $J$ = 2.1 Hz, 1H, arom H), 9.06 (d, $J$ = 2.01 Hz, 1H, arom H) ppm.

***N*-(5-Bromo-isothiazolo)[5,4-*b*]pyridine-3-yl)-cyclohexanecarboxamide (9).** To a solution of 3-amino-5-bromo-isothiazolo[5,4-*b*]pyridine (**7**, 1.28 g, 5.56 mmol) in dry pyridine (17 mL) cyclohexanecarboxylic acid chloride (6.12 mmol, 823 μL) was added dropwise at 0 °C and the reaction mixture was stirred at room temperature for 2 h. The solvent was removed under vacuum and the residue was partitioned between EtOAc and $H_2O$. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated in vacuo. The crude product was purified by flash chromatography on silicagel eluting with a mixture of cyclohexane and dichloromethane (in a ratio gradually ranging from 6:4 to 1:9) as mobile phase. After crystallization from MeOH, the title compound was obtained as a white solid (1.39 g, 74%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 1.32 (m, 3H, cyclohexyl), 1.61 (m, 2H, cyclohexyl), 1.75 (m, 1H, cyclohexyl), 1.88 (m, 2H, cyclohexyl), 2.05 (m, 2H, cyclohexyl), 2.43 (m, 1H, cyclohexyl H), 8.30 (br s, 1H, NH), 8.76 (d, 1H, $J$ = 2.2 Hz, arom), 8.81 (d, 1H, $J$ = 2.2 Hz, arom) ppm.

***N*-(5-Aryl-isothiazolo)[5,4-*b*]pyridine-3-yl)-cyclohexanecarboxamide analogues (10b−d and 10f−o).** *General Procedure B.* To a solution of *N*-(5-bromo-isothiazolo)[5,4-*b*]pyridine-3-yl)cyclohexanecarboxamide (**9**, 100 mg, 0.3 mmol) in dioxane or DME (1.5 mL) were added $(PPh_3)_4Pd$ (5 mol %, 17 mg), a 2 M aqueous $K_2CO_3$ solution (2.0 equiv, 0.3 mL, 0.6 mmol), and the appropriate boronic acid (2.0 equiv, 0.6 mmol). The resulting mixture was heated in microwave at 150 °C and the reaction monitored by TLC. Upon completion, the mixture was diluted with EtOAc, washed with $H_2O$ and brine, and dried over $MgSO_4$. After concentration in vacuo, the crude residue was purified by flash chromatography on silicagel to afford the title compounds.

*General procedure C.* To a solution of *N*-(5-bromobenzo[*d*]-isothiazol-3-yl)cycloalkanecarboxamide (**9**) in DME (2 mL) was added an appropriate boronic acid (1.5 equiv), potassium carbonate (2 equiv, 2 M solution in $H_2O$), and $Pd(PPh_3)_4$ (10 mol %) was added. The reaction was heated at 80 °C. After completion of reaction, solvents were evaporated. The crude residue was purified by silica gel flash chromatography, yielding the pure title compounds.

**Synthesis of *N*-(5-thiophen-2-yl-isothiazolo)[5,4-*b*]pyridine-3-yl)cyclohexane-carboxamide (10b).** To a solution of *N*-(5-bromo-isothiazolo)[5,4-*b*]pyridine-3-yl)cyclohexanecarboxamide (**9**, 100 mg, 0.3 mmol) in dioxane (1.5 mL) were added $(PPh_3)_4Pd$ (5 mol %, 17 mg), a 2 M aqueous $Na_2CO_3$ solution (0.3 mL, 0.6 mmol), and 2-thiopheneboronic acid (77 mg, 0.6 mmol). The resulting mixture was heated at reflux overnight. One more equivalent of 2-thiopheneboronic acid (38 mg, 0.3 mmol) was added and the reaction was stirred at reflux for another 8 h. The mixture was diluted with EtOAc, washed with $H_2O$ and brine, and dried over $MgSO_4$. After concentration in vacuo, the crude residue was purified by flash chromatography eluting with a mixture of cyclohexane−EtOAc, in a ratio of 9:1. The obtained compound was further purified by preparative TLC using a mixture of heptane−EtOAc (in a ratio of 7:3) as mobile phase, yielding the pure title compound (35 mg, 34%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 1.17−1.48 (m, 3H, cyclohexyl), 1.52−1.80 (m, 3H, cyclohexyl H), 1.82−1.96 (m, 2H, cyclohexyl), 2.00−2.14 (m, 2H, cyclohexyl), 2.39−2.57 (m, 1H, cyclohexyl), 7.15 (dd, 1H, $J$ = 3.7, $J$ = 5.2 Hz, arom H), 7.40 (dd, 1H, $J$ = 1.2, 5.2 Hz, arom), 7.44 (dd, 1H, $J$ = 1.2, $J$ = 4.7 Hz, arom), 8.32 (br s, 1H, NH), 8.76 (d, 1H, $J$ = 2.0 Hz, arom H), 8.99 (d, 1H, $J$ = 2.1 Hz, arom H) ppm. $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ = 25.28 ($CH_2$), 25.51 ($CH_2$), 29.13 ($CH_2$), 43.96 (CH), 122.09 ($C_q$), 125.83 (CH), 126.44 ($C_q$), 127.50 (CH), 129.02 (CH), 129.48 (CH), 139.17 ($C_q$), 148.85 (CH), 152.40 ($C_q$), 169.44 ($C_q$), 174.89 (CO) ppm. HRMS: calcd for $C_{17}H_{18}N_3OS_2$ 344.0885, found 344.0881.

***N*-(5-(Furan-3-yl)isothiazolo[5,4-*b*]pyridin-3-yl)-cyclohexanecarboxamide (10c).** This compound was prepared according to general procedure C from compound **9** (40 mg, 0.117 mmol) using 3-furanboronic acid (0.175 mmol, 20 mg), 2 M $K_2CO_3$


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(0.117 mL), and $Pd(PPh_3)_4$ (0.0117 mmol, 13 mg). The crude product was purified using a mixture of cyclohexane/ethyl acetate in a ratio of 5:1, affording the pure title compound (22 mg, 56%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 1.60−2.06 (m, 10H, 5 × $CH_2$), 2.50 (m, 1H, CH), 6.81 (m, 1H, arom H), 7.57 (m, 1H, arom H), 7.88 (s, 1H, arom H), 8.37 (bs, 1H, NH), 8.67 (d, $J$ = 1.95 Hz, 1H, arom H), 8.91 (d, $J$ = 2.07 Hz, 1H, arom H) ppm. $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ = 25.32 ($CH_2$), 25.52 ($CH_2$), 29.09 ($CH_2$), 44.00 (CH), 108.82 (CH), 122.30 ($C_q$), 122.44 ($C_q$), 124.57 ($C_q$), 129.40 (CH), 140.52 (CH), 145.08 (CH), 149.44 (CH), 152.44 ($C_q$), 169.14 ($C_q$), 174.93 (CO) ppm. HRMS: calcd for $C_{17}H_{18}N_3O_2S$ 328.1114, found 328.1116. HPLC purity 93.2%.

***N*-(5-Phenyl-isothiazolo)[5,4-*b*]pyridine-3-yl)-cyclohexanecarboxamide (10d).** This compound was prepared according to general procedure B from compound **9** using benzeneboronic acid (73 mg, 0.6 mmol) in dioxane. The reaction was stirred for 45 min and the crude residue was purified by flash chromatography eluting with a mixture of heptane−EtOAc (in a ratio of 94:6) as mobile phase, affording the title compound in 50% yield (50 mg, 0.15 mmol). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 1.26−1.45 (m, 3H, cyclohexyl), 1.55−1.76 (m, 3H, cyclohexyl), 1.86−1.90 (m, 2H, cyclohexyl), 2.04−2.09 (m, 2H, cyclohexyl), 2.39−2.55 (m, 1H, cyclohexyl), 7.41−7.56 (m, 3H, arom H), 7.62−7.69 (m, 2H, arom H), 8.32 (bs, 1H, NH), 8.76 (d, 1H, $J$ = 2.1 Hz, arom H), 8.98 (d, 1H, $J$ = 2.1 Hz, arom H) ppm. $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ = 25.30 ($CH_2$), 25.50 ($CH_2$), 29.12 ($CH_2$), 44.03 (CH), 122.17 ($C_q$), 127.46 (CH), 128.54 (CH), 129.46 (CH), 131.39 (CH), 132.28 ($C_q$), 136.67 ($C_q$), 150.22 (CH), 152.58 ($C_q$), 169.81 ($C_q$), 174.86 (CO) ppm. HRMS: calcd for $C_{19}H_{20}N_3O_1S_1$ 338.13215, found 338.1316.

***N*-(5-(3,4-Dimethyl)phenyl-isothiazolo)[5,4-*b*]pyridine-3-yl)-cyclohexanecarboxamide (10f).** This compound was made according to the general procedure B from compound **9** using 3,4-dimethylphenylboronic acid (90 mg, 0.6 mmol) in dioxane. The reaction was stirred for 30 min and the crude residue was purified by flash chromatography eluting with a mixture of heptane−EtOAc (in a ratio of 94:6) as mobile phase, affording the title compound in 38% yield (42 mg, 0.114 mmol). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 1.17−1.41 (m, 3H, cyclohexyl), 1.55−1.77 (m, 3H, cyclohexyl), 1.86−1.90 (m, 2H, cyclohexyl), 2.04−2.08 (m, 2H, cyclohexyl), 2.36 (d, 6H, $J$ = 9.5 Hz, 2$CH_3$), 7.28 (s, 1H, arom H), 7.37−7.41 (m, 2H, arom H), 8.32 (br s, 1H, NH), 8.76 (d, 1H, $J$ = 2.1 Hz, arom H), 8.98 (d, 1H, $J$ = 2.01 Hz, arom H) ppm. $^{13}$C NMR (125 MHz, DMSO-$d_6$): $\delta$ = 19.22 ($CH_3$), 19.65 ($CH_3$), 25.30 ($CH_2$), 25.50 ($CH_2$), 29.12 ($CH_2$), 44.00 (CH), 122.17 ($C_q$), 124.74 (CH), 128.34 (CH), 130.53 (CH), 130.74 (CH), 132.35 ($C_q$), 134.07 ($C_q$), 136.83 ($C_q$), 137.38 ($C_q$), 150.13 ($C_q$), 152.47 ($C_q$), 169.44 ($C_q$), 174.82 (CO) ppm. HRMS: calcd for $C_{21}H_{24}N_3O_1S_1$ 366.16345, found 366.1629.

***N*-(5-(2,5-Dimethoxyphenyl)isothiazolo[5,4-*b*]pyridin-3-yl)-cyclohexanecarboxamide (10g).** This compound was prepared according to the general procedure C from compound **9** (40 mg, 0.117 mmol) using 2,5-dimethoxyphenylboronic acid (0.175 mmol, 32 mg), 2 M $K_2CO_3$ (0.117 mL), and $Pd(PPh_3)_4$ (0.0117 mmol, 13 mg). Product was purified using a mixture of cyclohexane/ethyl acetate in a ratio of 4:1, affording the title compound in 64% yield (30 mg, 0.075 mmol). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 1.58−2.06 (m, 10H, 5 × $CH_2$), 2.52 (m, 1H, CH), 3.94 (s, 3H, $CH_3$), 3.80 (s, 3H, $CH_3$), 3.85 (s, 3H, $CH_3$), 6.96 (m, 3H, arom H), 8.67 (d, $J$ = 1.53 Hz, 1H, arom H), 8.72 (br s, 1H, NH), 8.94 (d, $J$ = 1.59 Hz, 1H, arom H) ppm. $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ = 25.26 ($CH_2$), 25.49 ($CH_2$), 29.11 ($CH_2$) 43.88 (CH), 55.73 ($OCH_3$), 56.33 ($OCH_3$), 113.40 (CH), 114.59 (CH), 116.72 (CH), 121.91 ($C_q$), 126.55 ($C_q$), 129.92 ($C_q$), 133.81 (CH), 150.51 ($C_q$), 151.97 (CH), 152.67 ($C_q$), 153.69 ($C_q$), 169.39 ($C_q$), 174.95 (CO) ppm. HRMS: calcd for $C_{21}H_{24}N_3O_3S$ 398.1532, found 398.1528.

***N*-(5-(3,5-Dimethoxyphenyl)isothiazolo[5,4-*b*]pyridin-3-yl)-cyclohexanecarboxamide (10h).** This compound was prepared according to the general procedure C from compound **9** (40 mg, 0.117 mmol) using 3,5-dimethoxyphenylboronic acid (0.175 mmol, 32 mg), 2 M $K_2CO_3$ (0.117 mL) and $Pd(PPh_3)_4$ (0.0117 mmol, 13 mg). The crude product was purified using a mixture of cyclohexane/ethyl acetate in a ratio of 4:1, affording the title compound in 18% yield (8.4 mg, 0.021 mmol). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 1.60−2.19 (m, 10H, 5 × $CH_2$), 2.50 (m, 1H, CH), 3.96 (s, 6H, 2 × $CH_3$), 6.55 (m, 1H, arom H), 6.76 (d, $J$ = 2.22 Hz, 2H, arom H), 8.53 (br s, 1H, NH), 8.71 (m, 1H, arom H), 8.95 (d, $J$ = 2.10 Hz, 1H, arom H) ppm. $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ = 25.29 ($CH_2$), 25.50 ($CH_2$), 29.10 ($CH_2$) 43.98 (CH), 55.61 ($OCH_3$), 100.07 (CH), 105.78 (CH), 123.13 ($C_q$), 131.47 (CH), 132.16 ($C_q$), 138.71 ($C_q$), 150.34 (CH), 152.54 ($C_q$), 161.31 ($C_q$), 169.96 ($C_q$), 174.88 (CO) ppm. HRMS: calcd for $C_{21}H_{24}N_3O_3S$ 398.1532, found 398.1537.

