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Design, synthesis and evaluation of 2-aminothiazole derivatives as sphingosine kinase inhibitors

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ABSTRACT

Sphingosine kinases (SphK1, SphK2) are main regulators of sphingosine-1-phosphate (S1P), which is a pleiotropic lipid mediator involved in numerous physiological and pathophysiological functions. SphKs are targets for novel anti-cancer and anti-inflammatory agents that can promote cell apoptosis and modulate autoimmune diseases. Herein, we describe the design, synthesis and evaluation of an aminothiazole class of SphK inhibitors. Potent inhibitors have been discovered through a series of modifications using the known **SKI-II** scaffold to define structure–activity relationships. We identified *N*-(4-methylthiazol-2-yl)-(2,4′-bithiazol)-2′-amine (**24**, **ST-1803**; IC_{50} values: 7.3 μM (SphK1), 6.5 μM (SphK2)) as a promising candidate for further in vivo investigations and structural development.

1. Introduction

Sphingolipids represent a major class of lipids that are ubiquitous localized and essential constituents of eukaryotic cells. Besides structural roles in membrane formation, they act as modulators in cell signaling processes. Sphingolipid metabolism displays a complex network of enzymatically controlled reactions with ceramide (Cer) in the center of biosynthesis and catabolism.[1] Most important sphingolipid mediators are sphingosine-1-phosphate (S1P), sphingosine (Sph) and Cer.[2] The balance between cellular concentrations of Cer and S1P (called 'sphingolipid rheostat') has been proposed to determine the physiological fate of cells. Cer and S1P elicit opposing cellular fates, for example, growth arrest and apoptosis versus proliferation and survival. Changes of intracellular S1P levels also affect the levels of Cer and Sph. The bioactive S1P is a potent mitogenic and migratory signaling lipid. It has been linked to the development and progression of numerous hyperproliferative and inflammatory diseases including cancer, asthma, atherosclerosis, sepsis, inflammatory bowel disease, rheumatic arthritis and multiple sclerosis.[3,4]

Therapeutic opportunities targeting the S1P/Cer rheostat are manifold. The major objective of drug discovery has focused on molecules that are capable of agonizing or antagonizing S1P receptors. S1P receptor modulator Fingolimod (FTY720), a Sph analogue, is the first oral therapeutic approved for the treatment of multiple sclerosis.[5] Another approach is to reduce the bioavailability of S1P at its receptors using S1P neutralizing antibodies. The anti-S1P monoclonal antibody Sonepcizumab (LT1009) is currently in clinical trials phase I for age-related macular degeneration (iSONEP™) and for advanced solid tumors (ASONEP™).[6] Sphingosine kinase (SphK) inhibitors are another therapeutic option.[7]

Sph is phosphorylated by SphK to form S1P. SphK exists in two isoforms, SphK1 and SphK2, which differ in their substrate preferences, subcellular localizations and tissue distributions, suggesting that they perform different physiological roles.[8] Studies using isoform-specific siRNA and knock-out mice have indicated that SphK1 and SphK2 have distinct and non-redundant functions involved in (patho)physiology. This promoted the search for isoform-specific inhibitors of SphK1 and SphK2.[9] The actions of SphK1 and S1P are complex and far away from being fully understood, especially with regard to the involvement in inflammation and cancer. Cancers of stomach, lung, brain, colon, kidney and breast as well as non-Hodgkin's lymphoma have increased SphK1 expression.[10] Up-regulation of SphK1 increases S1P production and correlates

Abbreviations: ATP, adenosine triphosphate; ADP, adenosine diphosphate; Cer, ceramide; ERK-1/2, extra-cellular signal-regulated kinase 1/2; HDAC, histone deacetylase; LDH, lactate dehydrogenase; S1P, sphingosine-1-phosphate; SAR, structure–activity relationship; Sph, sphingosine; SphK1, sphingosine kinase 1; SphK2, sphingosine kinase 2; TRAF2, TNFα receptor associated factor 2; TNFα, tumor necrosis factor alpha; TPSA, topological polar surface area.

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with poor cancer prognosis.[11] The activity and expression of SphK1 is increased in response to growth factors and pro-inflammatory cytokines.[8] TNFα-induced interaction of SphK1 with TNFα receptor associated factor 2 (TRAF2) is required for the extracellular signal-regulated kinase (ERK-1/2)-mediated phosphorylation of SphK1 and subsequent translocation of SphK1 to the plasma membrane, where it interacts with phosphatidylserine.[12] The physiological and pathophysiological relevance of this translocation remains to be determined. It might be required for the so-called 'inside-out' signaling of S1P.[13]

Less is known about the role and regulation of SphK2. Some studies have supported a role for SphK2 in survival and migration.[14] By contrast, other studies have demonstrated the suppression of cell cycle arrest and apoptosis.[15] The ambiguous nature might be determined by the subcellular localization of SphK2 and resulting intracellular S1P pools.[16] Recently, histone deacetylase (HDAC) has been claimed as an intracellular target for nuclear localized SphK2-derived S1P. S1P binds to and inhibits HDAC1 and HDAC2 within the repressor complexes that are enriched at the promoters of genes encoding the transcriptional regulator c-fos and cyclin-dependent kinase inhibitor p21. S1P promotes their expression by inhibiting HDACs and increasing histone acetylation (epigenetic S1P effect).[17]

There is a need for specific inhibitors of SphK1 and/or SphK2 to determine whether strategies targeting the isoenzymes alone or in combination offer the best therapeutic option for the diseases mentioned above. A major difficulty in the development of isoenzyme-selective inhibitors has been the lack of structural information of substrate recognition and catalysis. The recent elucidation of the X-ray crystal structure of SphK1 in 2013[18] will lead to a better understanding of the enzyme functionality. The crystal structure of SphK2 remains to be solved and would accelerate the development of selective inhibitors for therapeutic uses.

To date, most SphK inhibitors exhibit K_i or IC_{50} values in the micromolar range.[7] The roles of SphKs and S1P in different pathologies were first elucidated by the discovery of the non-selective SphK inhibitor *N*,*N*-dimethylsphingosine (DMS)[19] (Fig. 1). Due to its high structural similarity to the endogenous Sph, the therapeutic potential of this substrate analogue is limited. A milestone in the development of more 'drug like' molecules was the synthesis and evaluation of non-selective SphK inhibitor **SKI-II** (4-((4-(4-chlorophenyl)-2-thiazolyl)amino)phenol, compound **1**)[20] (Fig. 1). **SKI-II** has been used for in vitro and in vivo studies.[21,22] It reduces intracellular S1P, inhibits proliferation and induces apoptosis in various cancer cell lines.[23] **SKI-II** is orally bioavailable.[22] Its described potency may depend beside several non-Sph-related

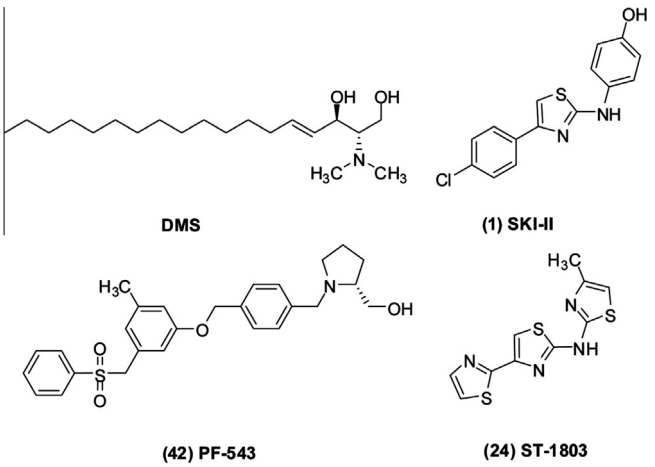


Figure 1. Representative SphK inhibitors and screening hit ST-1803.

effects in part on its ability to induce the proteasomal[24] or lysosomal[25] degradation of SphK1. **PF-543** (**42**) is the first nanomolar SphK1-selective inhibitor that rapidly reduces S1P in cells (Fig. 1).[26] It appears to be a useful tool for inhibiting SphK1 in vitro, but its in vivo effects were not described so far.

We focused on **SKI-II** as promising lead compound for the development of a small library of 2-aminothiazole derivatives as SphK inhibitors with different selectivity ratios. 2-Aminothiazoles and derivatives provide a wide spectrum of biological activities[27] and are seen in many bioactive scaffolds as 'privileged structures'.[28,29]

2. Chemistry

Compounds **1**–**37** were prepared as shown in Scheme 1. We started with the bromination of the corresponding ketone derivatives (**A**) that gave α-bromoketones (**B**). The substituted thioureas (**E**) that were not commercially available were synthesized by condensation of the appropriate aniline or amine derivative (**C**) with benzoyl chloride in the presence of ammonium thiocyanate, followed by saponification of the resultant *N*-aryl-*N*′-benzoylthioureas (**D**) to remove the benzoyl group.[30,31] In the final step, microwave-assisted Hantzsch thiazole synthesis, the condensation of α-bromoketones (**B**) and N-substituted thioureas (**E**), provided the desired 2-aminothiazole derivatives (**1**–**37**).[32]

For the synthesis of compounds **38**–**41** we used different amide bond formation procedures to couple 2-aminothiazole to activated cinnamic acid derivatives.[33] Acyl chlorides (for **38**) or carboxylic acids after previous activation by DIC/HOBt (**39**) or EDC/HOBt (**40**, **41**) were coupled to the aromatic amine.[34] Reference compound **PF-543** (**42**) was synthesized as described by Schnute et al. (cf. Supplementary material).[26]

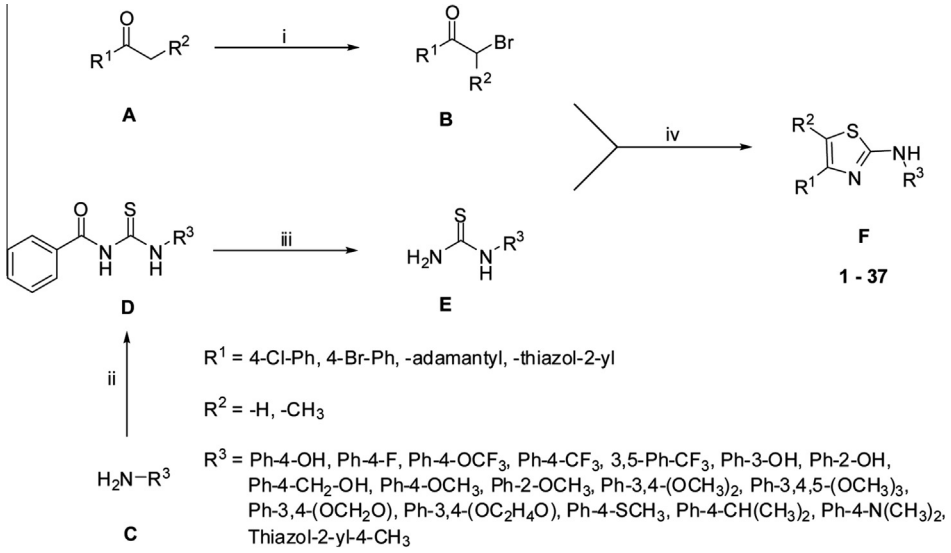
3. Results and discussion

3.1. Biological evaluation

Compounds were screened for SphK inhibitory activity at 10 μM (Table 1). An ADP-detecting fluorescence assay has been used as ADP and S1P are equimolar products of the enzymatic phosphorylation reaction. Inhibition of SphK1 or SphK2 was determined by incubating SphK1 or SphK2 in the presence of sphingosine, ATP and inhibitor or control (DMSO). The inhibitory potential of the lead structure **SKI-II** (**1**) has been shown to be only moderate under these assays conditions (15–25% inhibition), whereas **PF-543** (**42**) was able to inhibit SphK1 almost quantitatively at this concentration (94%).

The initial round of lead compound **SKI-II** (**1**) modifications aimed at improving its inhibitory potential at SphK1 and/or SphK2 via structural variations at R^3. Small functional groups were attached at different positions of the aromatic ring to affect the hydrogen-bond donor/acceptor ability of the moiety, thereby playing a role in the interactions with the target binding site.

The hydroxy group in 4-position of the phenyl ring seems to be crucial for inhibitory potential, as both its shift to the 2- and 3-position (**3**, **4**) and methylation (**6**) led to loss of inhibition. The thioether derivative **12** nevertheless was able to selectively inhibit SphK1. The insertion of a methylene spacer (**5**) between hydroxyl moiety and aromatic ring produced an inactive compound. Interestingly, the methoxy-group in 2-position of the phenyl ring (**7**) showed a slight tendency towards SphK1-selective inhibition. Analogues containing alkyloxy-substituents in 4- and 3-position as dimethoxy (**8**) as well as methylene or ethylene linked diethers (**10**, **11**) lost their ability to inhibit SphKs. By introducing a third methoxy group in 5-position (**9**, **ST-1780**), a substantial increase



Scheme 1. General procedure for the synthesis of 2-aminothiazole derivatives **1**–**37**. Reagents and conditions: (i) Br_2, chloroform, rt, 2 h; (ii) benzoyl chloride, NH_4SCN, acetone, 60 °C, 45 min; (iii) NaOH (2 M), H_2O/THF, 100 °C, 1 h; (iv) ethanol, microwave irradiation, 80 °C, 30 min.

in potency and isoenzyme preference could be achieved. Trifluoromethylation at different positions or different functionalities led to inactive compounds as well as isopropylation in 4-position (**13**–**16**). In contrast, the 4-dimethylamino group with **17** re-established inhibitory potential at SphK1.

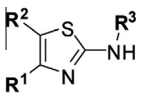
Since the variations on the phenyl substitution pattern for R^3 led to a potent compound only for **7**, **9**, **12**, we focused in the next step on the 5-methylation of the central thiazole core ring. It is described that 2-aminothiazoles without substituents at C-5 that are able to inhibit metabolism can undergo oxidation in vivo to generate potentially toxic reactive epoxide metabolites.[35] Recently, 2-aminothiazole derivatives were evaluated for microsomal stability and the regions of the scaffold predestined for phase I metabolism were determined. The observed thiazole ring modifications were either the 4,5-epoxide or the 5-hydroxythiazole whose tautomer is a thiazolone. Ring opening of the thiazolone via hydrolytic opening was determined as well. The introduction of a methyl-group at C-5 of the thiazole ring avoids this metabolic pathway.[36] Therefore, the presence of a substituent at the C-5 might be useful in terms of improved pharmacokinetics and toxicological aspects. Compound **18**, the methylated derivative of **SKI-II** (**1**), and compound **21** did not show any inhibition. In contrast, the methyl-group in compound **20** maintained inhibitory potential compared to compound **9** (**ST-1780**). Surprisingly, the methyl group in compound **19** provided selective inhibitory potential for SphK1 compared to the inactive compound **6** whereas the same modification in **12** compared to **21** led to an inactive derivative.

With structural modifications on the substituent at C-4 of the 2-aminothiazole we examined whether the 4-chloro-phenyl moiety could be replaced by other functionalities that are able to maintain or to improve selectivity and potency of the inhibitor. The slight modification of **SKI-II** (**1**) by introducing 4-bromo-phenyl (**2**) led to a comparable inhibition of SphK2 but a reduced inhibition of SphK1. The exchange into a thiazol-2-yl ring (**23**) resulted in selective SphK1 inhibition. Taking into consideration the hydrophobic properties that are supposed to be responsible for the binding of sphingosine in the active site, we prepared a series of adamantyl-containing derivatives (compounds **25**–**37**). The introduction of an adamantyl group provides a rigid scaffold and a larger volume compared to the aromatic phenyl ring. It confers higher lipophilicity to the entire molecule. This may completely change the absorption, distribution, metabolism or excretion (ADME) properties of a molecule. Oral efficacy of the drugs can be extended and more lipophilic interactions in the active site of an enzyme are provided as shown on other compound classes.[37] Adamantyl-containing compounds have already been successfully established as SphK inhibitors. Aryladamantane compound **ABC294640** (3-(4-chlor ophenyl)adamantane-1-carboxylic acid (pyridin-4-ylmethyl) amide) (Fig. 2) selectively inhibits SphK2 in vitro, competitive to Sph with a K_i of 9.8 μM. It attenuated S1P formation, suppressed cell proliferation in several cancer cell lines and promoted autophagy.[38] **ABC294640** has a good oral bioavailability and it showed promising inhibitory potency in tumor growth in vivo in several xenograft rodent models, such as different breast, kidney and pancreatic cancer models.[38,39] **ABC294640** is currently in a phase I clinical trial for patients with advanced solid tumors (ClinicalTrials.gov identifier, NCT01488513). In addition, this compound was successfully evaluated in animal models of ulcerative colitis[40] and Crohn's disease[41], were it decreased S1P levels and the levels of inflammatory cytokines. **ABC294735** (3-(4-chlorophenyl)adamantane-1-carboxylic acid 3,4-dihydroxybenzylamide) (Fig. 2) is a non-selective SphK inhibitor. It is Sph-competitive with K_i values for SphK1 and SphK2 of 3.1 and 4.2 μM, respectively.[42] Both, **ABC294640** and **ABC294735**, have been demonstrated to cause a comparable delay in tumor growth, an effect that was potentiated by the co-administration of Sorafenib.[39]

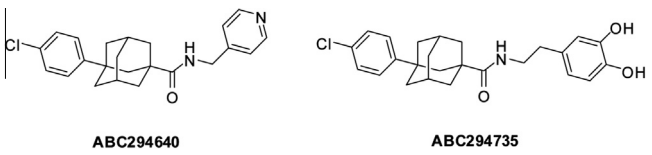
Taken together, these results indicate that adamantyl is a structural element in SphK inhibitors that is worth to be further investigated.

Within the adamantyl series, we also explored the previously successful optimization by methylation at C-5 of the 2-aminothiazole. The promising substitution patterns of compounds **7**, **9** (**ST-1780**), **20**, and **22** were confirmed. In combination with the adamantyl-moiety at C-4 of the aminothiazole, the trimethoxy-substitution of the phenyl ring resulted in an increase in potency, but SphK1 selectivity was lost (compounds **27** and **36**). Introduction of only one methoxy group in 2-position of the phenyl ring (compounds **28** and **35**) led to a significant increase in activity compared to that of compound **7**. SphK1 selectivity was only observed for compound **25**. Compound **29**, the thioether analogue of compound **25** was comparable potent at both isoenzymes. SphK1 selectivity was restored by additional introduction of the methyl group at C-5 of the aminothiazole ring (compound **37**). Dimethoxy-substituted derivative **26** is completely inactive like

Table 1
Human SphK1 and SphK2 screening for compounds **1**–**42**[a]

No.				% Inhibition[a] SphK1 @10 μM	% Inhibition[a] SphK2 @10 μM
	R^1	R^2	R^3		
1[b]	4-Cl-Ph	H	Ph-4-OH	**25.0** ± 6.1***	**15.5** ± 4.2**
2	4-Br-Ph	H	Ph-4-OH	**10.3** ± 4.7	**13.6** ± 7.0
3	4-Cl-Ph	H	Ph-3-OH	—[g]	—
4	4-Cl-Ph	H	Ph-2-OH	—	—
5	4-Cl-Ph	H	Ph-4-CH_2-OH	—	—
6	4-Cl-Ph	H	Ph-4-OCH_3	—	—
7	4-Cl-Ph	H	Ph-2-OCH_3	**19.1** ± 8.6	—
8	4-Cl-Ph	H	Ph-3,4-$(OCH_3)_2$	—	—
9[d]	4-Cl-Ph	H	Ph-3,4,5-$(OCH_3)_3$	**30.7** ± 9.1*	—
10	4-Cl-Ph	H	Ph-3-O-CH_2-O-4	—	—
11	4-Cl-Ph	H	Ph-3-O-C_2H_4-O-4	—	—
12	4-Cl-Ph	H	Ph-4-SCH_3	**29.8** ± 14.7	—
13	4-Cl-Ph	H	Ph-4-OCF_3	—	—
14	4-Cl-Ph	H	Ph-4-CF_3	—	—
15	4-Cl-Ph	H	Ph-3,5-$(CF_3)_2$	—	—
16	4-Cl-Ph	H	Ph-4-$CH(CH_3)_2$	—	—
17	4-Cl-Ph	H	Ph-4-$N(CH_3)_2$	**15.3** ± 7.3	—
18	4-Cl-Ph	CH_3	Ph-4-OH	—	—
19	4-Cl-Ph	CH_3	Ph-4-OCH_3	**21.8** ± 6.0	—
20	4-Cl-Ph	CH_3	Ph-3,4,5-$(OCH_3)_3$	**31.4** ± 15.8	—
21	4-Cl-Ph	CH_3	Ph-4-SCH_3	—	—
22	4-Cl-Ph	CH_3	Thiazol-2-yl-4-CH_3	**26.7** ± 11.1	**19.2** ± 5.2*
23	Thiazol-2-yl	H	Ph-4-OH	**29.5** ± 8. 6*	-
24[e]	Thiazol-2-yl	H	Thiazol-2-yl-4-CH_3	**59.4** ± 3.3***	**50.0** ± 9.9**
25	Adamantyl	H	Ph-4-OCH_3	**20.3 ±** 12.6	—
26	Adamantyl	H	Ph-3,4-$(OCH_3)_2$	—	—
27	Adamantyl	H	Ph-3,4,5-$(OCH_3)_3$	**43.1** ± 6.0	**20.8** ± 12.0
28	Adamantyl	H	Ph-2-OCH_3	**27.3** ± 5.8**	**19.0** ± 5.4*
29	Adamantyl	H	Ph-4-SCH_3	**16.7** ± 8.6	**18.6** ± 7.6
30	Adamantyl	H	Ph-4-$CH(CH_3)_2CH$	—	—
31	Adamantyl	H	Ph-4-$N(CH_3)_2$	**21.9** ± 12.7	—
32	Adamantyl	H	Ph-3-O-CH_2-O-4	**20.8** ± 10.8	**41.3** ± 17.1
33	Adamantyl	H	Ph-3-O-C_2H_4-O-4	—	**31.7** ± 18.6
34	Adamantyl	CH_3	Thiazol-2-yl-4-CH_3	**38.9** ± 10.9*	**20.9** ± 5.9*
35	Adamantyl	CH_3	Ph-2-OCH_3	**33.9** ± 5.9**	**41.6** ± 10.1*
36	Adamantyl	CH_3	Ph-3,4,5-$(OCH_3)_3$	**36.1** ± 3.8***	**21.5** ± 8.4
37	Adamantyl	CH_3	Ph-4-SCH_3	**19.2** ± 8.0	—
38	4-Cl-Ph	H	C(O)CH=CH—Ph-3,4-$(OCH_3)_2$	—	**31.7** ± 7.9*
39[f]	4-Cl-Ph	H	C(O)CH=CH—Ph-3,4,5-$(OCH_3)_3$	—	**32.0** ± 8.1*
40	H	H	C(O)CH=CH—Ph-3,4-$(OCH_3)_2$	**14.3** ± 8.3	**26.5** ± 3.3**
41	H	H	C(O)CH=CH—Ph-3,4,5-$(OCH_3)_3$	**17.7** ± 8.8	**26.6** ± 4.5**
42[c]	See Figure 1			**94.1** ± 3.2***	**14.7** ± 4.0**