**Synthesis of *N*-(5-(Benzo[*d*][1,3]dioxol-5-yl)isothiazolo[5,4-*b*]pyridin-3-yl)cyclohexanecarboxamide (10i).** This compound was prepared according to the general procedure B from compound **9** using 3,4-methylenedioxyphenylboronic acid (99 mg. 0.6 mmol) in DME. The crude residue was purified by flash chromatography eluting with DCM−MeOH (in a ratio of 99.5:0.5), followed by crystallization from MeOH, affording the title compound as a white powder (11 mg, 10%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 1.33 (m, 3H, cyclohexyl), 1.61 (m, 2H, cyclohexyl H), 1.75 (m, 1H, cyclohexyl), 1.88 (m, 2H, cyclohexyl), 2.06 (m, 2H, cyclohexyl), 2.47 (m, 1H, cyclohexyl), 6.04 (s, 2H, $CH_2$), 6.94 (d, $J$ = 8.5 Hz, 1H, arom H), 7.10 (m, 2H, arom H), 8.37 (br s, 1H, NH), 8.66 (d, $J$ = 2.0 Hz, 1H, arom), 8.90 (d, $J$ = 2.1 Hz, 1H, arom H) ppm. $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ = 25.31 ($CH_2$), 25.50 ($CH_2$), 29.12 ($CH_2$), 44.03 (CH), 101.60 ($CH_2$), 107.74 (CH), 109.21 (CH), 121.33 (CH), 122.13 ($C_q$), 130.69 ($C_q$), 130.88 (CH), 132.07 ($C_q$), 147.81 ($C_q$), 148.42 ($C_q$), 150.14 (CH), 152.49 ($C_q$), 169.32 ($C_q$), 174.82 (CO) ppm. HRMS: calcd for $C_{20}H_{20}N_3O_3S_1$ 382.12198, found 382.1223.

***N*-(5-(3,4,5-Trimethoxyphenyl)benzo[*d*]isothiazol-3-yl)-cyclohexanecarboxamide (10j).** This compound was prepared according to the general procedure C from compound **9** (68 mg, 0.2 mmol) using 3,4,5-trimethoxyphenylboronic acid (0.3 mmol, 63.6 mg), 2 M $K_2CO_3$ (0.2 mL), and $Pd(PPh_3)_4$ (0.02 mmol, 23 mg). The crude product was purified using a mixture of cyclohexane/ethyl acetate in a ratio of 4:1, affording the title compound as a white powder (44 mg, 51%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 1.60−2.03 (m, 10H, 5 × $CH_2$), 2.56 (m, 1H, CH), 3.94 (s, 3H, $CH_3$), 3.96 (s, 6H, 2 × $CH_3$), 6.81 (s, 2H, arom H), 8.70 (d, $J$ = 2.07 Hz, 1H, arom H), 8.86 (br s, 1H, NH), 8.95 (d, $J$ = 2.13 Hz, 1H, arom H) ppm. $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ = 25.26 ($CH_2$), 25.52 ($CH_2$), 29.07 ($CH_2$), 43.88 (CH), 56.35 ($OCH_3$), 60.28 ($OCH_3$), 105.28 (CH), 122.13 ($C_q$), 131.34 (CH), 132.39 ($C_q$), 132.53 ($C_q$), 150.53 (CH), 152.64 ($C_q$), 153.18 ($C_q$), 153.66 ($C_q$), 169.61 ($C_q$), 175.05 (CO) ppm. HRMS: calcd for $C_{22}H_{26}N_3O_4S$ 428.1638, found 428.1638.

***N*-(5-(4-Hydroxy)phenyl-isothiazolo)[5,4-*b*]pyridine-3-yl)-cyclohexanecarboxamide (10k).** This compound was prepared according to the general procedure B from compound **9** using 4-hydroxyphenylboronic acid (83 mg, 0.6 mmol) in DME. The crude residue was purified by flash chromatography eluting with DCM−MeOH 99:1, affording the title compound as a white powder (21 mg, 20%). $^1$H NMR (300 MHz, MeOD): $\delta$ = 1.36 (m, 3H, cyclohexyl), 1.59 (m, 2H, cyclohexyl), 1.75 (m, 1H, cyclohexyl), 1.87 (m, 2H, cyclohexyl), 2.47 (m, 2H, cyclohexyl), 2.59 (m, 1H, cyclohexyl), 6.94 (d, 2H, $J$ = 8.6 Hz, arom H), 7.56 (d, 2H, $J$ = 8.6 Hz, arom), 8.59 (d, 1H, $J$ = 2.0 Hz, arom H), 8.96 (d, 1H, $J$ = 2.0 Hz, arom H) ppm. $^{13}$C NMR (125 MHz, DMSO-$d_6$): $\delta$ = 25.31 ($CH_2$), 25.50 ($CH_2$), 29.13 ($CH_2$), 44.02 (CH), 116.30 (CH), 117.30 ($C_q$), 127.18 (CH), 128.62 (CH), 130.14 (CH), 132.21 ($C_q$), 149.94 (CH), 152.61 ($C_q$), 158.13 ($C_q$), 168.75 ($C_q$), 174.89 (CO) ppm. HRMS: calcd for $C_{19}H_{20}N_3O_2S_1$ 354.12706, found 354.1264.

***N*-(5-(3-Aminophenyl)isothiazolo[5,4-*b*]pyridin-3-yl)-cyclohexanecarboxamide (10l).** This compound was prepared according to the general procedure C from compound **9** (40 mg, 0.117 mmol) using 3-aminophenylboronic acid (0.175 mmol, 32 mg), 2 M $K_2CO_3$ (0.117 mL) and $Pd(PPh_3)_4$ (0.0117 mmol, 13 mg). The crude product was purified using a mixture of cyclohexane/ethyl acetate in a ratio of 4:1, affording the title compound as a white powder (25 mg, 60%). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ = 1.45−1.89 (m, 10H, 5 × $CH_2$), 2.59 (m, 1H, CH), 3.94 (s, 3H, $CH_3$), 5.31 (s, 2H, $NH_2$), 6.65 (d, $J$ = 7.59 Hz, 1H, arom H), 6.88 (m, 2H, arom H), 7.21 (t, $J$ = 7.71


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Hz, $J$ = 7.74 Hz, 1H, arom H), 8.62 (d, $J$ = 1.95 Hz, 1H, arom H), 9.02 (d, $J$ = 2.01 Hz, 1H, arom H), 10.91 (br s, 1H, NH) ppm. $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 25.31 ($CH_2$), 25.59 ($CH_2$), 29.14 ($CH_2$), 44.01 (CH), 112.44 (CH), 114.12 (CH), 114.90 (CH), 122.16 ($C_q$), 129.98 (CH), 130.95 ($C_q$), 133.21 ($C_q$), 137.29 ($C_q$), 149.63 ($C_q$), 150.06 (CH), 152.55 ($C_q$), 169.71 ($C_q$), 174.85 (CO) ppm. HRMS: calcd for $C_{19}H_{21}N_4OS$ 353.1430, found 353.1432.

**N-(5-(4-Aminophenyl)isothiazolo[5,4-*b*]pyridin-3-yl)-cyclohexanecarboxamide (10m).** This compound was prepared according to the general procedure C from compound **9** (40 mg, 0.117 mmol) using 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (0.175 mmol, 38 mg), 2 M $K_2CO_3$ (0.117 mL) and $Pd(PPh_3)_4$ (0.0117 mmol, 13 mg). Product was purified using a mixture of cyclohexane/ethyl acetate in a ratio of 3:2, affording the title compound as a white powder (15 mg, 36%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 1.64−2.05 (m, 10H, 5 × $CH_2$), 2.50 (m, 1H, CH), 6.82 (d, $J$ = 8.43 Hz, 2H, arom H), 7.62 (d, $J$ = 8.43 Hz, 2H, arom H), 8.42 (br s, 1H, NH), 8.65 (s, 1H, arom H), 8.94 (d, $J$ = 2.04 Hz, 1H, arom H) ppm. $^{13}$C NMR (125 MHz, DMSO-$d_6$): $\delta$ = 25.31 ($CH_2$), 25.51 ($CH_2$), 29.14 ($CH_2$), 44.01 (CH), 114.53 (CH), 122.32 ($C_q$), 123.29 ($C_q$), 127.97 (CH), 128.97 (CH), 132.94 ($C_q$), 149.47 (CH), 149.61 ($C_q$), 152.39 ($C_q$), 168.54 ($C_q$), 174.99 (CO) ppm. HRMS: calcd for $C_{19}H_{21}N_4OS$ 353.1430, found 353.1428.

***N*-(5-(4-Amino-3-methoxyphenyl)benzo[*d*]isothiazol-3-yl)-cyclohexanecarboxamide (10n).** This compound was prepared according to the general procedure C from compound **9** (68 mg, 0.2 mmol) using 4-amino-3-methoxyphenylboronic acid pinacol ester (0.3 mmol, 74.7 mg), 2 M $K_2CO_3$ (0.2 mL) and $Pd(PPh_3)_4$ (0.02 mmol, 23 mg). Product was purified using a mixture of cyclohexane/ethyl acetate in a ratio of 4:1, affording the title compound (25 mg, 33%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 1.65−2.08 (m, 10H, 5 × $CH_2$), 2.56 (m, 1H, CH), 3.96 (s, 3H, $CH_3$), 6.85 (d, $J$ = 7.95 Hz, 1H, arom H), 7.06 (d, $J$ = 1.89 Hz, 1H, arom H), 7.11 (dd, $J$ = 1.92 Hz, $J$ = 7.95 Hz, 1H, arom H), 8.64 (d, $J$ = 2.01 Hz, 1H, arom H), 8.74 (br s, 1H, NH), 8.95 (d, $J$ = 2.13 Hz, 1H, arom H) ppm. $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 25.29 ($CH_2$), 25.52 ($CH_2$), 29.12 ($CH_2$), 43.94 (CH), 55.67 ($OCH_3$), 109.58 (CH), 114.18 (CH), 120.21 (CH), 122.31 ($C_q$), 124.04 ($C_q$), 129.36 (CH), 133.13 ($C_q$), 138.62 ($C_q$), 146.93 ($C_q$), 149.95 (CH), 152.42 ($C_q$), 168.28 ($C_q$), 174.90 (CO) ppm. HRMS: calcd for $C_{20}H_{23}N_4O_2S$ 381.1390, found 381.1387.

***N*-(5-(4-Hydroxy-3-methoxyphenyl)isothiazolo[5,4-*b*]-pyridin-3-yl)cyclohexanecarboxamide (10o).** This compound was prepared according to the general procedure C from compound **9** (68 mg, 0.2 mmol) using 2-methoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol (75 mg, 0.3 mmol), 2 M $K_2CO_3$ (0.2 mL) and $Pd(PPh_3)_4$ (0.02 mmol, 23 mg). Product was purified using a mixture of cyclohexane/ethyl acetate in a ratio of 4:1, affording the title compound as a white powder (27 mg, 32%). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ = 1.29−1.94 (m, 10H, 5 × $CH_2$), 2.59 (m, 1H, CH), 3.84 (s, 3H, $CH_3$), 7.07−7.21 (m, 3H, arom H), 8.61 (d, $J$ = 2.07 Hz, 1H, arom H), 9.04 (d, $J$ = 2.10 Hz, 1H, arom H), 9.28 (bs, 1H, NH), 10.88 (s, 1H, OH) ppm. $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 25.62 ($CH_2$), 25.82 ($CH_2$), 29.46 ($CH_2$), 44.35 (CH), 56.18 ($OCH_3$), 113.28 (CH), 114.68 (CH), 118.68 (CH), 122.50 ($C_q$), 129.60 ($C_q$), 130.74 (CH), 132.57 ($C_q$), 147.64 ($C_q$), 148.68 ($C_q$), 150.21 (CH), 152.80 ($C_q$), 169.45 ($C_q$), 174.18 (CO) ppm. HRMS: calcd for $C_{20}H_{22}N_3O_3S$ 384.1376, found 384.1377.

**Synthesis of 3-(Amino-acyl)-5-aryl-isothiazolo[5,4-*b*]-pyridines (10a, 10e, 10p-10af).** *General Procedure D.* To a solution of an appropriate 3-amino-5-aryl-isothiazolo[5,4-*b*]pyridine (0.32 mmol) in pyridine (10 mL) was added a suitable acid chloride (1.5 equiv). The reaction mixture was stirred at room temperature overnight. The solvents were evaporated in vacuo and the crude residue was purified by flash chromatography on silicagel yielding the pure title compounds.

*General Procedure E.* To a solution of an appropriate 3-amino-5-(3,4-dimethoxyphenyl)isothiazolo[5,4-*b*]pyridine (72 mg, 0.25 mmol) in dichloromethane (10 mL) was added diisopropylethlyamine (0.5 mmol) and an appropriate acid chloride (0.3 mmol). The resulting mixture was stirred at room temperature for 1.5 h. After concentration under reduced pressure, the crude residue was purified by flash chromatography on silica, yielding the title compounds.

*General Procedure F.* A solution of the carboxylic acid analogue (0.3 mmol) in thionyl chloride (2 mL) was heated under reflux for 30 min. The excess of thionyl chloride was removed under reduced pressure. The residue was dissolved in dichloromethane (2 mL) and added to a solution of 3-amino-5-(3,4-dimethoxyphenyl)isothiazolo-[5,4-*b*]pyridine (57 mg, 0.2 mmol) in pyridine (2 mL). The mixture was stirred at room temperature for 30 min. After concentration under reduced pressure, the residue was purified by flash chromatography on silica, yielding the pure title compounds.