[a] Compounds were screened at 10 μM. Results are presented as % inhibition of SphK1 and SphK2 compared to control (DMSO). Results were calculated as mean ± SEM of five independent experiments and were analyzed using one sample *t*-test. Significant inhibition is illustrated as follows: $^{*}p \leqslant 0.05$, $^{**}p \leqslant 0.01$ and $^{***}p \leqslant 0.0001$. Two-tailed Student's *t*-tests were performed (c.f. Supplementary material): for **PF-453** selectivity profile towards SphK1 was highly significant ($p \leqslant 0.05$).
[b] Reference compound: **SKI-II**.
[c] Reference compound: **PF-543**.
[d] Reference compound: **ST-1780**.
[e] Reference compound: **ST-1803**.
[f] Reference compound: **ST-1577**.
[g] (—) no significant inhibition at the concentration tested.



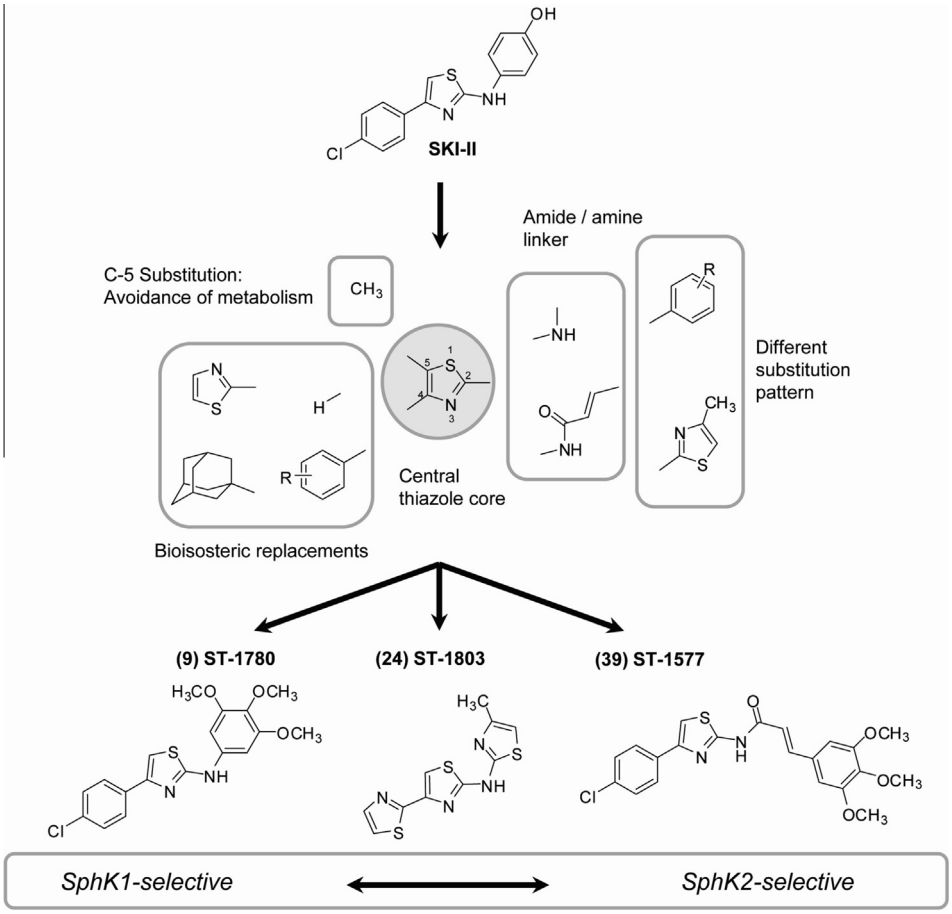
ABC294640 **ABC294735**

Figure 2. Adamantyl-containing SphK inhibitors **ABC294640** and **ABC294735**.

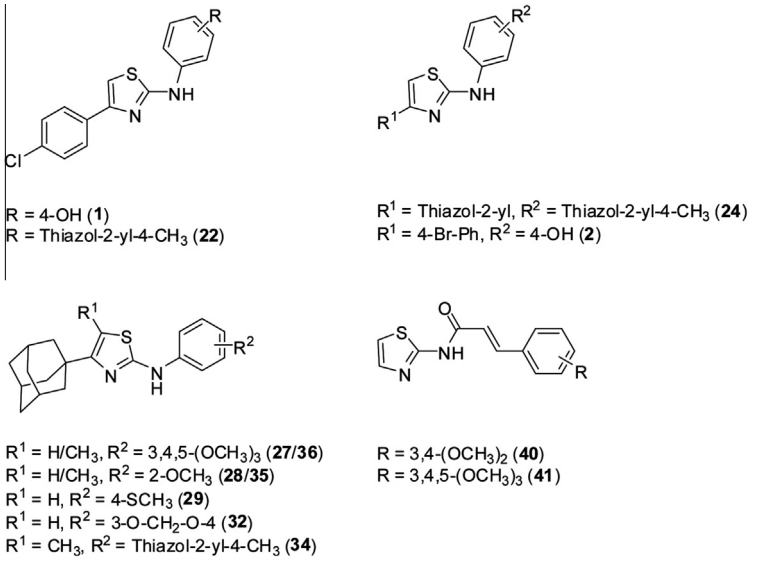
compound **8**, but interestingly compound **33** with dioxin partial structure and adamantyl moiety instead of 4-chloro-phenyl showed preferred SphK2 inhibition, compared to non-selective dioxole-containing non-selective compound **32**. The positive trend towards selective SphK1 inhibition via replacement of isopropyl- by *N*,*N*-dimethyl moieties (compounds **16** and **17**) was confirmed by compounds **30** and **31**.

Based on the modelling results discussed below we decided to evaluate the role of an amide linkage between the central thiazole and the aromatic system, wherein the carbonyl provides a further hydrogen bond acceptor. The amide group is additionally part of a substituted acrylamide as electrophile. Acrylamide-based compounds, also known as Michael acceptors, are targeting cysteine residues in the enzyme that display nucleophilic thiol groups. This potentially covalent and irreversible inhibition is a powerful approach for enhancing pharmacological potency and selectivity. It may have advantages for the extent and duration of the therapeutic effect, since restoration of pharmacological activity requires re-synthesis of the protein target.[43]

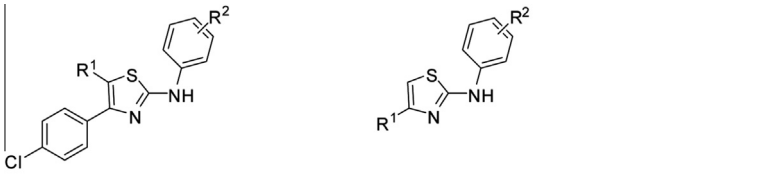
We discovered four candidates as potential covalent compounds through our screening in the biological assay. The proof of concept—the investigation of the covalent molecular mechanism—has to be elucidated in future experiments beyond this structure–activity relationships evaluation. Among the acrylamide-containing compounds, **38** and **39** (**ST-1577**) emerged as SphK2-selective inhibitors. To assess the importance of the 4-chloro-phenyl moiety, we tested compounds **40** and **41**. Both lost their isoenzyme preference, thereby indicating that a further substitution at C-4 of the aminothiazole is crucial for SphK2 selectiv-



Scheme 2. Summary of molecular profiling of 2-aminothiazole derivatives as SphK inhibitors and development of SphK1-selective **ST-1780**, non-selective **ST-1803** and SphK2-selective **ST-1577** as promising new lead compounds.



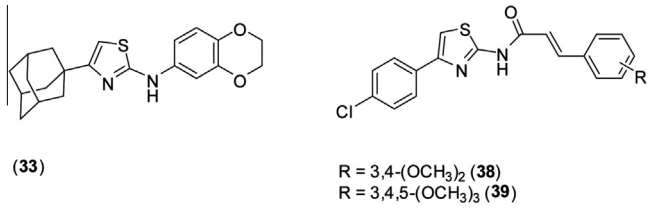
Scheme 3. Structure–activity relationships of non-selective SphK inhibitors.



Scheme 4. Structure–activity relationships of inhibitors with preference for SphK1.

ity. Taken together, the results of the four amide-containing derivatives demonstrate that the amino moiety could also be embodied into an amide bond without loss of inhibitory activity at SphKs.

To further define functionalities tolerated by SphK1 and SphK2, we replaced the characteristic 4-hydroxy-phenyl and/or the 4-chloro-phenyl substituent of **SKI-II** (**1**) by thiazole and methyl-thiazole moieties (compounds **22**, **23**, **24** and **34**). This bioisosteric replacement was successful as all four derivatives were active as SphK inhibitors. Whereas non-selective compound **22** showed comparable inhibition to that of **SKI-II** (**1**), compound **34** had a 2-fold higher inhibitory potency at SphK1. With compound **23**, we identified a SphK1-selective inhibitor. A summary of the molecular profiling and the observed structure activity relationships of 2-aminothiazole derivatives as SphK inhibitors is shown in Schemes 2–5. Tri-thiazole compound **24** (**ST-1803**) was found to be the most active compound among all screened 2-aminothiazole derivatives with 59.4 ± 3.3% inhibition of SphK1 and 50.0 ± 9.9% inhibition of SphK2 at 10 μM. Extensive screening allowed the determination of IC_{50} values for **24** (**ST-1803**) at SphK1 and SphK2 of 7.3 μM and 6.5 μM, respectively (95% confidence interval 4.9 to 10.8 μM for SphK1 and 4.5 to 9.4 μM for SphK2). **ST-1803** fulfills Lipinski's Rules of Five for oral bioavailability,[44–46] which generally simplifies the administration and drug formulation systems. A calculated log *P* value of 2.9 and a calculated TPSA value of 38.7 Å^2 for **ST-1803** suggest a good permeation of cell membranes and even the blood brain barrier (*c* log *P* <5, TPSA <60 Å^2).[47] **ST-1803** was also tested to assess its cytotoxicity. We measured the leakage of LDH which points to a loss of cell membrane integrity. U937 cells were treated with **ST-1803** up to a concentration of 30 μM for 24 h. **ST-1803** showed no signs of cell toxicity with an LDH release of 0.69 ± 0.12% at 30 μM.



Scheme 5. Structure–activity relationships of inhibitors with preference for SphK2.