***N*-(5-(Thiophen-3-yl)isothiazolo[5,4-*b*]pyridin-3-yl)-cyclohexanecarboxamide (10a).** This compound was prepared according to the general procedure D from 3-amino-5-(3-thienyl)-isothiazolo[5,4-*b*]pyridine (**8a**, 0.41 mmol, 95 mg) and cyclohexanecarbonyl chloride (0.147 mL, 1.09 mmol). The crude residue was purified by flash chromatography on silicagel using a mixture of cyclohexane and ethyl acetate (in a ratio of 5:1) as mobile phase, affording the pure title compound as a white powder (62 mg, 44%). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ = 1.25 (m, 2H, cyclohexyl H), 1.29 (br s, 1H, cyclohexyl H), 1.33 (br s, 1H, cyclohexyl H), 1.48 (m, 2H, cyclohexyl H), 1.68 (m, 1H, cyclohexyl H), 1.79 (m, 2H, cyclohexyl H), 1.93 (m, 2H, cyclohexyl H), 7.67 (dd, $J$ = 1.35 and 5.04 Hz, 1H, arom H), 7.78 (dd, $J$ = 2.91 and 5.01 Hz, 1H, arom H), 8.11 (dd, $J$ = 1.35 and 2.88 Hz, 1H, arom H), 8.70 (d, $J$ = 2.04 Hz, arom H), 9.23 (d, $J$ = 2.13 Hz, arom H), 10.85 (s, 1H, NH) ppm. $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ = 25.32 ($CH_2$), 25.52 ($CH_2$), 29.11 ($CH_2$), 44.02 (CH), 122.25 ($C_q$), 123.01 (CH), 126.34 (CH), 127.51 ($C_q$), 128.21 (CH), 130.11 (CH), 137.59 ($C_q$), 149.86 (CH), 152.54 ($C_q$), 169.24 ($C_q$), 174.90 (CO) ppm. HRMS: calcd for $C_{17}H_{18}N_3OS_2$ 344.0885, found 344.0887.

***N*-(5-(3,4-Dimethoxyphenyl)isothiazolo[5,4-*b*]pyridin-3-yl)-cyclohexanecarboxamide (10e).** This compound was prepared according to the general procedure D from 3-amino-5-(3,4-dimethoxyphenyl)-isothiazolo[5,4-*b*]pyridine (**8b**, 0.33 mmol, 95 mg) and cyclohexanecarbonyl chloride (0.102 mL, 0.76 mmol). The crude residue was purified by flash chromatography on silicagel using a mixture of cyclohexane and ethyl acetate (in a ratio of 5:1) as mobile phase, affording the pure title compound as a white powder (113 mg, 86%). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ = 1.24 (m, 3H, cyclohexyl H), 1.33 (br s, 1H, cyclohexyl H), 1.46 (m, 2H, cyclohexyl H), 1.67 (m, 1H, cyclohexyl H), 1.78 (m, 2H, cyclohexyl H), 1.92 (m, 2H, cyclohexyl H), 3.82 (s, 3H, $OCH_3$), 3.88 (s, 3H, $OCH_3$), 7.14 (d, $J$ = 8.37 Hz, 1H, arom H), 7.30 (dd, $J$ = 2.01 and 8.31 Hz, 1H, arom H), 7.37 (d, $J$ = 8.37 Hz, 1H, arom H), 8.63 (d, $J$ = 2.01 Hz, arom H), 9.15 (d, $J$ = 2.13 Hz, arom H), 10.87 (s, 1H, NH) ppm. $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ = 25.29 ($CH_2$), 25.51 ($CH_2$), 29.96 ($CH_2$) 43.95 (CH), 55.79 ($OCH_3$), 55.92 ($OCH_3$), 111.18 (CH), 112.66 (CH), 119.88 (CH), 122.18 ($C_q$), 129.21 ($C_q$), 130.68 (CH), 132.30 ($C_q$), 149.44 ($C_q$), 149.49 ($C_q$), 150.23 (CH), 152.54 ($C_q$), 169.20 ($C_q$), 174.93 (CO) ppm. HRMS: calcd for $C_{21}H_{24}N_3O_3S$ 398.1532, found 398.1535.

***N*-(5-(3,4-Dimethoxyphenyl)isothiazolo[5,4-*b*]pyridin-3-yl)-cyclopropanecarboxamide (10p).** This compound was prepared according to the general procedure E from 3-amino-5-(3,4-dimethoxyphenyl)-isothiazolo[5,4-*b*]pyridine (**8b**, 0.25 mmol, 72 mg) and cyclopropanecarbonyl chloride (1.2 equiv, 0.3 mmol). The crude residue was purified by flash chromatography on silica gel using a mixture of cyclohexane and ethyl acetate (in a ratio of 5:1) as mobile phase, affording the pure title compound as a white powder (50 mg, 56%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 1.00−1.70 (m, 4H, $CH_2$), 1.91 (m, 1H, CH), 3.97 (s, 3H, $OCH_3$), 3.99 (s, 3H, $OCH_3$), 7.01 (d, $J$ = 8.3 Hz), 7.14 (d, $J$ = 2.1 Hz, 1H, arom H), 7.21 (dd, $J$ = 8.3 Hz and $J$ = 2.1 Hz, 1H, arom H), 8.69 (d, $J$ = 2.3 Hz, 1H, arom H), 8.90 (br s, 1H, NH), 8.97 (d, $J$ = 2.3 Hz, 1H, arom H) ppm. $^{13}$C NMR (150 MHz, DMSO-$d_6$): $\delta$ = 175.50, 169.20, 152.29, 150.22, 149.41, 149.33, 132.26, 130.88, 129.14, 122.04, 119.82, 112.54, 111.05, 55.84, 55.72, 14.05, 8.31 ppm. HRMS: calcd for $C_{18}H_{17}N_3O_3S_1$ 356.1063, found 356.1069.


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***N*-(5-(3,4-Dimethoxyphenyl)isothiazolo[5,4-*b*]pyridin-3-yl)-cyclobutanecarboxamide (10q).** This compound was prepared according to the general procedure D from 3-amino-5-(3,4-dimethoxyphenyl)-isothiazolo[5,4-*b*]pyridine (**8b**, 0.20 mmol, 57 mg) and cyclobutanecarbonyl chloride (0.2 mmol). The crude residue was purified by flash chromatography on silicagel using a mixture of cyclohexane and ethyl acetate (in a ratio of 5:1) as mobile phase, affording the pure title compound as a yellowish solid (60 mg, 81%). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ = 1.96 (m, 2H, $CH_2$), 2.7 (m, 4H, $CH_2$), 3.50 (m, 1H, CH), 3.83 (s, 3H, $OCH_3$), 3.88 (s, 3H, $OCH_3$), 7.14 (d, $J$ = 8.3 Hz, 1H, arom H), 7.32 (d, $J$ = 8.3 Hz, 1H, arom H), 7.39 (s, 1H, arom H), 8.72 (d, $J$ = 1.9 Hz, 1H, arom H), 9.16 (d, $J$ = 1.9 Hz, 1H, arom H), 10.85 (s, 1H, NH) ppm. $^{13}$C NMR (150 MHz, DMSO-$d_6$): $\delta$ = 173.75, 169.14, 152.37, 150.22, 149.41, 149.36, 132.27, 130.72, 129.13, 122.01, 119.83, 112.55, 111.08, 55.85, 55.72, 38.94, 24.67, 17.79 ppm. HRMS: calcd for $C_{19}H_{19}N_3O_3S_1$ 370.1220, found 370.1219.

***N*-(5-(3,4-Dimethoxyphenyl)isothiazolo[5,4-*b*]pyridin-3-yl)-cyclopentanecarboxamide (10r).** This compound was prepared according to the general procedure D from 3-amino-5-(3,4-dimethoxyphenyl)-isothiazolo[5,4-*b*]pyridine (**8b**, 0.30 mmol, 86 mg) and cyclopentanecarbonyl chloride (0.30 mmol). The crude residue was purified by flash chromatography on silica gel using a mixture of cyclohexane and ethyl acetate (in a ratio of 10:1) as mobile phase, affording the pure title compound as a white solid (90 mg, 84%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 1.69−2.10 (m, 8H, $CH_2$), 2.99 (m, 1H, CH), 3.97 (s, 3H, $OCH_3$), 3.99 (s, 3H, $OCH_3$), 7.02 (d, $J$ = 8.3 Hz), 7.15 (d, $J$ = 2.1 Hz, 1H, arom H), 7.23 (dd, $J$ = 8.3 Hz and $J$ = 2.1 Hz, 1H, arom H), 8.64 (br s, 1H, NH), 8.71 (d, $J$ = 2.3 Hz, 1H, arom H), 8.97 (d, $J$ = 2.3 Hz, 1H, arom H) ppm. $^{13}$C NMR (150 MHz, DMSO-$d_6$): $\delta$ = 175.14, 169.16, 152.50, 150.20, 149.41, 149.35, 132.24, 130.74, 129.13, 122.11, 119.79, 112.56, 111.05, 55.82, 55.72, 44.43, 30.12, 25.79 ppm. HRMS: calcd for $C_{20}H_{21}N_3O_2S_1$ 384.1376, found 384.1374.

***N*-(5-(3,4-Dimethoxyphenyl)isothiazolo[5,4-*b*]pyridin-3-yl)-cycloheptanecarboxamide (10s).** This compound was prepared according to the general procedure F from 3-amino-5-(3,4-dimethoxyphenyl)-isothiazolo[5,4-*b*]pyridine (**8b**, 0.20 mmol, 57 mg) and cycloheptanecarbonic acid (0.30 mmol). The crude residue was purified by flash chromatography on silica gel using a mixture of cyclohexane and ethyl acetate (in a ratio of 10:1) as mobile phase, affording the pure title compound as a white solid (70 mg, 85%). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ = 1.40−2 (m, 12H, CH and $CH_2$), 2.79 (m, 1H, CH), 10.88 (s, 1H, NH), 3.83 (s, 3H, $OCH_3$), 3.88 (s, 3H, $OCH_3$), 7.14 (d, $J$ = 8.3 Hz, 1H, arom H), 7.29 (d, $J$ = 8.3 Hz, 1H, arom H), 7.38 (s, 1H, arom H), 8.63 (d, $J$ = 2.0 Hz, 1H, arom H), 9.16 (d, $J$ = 2.0 Hz, 1H, arom H) ppm. HRMS: calcd for $C_{22}H_{25}N_3O_3S_1$ 412.1689, found 412.1683.

***N*-(5-(3,4-Dimethoxyphenyl)isothiazolo[5,4-*b*]pyridin-3-yl)-cyclohex-3-enecarboxamide (10t).** This compound was prepared according to the general procedure D from 3-amino-5-(3,4-dimethoxyphenyl)-isothiazolo[5,4-*b*]pyridine (**8b**, 0.20 mmol, 57 mg) and cyclohex-3-enecarbonyl chloride (0.2 mmol). The crude residue was purified by flash chromatography on silicagel using a mixture of dichloromethane and ethyl acetate (in a ratio of 8:1) as mobile phase, affording the pure title compound as a yellowish solid (70 mg, 89%). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ = 1.60−2.50 (m, 6H, $CH_2$), 2.83 (m, 1H, CH), 3.83 (s, 3H, $OCH_3$), 3.88 (s, 3H, $OCH_3$), 5.67 (d, $J$ = 11.8 Hz, 1H, C═CH), 5.73 (s, 1H, C═CH), 7.14 (d, $J$ = 8.3 Hz, 1H, arom H), 7.32 (d, $J$ = 8.3 Hz, 1H, arom H), 7.38 (s, 1H, arom H), 8.81 (d, $J$ = 1.9 Hz, 1H, arom H), 9.16 (d, $J$ = 1.9 Hz, 1H, arom H), 10.99 (s, 1H, NH) ppm. $^{13}$C NMR (150 MHz, DMSO-$d_6$): $\delta$ = 176.58, 169.15, 152.41, 150.23, 149.37, 132.27, 130.67, 129.12, 126.60, 125.61, 122.12, 119.83, 112.57, 111.08, 55.85, 55.73, 38.49, 27.52, 25.39, 24.32 ppm. HRMS: calcd for $C_{21}H_{21}N_3O_3S_1$ 396.1376, found 396.1371.

**2-Cyclohexyl-*N*-(5-(3,4-dimethoxyphenyl)isothiazolo[5,4-*b*]-pyridin-3-yl)acetamide (10u).** This compound was prepared according to the general procedure D from 3-amino-5-(3,4-dimethoxyphenyl)-isothiazolo[5,4-*b*]pyridine (**8b**, 0.20 mmol, 57 mg) and 2-cyclohexylacetyl chloride (0.2 mmol). The crude residue was purified by flash chromatography on silica gel using a mixture of dichloromethane and ethyl acetate (in a ratio of 8:1) as mobile phase, affording the pure title compound as a yellowish solid (60 mg, 73%). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ = 1.20 (m, 5H, $CH_2$) 1.7 (m, 6H, $CH_2$), 2.42 (d, $J$ = 6.5 Hz, 2H, $CH_2$), 3.83 (s, 3H, $OCH_3$), 3.88 (s, 3H, $OCH_3$), 7.14 (d, $J$ = 8.3 Hz, 1H, arom H), 7.32 (d, $J$ = 8.3 Hz, 1H, arom H), 7.36 (s, 1H, arom H), 10.96 (s, 1H, NH), 8.67 (d, $J$ = 1.9 Hz, 1H, arom H), 9.16 (d, $J$ = 1.9 Hz, 1H, arom H) ppm. $^{13}$C NMR (150 MHz, DMSO-$d_6$): $\delta$ = 171.27, 169.17, 152.46, 150.19, 149.43, 149.37, 132.21, 130.73, 129.08, 122.12, 119.75, 112.54, 110.96, 55.78, 55.73, 43.29, 34.66, 32.61, 25.90, 25.70 ppm. HRMS: calcd for $C_{22}H_{25}N_3O_3S_1$ 412.1689, found 412.1681.