3.2. Molecular modelling

We identified the distinction between the binding sites of SphK1 and SphK2 by building a homology model of non-crystallized SphK2, based on the sequences of both molecules and the crystal structure of SphK1 (28.1% sequence identity). We identified three amino acid substitutions (Ille174 → Val340, Met272 → Leu272, Phe288 → Cys589) within a radius of 4.5 Å to the reference ligand **SKI-II** (**1**). It is conspicuous that all amino acids substituted in SphK2 are of a smaller size, than the corresponding amino acids in SphK1. These findings indicate that the binding pocket of SphK2 is larger as compared to that of SphK1. Furthermore, the lipophilicity seems not to be affected by the substitutions (see Fig. 3). Molecular docking studies suggest that the lacking activity for SphK1 of compounds **38** and **39** (**ST-1577**) arises from steric hindrance as the molecules are too big to fit into the binding site. The measured inhibition of compounds **38** and **39** (**ST-1577**) on SphK2 is a second indicator for a bigger binding site. It is apparent that the SphK2 selective compounds **38** and **39** both have an acrylamide function-

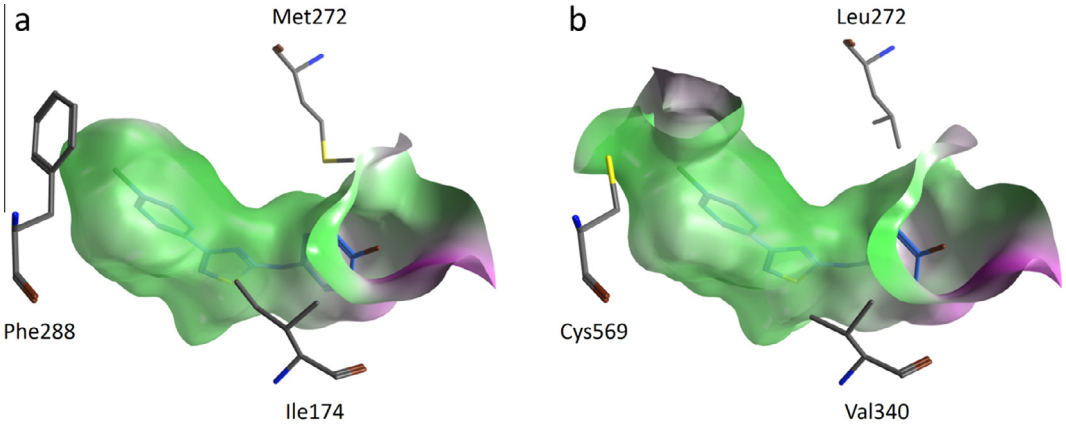


Figure 3. Binding site comparison of SphK1 (a) and SphK2 homology model (b). The homology model was built on the basis of the co-crystallized structure of SphK1 and **SKI-II** (PDB code: 3VZD). **SKI-II** (blue) is shown within both binding sites as well as the substituted amino acids (Ille174 → Val340, Met272 → Leu272, Phe288 → Cys569) determined by the sequence alignment and the shape of the binding pocket colored by lipophilicity (green: lipophilic; magenta: hydrophilic; white: neutral).

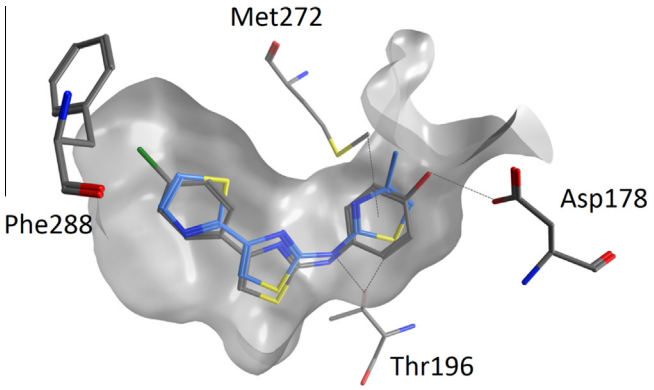


Figure 4. Reference SphK inhibitor **SKI-II** (grey) and docking pose of **ST-1803** (blue). Close interactions (4.5 Å distance cutoff) between **ST-1803** and the corresponding amino acids are shown as dashed lines.

ality. A hypothetical mode of action could be the acrylamide forming a covalent bond as Michael acceptor with the Cys569 residue which is only present within the binding site of SphK2. Additional studies to investigate covalent binding potential of the SphK2 selective inhibitors are projected. The docking pose of the most potent compound **24** (**ST-1803**) is shown in Figure 4. The compound forms mostly hydrophobic interactions and π-stacking which should not be affected by the given amino acid substitutions and therefore explains the nonselective behavior of the compound. A common problem with kinase inhibitors is their tendency towards nonselective and undesired inhibition of other ATP-dependent enzymes because of an interaction with the highly conserved nucleotide binding site. Binding mode of SphK inhibitor **SKI-II** turned out to be non-competitive with ATP.[18,20] As we could show in the docking pose compound **24** (**ST-1803**) binds congruent with **SKI-II** to SphKs (Fig. 4). The structural similarity suggests a similar non-competitive binding mechanism.

4. Conclusion

The aim of the present study was to identify inhibitors of SphK1 and/or SphK2 with an improved inhibition profile compared to non-selective SphK inhibitor **SKI-II**. Described is the optimization and SAR of 2-aminothiazole-based inhibitors with low micromolar activity. Modifications led to a variable range of inhibitory activities and allowed us to construct a plausible SAR as described above. Microwave-assisted Hantzsch thiazole synthesis demonstrates a fast and facile synthesis of 2-aminothiazole derivatives (Scheme 1).

The 3,4,5-trimethoxyphenyl moiety is an auspicious substitution pattern for inhibitory activity. Isoenzyme preference or selectivity depend on further functionalities that are part of the 2-aminothiazole scaffold. From this series, SphK1-selective inhibitors **9** (**ST-1780**) and **20** are candidates for continuative structural modifications and in vivo experiments, same for non-selective SphK inhibitors **27** and **36**. Compounds **38** and **39** (**ST-1577**), characterized by the substituted acrylamide were identified as selective SphK2 inhibitors. Finally, we successfully introduced the di- and tri-thiazole pattern as completely novel functionality for the design of potent SphK inhibitors (compounds **18**, **23**, **24** and **34**). Most promising screening hit **24** (**ST-1803**, Fig. 1) showed an encouraging inhibition of over 50% (at 10 μM) for both isoenzymes, with an IC_{50} of 7.3 μM for SphK1 and 6.5 μM for SphK2, respectively. Moreover, **ST-1803** is completely devoid of apparent cytotoxicity (LDH assay: 0.69 ± 0.12% LDH release at 30 μM). These favorable properties qualify **ST-1803** for further physicochemical investigations. If the inhibitory effect of this potent drug candidate proves to be reproducible in vivo, it may be useful not only in the investigation of S1P metabolism, but also in a clinical setting. Current efforts are aimed at modifying several regions of this lead compound to achieve selectivity and increased potency.

Overall, our study supports the continued and future design of SphK inhibitors. Ongoing development will enhance treatment options for diseases possessing S1P-dependent components. As these molecular targets are distinct from those of conventional drugs in the clinical management of cancer, inflammatory disorders and autoimmune diseases, they are a further step towards progress in therapy.

5. Experimental section

5.1. Chemistry

All commercial chemicals were reagent grade and used as purchased by Sigma–Aldrich (Steinheim/Germany), ABCR (Karlsruhe/Germany), Acros Organics (Geel/Belgium), Alfa-Aesar (Karlsruhe/Germany) and Fluorochem (Derbyshire/UK) unless otherwise indicated. Only dried solvents were used. Reactions were monitored by thin-layer chromatography using silica gel 60 F_{254} aluminum-backed plate from Merck (Darmstadt/Germany) with detection using a UV lamp and ninhydrin. Column chromatography was performed on silica gel (SiO_2, 40–63 μM). Microwave-assisted synthesis was performed using a Biotage Initiator 2.0, 400 Watt microwave synthesizer (Biotage, Uppsala/Sweden). All products were characterized by ^1H NMR and ^{13}C NMR. NMR spectra were recorded on a Bruker AV 250 (^1H: 250 MHz; ^{13}C: 63 MHz) spectrometer (Bruker, Karlsruhe/Germany). Chemical shifts (δ) are reported in ppm relative to tetramethylsilane (TMS) as internal standard. Multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet; coupling constants (J) are shown in hertz (Hz); number and assignment of protons. Mass spectra were obtained on a VG Platform II (Fisons Instruments, Ipswich/UK) using electrospray ionization (ESI). Data are listed as mass number $[M+H]^+$ or $[M-H]^-$. High resolution MS (HR-MS) were achieved on a LTQ Orbitrap XL (Thermo Fisher Scientific, Waltham/USA). For final compounds the analyses were within ±5 ppm of the theoretical values. The purity of the compounds was determined by elemental analysis (C, H, N, S) on a MicroCube instrument (Elementar, Hanau/Germany) and was within ±0.4% of the theoretical values for all final compounds, which corresponds to ⩾95% purity. Melting points were recorded using a Buchi B-540 melting point instrument (Buchi, Flawil/Switzerland) and are uncorrected.

5.1.1. General procedure for the synthesis of α-bromoketones (B, Scheme 1)

To a solution of the appropriate ketone (**A**, 1 equiv) in chloroform, bromine (1 equiv) in chloroform was added dropwise at 0 °C. The mixture was stirred at room temperature for 2 h and was washed with H_2O (3 × 50 ml) and saturated $Na_2S_2O_3$ solution (2 × 50 ml). The organic phase was dried over Na_2SO_4, filtered and the solvent was removed in vacuum. The crude α-bromoketone (**B**) was recrystallized from petrolether.[48]

5.1.2. General procedure for the synthesis of *N*-aryl-*N'*-benzoylthioureas (D, Scheme 1)

Benzoyl chloride (1 equiv) was added over 5 min to a freshly prepared solution of NH_4SCN (1.1 equiv) in acetone and the mixture was heated to reflux for 15 min. The in situ generated benzoyl isothiocyanate reacted with added amine/aniline derivatives (**C**, 1 equiv) in acetone. The mixture was heated to reflux for 30 min, and then poured on ice with vigorous stirring. The resulting solid (**D**) was collected and washed with H_2O, followed by cold acetone.[30]

5.1.3. General procedure for the synthesis of N-substituted thioureas (E, Scheme 1)

A solution of *N*-aryl-*N*′-benzoylthiourea (**D**) in aqueous sodium hydroxide (2 M)/THF (1:1) was heated to 100 °C for 1 h. The precipitating solid N-substituted thiourea (**E**) was collected and washed with H_2O.[30]

5.1.4. General procedure for the synthesis of 2-aminothiazole derivatives (F, Scheme 1, compounds 1–37)

2-Aminothiazole derivatives **1**–**37** were prepared by Hantzsch thiazole synthesis: The cyclic condensation of an α-bromoketone (1 equiv) and N-substituted thiourea (1 equiv) in anhydrous ethanol, stirring under microwave irradiation at 80 °C for 30 min. The precipitating hydrobromide salts were collected and washed with H_2O and cold ethanol. If needed, further purification was done after neutralization with saturated NH_4OH solution, stirring at room temperature, filtration of the precipitate and washing with cold ethanol. Colum chromatography on silica gel (eluent: hexane/ethylacetate 2:1 or dichloromethane/methanol 9:1) afforded the title compound.[30]

5.1.4.1. 4-(4-(4-Chlorophenyl)thiazol-2-ylamino)phenol hydrobromide (1, SKI-II). General procedure 5.1.4 was used to couple 2-bromo-1-(4-chlorophenyl)-ethanone (1.39 g, 5.95 mmol) and 1-(4-hydroxyphenyl)thiourea (1.00 g, 5.94 mmol) in 30 ml ethanol to yield the title compound. White solid, yield: 1.65 g (92%). ^{1}H NMR (250 MHz, DMSO-d_6) δ = 9.99 (s, 1H, –N*H*–), 9.20 (s, 1H, Ph-O*H*), 7.97 (d, *J* = 8.6 Hz, 2H, 3*H*,5*H*-Ph-Cl), 7.56 (d, *J* = 8.9 Hz, 2H, 3*H*,5*H*-Ph-OH), 7.50 (d, *J* = 8.5 Hz, 2H, 2*H*,6*H*-Ph-Cl), 7.35 (s, 1H, 5*H*-thiazole), 6.81 (d, *J* = 8.9 Hz, 2H, 2*H*,6*H*-Ph-OH); ^{13}C NMR (63 MHz, DMSO-d_6) δ = 164.39, 152.40, 148.99, 133.52, 133.17, 131.81, 128.57, 127.27, 119.32, 115.43, 102.63; ESI-MS (*m*/*z*) = 303.0 [M+H]$^+$; Anal. Calcd for $C_{15}H_{11}ClN_2OS{\cdot}HBr$: C (46.95) H (3.15) N (7.30) S (8.36); found: C (46.70) H (2.96) N (7.28) S (8.09); mp = 171.0 °C.

5.1.4.2. 4-(4-(4-Bromophenyl)thiazol-2-ylamino)phenol (2). General procedure 5.1.4 was used to couple 2-bromo-1-(4-bromophenyl)ethanone (0.30 g, 1.08 mmol) and 1-(4-hydroxyphenyl)thiourea (0.18 g, 1.08 mmol) in 4 ml ethanol to yield the title compound. White solid, yield: 299 mg (80%). ^{1}H NMR (250 MHz, DMSO-d_6) δ = 9.91 (s, 1H, –N*H*–), 9.11 (s, 1H, Ph-O*H*), 7.84 (d, *J* = 8.6 Hz, 2H, 2*H*,6*H*-Ph-Br), 7.60 (d, *J* = 8.6 Hz, 2H, 3*H*,5*H*-Ph-Br), 7.46 (d, *J* = 8.9 Hz, 2H, 2*H*,6*H*-Ph-OH), 7.29 (s, 1H, 5*H*-thiazole), 6.75 (d, *J* = 8.8 Hz, 2H, 3*H*,5*H*-Ph-OH); ^{13}C NMR (63 MHz, DMSO-d_6) δ = 164.3, 152.3, 148.7, 133.7, 133.1, 131.4, 127.5, 120.3, 119.2, 115.4, 102.6; ESI-MS (*m*/*z*) = 347.0 (100.0%), 349.0 (95.0%) [M+H]$^+$; Anal. Calcd for $C_{15}H_{11}BrN_2OS$: C (51.89) H (3.19) N (8.07) S (9.42); found: C (51.70) H (3.23) N (7.97) S (9.42); mp = 190.3 °C.

5.1.4.3. 3-(4-(4-Chlorophenyl)thiazol-2-ylamino)phenol hydrobromide (3). General procedure 5.1.4 was used to couple 2-bromo-1-(4-chlorophenyl)ethanone (0.42 g, 1.78 mmol) and 1-(3-hydroxyphenyl)thiourea (0.30 g, 1.78 mmol) in 4 ml ethanol to yield the title compound. White solid, yield: 519 mg (76%). ^{1}H NMR (250 MHz, DMSO-d_6) δ = 10.16 (s, 1H, –N*H*–), 9.42 (s, 1H, Ph-O*H*), 7.96 (d, *J* = 8.6 Hz, 2H, 2*H*,6*H*-Ph-Cl), 7.49 (d, *J* = 8.6 Hz, 2H, 3*H*,5*H*-Ph-Cl), 7.39 (s, 1H, 5*H*-thiazole), 7.31 (s, 1H, 2*H*-Ph-OH), 7.10 (t, 1H, 5*H*-Ph-OH), 7.02 (d, *J* = 8.7 Hz, 1H, 4*H*-Ph-OH), 6.38 (d, *J* = 8.9 Hz, 1H, 6*H*-Ph-OH); ^{13}C NMR (63 MHz, DMSO-d_6) δ = 163.2, 157.9, 154.2, 148.8, 142.0, 133.3, 131.9, 128.5, 127.3, 108.6, 107.9, 104.1, 100.4; ESI-MS (*m*/*z*) = 303.3 (100.0%), 305.3 (35.0%) [M+H]$^+$; Anal. Calcd for $C_{15}H_{11}ClN_2OS{\cdot}HBr$: C (46.95) H (3.15) N (7.30) S (8.36); found: C (46.98) H (3.17) N (7.20) S (8.61); mp = 278.9 °C.

5.1.4.4. 2-(4-(4-Chlorophenyl)thiazol-2-ylamino)phenol (4). General procedure 5.1.4 was used to couple 2-bromo-1-(4-chlorophenyl)ethanone (0.55 g, 2.38 mmol) and 1-(2-hydroxyphenyl)thiourea (0.40 g, 2.38 mmol) in 4 ml ethanol to yield the title compound. Beige powdered solid, yield: 498 mg (69%). ^{1}H NMR (250 MHz, DMSO-d_6) δ = 9.89 (s, 1H, –O*H*), 9.50 (s, 1H, –N*H*–), 8.26 (t, *J* = 4.4 Hz, 1H, 4*H*-Ph-OH), 7.90 (d, *J* = 8.6 Hz, 2H, 2*H*,6*H*-Ph-Cl), 7.48 (d, *J* = 8.6 Hz, 2H, 3*H*,5*H*-Ph-Cl), 7.34 (s, 1H, 5*H*-thiazole), 6.88–6.84 (m, 3H, 3*H*,5*H*,6*H*-Ph-OH); ^{13}C NMR (63 MHz, DMSO-d_6) δ = 164.2, 148.2, 146.3, 133.4, 131.7, 129.1, 128.5, 127.1, 122.4, 119.2, 119.0, 114.9, 103.9; ESI-MS (*m*/*z*) = 303.1 (100.0%), 305.1 (79.0%) [M+H]$^+$; Anal. Calcd for $C_{15}H_{11}ClN_2OS$: C (59.50) H (3.66) N (9.25) S (10.59); found: C (59.17) H (3.72) N (8.85) S (10.63); mp = 198.9 °C.

5.1.4.5. (4-(4-(4-Chlorophenyl)thiazol-2-ylamino)phenyl)methanol (5). General procedure 5.1.4 was used to couple 2-bromo-1-(4-chlorophenyl)ethanone (0.26 g, 1.10 mmol) and 1-(4-(hydroxymethyl)phenyl)thiourea (0.20 g, 1.10 mmol) in 4 ml ethanol to yield the title compound. Pale brown solid, yield: 226 mg (65%). ^{1}H NMR (250 MHz, DMSO-d_6) δ = 10.28 (s, 1H, –N*H*), 7.93 (d, *J* = 8.6 Hz, 2H, 2*H*,6*H*-Ph-Cl), 7.65 (d, *J* = 8.5 Hz, 2H, 2*H*,6*H*-Ph-CH_2OH), 7.49 (d, *J* = 8.6 Hz, 2H, 3*H*,5*H*-Ph-Cl), 7.39 (s, 1H, 5*H*-thiazole), 7.28 (d, *J* = 8.5 Hz, 2H, 3*H*,5*H*-Ph-CH_2OH), 4.79 (s, 1H, –O*H*–), 4.44 (s, 2H, -C*H*$_2$-); ^{13}C NMR (63 MHz, DMSO-d_6) δ = 160.6, 150.2, 139.4, 134.3, 131.2, 131.1, 129.3, 129.2, 128.9, 120.6, 105.0, 64.7; ESI-MS (*m*/*z*) = 317.3 (100.0%), 319.3 (43.0%) [M+H]$^+$; Anal. Calcd for $C_{16}H_{13}ClN_2OS$: C (60.66) H (4.14) N (8.84) S (10.12); found: C (60.65) H (4.27) N (8.58) S (9.97); mp = 167.0 °C.

5.1.4.6. 4-(4-Chlorophenyl)-*N*-(4-methoxyphenyl)thiazol-2-amine (6). General procedure 5.1.4 was used to couple 2-bromo-1-(4-chlorophenyl)ethanone (0.64 g, 2.74 mmol) and 1-(4-methoxyphenyl)thiourea (0.50 g, 2.74 mmol) in 5 ml ethanol to yield the title compound. Grey crystalline solid, yield: 396 mg (46%). ^{1}H NMR (250 MHz, DMSO-d_6) δ = 10.09 (s, 1H, –N*H*), 7.92 (d, *J* = 8.6 Hz, 2H, 2*H*,6*H*-Ph-Cl), 7.62 (d, *J* = 9.0 Hz, 2H, 2*H*,6*H*-Ph-OCH_3), 7.47 (d, *J* = 8.6 Hz, 2H, 3*H*,5*H*-Ph-Cl), 7.33 (s, 1H, 5*H*-thiazole), 6.94 (d, *J* = 9.0 Hz, 2H, 3*H*,5*H*-Ph-OCH_3), 3.74 (s, 3H, –O*CH*$_3$); ^{13}C NMR (63 MHz, DMSO-d_6) δ = 163.8, 154.1, 148.7, 134.5, 133.3, 131.7, 128.5, 127.2, 118.6, 114.2, 102.9, 55.1; ESI-MS (*m*/*z*) = 317.2 (100.0%), 319.3 (38.5%) [M+H]$^+$; Anal. Calcd for $C_{16}H_{13}ClN_2OS$: C (60.66) H (4.14) N (8.84) S (10.12); found: C (60.43) H (4.08) N (8.56) S (10.27); mp = 193.0 °C.

5.1.4.7. 4-(4-Chlorophenyl)-*N*-(2-methoxyphenyl)thiazol-2-amine hydrobromide (7). General procedure 5.1.4 was used to couple 2-bromo-1-(4-chlorophenyl)ethanone (1.28 g, 5.49 mmol) and 1-(2-methoxyphenyl)thiourea (1.00 g, 5.49 mmol) in 5 ml ethanol to yield the title compound. Pale yellow solid, yield: 1.767 g (81%). ^{1}H NMR (250 MHz, DMSO-d_6) δ = 9.70 (s, 1H, –N*H*), 8.42 (m, 1H, 3*H*-Ph-OCH_3), 7.90 (d, *J* = 8.6 Hz, 2H, 2*H*,6*H*-Ph-Cl), 7.48 (d, *J* = 8.6 Hz, 2H, 3*H*,5*H*-Ph-Cl), 7.37 (s, 1H, 5*H*-thiazole), 7.03–6.97 (m, 3H, 4*H*,5*H*,6*H*-Ph-OCH_3), 3.86 (s, 3H, –O*CH*$_3$); ^{13}C NMR (63 MHz, DMSO-d_6) δ = 162.5, 148.2, 145.3, 133.0, 132.6, 132.3, 129.4, 128.3, 127.2, 122.6, 120.5, 118.5, 110.9, 104.3, 91.4, 55.6; ESI-MS (*m*/*z*) = 317.3 (100.0%), 319.3 (38.4%) [M+H]$^+$; Anal. Calcd for $C_{16}H_{13}ClN_2OS{\cdot}HBr$: C (48.32) H (3.55) N (7.04) S (8.06); found: C (48.13) H (3.41) N (7.07) S (8.09); mp = 214.0 °C.