***N*-(5-(3,4-Dimethoxyphenyl)isothiazolo[5,4-*b*]pyridin-3-yl)-3-noradamantanecarboxamide (10v).** This compound was prepared according to the general procedure D from 3-amino-5-(3,4-dimethoxyphenyl)-isothiazolo[5,4-*b*]pyridine (**8b**, 0.20 mmol, 57 mg) and 3-noradamantanecarbonyl chloride (0.2 mmol). The crude residue was purified by flash chromatography on silica gel using a mixture of dichloromethane and ethyl acetate (in a ratio of 6:1) as mobile phase, affording the pure title compound as a white solid (70 mg, 80%). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ = 1.60−2.30 (m, 13H, CH and $CH_2$), 3.83 (s, 3H, $OCH_3$), 3.87 (s, 3H, $OCH_3$), 7.14 (d, $J$ = 8.3 Hz, 1H, arom H), 7.32 (d, $J$ = 8.3 Hz, 1H, arom H), 7.37 (s, 1H, arom H), 8.38 (d, $J$ = 1.9 Hz, 1H, arom H), 9.16 (d, $J$ = 1.9 Hz, 1H, arom H), 10.41 (s, 1H, NH) ppm. $^{13}$C NMR (150 MHz, DMSO-$d_6$): $\delta$ = 176.20, 169.22, 153.32, 150.32, 149.40, 149.35, 132.36, 131.01, 129.14, 122.95, 119.87, 112.60, 111.14, 55.78, 55.72, 46.65, 43.25, 42.53, 37.15, 34.29 ppm. HRMS: calcd for $C_{24}H_{25}N_3O_3S_1$ 436.1689, found 436.1686.

***N*-(5-(3,4-Dimethoxyphenyl)isothiazolo[5,4-*b*]pyridin-3-yl)-1-adamantanecarboxamide (10w).** This compound was prepared according to the general procedure D from 3-amino-5-(3,4-dimethoxyphenyl)-isothiazolo[5,4-*b*]pyridine (**8b**, 0.20 mmol, 57 mg) and adamantanecarbonyl chloride (0.2 mmol). The crude residue was purified by flash chromatography on silica gel using a mixture of dichloromethane and ethyl acetate (in a ratio of 6:1) as mobile phase, affording the pure title compound as a white solid (70 mg, 78%). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ = 2.02−1.73 (m, 15H, CH and $CH_2$), 3.83 (s, 3H, $OCH_3$), 3.88 (s, 3H, $OCH_3$), 7.14 (d, $J$ = 8.3 Hz, 1H, arom H), 7.29 (d, $J$ = 8.3 Hz, 1H, arom H), 7.36 (s, 1H, arom H), 8.32 (d, $J$ = 2.0 Hz, 1H, arom H), 9.16 (d, $J$ = 2.0 Hz, 1H, arom H), 10.42 (s, 1H, NH) ppm. $^{13}$C NMR (150 MHz, DMSO-$d_6$): $\delta$ = 177.13, 169.20, 153.40, 150.32, 149.40, 149.35, 132.37, 130.87, 129.14, 122.91, 119.88, 112.63, 111.16, 55.80, 55.73, 40.97, 38.23, 36.03, 27.69 ppm. HRMS: calcd for $C_{25}H_{27}N_3O_3S_1$ 450.1846, found 450.1841.

***trans*-*N*-(5-(3,4-Dimethoxyphenyl)isothiazolo[5,4-*b*]pyridin-3-yl)-4-methylcyclohexanecarboxamide (10x).** This compound was prepared according to the general procedure F from 3-amino-5-(3,4-dimethoxyphenyl)-isothiazolo[5,4-*b*]pyridine (**8b**, 0.20 mmol, 57 mg) and trans-4-methylcyclohexanecarboxylic acid (0.3 mmol). The crude residue was purified by flash chromatography on silica gel using a mixture of dichloromethane and ethyl acetate (in a ratio of 7:1) as mobile phase, affording the pure title compound as a white solid (70 mg, 85%). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ = 0.90 (d, $J$ = 6.5 Hz, 3H, $CH_3$), 1.44−2.00 (m, 10H, CH and $CH_2$), 3.83 (s, 3H, $OCH_3$), 3.88 (s, 3H, $OCH_3$), 7.14 (d, $J$ = 8.3 Hz, 1H, arom H), 7.30 (d, $J$ = 8.3 Hz, 1H, arom H), 7.38 (s, 1H, arom H), 8.64 (d, $J$ = 2.0 Hz, 1H, arom H), 9.16 (d, $J$ = 2.0 Hz, 1H, arom H), 10.90 (s, 1H, NH) ppm. $^{13}$C NMR (150 MHz, DMSO-$d_6$): $\delta$ = 174.97, 169.13, 152.47, 150.21, 149.40, 149.36, 132.26, 130.65, 129.13, 122.12, 119.83, 112.57, 111.09, 55.85, 55.72, 43.77, 34.00, 31.64, 29.03, 22.60 ppm. HRMS: calcd for $C_{22}H_{25}N_3O_3S_1$ 412.1689, found 412.1687.

***N*-(5-(3,4-Dimethoxyphenyl)isothiazolo[5,4-*b*]pyridin-3-yl)-pivalamide (10aa).** This compound was prepared according to the general procedure E from 3-amino-5-(3,4-dimethoxyphenyl)-isothiazolo[5,4-*b*]pyridine (**8b**, 72 mg, 0.25 mmol) and pivaloyl chloride (0.3 mmol). The residue was purified by flash chromatography on silica, the mobile phase being a mixture of ethyl acetate and dichloromethane (in a ratio of 1/8), yielding the title compound (60


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mg, 65%) as white solid. $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 1.42 (s, 9H, $CH_3$), 3.97 (s, 3H, $OCH_3$), 3.99 (s, 3H, $OCH_3$), 7.01 (d, $J$ = 8.3 Hz), 7.15 (d, $J$ = 2.1 Hz, 1H, arom H), 7.22 (dd, $J$ = 8.3 Hz, 2.1 Hz, 1H, arom H), 8.40 (br s, 1H, NH), 8.63 (d, $J$ = 2.1 Hz, 1H, arom H), 8.95 (d, $J$ = 2.1 Hz, 1H, arom H) ppm. $^{13}$C NMR (150 MHz, DMSO-$d_6$): $\delta$ = 177.70, 169.21, 153.36, 150.32, 149.41, 149.36, 132.37, 130.90, 129.12, 122.89, 119.86, 112.60, 111.14, 55.79, 55.73, 33.43, 27.11 ppm. HRMS: calcd for $C_{19}H_{21}N_3O_3S_1$ 372.1376, found 372.1377.

***N*-(5-(3,4-Dimethoxyphenyl)isothiazolo[5,4-*b*]pyridin-3-yl)-propionamide (10ab).** This compound was prepared according to general procedure E from 3-amino-5-(3,4-dimethoxyphenyl)-isothiazolo[5,4-*b*]pyridine and propionyl chloride. The crude residue was purified by flash chromatography on silica gel using a mixture of cyclohexane and ethyl acetate (in a ratio of 2:1) as mobile phase, affording the pure title compound in 80% yield. $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ = 1.13 (t, $J$ = 7.47 Hz, 3H, $CH_3$), 2.57 (q, $J$ = 7.41 Hz, 2H, $CH_2$), 3.82 (s, 3H, $OCH_3$), 3.88 (s, 3H, $OCH_3$), 7.13 (d, $J$ = 8.31 Hz, 1H, arom H), 7.32 (dd, $J$ = 2.01 and 8.22 Hz, 1H, arom H), 7.38 (d, $J$ = 2.01 Hz, 1H, arom H), 8.73 (d, $J$ = 1.89 Hz, arom H), 9.15 (d, $J$ = 2.07 Hz, 1H, arom H), 10.94 (s, 1H, NH) ppm.

***N*-(5-(3,4-Dimethoxyphenyl)isothiazolo[5,4-*b*]pyridin-3-yl)-benzamide (10ac).** This compound was prepared according to the general procedure E from 3-amino-5-(3,4-dimethoxyphenyl)-isothiazolo[5,4-*b*]pyridine (**8b**, 0.20 mmol, 57 mg) and benzoyl chloride (0.3 mmol). The crude residue was purified by flash chromatography on silica gel using a mixture of dichloromethane and ethyl acetate (in a ratio of 6:1) as mobile phase, affording the pure title compound as a white solid in (50 mg, 51%).

$^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 3.74 (s, $OCH_3$), 3.76 (s, $OCH_3$), 3.95 (s, $OCH_3$), 3.99 (s, $OCH_3$), 6.70−7.70 (m, 8H, Ar−H), 8.02 (d, $J$ = 2.3 Hz, 1H, arom H), 8.76 (d, $J$ = 2.3 Hz, 1H, arom H), 8.90 (br., 1H, NH) ppm. $^{13}$C NMR (150 MHz, DMSO-$d_6$): $\delta$ = 169.25, 166.13, 152.89, 150.38, 149.38, 149.34, 132.48, 131.08, 129.33, 129.13, 129.04, 128.59, 128.40, 122.03, 119.55, 112.50, 111.26, 55.89, 55.71 ppm. HRMS: calcd for $C_{21}H_{17}N_3O_3S_1$ 392.1063, found 392.1060.

***N*-(5-(3,4-Dimethoxyphenyl)isothiazolo[5,4-*b*]pyridin-3-yl)-furan-2-carboxamide (10ad).** This compound was prepared according to the general procedure E from 3-amino-5-(3,4-dimethoxyphenyl)-isothiazolo[5,4-*b*]pyridine (**8b**, 0.20 mmol, 57 mg) and furan-2-carbonyl chloride (0.2 mmol). The residue was purified by flash chromatography on silica, the mobile phase being a mixture of ethyl acetate and dichloromethane (in a ratio of 1/20), yielding the title compound 50 mg (53%) as a white solid. $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 3.96 (s, 3H, $OCH_3$), 3.99 (s, 3H, $OCH_3$), 6.50−7.70 (m, 6H, arom H), 8.84 (d, $J$ = 2.3 Hz, 1H, arom H), 8.98 (d, $J$ = 2.3 Hz, 1H, arom H), 9.12 (br s, 1H, NH) ppm. HRMS: calcd for $C_{19}H_{15}N_3O_4S_1$ 382.0856, found 382.0863.

***N*-(5-(3,4-Dimethoxyphenyl)isothiazolo[5,4-*b*]pyridin-3-yl)-nicotinamide (10ae).** This compound was prepared according to the general procedure E from 3-amino-5-(3,4-dimethoxyphenyl)-isothiazolo[5,4-*b*]pyridine (**8b**, 0.20 mmol, 57 mg) and nicotinoyl chloride (0.2 mmol). The residue was purified by flash chromatography on silica, the mobile phase being a mixture of ethyl acetate and dichloromethane (in a ratio of 1/20), yielding the title compound (50 mg, 51%) as white solid. $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ = 3.81 (s, 3H, $OCH_3$), 3.87 (s, 3H, $OCH_3$), 7.11 (d, $J$ = 8.3 Hz), 7.34 (dd, $J$ = 8.3 and 2.1 Hz, 1H, Ar−H), 7.40 (d, $J$ = 2.1 Hz, 1H, arom H), 7.62 (dd, $J$ = 8.0 and 5.0 Hz, 1H, arom H), 8.41 (dt, $J$ = 8.0 and 1.9 Hz, 1H, arom H), 8.73 (d, $J$ = 2.1 Hz, 1H, arom H), 8.83 (dd, $J$ = 5.0 and 1.9 Hz, 1H, arom H), 9.19 (d, $J$ = 2.1 Hz, 1H, arom H), 9.23 (d, $J$ = 1.9 Hz, 1H, arom H), 11.63 (br s, 1H, NH) ppm. $^{13}$C NMR (150 MHz, DMSO-$d_6$): $\delta$ = 169.26, 164.83, 152.83, 152.47, 150.44, 149.38, 136.15, 132.51, 131.09, 129.20, 129.06, 123.65, 122.66, 120.05, 112.48, 111.27, 55.90, 55.71 ppm. HRMS: calcd for $C_{20}H_{16}N_4O_3S_1$ 393.1016, found 393.1014.

**2-(4-Chlorophenoxy)-*N*-(5-(3,4-dimethoxyphenyl)-isothiazolo[5,4-*b*]pyridin-3-yl)acetamide (10af).** This compound was prepared according to the general procedure E from 3-amino-5-(3,4-dimethoxyphenyl)-isothiazolo[5,4-*b*]pyridine (**8b**, 0.20 mmol, 57 mg) and 2-(4-chlorophenoxy)acetyl chloride (0.2 mmol). The residue was purified by flash chromatography on silica, the mobile phase being a mixture of ethyl acetate and dichloromethane (in a ratio of 1/10), yielding the title compound (90 mg, 79%) as a white solid. $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 3.97 (s, 3H, $OCH_3$), 4.00 (s, 3H, $OCH_3$), 4.76 (s, 2H, $OCH_2$), 6.85−7.40 (m, 7H, arom H), 8.75 (d, $J$ = 2.1 Hz, 1H, arom H), 9.00 (d, $J$ = 2.1 Hz, 1H, arom H), 9.27 (br s, 1H, NH) ppm. HRMS: calcd for $C_{22}H_{18}N_3O_4S_1$ 456.0779, found 456.0775.

**1-Acetyl-*N*-(5-(3,4-dimethoxyphenyl)isothiazolo[5,4-*b*]-pyridin-3-yl)piperidine-4-carboxamide (10y).** To a suspension of 1-acetylpiperidine-4-carboxylic acid (0.3 mmol) in dichloromethane (10 mL) were added *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU, 0.3 mmol) and DIPEA (0.4 mmol), respectively. After stirring for 20 min, 3-amino-5-(3,4-dimethoxyphenyl)isothiazolo[5,4-*b*]pyridine (57 mg, 0.2 mmol) was added. The resulting reaction mixture was stirred at room temperature for 12 h. After concentration under reduced pressure, the crude residue was purified by flash chromatography on silica, the mobile phase being a mixture of methanol and dichloromethane (in a ratio of 1/40), yielding the pure title as a white solid (60 mg, 68).

$^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ = 2.02 (s, 3H, $CH_3$), 2.00−1.45 (m, 4H), 3.01, 2.65 (m, 4H, $CH_2$), 3.45 (m, 1H, CH), 3.83 (s, 3H, $OCH_3$), 3.88 (s, 3H, $OCH_3$), 4.40 (m, 1H, CH), 7.14 (d, $J$ = 8.3 Hz, 1H, arom H), 7.31 (d, $J$ = 8.3 Hz, 1H, arom H), 7.38 (s, 1H, arom H), 8.65 (d, $J$ = 2.0 Hz, 1H, arom H), 9.16 (d, $J$ = 2.0 Hz, 1H, arom H), 11.02 (s, 1H, NH) ppm. $^{13}$C NMR (150 MHz, DMSO-$d_6$): $\delta$ = 173.65, 169.15, 168.13, 152.31, 150.27, 149.41, 149.37, 132.29, 130.66, 129.09, 122.10, 119.83, 112.56, 111.09, 55.85, 55.73, 45.24(41.78), 40.33, 28.63(28.07), 21.38 ppm. HRMS: calcd for $C_{22}H_{24}N_4O_4S_1$ 441.1591, found 441.1592.

***tert*-Butyl-4-(5-(3,4-dimethoxyphenyl)isothiazolo[5,4-*b*]-pyridin-3-ylcarbamoyl)piperidine-1-carboxylate.** This compound was prepared according to the procedure, described for the synthesis of compound **10y** from 3-amino-5-(3,4-dimethoxyphenyl)-isothiazolo[5,4-*b*]pyridine (**8b**, 1.0 mmol, 287 mg) and 1-(*tert*-butoxycarbonyl)piperidine-4-carboxylic acid (1.0 mmol). The crude residue was purified by flash chromatography on silica gel using a mixture of dichloromethane and ethyl acetate (in a ratio of 7:1) as mobile phase, affording the pure intermediate as a yellowish solid (375 mg, 85%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 1.46 (s, 9H, $CH_3$), 1.40−1.80 (m, 4H, $CH_2$), 3.90−2.06 (m, 5H, CH and $CH_2$), 3.97 (s, 3H, $OCH_3$), 4.00 (s, 3H, $OCH_3$), 7.02 (d, $J$ = 8.3 Hz, 1H, arom H), 7.16 (s, 1H, arom H), 7.22 (d, $J$ = 8.3 Hz, 1H, arom H), 8.67 (d, $J$ = 2.0 Hz, 1H, arom H), 8.97 (d, $J$ = 2.0 Hz, 1H, arom H), 9.17 (br s, 1H, NH) ppm. MS $m/z$ (%): 499.2 ([M + H]$^+$, 100).

***N*-(5-(3,4-Dimethoxyphenyl)isothiazolo[5,4-*b*]pyridin-3-yl)-piperidine-4-carboxamide (10z).** To a solution of *tert*-butyl-4-(5-(3,4-dimethoxyphenyl)isothiazolo[5,4-*b*]pyridin-3-ylcarbamoyl)-piperidine-1-carboxylate (220 mg, 0.5 mmol) in dichloromethane (5 mL) was added trifluoroacetic acid (2 mL). The mixture was stirred at room temperature for 30 min. 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 compound as a yellowish solid (190 mg, 95%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 1.40−1.80 (m, 4H, $CH_2$), 2−17−3.90 (m, 5H, CH and $CH_2$), 3.86 (s, 3H, $OCH_3$), 3.89 (s, 3H, $OCH_3$), 6.83 (d, $J$ = 8.3 Hz, 1H, Ar−H), 7.01 (s, 1H, arom H), 7.02 (d, $J$ = 8.3 Hz, 1H, arom H), 8.72 (d, $J$ = 2.0 Hz, 1H, arom H), 8.77 (d, $J$ = 2.0 Hz, 1H, arom H), 9.30 (br s, 1H, NH) ppm. $^{13}$C NMR (150 MHz, DMSO-$d_6$): $\delta$ = 171.54, 169.11, 151.84, 150.30, 149.44, 149.39, 132.31, 130.66, 129.02, 122.07, 119.83, 112.52, 111.10, 55.87, 55.75, 43.95, 43.03, 26.14 ppm. HRMS: calcd for $C_{20}H_{22}N_4O_3S_1$ 399.1485, found 399.1476.

**3-Chloro-6-(3,4-dimethoxyphenyl)pyrazine-2-carbonitrile (12).** This compound was prepared starting from 6-bromo-3-chloropyrazine-2-carbonitrile (218 mg, 1.0 mmol) and 3,4-dimethoxyphenylboronic acid (1.2 equiv) according to general procedure A. The crude residue was purified by flash chromatography on silica, the mobile phase being a mixture of methanol and dichloromethane (in a ratio of 1/15), yielding the title compound as a yellow solid (170 mg,


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62%). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ = 3.86 (s, 3H, $OCH_3$), 3.88 (s, 3H, $OCH_3$), 7.15 (d, $J$ = 8.5 Hz, 1H, arom H), 7.68 (s, 1H, arom H), 7.79 (d, $J$ = 8.5 Hz, 1H, arom H), 9.42 (s, 1H, arom H) ppm.

**5-(3,4-Dimethoxyphenyl)isothiazolo[5,4-*b*]pyrazin-3-amine (14).** This compound was prepared starting from 3-chloro-6-(3,4-dimethoxyphenyl)pyrazine-2-carbonitrile (**12**, 83 mg, 0.3 mmol), following the same reaction pathway as described for the synthesis of compound **7**. The crude residue was purified by flash chromatography on silica, the mobile phase being a mixture of ethyl acetate and dichloromethane (in a ratio of 1/20), yielding the title compound as a yellow solid (57 mg, 86%). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ = 3.86 (s, 3H, $OCH_3$), 3.93 (s, 3H, $OCH_3$), 7.14 (d, $J$ = 8.5 Hz, 1H, arom H), 7.22 (s, 2H, $NH_2$), 7.94 (d, $J$ = 8.5 Hz, 1H, arom H), 7.98 (s, 1H, arom H), 9.44 (s, 1H, arom H) ppm.

***N*-(5-(3,4-Dimethoxyphenyl)isothiazolo[4,5-*b*]pyridin-3-yl)-cyclohexanecarboxamide (15).** This compound was synthesized starting from compound **14** (50 mg, 0.17 mmol) and cyclohexanecarbonyl chloride (0.17 mmol), according to general procedure E. The crude residue was purified by flash chromatography on silica, the mobile phase being a mixture of ethyl acetate and dichloromethane (in a ratio of 1/15), yielding the title compound as a yellow solid (55 mg, 81%). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ = 1.23−1.98 (m, 10H, $CH_2$), 2.71 (m, 1H, CH), 3.87 (s, 3H, $OCH_3$), 3.91 (s, 3H, $OCH_3$), 7.16 (d, $J$ = 8.5 Hz, 1H, arom H), 7.91 (s, 1H, arom H), 7.97 (d, $J$ = 8.5 Hz, 1H, arom H), 9.54 (s, 1H, arom H), 10.73 (s, 1H, NH) ppm. $^{13}$C NMR (150 MHz, DMSO-$d_6$): $\delta$ = 174.73, 161.26, 152.58, 151.20, 149.60, 149.28, 142.94, 136.50, 127.73, 120.72, 111.99, 110.46, 55.75, 55.71, 43.80, 29.19, 25.50, 25.19 ppm. HRMS: calcd for $C_{20}H_{22}N_4O_3S_1$ 399.1485, found 399.1479.

**5-(3,4-Dimethoxyphenyl)-1*H*-pyrazolo[3,4-*b*]pyrazin-3-amine (16).** To a solution of 3-chloro-6-(3,4-dimethoxyphenyl)-pyrazine-2-carbonitrile (**12**, 138 mg, 0.5 mmol) in EtOH (5 mL) was added a 60% solution of hydrazine in water (2.5 mmol). The mixture was refluxed for 2 h. After concentration under reduced pressure, the crude residue was purified by flash chromatography on silicagel, the mobile phase being a mixture of methanol and dichloromethane (in a gradient, gradually ranging from 1:40 to 1:10), yielding the pure title compound as yellow solid (40 mg, 29%). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ = 3.83 (s, 3H, $OCH_3$), 3.89 (s, 3H, $OCH_3$), 7.09 (d, $J$ = 8.5 Hz, 1H, arom H), 7.70 (d, $J$ = 8.5 Hz, 1H, arom H), 7.75 (s, 1H, arom H), 9.02 (s, 1H, arom H), 12.35 (s, 1H, NH) ppm.

***N*-(5-(3,4-Dimethoxyphenyl)-1*H*-pyrazolo[3,4-*b*]pyrazin-3-yl)cyclohexanecarboxamide (17).** This compound was prepared from 5-(3,4-dimethoxyphenyl)-1*H*-pyrazolo[3,4-*b*]pyrazin-3-amine (**16**, 40 mg, 0.15 mmol) and cyclohexanecarbonyl chloride (0.2 mmol) according to general procedure E. The crude residue was purified by flash chromatography on silica gel using a mixture of dichloromethane and ethyl acetate (in a ratio of 15:1) as mobile phase, affording the pure title compound as a yellowish solid (31 mg, 55%). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ = 1.2−2.0 (m, 11H, CH and $CH_2$), 3.83 (s, 3H, $OCH_3$), 3.88 (s, 3H, $OCH_3$), 7.08 (d, $J$ = 8.5 Hz, 1H, arom H), 7.73 (d, $J$ = 8.5 Hz, 1H, arom H), 7.77 (s, 1H, arom H), 8.18 (s, 1H, NH) 9.25 (s, 1H, arom H) ppm. HRMS: calcd for $C_{20}H_{23}N_5O_3$ 382.1874, found 382.1870.

**Ethyl 3-Amino-5-bromobenzo[*b*]thiophene-2-carboxylate (19a).** 5-Bromo-2-chlorobenzonitrile (**18a**, 864 mg, 4.0 mmol) and ethyl 2-mercaptoacetate (393 μL, 4.4 mmol) were dissolved in dry DMF (20 mL) and the mixture was cooled to 0 °C. To this mixture, tBuOK (985 mg, 8.8 mmol) was added portionwise over a period of 45 min. The reaction mixture was stirred an additional hour at 0 °C. The solvent was evaporated and the crude residue was purified by silicagel flash chromatography, the mobile phase being a mixture of cyclohexane/ethyl acetate in a ratio 3:2, yielding the pure title compound as a white powder (450 mg, 38%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 1.40 (t, $J$ = 7.11 Hz, 3H, $CH_3$), 4.36 (q, $J$ = 7.11 Hz, 2H, $CH_2$), 5.86 (br s, 1H, NH), 7.54 (m, 2H, arom H), 7.76 (d, $J$ = 1.38 Hz, 1H, arom H) ppm. HRMS: calcd for $C_{11}H_{11}BrNO_2S$ 299.9688, found 299.9692.

**Ethyl 3-Amino-5-bromothieno[2,3-*b*]pyridine-2-carboxylate (19b).** 5-Bromo-2-chloro-3-cyanopyridine (**18b**, 617 mg, 2.84 mmol) and ethyl 2-mercaptoacetate (344 μL, 3.12 mmol) were dissolved in dry DMF (20 mL) and the mixture was cooled to 0 °C. To this mixture tBuOK (699 mg, 6.25 mmol) was added portionwise over a period of 45 min. The reaction mixture was stirred an additional 30 min at 0 °C, and then 1 h at room temperature. After the reaction was completed, ice (20 mL) was added to the mixture. The yellow precipitate was filtered off and dried affording the title compound as a yellow powder (692 mg, 80%). HRMS: calcd for $C_{10}H_{10}BrN_2O_2S$ 300.9641, found 300.9638.

**Ethyl 7-Amino-2-bromothieno[2,3-*b*]pyrazine-6-carboxylate (19c).** 5-Bromo-2-chloro-3-cyanopyrazine (**11**, 1.06 g, 5.0 mmol) and ethyl 2-mercaptoacetate (610 μL, 11.0 mmol) were dissolved in dry DMF (30 mL) and the mixture was cooled to 0 °C. To this mixture tBuOK (1.24 mg, 11.0 mmol) was added portionwise over a period of 60 min. Mixture was stirred additional 60 min at 0 °C. After the reaction was complete, the solvent was evaporated and the residue was purified on silicagel using a mixture of cyclohexane/ethyl acetate (in a ratio of 3:1) as mobile phase, yielding the pure title compound (303 mg, 20%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 1.42 (t, $J$ = 7.14 Hz, 3H, $CH_3$), 4.39 (q, $J$ = 7.14 Hz, 2H, $CH_2$), 6.14 (br s, 2H, $NH_2$), 8.58 (s, 1H, arom H) ppm. HRMS: calcd for $C_9H_9BrN_3O_2S$ 301.9593, found 301.9596.

**5-Bromobenzo[*b*]thiophen-3-amine (20a).** Ethyl 3-amino-5-bromobenzo[*b*]thiophene-2-carboxylate (**19a**, 450 mg, 1.5 mmol) was treated with a 30% aqueous NaOH solution (3 mL) in refluxing ethanol (10 mL). After completion of the reaction (2 h), solvents were evaporated. The residue was dissolved in water (45 mL) and the mixture was carefully neutralized with a 1 M HCl solution. The intermediate was then extracted into ethyl acetate. A light yellow powder was obtained upon evaporation. This residue was suspended in $H_3PO_4$ (3 mL). The mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with water/ice and the pH was adjusted to 7−8 with 5 M NaOH. The product was then extracted into ethyl acetate. The organic phase was dried over $MgSO_4$ and finally filtered. Upon evaporation the crude product **20a** was obtained as a dark yellow powder (50 mg, 15%) and was used in the next step without further purification. $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 3.78 (bs, 1H, $NH_2$), 6.37 (s, 1H, arom H), 7.43 (dd, $J$ = 1.83 Hz, $J$ = 8.55 Hz, 1H, arom H), 7.63 (d, $J$ = 8.55 Hz, 1H, arom H), 7.76 (d, $J$ = 1.80 Hz, 1H, arom H) ppm.