5.1.4.8. 4-(4-Chlorophenyl)-*N*-(3,4-dimethoxyphenyl)thiazol-2-amine (8). General procedure 5.1.4 was used to couple 2-bromo-1-(4-chlorophenyl)ethanone (0.20 g, 0.86 mmol) and 1-(3,4-dimethoxyphenyl)thiourea (0.18 g, 0.86 mmol) in 3 ml ethanol to yield the title compound. Yellow powdered solid, yield: 253 mg (85%). ^{1}H NMR (250 MHz, DMSO-d_6) δ = 10.12 (s, 1H, –

N*H*–), 7.92 (d, *J* = 8.6 Hz, 2H, 3*H*,5*H*-Ph-Cl), 7.50–7.46 (m, 3H, 2*H*,6*H*-Ph-Cl+5*H*-thiazole), 7.35 (s, 1H, 2*H*-Ph-$(OCH_3)_2$), 7.15 (dd, 1H, ^4J = 2.4 Hz, ^3J = 8.6 Hz, 6*H*-Ph-$(OCH_3)_2$), 6.94 (d, 1H, 5*H*-Ph-$(OCH_3)_2$), 3.80 (s, 3H, 3-(OCH_3)-Ph-), 3.73 (s, 3H, 4-(OCH_3)-Ph); ^{13}C NMR (63 MHz, DMSO-d_6) δ = 163.7, 148.9, 148.6, 143.6, 135.0, 133.4, 131.8, 128.5, 127.2, 112.7, 108.8, 103.0, 102.7, 55.9, 55.3; ESI-MS (*m*/*z*) = 347.2 (100.0%), 348.7 (37.9%) $[M+H]^+$; Anal. Calcd for $C_{17}H_{15}ClN_2O_2S$: C (58.87) H (4.36) N (8.08) S (9.25); found: C (58.58) H (4.28) N (7.94) S (9.30); mp = 155.0 °C.

5.1.4.9. 4-(4-Chlorophenyl)-*N*-(3,4,5-trimethoxyphenyl)thiazol-2-amine hydrobromide (9, ST-1780). General procedure 5.1.4 was used to couple 2-bromo-1-(4-chlorophenyl)ethanone (0.19 g, 0.83 mmol) and 1-(3,4,5-trimethoxyphenyl)thiourea (0.20 g, 0.83 mmol) in 3 ml ethanol to yield the title compound. White solid, yield: 378 mg (78%). ^{1}H NMR (250 MHz, DMSO-d_6) δ = 10.29 (s, 1H, –N*H*–), 7.92 (d, *J* = 8.6 Hz, 2H, 2*H*,6*H*-Ph-Cl), 7.47 (d, *J* = 8.6 Hz, 2H, 3*H*,5*H*-Ph-Cl), 7.40 (s, 1H, 5*H*-thiazole), 7.12 (s, 2H, 6*H*-Ph-$(OCH_3)_3$), 3.80 (s, 6H, 3-OCH_3, 5-OCH_3), 3.62 (s, 3H, 4-OCH_3); ^{13}C NMR (63 MHz, DMSO-d_6) δ = 163.2, 161.9, 152.9, 148.5, 145.5, 137.1, 129.3, 128.3, 127.1, 103.4, 95.2, 60.0, 55.6; ESI-MS (*m*/*z*) = 377.2 (100.0%), 379.2 (62.5%) $[M+H]^+$; Anal. Calcd for $C_{18}H_{17}ClN_2O_3S{\cdot}HBr$: C (47.23) H (3.96) N (6.12) S (7.00); found: C (47.22) H (3.98) N (6.19) S (7.18); mp = 209.0 °C.

5.1.4.10. *N*-(Benzo[*d*][1,3]dioxol-5-yl)-4-(4-chlorophenyl)thiazol-2-amine hydrobromide (10). General procedure 5.1.4 was used to couple 2-bromo-1-(4-chlorophenyl)ethanone (0.15 g, 0.64 mmol) and 1-(benzo[*d*][1,3]dioxol-5-yl)thiourea (0.13 g, 0.64 mmol) in 2.5 ml ethanol to yield the title compound. White solid, yield: 182 mg (69%). ^{1}H NMR (250 MHz, DMSO-d_6) δ = 10.17 (s, 1H, –N*H*–), 7.90 (d, *J* = 8.5, 2H 2*H*,6*H*-Ph-Cl), 7.50–7.46 (m, 3H, 3*H*,5*H*-Ph-Cl + 5*H*-thiazole), 7.36 (s, 1H, 4*H*-benzo[*d*][1,3]dioxol), 7.05 (dd, 1H, ^4J = 2.3 Hz, ^3J = 8.5 Hz, 6*H*-benzo[*d*][1,3]dioxol), 6.89 (d, 1H, *J* = 8.4 Hz, 7*H*-benzo[*d*][1,3]dioxol), 5.99 (s, 2H, –CH_2–); ^{13}C NMR (63 MHz, DMSO-d_6) δ = 163.6, 148.6, 147.3, 141.6, 135.8, 133.3, 131.9, 128.6, 127.2, 109.6, 108.3, 103.3, 100.8, 99.5; ESI-MS (*m*/*z*) = 321.2 (100.0%), 333.5 (31.2%) $[M+H]^+$; Anal. Calcd for $C_{16}H_{11}ClN_2O_2S{\cdot}HBr$: C (46.68) H (2.94) N (6.80) S (7.79); found: C (46.63), H (3.02), N (6.87), S (7.75); mp = 251.0 °C.

5.1.4.11. 4-(4-Chlorophenyl)-*N*-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)thiazol-2-amine hydrobromide (11). General procedure 5.1.4 was used to couple 2-bromo-1-(4-chlorophenyl)ethanone (0.22 g, 0.95 mmol) and 1-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)thiourea (0.20 g, 0.95 mmol) in 3 ml ethanol to yield the title compound. Grey powdered solid, yield: 263 mg (65%). ^{1}H NMR (250 MHz, DMSO-d_6) δ = 10.09 (s, 1H, –N*H*–), 7.89 (d, *J* = 8.6 Hz, 2H, 2*H*,6*H*-Ph-Cl), 7.49 (d, *J* = 8.6 Hz, 2H, 3*H*,5*H*-Ph-Cl), 7.40 (d, *J* = 2.5 Hz, 1H, 7*H*-2,3-dihydrobenzo[*b*][1,4]dioxin), 7.35 (s, 1H, 5*H*-thiazole), 7.01 (dd, ^4J = 2.5 Hz, ^3J = 8.8 Hz, 1H, 8*H*-2,3-dihydrobenzo[*b*][1,4]dioxin), 6.82 (s, 1H, 5*H*-2,3-dihydrobenzo[*b*][1,4]dioxin), 4.24–4.22 (m, 4H, –CH_2–CH_2–); ^{13}C NMR (63 MHz, DMSO-d_6) δ = 163.6, 148.6, 143.1, 138.0, 135.0, 133.3, 131.9, 138.6, 127.2, 117.1, 110.4, 106.1, 103.1, 64.1; ESI-MS (*m*/*z*) = 344.7 (100.0%) $[M+H]^+$; Anal. Calcd for $C_{17}H_{13}ClN_2O_2S{\cdot}HBr$: C (47.96) H (3.31) N (6.58) S (7.53); found: C (47.84) H (3.29) N (6.51) S (7.43); mp = 247.0 °C.

5.1.4.12. 4-(4-Chlorophenyl)-*N*-(4-(methylthio)phenyl)thiazol-2-amine (12). General procedure 5.1.4 was used to couple 2-bromo-1-(4-chlorophenyl)ethanone (0.41 g, 1.77 mmol) and 1-(4-(methylthio)phenyl)thiourea (0.35 g, 1.77 mmol) in 5 ml ethanol to yield the title compound. Pale yellow crystalline solid, yield: 335 mg (57%). ^{1}H NMR (250 MHz, DMSO-d_6) δ = 10.32 (s, 1H, –N*H*), 7.94 (d, *J* = 8.6 Hz, 2H, 2*H*,6*H*-Ph-Cl), 7.69 (d, *J* = 8.8 Hz, 2H, 3*H*,5*H*-Ph-SCH_3), 7.49 (d, *J* = 8.6 Hz, 2H, 3*H*,5*H*-Ph-Cl), 7.40 (s, 1H, 5*H*-thiazole), 7.29 (d, *J* = 8.7 Hz, 2H, 2*H*,6*H*-Ph-SCH_3), 2.45 (s, 3H, –SCH_3); ^{13}C NMR (63 MHz, DMSO-d_6) δ = 163.0, 148.7, 138.8, 133.2, 131.9, 129.2, 128.5, 128.0, 127.2, 117.5, 103.6, 16.0; ESI-MS (*m*/*z*) = 333.2 (100.0%), 335.3 (43.8%) $[M+H]^+$; Anal. Calcd for $C_{16}H_{13}ClN_2S_2$: C (57.73) H (3.94) N (8.42) S (19.27); found: C (57.89) H (3.94) N (8.32) S (19.28); mp = 180.0 °C.

5.1.4.13. 4-(4-Chlorophenyl)-*N*-(4-(trifluoromethoxy)phenyl)thiazol-2-amine (13). General procedure 5.1.4 was used to couple 2-bromo-1-(4-chlorophenyl)ethanone (0.18 g, 0.85 mmol) and 1-(4-(trifluoromethoxy)phenyl)thiourea (0.20 g, 0.85 mmol) in 2 ml ethanol to yield the title compound. Pale yellow solid, yield: 188 mg (60%). ^{1}H NMR (250 MHz, DMSO-d_6) δ = 10.52 (s, 1H, –N*H*–), 7.95 (d, *J* = 8.6 Hz, 2H, 2*H*,6*H*-Ph-Cl), 7.84 (d, *J* = 9.1 Hz, 2*H*,6*H*-Ph-OCF_3), 7.50 (s, 1H, 5*H*-thiazole), 7.46 (d, *J* = 8.5 Hz, 2H, 3*H*,5*H*-Ph-Cl), 7.35 (d, *J* = 8.4 Hz, 2H, 3*H*,5*H*-Ph-OCF_3); ^{13}C NMR (63 MHz, DMSO-d_6) δ = 162.8, 148.7, 141.9, 140.2, 133.2, 132.0, 128.6, 127.3, 121.9, 117.8, 104.2; ESI-MS (*m*/*z*) = 371.6 (100.0%), 371.6 (70.0%) $[M+H]^+$; Anal. Calcd for $C_{16}H_{10}ClF_3N_2OS$: C (51.83) H (2.72) N (7.56) S (8.65); found: C (51.50) H (2.66) N (7.42) S (8.81); mp = 134.1 °C.