**5-Bromothieno[2,3-*b*]pyridin-3-amine (20b).** This compound was obtained from ethyl 3-amino-5-bromothieno[2,3-*b*]pyridine-2-carboxylate (**19b**, 117 mg, 0.32 mmol) according to the procedure described for the synthesis of **20a**. The crude product **20b** was obtained as yellow powder (75 mg, 100%) and was used in the next step without further purification.

**2-Bromothieno[2,3-*b*]pyrazin-7-amine (20c).** This compound was obtained from ethyl 7-amino-2-bromothieno[2,3-*b*]pyrazine-6-carboxylate (**19c**, 303 mg, 1.0 mmol) according to the procedure described for the synthesis of **20a**. The crude product **20c** was obtained as yellow powder (40 mg, 17%) and was used in the next step without further purification. $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 3.85 (bs, 1H, $NH_2$), 6.67 (s, 1H, arom H), 8.58 (s, 1H, arom H) ppm.

***N*-(5-Bromobenzo[*b*]thiophen-3-yl)-cyclohexanecarboxamide (21a).** Cyclohexanecarboxylic acid chloride (33 μL, 0.24 mmol) was added in one portion to a stirred mixture of 5-bromobenzo[*b*]thiophen-3-amine (**20a**, 50 mg, 0.21 mmol) in dichloromethane (4 mL). After completion of reaction (24 h) solvents were evaporated. The crude residue was purified on silicagel using a mixture of cyclohexane/ethyl acetate in a ratio of 2:1, affording the pure title compound (46 mg, 62%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 1.59−2.06 (m, 10H, 5 × $CH_2$), 2.38 (m, 1H, CH), 7.46−7.52 (m, 2H, arom H, NH), 7.70−7.73 (m, 2H, arom H), 8.04 (s, 1H, arom H) ppm.

***N*-(5-Bromothieno[2,3-*b*]pyridin-3-yl)-cyclohexanecarboxamide (21b).** This compound was prepared according to the procedure for the synthesis of compound **21a**, starting from 5-bromothieno[2,3-*b*]pyridin-3-amine (**20b**, 75 mg, 0.329 mmol). The crude product was purified on silicagel using a mixture of cyclohexane/ethyl acetate as mobile phase (in a ratio of


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9:1), affording the title compound as white powder (74 mg, 66%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 1.63−2.05 (m, 10H, 5 × $CH_2$), 2.37 (m, 1H, CH), 7.55 (br s, 1H, NH), 7.97 (s, 1H, arom H), 8.03 (d, $J$ = 2.07 Hz, 1H, arom H), 8.62 (d, $J$ = 1.98 Hz, 1H, arom H) ppm.

***N*-(2-Bromothieno[2,3-*b*]pyrazin-7-yl)-cyclohexanecarboxamide (21c).** This compound was prepared according to the procedure for the synthesis of compound **21a**, starting from crude 2-bromothieno[2,3-*b*]pyrazin-7-amine (**20c**, 40 mg, 0.17 mmol) The crude product was purified on silicagel using a mixture of cyclohexane/ethyl acetate (in a ratio of 9:1) as mobile phase, affording the pure title (17 mg, 27%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 1.63−2.04 (m, 10H, 5 × $CH_2$), 2.47 (m, 1H, CH), 8.28 (br s, 1H, NH), 8.45 (s, 1H, arom H), 8.64 (s, 1H, arom H) ppm.

***N*-(5-(3,4-Dimethoxyphenyl)benzo[*b*]thiophen-3-yl)-cyclohexanecarboxamide (22a).** To a solution of *N*-(5-bromobenzo[*b*]thiophen-3-yl)cyclohexanecarboxamide (**21a**, 46 mg, 0.13 mmol) in DME (2 mL) was added 3,4-dimethoxyphenyl boronic acid (47 mg, 0.26 mmol) and potassium carbonate (0.13 mL, 2 M solution in $H_2O$). The mixture was degassed and $Pd(PPh_3)_4$ (15 mg, 0.013 mmol) was added. The reaction was heated to 80 °C. After completion of the reaction (24 h), solvents were evaporated. The crude product was purified on silicagel using a mixture of cyclohexane/ethyl acetate (in a ratio of 1:1) as mobile phase, affording the pure title compound (12.2 mg, 23%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 1.61−2.06 (m, 10H, 5 × $CH_2$), 2.40 (m, 1H, CH), 3.95 (s, 3H, $CH_3$), 3.99 (s, 3H, $CH_3$), 6.98 (d, $J$ = 8.28 Hz, 1H, arom H), 7.16 (d, $J$ = 2.04 Hz, 1H, arom H), 7.22 (dd, $J$ = 2.10 Hz, $J$ = 8.19 Hz, 1H, arom H), 7.57 (d, $J$ = 8.34 Hz, arom H), 7.67 (bs, 1H, NH), 7.88 (d, $J$ = 8.37 Hz, 1H, arom H), 8.03 (s, 1H, arom H) ppm. $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 25.32 ($CH_2$), 29.35 ($CH_2$), 29.49 ($CH_2$) 45.75 (CH), 55.71 ($OCH_3$), 55.83 ($OCH_3$), 110.84 (CH), 111.32 (CH), 112.77 (CH), 116.46 (CH), 119.57 (CH), 123.10 (CH), 124.18 (CH), 128.19 ($C_q$), 132.69 ($C_q$), 134.01 ($C_q$), 136.33 ($C_q$), 137.39 ($C_q$), 148.50 ($C_q$), 148.97 ($C_q$), 173.76 (CO) ppm. HRMS: calcd for $C_{23}H_{26}NO_3S$ 396.1627, found 396.1626.

***N*-(5-(3,4-Dimethoxyphenyl)thieno[2,3-*b*]pyridin-3-yl)-cyclohexanecarboxamide (22b).** This compound was prepared according to the procedure described for the preparation of **22a**, starting from *N*-(5-bromothieno[2,3-*b*]pyridin-3-yl)-cyclohexanecarboxamide (**21b**, 74 mg, 0.21 mmol). The crude residue was purified on silicagel using a mixture of cyclohexane/ethyl acetate (in a ratio of 3:2) as mobile phase, affording the title compound as white powder (34 mg, 39%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 1.64−2.00 (m, 10H, 5 × $CH_2$), 2.39 (m, 1H, CH), 3.94 (s, 3H, $CH_3$), 3.96 (s, 3H, $CH_3$), 6.97 (d, $J$ = 8.28 Hz, 1H, arom H), 7.10 (d, $J$ = 2.01 Hz, 1H, arom H), 7.16 (dd, $J$ = 2.01 Hz, $J$ = 8.22 Hz, 1H, arom H), 7.75 (br s, 1H, NH), 7.97 (d, $J$ = 1.95 Hz, 1H, arom H), 8.03 (s, 1H, arom H), 8.78 (d, $J$ = 1.86 Hz, 1H, arom H) ppm. $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 25.62 ($CH_2$), 29.69 ($CH_2$), 29.78 ($CH_2$), 45.96 (CH), 56.04 ($OCH_3$), 56.16 ($OCH_3$), 110.89 (CH), 111.87 (CH), 114.09 (CH), 119.06 (CH), 124.36 (CH), 126.30 ($C_q$), 126.70 ($C_q$), 130.83 ($C_q$), 132.68 ($C_q$), 146.32 (CH), 149.29 ($C_q$), 149.50 ($C_q$), 158.07 ($C_q$), 174.55 (CO) ppm. HRMS: calcd for $C_{22}H_{23}N_2O_3S$ 395.1434, found 395.1434.

***N*-(2-(3,4-Dimethoxyphenyl)thieno[2,3-*b*]pyrazin-7-yl)-cyclohexanecarboxamide (22c).** This compound was prepared according to the procedure described for the preparation of **22a**, starting from *N*-(2-bromothieno[2,3-*b*]pyrazin-7-yl)-cyclohexanecarboxamide (**21c**, 17 mg, 0.05 mmol). The crude residue was purified on silica gel using a mixture of cyclohexane/ethyl acetate (in a ratio of 3:2), affording the title compound as a white powder (12.5 mg, 62%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 1.61−2.09 (m, 10H, 5 × $CH_2$), 2.45 (m, 1H, CH), 3.99 (s, 3H, $CH_3$), 4.04 (s, 3H, $CH_3$), 6.05 (d, $J$ = 8.22 Hz, 1H, arom H), 7.66 (m, 2H, arom H), 8.39 (s, 1H, arom H), 8.51 (br s, 1H, NH), 8.98 (s, 1H, arom H) ppm. $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 25.28 ($CH_2$), 25.34 ($CH_2$) 29.31 ($CH_2$), 45.45 (CH), 55.75 ($OCH_3$), 55.83 ($OCH_3$), 109.96 (CH), 111.24 (CH), 113.90 ($C_q$), 120.00 (CH), 126.82 ($C_q$), 128.88 ($C_q$), 139.00 (CH), 148.53 ($C_q$), 149.32 (CH), 150.61 ($C_q$), 151.08 ($C_q$), 174.90 (CO) ppm. HRMS: calcd for $C_{21}H_{24}N_3O_3S$ 398.1532, found 398.1529.

**5-(3,4-Dimethoxyphenyl)-3-nitro-1*H*-pyrrolo[2,3-*b*]pyridine (24).** A mixture of 5-bromo-3-nitro-1H-pyrrolo[2,3-*b*]pyridine **23** (242 mg, 1.0 mmol), 3,4-dimethoxyphenylboronic acid (1.2 mmol), $K_2CO_3$ (2.0 mmol), and tetrakis(triphenylphosphine)palladium(0) (0.03 mmol) in 1,4-dioxane (4 mL) and water (1 mL) was refluxed for 12 h. After cooling to room temperature, the precipitate was filtered off, and the crude product **24** was used as such in the following step. $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ = 3.81 (s, 3H, $OCH_3$), 3.88 (s, 3H, $OCH_3$), 7.09 (d, $J$ = 8.3 Hz, 1H, arom H), 7.32 (s, 1H, arom H), 7.32 (d, $J$ = 8.3 Hz, 1H, arom H), 13.4 (br s, 1H, NH), 8.84 (s, 1H, arom H), 8.73 (d, $J$ = 2.0 Hz, 1H, arom H), 8.55 (d, $J$ = 2.0 Hz, 1H, arom H) ppm.

***N*-(5-(3,4-Dimethoxyphenyl)-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)-cyclohexanecarboxamide (25).** To a suspension of 5-(3,4-dimethoxyphenyl)-3-nitro-1*H*-pyrrolo[2,3-*b*]pyridine **24** (100 mg, 0.33 mmol) in methanol (10 mL) was added Raney Ni (100 mg). The mixture was stirred at room temperature under an atmosphere of hydrogen for 48 h. The reaction mixture was filtered through Celite. After concentration under reduced pressure, the crude residue was treated with cyclohexanecarbonyl chloride according to general procedure E. The crude residue was purified by silicagel flash chromatography, the mobile phase being a mixture of methanol and dichloromethane (in a ratio gradually ranging from 1/50 to 1/25), yielding the pure title compound as a white solid (40 mg, 32%). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ = 1.98−1.23 (m, 10H, $CH_2$), 2.48 (m, 1H, CH), 3.81 (s, 3H, $OCH_3$), 3.88 (s, 3H, $OCH_3$), 7.08 (d, $J$ = 8.3 Hz, 1H, arom H), 7.22 (d, $J$ = 8.3 Hz, 1H, arom H), 7.26 (s, 1H, arom H), 7.82 (s, 1H, arom H), 11.31 (s, 1H, NH), 8.44 (d, $J$ = 2.0 Hz, 1H, arom H), 8.51 (d, $J$ = 2.0 Hz, 1H, arom H), 9.88 (s, 1H, arom H) ppm. $^{13}$C NMR (150 MHz, DMSO-$d_6$): $\delta$ = 173.34, 149.26, 148.25, 144.94, 141.99, 131.98, 127.44, 124.13, 119.01, 115.54, 114.38, 112.67, 112.49, 110.76, 55.78, 55.69, 44.06, 29.45, 25.54, 25.41 ppm. HRMS: calcd for $C_{22}H_{26}N_3O_3$ 380.1969, found 380.1967.

**5-Bromo-2-mercaptobenzonitrile (27).** Into an oven-dried flask was added 5-bromo-2-fluorobenzonitrile (**26**, 400 mg, 2 mmol), $Na_2S$ (172 mg, 2.2 mmol), and DMF (2 mL) under nitrogen gas. The reaction mixture was stirred at room temperature for 2.5 h. Then, a 1 M NaOH solution (25 mL) was added and the aqueous phase was washed with dichloromethane (2 × 25 mL). The aqueous layer was acidified to pH 1−2 with a 6 M HCl solution and extracted with dichloromethane (2 × 25 mL). The combined organic layers were washed with brine (50 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure to provide a crude residue. To the residue was added 10% HCl (20 mL) and cooled with an ice−water bath. Then, zinc dust (1 g) was added and the mixture was stirred for 1 h. Then, ethyl acetate (100 mL) was added and the mixture was stirred for an additional 30 min. The organic layer was separated and washed with water (20 mL) and brine (20 mL), dried over $MgSO_4$, filtered, and concentrated to provide the title compound (209 mg, 98%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$= 4.11 (br s, 1H, SH), 7.28 (d, $J$ = 8.55 Hz, 1H, arom H), 7.55 (dd, $J$ = 8.49 Hz, $J$ = 1.47 Hz, 1H, arom H), 7.70 (s, 1H, arom H) ppm.

**5-Bromobenzo[*d*]isothiazol-3-amine (28).** To a solution of 5-bromo-2-mercaptobenzonitrile (**27**, 446 mg, 1.08 mL) in a 25% aqueous ammonia solution (13 mL) were added 3% NaOH (5 mL) and NaOCl (13% active chlorine, 1.3 mL) at 0 °C. The mixture was stirred at 0 °C for 4 h. The precipitate was filtered off, washed with water, and dried, yielding the pure title compound (244 mg, 51%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 5.31 (br s, 2H, $NH_2$), 7.53−7.60 (m, 2H, arom H), 7.63 (d, $J$ = 1.35 Hz, 1H, arom H) ppm.