5.1.4.14. 4-(4-Chlorophenyl)-*N*-(4-(trifluoromethyl)phenyl)thiazol-2-amine (14). General procedure 5.1.4 was used to couple 2-bromo-1-(4-chlorophenyl)ethanone (0.21 g, 0.91 mmol) and 1-(4-(trifluoromethyl)phenyl)thiourea (0.20 g, 0.91 mmol) in 2 ml ethanol to yield the title compound. Yellow powdered solid, yield: 236 mg (73%). ^{1}H NMR (250 MHz, DMSO-d_6) δ = 10.73 (s, 1H, –N*H*–), 7.98 (d, *J* = 8.6 Hz, 2H, 2*H*,6*H*-Ph-Cl), 7.91 (d, *J* = 8.6 Hz, 2H, 2*H*,6*H*-Ph-CF_3), 7.69 (d, *J* = 8.6 Hz, 2H, 3*H*,5*H*-Ph-CF_3), 7.52 (s, 1H, 5*H*-thiazole), 7.49 (d, *J* = 8.6 Hz, 2H, 3*H*,5*H*-Ph-Cl); ^{13}C NMR (63 MHz, DMSO-d_6) δ = 162.4, 148.9, 144.2, 133.1, 132.1, 128.6, 127.4, 126.2, 121.2, 120.7, 116.5, 104.9; ESI-MS (*m*/*z*) = 355.6 (100.0%), 357.6 (70.0%) $[M+H]^+$; Anal. Calcd for $C_{16}H_{10}ClF_3N_2S$: C (54.17) H (2.84) N (7.90) S (9.04); found: C (53.79) H (2.98) N (7.63) S (9.34); mp = 123.4 °C.

5.1.4.15. *N*-(3,5-Di(trifluoromethyl)phenyl)-4-(4-chlorophenyl)thiazol-2-amine (15). General procedure 5.1.4 was used to couple 2-bromo-1-(4-chlorophenyl)ethanone (0.24 g, 1.04 mmol) and 1-(3,5-di(trifluoromethyl)phenyl)thiourea (0.30 g, 1.04 mmol) in 3 ml ethanol to yield the title compound. Pale yellow powdered solid, yield: 313 mg (71%). ^{1}H NMR (250 MHz, DMSO-d_6) δ = 11.12 (s, 1H, –N*H*–), 8.43 (s, 2H, 2*H*,6*H*-Ph-$(CF_3)_2$), 7.90 (d, *J* = 8.6 Hz, 2H, 2*H*,6*H*-Ph-Cl), 7.60 (s, 1H, 4*H*-Ph-$(CF_3)_2$), 7.58 (s, 1H, 5*H*-thiazole), 7.51 (d, *J* = 8.6 Hz, 2H, 3*H*,5*H*-Ph-Cl); ^{13}C NMR (63 MHz, DMSO-d_6) δ = 162.2, 148.7, 142.4, 132.9, 132.3, 130.6, 128.7, 127.0, 125.4, 121.1, 116.2, 105.5; ESI-MS (*m*/*z*) = 423.1$[M+H]^+$; Anal. Calcd for $C_{17}H_9ClF_6N_2S$: C (48.30) H (2.15) N (6.63) S (7.58); found: C (47.91) H (2.23) N (6.47) S (7.75); mp = 139.5 °C.

5.1.4.16. 4-(4-Chlorophenyl)-*N*-(4-isopropylphenyl)thiazol-2-amine hydrobromide (16). General procedure 5.1.4 was used to couple 2-bromo-1-(4-chlorophenyl)ethanone (0.30 g, 1.29 mmol) and 1-(4-isopropylphenyl)thiourea (0.25 g, 1.29 mmol) in 3 ml ethanol to yield the title compound. White solid, yield: 410 mg (78%). ^{1}H NMR (250 MHz, DMSO-d_6) δ = 10.23 (s, 1H, –N*H*–), 7.94 (d, *J* = 8.6 Hz, 2H, 2*H*,6*H*-Ph-Cl), 7.62 (d, *J* = 8.6 Hz, 2H, 2*H*,6*H*-Ph-$CH(CH_3)_2$), 7.49 (d, *J* = 8.6 Hz, 2H, 3*H*,5*H*-Ph-Cl), 7.38 (s, 1H, 5*H*-thiazole), 7.22 (d, *J* = 8.5 Hz, 2H, 3*H*,5*H*-Ph-$CH(CH_3)_2$), 2.86 (septet, 1H, –$CH(CH_3)_2$), 1.20 (d, *J* = 6.9 Hz, 6H, –$CH(CH_3)_2$); ^{13}C NMR (63 MHz, DMSO-d_6) δ = 163.4, 162.1, 148.6, 142.0, 138.2, 132.2, 129.5, 128.3, 126.7, 117.4, 103.3, 32.6, 23.6; ESI-MS (*m*/*z*) = 329.3 (100.0%), 331.4

(35.0%) $[M+H]^+$; Anal. Calcd for $C_{18}H_{17}ClN_2S{\cdot}HBr$: C (52.76) H (4.43) N (6.84) S (7.83); found: C (53.07) H (4.45) N (6.62) S (7.95); mp = 214.0 °C.

5.1.4.17. N^1-(4-(4-Chlorophenyl)thiazol-2-yl)-N^4,N^4-dimethylbenzene-1,4-diamine hydrobromide (17). General procedure 5.1.4 was used to couple 2-bromo-1-(4-chlorophenyl)-ethanone (0.36 g, 1.54 mmol) and 1-(4-(dimethylamino) phenyl)-2-thiourea (0.30 g, 1.54 mmol) in 3 ml ethanol to yield the title compound. White solid, yield: 515 mg (81%). ^{1}H NMR (250 MHz, DMSO-d_6) δ = 11.58 (s, 1H, –N*H*–), 7.95 (d, J = 8.6 Hz, 2H, 2*H*,6*H*-Ph-Cl), 7.95 (m, 2H, 3*H*,5*H*-Ph-N(CH_3)$_2$), 7.61 (m, 2H, 2*H*,6*H*-Ph-N(CH_3)$_2$), 7.50–7.48 (m, 3H, 5*H*-thiazole, 3*H*,5*H*-Ph-Cl), 3.15 (s, 6H, –N(*C*H_3)$_2$); ^{13}C NMR (63 MHz, DMSO-d_6) δ = 164.1, 155.7, 153.8, 150.3, 141.6, 135.9, 133.1, 131.9, 129.5, 127.2, 118.5, 45.3; ESI-MS (m/z) = 330.9 (100.0%), 332.8 (38.0%) $[M+H]^+$; Anal. Calcd for $C_{17}H_{16}ClN_3S{\cdot}HBr$: C (49.71) H (4.17) N (10.23) S (7.81); found: C (49.67) H (4.23) N (10.05) S (7.83); mp = 213.0 °C.

5.1.4.18. 4-(4-(4-Chlorophenyl)-5-methylthiazol-2-ylamino)phenol (18). General procedure 5.1.4 was used to couple 2-bromo-1-(4-chlorophenyl)propan-1-one (0.25 g, 1.01 mmol) and 1-(4-hydroxyphenyl)thiourea (0.17 g, 1.01 mmol) in 3 ml ethanol to yield the title compound. Brown solid, yield: 286 mg (89%). ^{1}H NMR (250 MHz, DMSO-d_6) δ = 9.70 (s, 1H, –N*H*–), 9.10 (s, 1H, O*H*), 7.67 (d, J = 8.6 Hz, 2H, 2*H*,6*H*-Ph-Cl), 7.49 (d, J = 8.6 Hz, 2H, 3*H*,5*H*-Ph-Cl), 7.40 (d, J = 8.9 Hz, 2H, 2*H*,6*H*-Ph-OH), 6.71 (d, J = 8.9 Hz, 2H, 3*H*,5*H*-Ph-OH), 2.38 (s, 3H, –*C*H_3); ^{13}C NMR (63 MHz, DMSO-d_6) δ = 160.4, 152.1, 143.8, 134.0, 133.2, 131.3, 129.4, 128.2, 119.0, 115.8, 115.3, 11.9; ESI-MS (m/z) = 317.3 (100.0%), 319.3 (38.0%) $[M+H]^+$; Anal. Calcd for $C_{16}H_{13}ClN_2OS$: C (60.66) H (4.14) N (8.84) S (10.12); found: C (60.35) H (3.96) N (8.57) S (9.71); mp = 163.0 °C.

5.1.4.19. 4-(4-Chlorophenyl)-*N*-(4-methoxyphenyl)-5-methylthiazol-2-amine hydrobromide (19). General procedure 5.1.4 was used to couple 2-bromo-1-(4-chlorophenyl)propan-1-one (0.30 g, 1.21 mmol) and 1-(4-methoxyphenyl)thiourea (0.22 g, 1.21 mmol) in 3 ml ethanol to yield the title compound. White crystalline solid, yield: 425 mg (85%). ^{1}H NMR (250 MHz, DMSO-d_6) δ = 10.21 (s, 1H, –N*H*–), 7.65 (d, J = 8.6 Hz, 2H, 2*H*,6*H*-Ph-Cl), 7.54–7.50 (m, (2+2)H, 3H,5H-Ph-Cl, 2H,6H-Ph-OCH$_3$), 6.94 (d, J = 9.0 Hz, 2H, 3*H*,5*H*-Ph-OCH$_3$), 3.74 (s, 3H, Ph-O*C*H$_3$), 2.36 (s, 3H, 5-CH$_3$-thiazole); ^{13}C NMR (63 MHz, DMSO-d_6) δ = 159.6, 153.8, 147.9, 142.4, 134.6, 131.9, 129.6, 128.8, 121.7, 117.6, 114.7, 55.5, 15.8; ESI-MS (m/z) = 331.3 (100.0%), 333.3 (37.6%) $[M+H]^+$; Anal. Calcd for $C_{17}H_{15}ClN_2OS{\cdot}HBr$: C (49.59) H (3.92) N (6.80) S (7.79); found: C (49.86) H (3.88) N (6.65) S (7.47); mp = 238.0 °C.

5.1.4.20. 4-(4-Chlorophenyl)-5-methyl-*N*-(3,4,5-trimethoxyphenyl)thiazol-2-amine hydrobromide (20). General procedure 5.1.4 was used to couple 2-bromo-1-(4-chlorophenyl) propan-1-one (0.20 g, 0.83 mmol) and 1-(3,4,5-trimethoxyphenyl)thiourea (0.20 g, 0.83 mmol) in 2 ml ethanol to yield the title compound. White crystalline solid, yield: 290 mg (75%). ^{1}H NMR (250 MHz, DMSO-d_6) δ = 10.34 (s, 1H, –N*H*–), 7.70 (d, J = 8.4 Hz, 2H, 2H,6H-PhCl), 7.51 (d, J = 8.6 Hz, 2H, 3H,5H-Ph-Cl), 7.04 (s, 2H, 2H,6H-Ph(OCH$_3$)$_3$), 3.76 (s, 6H, 3-OCH$_3$, 5-OCH$_3$), 3.61 (s, 3H, 4-OCH$_3$); ^{13}C NMR (63 MHz, DMSO-d_6) δ = 168.7, 161.7, 152.8, 150.5, 146.5, 131.9, 129.6, 128.7, 128.3, 118.0, 95.2, 60.7, 55.9, 11.7; ESI-MS (m/z) = 391.9 (100.0%), 393.9 (40.1%) $[M+H]^+$; Anal. Calcd for $C_{19}H_{19}ClN_2O_3S{\cdot}HBr$: C (48.37) H (4.27) N (5.94) S (6.80); found: C (48.53) H (4.23) N (6.02) S (7.18); mp = 206.0 °C.