***N*-(5-Bromobenzo[*d*]isothiazol-3-yl)-cyclohexanecarboxamide (29).** This compound was synthesized according to the procedure for the synthesis of compound **21a**, starting from 5-bromobenzo[*d*]isothiazol-3-amine (**28**, 229 mg, 1 mmol) The crude product was purified by silicagel flash chromatography, using a mixture of cyclohexane/ethyl acetate (in a ratio of 1:1) as mobile phase, affording the title compound as a white powder (116 mg, 34%). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ = 1.60−1.91 (m, 10H,


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5 × $CH_2$), 2.51 (m, 1H, CH), 7.73 (dd, $J$ = 2.13 Hz, $J$ = 8.28 Hz, 1H, arom H), 7.74 (dd, $J$ = 8.70 Hz, $J$ = 1.80 Hz, 1H, arom H), 8.13 (d, $J$ = 8.70 Hz, 1H, arom H), 8.26 (d, $J$ = 1.26 Hz, 1H, arom H), 10.65 (s, 1H, NH) ppm.

***N*-(5-(3,4-Dimethoxyphenyl)benzo[*d*]isothiazol-3-yl)-cyclohexanecarboxamide (30).** This compound was synthesized according to the procedure for the synthesis of compound **22a**, starting from *N*-(5-bromobenzo[*d*]isothiazol-3-yl)-cyclohexanecarboxamide (**29**, 100 mg, 0.29 mmol). The crude product was purified on silicagel using a mixture of cyclohexane/ethyl acetate as mobile phase (in a ratio of 3:2), affording the title compound as a white powder (40 mg, 34%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 1.60−2.04 (m, 10H, 5 × $CH_2$), 2.64 (m, 1H, CH), 3.95 (s, 3H, $CH_3$), 3.98 (s, 3H, $CH_3$), 6.97 (d, $J$ = 8.28 Hz, 1H, arom H), 7.00 (d, $J$ = 2.04 Hz, 1H, arom H), 7.22 (dd, $J$ = 2.13 Hz, $J$ = 8.28 Hz, 1H, arom H), 7.73 (dd, $J$ = 8.49, $J$ = 1.56 Hz, 1H, arom H), 7.87 (d, $J$ = 8.46 Hz, 1H, arom H), 8.17 (s, 1H, arom H), 8.52 (s, 1H, NH) ppm. $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ = 25.31 ($CH_2$), 25.55 ($CH_2$), 29.22 ($CH_2$), 43.93 (CH), 55.78 ($OCH_3$), 55.81 ($OCH_3$), 111.00 (CH), 112.53 (CH), 119.42 (CH), 121.13 (CH), 121.48 (CH), 127.52 (CH), 129.73 ($C_q$), 132.40 ($C_q$), 137.20 ($C_q$), 148.95 ($C_q$), 149.32 ($C_q$), 149.95 ($C_q$), 153.31 ($C_q$), 174.98 (CO) ppm. HRMS: calcd for $C_{22}H_{25}N_2O_3S$ 397.1580, found 397.1583.

**5-Bromobenzo[*d*]isoxazol-3-amine (31).** Acetohydroxamic acid (150 mg, 2 mmol) and *t*BuOK (224 mg, 2 mmol) were suspended in dry DMF and the resulting reaction mixture was stirred at room temperature for 40 min. Then 5-bromo-2-fluorobenzonitrile (**26**, 400 mg, 2 mmol) was added in one portion. After completion of the reaction (24 h), the solvent was evaporated. The crude product was purified on silica gel using a mixture of cyclohexane/ethyl acetate as mobile phase (in a ratio of 5:1), affording the pure title compound (214 mg, 50%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 4.47 (br s, 2H, $NH_2$), 7.32 (d, $J$ = 8.62 Hz, 1H, arom H), 7.60 (dd, $J$ = 8.82 Hz, $J$ = 1.35 Hz, 1H, arom H), 7.69 (d, $J$ = 1.33 Hz, 1H, arom H) ppm.

***N*-(5-Bromobenzo[*d*]isoxazol-3-yl)cyclohexanecarboxamide (32).** This compound was prepared according to the procedure for the synthesis of compound **21a**, starting from 5-bromobenzo[d]isoxazol-3-amine (**31**, 200 mg, 0.93 mmol). The mixture was heated to 50 °C. The crude residue was purified by flash chromatography on silica gel using a mixture of cyclohexane/ethyl acetate (in a ratio of 9:1), affording the title compound (110 mg, 36%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 1.58−2.07 (m, 10H, 5 × $CH_2$), 2.47 (m, 1H, CH), 7.39 (d, $J$ = 8.91 Hz, 1H, arom H), 7.66 (dd, $J$ = 8.88 Hz, $J$ = 1.98 Hz, 1H, arom H), 8.45 (br s, 1H, NH), 7.49 (d, $J$ = 1.74 Hz, 1H, arom H) ppm.

***N*-(5-(3,4-Dimethoxyphenyl)benzo[*d*]isoxazol-3-yl)-cyclohexanecarboxamide (33).** To a solution of *N*-(5-bromobenzo[*d*]isoxazol-3-yl)cyclohexanecarboxamide (**32**, 110 mg, 0.34 mmol) in DME (2 mL) was added an 3,4-dimethoxyphenyl boronic acid (92 mg, 0.51 mmol) and potassium carbonate (0.68 mL, 2 M solution in $H_2O$). Mixture was degassed and $Pd(PPh_3)_4$ (36 mg, 0.034 mmol) was added. The reaction was heated to 80 °C. After the completion of reaction (6 h), solvents were evaporated. Product was purified on silica gel using a mixture of cyclohexane/ethyl acetate in a ratio of 3:2, affording the title compound in 21% yield (27 mg, 0.07 mmol). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 1.59−2.17 (m, 10H, 5 × $CH_2$), 3.09 (m, 1H, CH), 3.95 (s, 3H, $CH_3$), 3.94 (s, 3H, $CH_3$), 6.98 (d, $J$ = 8.28 Hz, 1H, arom H), 7.11−7.18 (m, 3H, arom H), 7.62 (dd, $J$ = 2.37 Hz, $J$ = 8.64 Hz, 1H, arom H), 8.21 (d, $J$ = 2.34 Hz, 1H, arom H), 8.52 (s, 1H, NH) ppm. $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 24.94 ($CH_2$), 25.10 ($CH_2$), 29.86 ($CH_2$), 35.94 (CH), 55.68 ($OCH_3$), 55.71 ($OCH_3$), 109.87 (CH), 110.51 ($C_q$), 111.25 (CH), 117.67 (CH), 118.74 (CH), 125.53 (CH), 131.28 (CH), 132.79 ($C_q$), 132.88 ($C_q$), 148.13 ($C_q$), 148.90 ($C_q$), 155.96 ($C_q$), 166.51 ($C_q$), 181.98 (CO) ppm. HRMS: calcd for $C_{22}H_{25}N_2O_4$ 381.1808, found 381.1808.

***tert*-Butyl 3-amino-5-bromo-1*H*-indazole-1-carboxylate.** To a solution of 5-bromo-1*H*-indazol-3-amine (**34**, 212 mg, 1 mmol) in dichloromethane (5 mL) was added dropwise a solution of di-*tert*-butyl dicarbonate (218 mg, 1 mmol) in dichloromethane (5 mL) at room temperature. After completion of the reaction (2 h), the solvents were evaporated. The crude product was purified by silicagel flash chromatography, using a mixture of cyclohexane/ethyl acetate as mobile phase (in a ratio of 3:1), affording the pure title compound (200 mg, 64%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 1.70 (s, 9H, 3 × $CH_3$), 4.50 (s, 2H, $NH_2$), 7.60 (dd, $J$ = 1.86 Hz, $J$ = 8.88 Hz, 1H, arom H), 7.69 (d, $J$ = 1.77 Hz, 1H, arom H), 7.96 (s, 1H, arom H) ppm.

***tert*-Butyl 5-bromo-3-(cyclohexanecarboxamido)-1*H*-indazole-1-carboxylate (35).** This compound was prepared according to the procedure for the synthesis of compound **21a**, starting from *tert*-butyl 3-amino-5-bromo-1*H*-indazole-1-carboxylate (200 mg, 0.64 mmol). After reaction completion (3 h), solvents were evaporated. The product was purified on silica gel using a mixture of cyclohexane/ethyl acetate (in a ratio of 7:1), affording the title compound (178 mg, 65%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 1.60−2.01 (m, 19H, 3 × $CH_3$, 5 × $CH_2$), 2.39 (m, 1H, CH), 7.62 (dd, $J$ = 1.92 Hz, $J$ = 8.97 Hz, 1H, arom H), 7.98 (d, $J$ = 9.03 Hz, 1H, arom H), 8.34 (s, 1H, arom H), 8.41 (s, 1H, NH) ppm.

***tert*-Butyl 5-bromo-3-(cyclohexanecarboxamido)-1*H*-indazole-1-carboxylate.** This compound was prepared according to the procedure described for the preparation of **22a**, starting from *tert*-butyl 5-bromo-3-(cyclohexanecarboxamido)-1*H*-indazole-1-carboxylate (**35**, 178 mg, 0.42 mmol). The crude residue was purified on silica gel using a mixture of cyclohexane/ethyl acetate (in a ratio of 3:2) as mobile phase, affording the pure title compound (101 mg, 50%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 1.61−2.07 (m, 19H, 3 × $CH_3$, 5 × $CH_2$), 2.42 (m, 1H, CH), 3.94 (s, 3H, $CH_3$), 3.98 (s, 3H, $CH_3$), 6.96 (d, $J$ = 8.31 Hz, 1H, arom H), 7.16 (d, $J$ = 2.04 Hz, 1H, arom H), 7.21 (dd, $J$ = 2.10 Hz, $J$ = 8.25 Hz, 1H, arom H), 7.74 (dd, $J$ = 8.82, $J$ = 1.77 Hz, 1H, arom H), 8.11 (d, J = 8.76 Hz, 1H, arom H), 8.14 (s, 1H, arom H), 8.25 (s, 1H, NH) ppm.

***N*-(5-(3,4-Dimethoxyphenyl)-1*H*-indazol-3-yl)-cyclohexanecarboxamide (36).** *Tert*-butyl 5-bromo-3-(cyclohexanecarboxamido)-1*H*-indazole-1-carboxylate (101 mg, 0.21 mmol) was suspended in 20% TFA/DCM (10 mL). The reaction was stirred for 20 min at room temperature. Then, the mixture was carefully neutralized with solid $NaHCO_3$. The solids were filtered off and the solvents were evaporated. The crude product was purified on silica gel using a mixture of cyclohexane/ethyl acetate (in a ratio of 1:6) as mobile phase, affording the pure title compound (27 mg, 34%). $^1$H NMR (300 MHz, MeOD): $\delta$ = 1.60−2.03 (m, 10H, 5 × $CH_2$), 2.56 (m, 1H, CH), 3.88 (s, 3H, $CH_3$), 3.93 (s, 3H, $CH_3$), 7.05 (d, $J$ = 8.13 Hz, 1H, arom H), 7.20 (m, 2H, arom H), 7.49 (d, $J$ = 8.73 Hz, 1H, arom H), 7.64 (d, $J$ = 8.40 Hz, 1H, arom H), 7.91 (s, 1H, arom H) ppm. $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ = 25.39 ($CH_2$), 25.59 ($CH_2$), 29.37 ($CH_2$), 43.99 (CH), 55.77 ($OCH_3$), 110.50 (CH), 110.96 (CH), 112.56 (CH), 117.12 ($C_q$), 119.06 (CH), 119.43 ($C_q$), 126.17 (CH), 132.16 ($C_q$), 134.10 (CH), 140.38 ($C_q$), 140.85 ($C_q$), 148.20 ($C_q$), 149.17 (CH), 174.62 (CO) ppm. HRMS: calcd for $C_{22}H_{26}N_3O_3$ 380.1968, found 380.1974.

***N*-(6-(3,4-Dimethoxyphenyl)pyrido[2,3-*d*]pyrimidin-4-yl)-cyclohexanecarboxamide (40a).** *Step a.* A mixture of 2-amino-5-bromonicotinonitrile **37a** (200 mg, 1.0 mmol) and $NH_4OAc$ (5 mmol) in triethyl orthoformate (5 mL) was refluxed for 2 h. After cooling to room temperature, the precipitate was filtered off, washed with water, and dried affording crude 6-bromopyrido[2,3-*d*]pyrimidin-4-amine **38a** as a gray solid (225 mg, 99%).

*Step b.* The crude product **38a** (225 mg, 1.0 mmol), 3,4-dimethoxyphenylboronic acid (1.2 mmol), $K_2CO_3$ (2.0 mmol), and tetrakis(triphenylphosphine)palladium(0) (0.03 mmol) in a mixture of 1,4-dioxane (4 mL) and water (1 mL) was heated at 90 °C for 2 h. After cooling to room temperature, the precipitate was filtered off, yielding crude 6-(3,4-dimethoxyphenyl)pyrido[2,3-*d*]pyrimidin-4-amine **39a**, which was used for further reaction without any further purification.

*Step c.* Compound **39a** was treated with cyclohexanecarbonyl chloride according to the general procedure E. The crude residue was purified by flash chromatography on silicagel using a mixture of dichloromethane and methanol (in a ratio of 50:1) as mobile phase, affording the pure title compound as a yellowish solid (290 mg, 74% over 3 steps). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ = 1.23−1.98 (m,


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11H, CH and $CH_2$), 3.85 (s, 3H, $OCH_3$), 3.90 (s, 3H, $OCH_3$), 7.16 (d, $J$ = 8.2 Hz, 1H, arom H), 7.47 (d, $J$ = 8.2 Hz, 1H, arom H), 7.48 (s, 1H, arom H), 8.76 (d, $J$ = 2.5 Hz, 1H, arom H), 9.12 (s, 1H, arom H), 9.58 (d, $J$ = 2.5 Hz, 1H, arom H), 10.94 (s, 1H, NH) ppm. $^{13}$C NMR (150 MHz, DMSO-$d_6$): $\delta$ = 171.27, 169.17, 152.46, 150.19, 149.43, 149.37, 132.21, 130.73, 129.08, 122.12, 119.75, 112.54, 110.96, 55.78, 55.73, 43.29, 32.61, 25.70, 25.20 ppm. HRMS: calcd for $C_{22}H_{24}N_4O_3$ 393.1921, found 393.1919.

***N*-(6-(3,4-Dimethoxyphenyl)pyrido[3,2-*d*]pyrimidin-4-yl)-cyclohexanecarboxamide (40b).** This compound was prepared using the 3-step procedure, as described for the preparation of compound **40a**, starting from 3-amino-6-chloropicolinonitrile **37b** (154 mg, 1.0 mmol). The final crude residue was purified by flash chromatography on silica gel using a mixture of dichloromethane and methanol (in a ratio of 50:1) as the mobile phase, yielding the title compound as a yellowish solid (134 mg, 34% over 3 steps). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ = 1.98−1.23 (m, 10H, $CH_2$), 3.05 (m, 1H, CH), 3.87 (s, 3H, $OCH_3$), 3.95 (s, 3H, $OCH_3$), 7.14 (d, $J$ = 8.5 Hz, 1H, arom H), 7.94 (d, $J$ = 8.5 Hz, 1H, arom H), 8.05 (s, 1H, arom H), 8.34 (d, $J$ = 8.9 Hz, 1H, arom H), 8.60 (d, $J$ = 8.9 Hz, 1H, arom H), 8.93 (s, 1H, arom H), 10.45 (s, 1H, NH) ppm. $^{13}$C NMR (150 MHz, DMSO-$d_6$): $\delta$ = 174.17, 156.08, 154.45, 151.19, 149.20, 144.59, 136.96, 130.75, 129.58, 126.23, 120.88, 111.74, 110.77, 55.73, 55.52, 44.66, 28.97, 25.48, 25.24 ppm. HRMS: calcd for $C_{22}H_{24}N_4O_3$ 393.1921, found 393.1914.

***N*-(6-(3,4-Dimethoxyphenyl)quinazolin-4-yl)-cyclohexanecarboxamide (45a).** *Step a.* A mixture of ethyl 2-amino-5-bromobenzoate **41a** (230 mg, 1.0 mmol) and $NH_4OAc$ (5 mmol) in triethyl orthoformate (5 mL) was refluxed for 1 h. After cooling to room temperature, the precipitate was filtered off, washed with water, and dried, yielding crude 6-bromoquinazolin-4(3H)-one **42a** as a gray solid (225 mg, 99%).

*Step b.* A mixture consisting of crude **42a** (225 mg, 1.0 mmol), 3,4-dimethoxyphenylboronic acid (1.2 mmol), $K_2CO_3$ (2.0 mmol), and tetrakis(triphenylphosphine)palladium(0) (0.03 mmol) in 1,4-dioxane (4 mL) and water (1 mL) was refluxed for 12 h. After cooling to room temperature, the precipitate was filtered off, affording crude **43a**, which was used as such in the following step.

*Step c.* Crude **43a** (1 mmol) in $POCl_3$ (2 mL) was heated at 125 °C for 2 h. After concentration under reduced pressure, the residue was treated with a 7 N ammonia solution in methanol at room temperature for 30 min. The solvents were evaporated affording crude **44a** as a gray residue, which was used as such for further reaction.

*Step d.* Crude **44a** was treated with cyclohexanecarbonyl chloride according to the general procedure D. The final crude residue was purified by flash chromatography on silica gel using a mixture of dichloromethane and methanol (in a ratio of 40:1) as mobile phase, yielding the pure title compound as white solid (240 mg, 61% over 4 steps).

$^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ = 1.98−1.23 (m, 10H, $CH_2$), 2.89 (m, 1H, CH), 3.83 (s, 3H, $OCH_3$), 3.89 (s, 3H, $OCH_3$), 7.13 (d, $J$ = 8.6 Hz, 1H, arom H), 7.38 (d, $J$ = 8.6 Hz, 1H, arom H), 7.39 (s, 1H, arom H), 8.00 (d, $J$ = 8.6 Hz, 1H, arom H), 8.30 (d, $J$ = 2.0 Hz, 1H, arom H), 8.35 (dd, $J$ = 8.6, $J$ = 2.0 Hz, 1H, arom H), 8.97 (s, 1H, Ar−H), 10.78 (s, 1H, NH) ppm. $^{13}$C NMR (150 MHz, DMSO-$d_6$): $\delta$ = 176.05, 157.69, 155.23, 153.98, 150.02, 149.31, 138.66, 133.07, 131.59, 128.40, 121.58, 119.72, 117.90, 112.40, 110.90, 55.75, 55.72, 44.22, 29.10, 25.50, 25.23 ppm. HRMS: calcd for $C_{23}H_{25}N_3O_3$ 392.1969, found 392.1963.

***N*-(6-(3,4-Dimethoxyphenyl)pteridin-4-yl)-cyclohexanecarboxamide (45b).** This compound was prepared using the 4-step procedure as described for the synthesis of **45a**, starting from ethyl 3-amino-6-bromopyrazine-2-carboxylate **41b** (116 mg, 0.5 mmol). The final residue was purified by flash chromatography on silica gel using a mixture of dichloromethane and methanol (in a ratio of 60:1) as mobile phase, yielding the title compound as a white solid (60 mg, 30% yield over 4 steps). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ = 1.98−1.23 (m, 10H, CH and $CH_2$), 3.02 (m, 1H, CH), 3.88 (s, 3H, $OCH_3$), 3.94 (s, 3H, $OCH_3$), 7.16 (d, $J$ = 8.5 Hz, 1H, arom H), 8.07 (s, 1H, arom H), 8.10 (d, $J$ = 8.5 Hz, 1H, arom H), 9.06 (s, 1H, arom H), 9.91 (s, 1H, arom H), 10.67 (s, 1H, NH) ppm.

$^{13}$C NMR (150 MHz, DMSO-$d_6$): $\delta$ = 174.61, 158.00, 157.00, 152.46, 151.83, 151.12, 150.70, 149.39, 127.28, 125.21, 121.66, 111.92, 110.99, 55.81, 55.76, 44.52, 28.99, 25.48, 25.21 ppm. HRMS: calcd for $C_{21}H_{23}N_5O_3$ 394.1874, found 394.1870.

**Binding Assays for DRAK1, DRAK2, DAPK1, DAPK2, and DAPK3.** Compounds were screened at a single concentration of 10 $\mu$M, using binding assays previously described.[14] Briefly, T7 kinase-tagged phage strains were grown in parallel in 24- or 96-well blocks in an *E. coli* host derived from the BL21 strain. *E. coli* were grown to log phase and infected with T7 phage from a frozen stock (multiplicity of infection ~0.1) and incubated with shaking at 32 °C until lysis (~90 min). The lysates were centrifuged (6000*g*) and filtered (0.2 $\mu$M) to remove cell debris. Streptavidin-coated magnetic beads were treated with biotinylated small molecule ligands for 30 min at 25 °C to generate affinity resins for kinase assays. The liganded beads were blocked with excess biotin and washed with blocking buffer (SeaBlock (Pierce), 1% BSA, 0.05% Tween 20, 1 mM DTT) to remove unbound ligand and to reduce nonspecific phage binding. Binding reactions were assembled by combining phage lysates, liganded affinity beads, and test compounds in 1 × binding buffer (20% SeaBlock, 0.17 × PBS, 0.05% Tween 20, 6 mM DTT). Test compounds were prepared as 1000× stocks in DMSO and rapidly diluted into the aqueous environment (0.1% DMSO final). DMSO (0.1%) was added to control assays lacking a test compound. All reactions were carried out in polystyrene 96-well plates that had been pretreated with blocking buffer in a final volume of 0.1 mL. The assay plates were incubated at 25 °C with shaking for 1 h, long enough for binding reactions to reach equilibrium, and the affinity beads were washed four times with wash buffer (1 × PBS, 0.05% Tween 20, 1 mM DTT) to remove unbound phage. After the final wash, the beads were resuspended in elution buffer (1 × PBS, 0.05% Tween 20, 2 mM nonbiotinylated affinity ligand) and incubated at 25 °C with shaking for 30 min. The phage titer in the eluates was measured by standard plaque assays or by quantitative PCR. The compounds were tested at a single concentration of 10 $\mu$M. The results of this primary screening is reported as '% Ctrl', where lower numbers indicate stronger hits.

**Binding Constant Measurements for DRAK1 and DRAK2.** For selected compounds where single concentration results at 10 $\mu$M had shown % control <5, $K_d$ values were determined. The equilibrium binding equations yield the following expression for the binding constant for the interaction between the free test compound and the kinase ($K_d$(test)), assuming that the phage concentration is below $K_d$(test): $K_d(\text{test}) = (K_{d(\text{probe})}/(K_{d(\text{probe})} + [\text{Probe}])) \times [\text{test}]_{1/2}$. $K_{d(\text{probe})}$ is the binding constant for the interaction between the kinase and the immobilized ligand, [Probe] is the concentration of the immobilized ligand, and $[\text{test}]_{1/2}$ is the concentration of the free test compound at the midpoint of the transition. If [Probe] is below $K_{d(\text{probe})}$ the expression simplifies to $K_{d(\text{test})} = [\text{test}]_{1/2}$. Under these conditions the binding constants measured for the interaction between kinases and test compounds ($K_{d(\text{test})}$) are therefore independent of the affinity of the immobilized ligand for the kinase ($K_{d(\text{probe})}$). T7 phage grow to a titer of $10^8$−$10^{10}$ plaque forming units (PFU)/mL, and the concentration of phage-tagged kinase in the binding reaction is therefore in the low picomolar range. The concentration of the immobilized ligand is kept in the low nanomolar range, below its binding constant for the kinase. Binding data were fit to the equation $\text{PFU} = L + ((H - L) \times (K_{d(\text{test})}/(K_{d(\text{test})} + [\text{test}])))$, where $L$ is the lower baseline, $H$ is the upper baseline, $K_{d(\text{test})}$ is the binding constant for the interaction between the test compound and the kinase, and [test] is the free test compound concentration. Binding constants measured in duplicate on the same day as part of the same experiment generally were within 2-fold. Duplicate measurements performed on separate days generally varied by no more than 4-fold. Clustering and visualization was performed with Cluster 3.0 (M. Eisen, Stanford University) and Mapletree software (M. Eisen, Stanford University; L. Simirenko, Lawrence Berkeley National Lab). For kinase/compound combinations where no interaction was observed, the binding constant was arbitrarily set to 1 M.


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**DRAK1 and DRAK2 Functional, Biochemical Assays.** The DRAK1 and DRAK2 to be employed in the compound profiling process were cloned, expressed, and purified in-house at SignalChem using proprietary methods (www.signalchem.com). The enzymes are generated from the full-length human genes and are mutation free. Quality control testing is routinely performed on each of the targets to ensure compliance to acceptable standards. $^{33}$P-ATP was purchased from PerkinElmer. All other materials were of standard laboratory grade. A stock solution of the compound in DMSO was prepared. The stock solution was then diluted to form an assay stock solution and this was used to profile against DRAK1 and DRAK2.

SignalChem uses a radioisotope assay format for profiling evaluation of protein kinase target and all assays are performed in a designated radioactive working area. Protein kinase assays (in duplicate) were performed at ambient temperature for 30 min in a final volume of 25 $\mu$L according to the following assay reaction recipe:

Component 1: 5 $\mu$L of diluted active DRAK1 or DRAK2 (~10−50 nM final protein concentration in the assay)

Component 2: 5 $\mu$L of stock solution of substrate (1−5 $\mu$g of peptide substrate)

Component 3: 5 $\mu$L of kinase assay buffer

Component 4: 5 $\mu$L of compound (various concentration) or 10% DMSO

Component 5: 5 $\mu$L of $^{33}$P-ATP (50 $\mu$M stock solution, 0.8 $\mu$Ci)

The assay was initiated by the addition of $^{33}$P-ATP and the reaction mixture incubated at ambient temperature for 30 min. After the incubation period, the assay was terminated by spotting 10 $\mu$L of the reaction mixture onto Multiscreen phosphocellulose P81 plate. The Multiscreen phosphocellulose P81 plate was washed 3 times for approximately 15 min each in a 1% phosphoric acid solution. The radioactivity on the P81 plate was counted in the presence of scintillation fluid in a Trilux scintillation counter. Blank control was set up which included all the assay components except the addition of the appropriate substrate (replace with equal volume of assay dilution buffer). The corrected activity for DRAK1 or DRAK2 was determined by removing the blank control value.

**DRAK1 and DRAK2 Kinetic, Biochemical Assays.** The same protocol as for the DRAK1 and DRAK2 biochemical assays is followed, with the exception that 8 different concentrations of ATP are being used (1 $\mu$M ATP, 5 $\mu$M ATP, 10 $\mu$M ATP, 25 $\mu$M ATP, 50 $\mu$M ATP, 100 $\mu$M ATP, 250 $\mu$M ATP, and 500 $\mu$M) and 4 different concentrations of compound **22b** (0.5 $\mu$M, 1 $\mu$M, 5 $\mu$M, 10 $\mu$M).

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### Notes

The authors declare no competing financial interest.

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## ABBREVIATIONS

DAPK, death-associated protein kinase; DRAK, DAPk related apoptosis inducing protein kinase; Boc, *tert*-butyloxycarbonyl; BOP, benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate; DCM, dichloromethane; DIPEA, diisopropylethylamine; DME, dimethoxyethane; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; $IC_{50}$, half maximal (50%) inhibitory concentration; $K_d$, dissociation constant; ND, not determined; rt, room temperature; SAR, structure−activity relationship; TBTU, *N,N,N′,N′*-tetramethyl-*O*-(benzotriazol-1-yl)uronium tetrafluoroborate; TFA, trifluoroacetic acid; THF, tetrahydrofuran

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