5.1.4.21. 4-(4-Chlorophenyl)-5-methyl-*N*-(4-(methylthio)phenyl)thiazol-2-amine hydrobromide (21). General procedure 5.1.4 was used to couple 2-bromo-1-(4-chlorophenyl)propan-1-one (0.35 g, 1.77 mmol) and 1-(4-(methylthio)phenyl)thiourea (0.44 g, 1.77 mmol) in 5 ml ethanol to yield the title compound. White crystalline solid, yield: 580 mg (77%). ^{1}H NMR (250 MHz, DMSO-d_6) δ = 10.33 (s, 1H, –N*H*–), 7.67 (d, J = 8.6 Hz, 2H, 2H,6H-Ph-Cl), 7.60 (d, J = 8.7 Hz, 2H, 3H,5H-Ph-Cl), 7.51 (d, J = 8.6 Hz, 2H, 4H-Ph-SCH$_3$), 7.24 (d, J = 8.8 Hz, 2H, 2H,6H-Ph-SCH$_3$), 2.43 (s, 3H, 5H-thiazole), 2.39 (s, 3H, Ph-SCH$_3$); ^{13}C NMR (63 MHz, DMSO-d_6) δ = 179.3, 142.5, 131.9, 130.7, 129.7, 128.8, 128.6, 128.3, 128.0, 126.5, 125.9, 118.3, 118.2, 117.0, 16.0, 14.5, 11.8; ESI-MS (m/z) = 347.4 (100.0%), 349.2 (42.5%) $[M+H]^+$; Anal. Calcd for $C_{17}H_{15}ClN_2S_2{\cdot}HBr$: C (47.73) H (3.77) N (6.55) S (14.99); found: C (47.68) H (3.80) N (6.54) S (15.17); mp = 238.0 °C.

5.1.4.22. 4-(4-Chlorophenyl)-5-methyl-*N*-(4-methylthiazol-2-yl)thiazol-2-amine (22). General procedure 5.1.4 was used to couple 2-bromo-1-(4-chlorophenyl)propan-1-one (0.36 g, 1.44 mmol) and 1-(4-methylthiazol-2-yl)thiourea (0.25 g, 1.44 mmol) in 4 ml ethanol to yield the title compound. White crystalline solid, yield: 289 mg (62%). ^{1}H NMR (250 MHz, DMSO-d_6) δ = 12.00 (s, 1H, –N*H*–), 7.74 (d, J = 8.6 Hz, 2H, 2*H*,6*H*-Ph-Cl), 7.51 (d, J = 8.6 Hz, 2H, 3*H*,5*H*-Ph-Cl), 6.54 (s, 1H, 5*H*-thiazole), 2.47 (s, 3H, 5-*C*H_3-thiazole), 2.22 (s, 3H, 4-*C*H_3-thiazole; ^{13}C NMR (63 MHz, DMSO-d_6) δ = 162.3, 159.8, 152.4, 150.0, 133.6, 131.5, 129.3, 128.3, 105.6, 16.8, 12.0; ESI-MS (m/z) = 322.2 (10.0%) $[M+H]^+$, 344.2 (100.0%) $[M+Na]^+$; Anal. Calcd for $C_{14}H_{12}ClN_3S_2$: C (52.25) H (3.76) N (13.06) S (19.93); found: C (52.30) H (3.87) N (13.06) S (20.24); mp = 256.0 °C.

5.1.4.23. 4-(2,4′-Bithiazol-2′-ylamino)phenol (23). General procedure 5.1.4 was used to couple 2-(bromoacetyl)-1,3-thiazole (0.15 g, 0.73 mmol) and 1-(4-hydroxyphenyl)thiourea (0.12 g, 0.73 mmol) in 2 ml ethanol to yield the title compound. Pale brown solid, yield: 130 mg (65%). ^{1}H NMR (250 MHz, DMSO-d_6) δ = 10.08 (s, 1H, –N*H*–), 9.19 (s, 1H, –O*H*), 7.85 (d, J = 3.2 Hz, 1H, 4*H*-thiazole), 7.70 (d, J = 3.2 Hz, 1H, 5*H*-thiazole), 7.41 (d, J = 8.8 Hz, 2H, 2*H*,6*H*-Ph), 7.36 (s, 1H, 5*H*-aminothiazole), 6.76 (d, J = 8.8 Hz, 2H, 3*H*,5*H*-Ph); ^{13}C NMR (63 MHz, DMSO-d_6) δ = 164.9, 162.5, 152.7, 144.2, 143.6, 132.7, 119.9, 119.8, 115.4, 104.2; ESI-MS (m/z) = 274.0 (100.0%) $[M-H]^-$; Anal. Calcd for $C_{12}H_9N_3OS_2$: C (52.34) H (3.29) N (15.26) S (23.29); found: C (52.14) H (3.44) N (15.04) S (23.66); mp = 204.0 °C.

5.1.4.24. *N*-(4-Methylthiazol-2-yl)-2,4′-bithiazol-2′-amine hydrobromide (24). General procedure 5.1.4 was used to couple 2-(bromoacetyl)-1,3-thiazole (0.30 g, 1.46 mmol) and 1-(4-methylthiazol-2-yl)thiourea (0.25 g, 1.46 mmol) in 3 ml ethanol to yield the title compound. Pale brown powdered solid, yield: 387 mg (73%). ^{1}H NMR (250 MHz, DMSO-d_6) δ = 7.89 (d, J = 3.2 Hz, 1H, 4*H*-thiazol-2-yl), 7.75 (d, J = 3.2 Hz, 1H, 5*H*-thiazol-2-yl), 7.63 (s, 1H, 5*H*-aminothiazole), 7.58 (s, 1H, –N*H*–), 6.62 (s, 1H, 5*H*-(4-methylthiazol-2-yl)), 2.24 (s, 3H, -C*H*$_3$); ^{13}C NMR (63 MHz, DMSO-d_6) δ = 162.3, 161.9, 158.9, 143.6, 143.3, 120.4, 120.2, 108.6, 108.2, 14.6; ESI-MS (m/z) = 281.1 (100.0%) $[M+H]^+$; Anal. Calcd for $C_{10}H_8N_4S_3{\cdot}HBr$: C (33.24) H (2.51) N (15.51) S (26.62); found: C (33.47) H (2.87) N (15.42) S (26.48); mp = 261.0 °C.

5.1.4.25. 4-Adamantyl-*N*-(4-methoxyphenyl)thiazol-2-amine hydrobromide (25). General procedure 5.1.4 was used to couple 1-adamantylbromomethylketone (0.30 g, 1.17 mmol) and 1-(4-methoxyphenyl)thiourea (0.21 g, 1.17 mmol) in 3 ml ethanol to yield the title compound. White crystalline solid, yield: 378 mg (77%). ^{1}H NMR (250 MHz, DMSO-d_6) δ = 10.45 (s, 1H, –

N*H*–), 7.46 (d, *J* = 8.9 Hz, 2H, 2*H*,6*H*-Ph-Cl), 7.00 (d, *J* = 8.9 Hz, 2H, 3*H*,5*H*-Ph-Cl), 6.44 (s, 1H, 5*H*-thiazole), 3.78 (s, 3H, –OCH_3), 2.03 (m, 3H, –C*H*–(adamantyl)), 1.87 (m, 6H, –CH_2–(adamantyl)), 1.71 (m, 6H, –CH_2–(adamantyl)); ^{13}C NMR (63 MHz, DMSO-d_6) δ = 161.3, 154.6, 134.2, 118.6, 114.1, 99.1, 85.0, 55.2, 40.6, 37.6, 36.0, 28.0; ESI-MS (*m*/*z*) = 341.4 (100.0%) $[M+H]^+$; Anal. Calcd for $C_{20}H_{24}N_2OS$·HBr: C (57.00) H (5.98) N (6.65) S (7.61); found: C (57.22) H (5.62) N (6.43) S (7.28); mp = 255.0 °C.

5.1.4.26. 4-Adamantyl-*N*-(3,4-dimethoxyphenyl)thiazol-2-amine (26). General procedure 5.1.4 was used to couple 1-adamantylbromomethylketone (0.50 g, 1.94 mmol) and 1-(3,4-dimethoxyphenyl)thiourea (0.41 g, 1.94 mmol) in 8 ml ethanol to yield the title compound. Beige solid, yield: 502 mg (70%). ^{1}H NMR (250 MHz, DMSO-d_6) δ = 9.87 (s, 1H, –N*H*–), 7.66 (d, *J* = 2.2 Hz, 1H, 6*H*-Ph), 6.94 (dd, ^4J = 2.2 Hz, ^3J = 8.7 Hz, 1H, 5*H*-Ph), 6.88 (s, 1H, 2*H*-Ph), 6.27 (s, 1H, 5*H*-thiazole), 3.77 (s, 3H, 3-OCH_3), 3.70 (s, 3H, 4-OCH_3), 2.02 (m, 3H, –C*H*–(adamantyl)), 1.89 (m, 6H, –CH_2–(adamantyl)), 1.71 (m, 6H, –CH_2–(adamantyl)); ^{13}C NMR (63 MHz, DMSO-d_6) δ = 162.9, 161.7, 148.8, 143.0, 135.6, 112.8, 108.2, 102.4, 98.2, 55.9, 55.0, 41.5, 36.4, 36.7, 27.9; ESI-MS (*m*/*z*) = 371.3 (100.0%) $[M+H]^+$; Anal. Calcd for $C_{21}H_{26}N_2O_2S$: C (68.08) H (7.07) N (7.56) S (8.65); found: C (67.88) H (7.05) N (7.38) S (8.84); mp = 195.0 °C.

5.1.4.27. 4-Adamantyl-*N*-(3,4,5-trimethoxyphenyl)thiazol-2-amine (27). General procedure 5.1.4 was used to couple 1-adamantylbromomethylketone (0.30 g, 1.17 mmol) and 1-(3,4,5-trimethoxyphenyl)thiourea (0.28 g, 1.17 mmol) in 3 ml ethanol to yield the title compound. Pale yellow powdered solid, yield: 276 mg (59%). ^{1}H NMR (250 MHz, DMSO-d_6) δ = 10.00 (s, 1H, –N*H*–), 7.11 (s, 2H, 2*H*,6*H*-Ph), 6.31 (s, 1H, 5*H*-thiazole), 3.77 (s, 3H, 3-OCH_3, 5-OCH_3), 3.60 (s, 3H, 4-OCH_3), 2.01 (m, 3H, –C*H*–(adamantyl)), 1.90 (m, 6H, –CH_2–(adamantyl)), 1.70 (m, 6H, –CH_2–(adamantyl)); ^{13}C NMR (63 MHz, DMSO-d_6) δ = 162.4, 161.6, 152.7, 137.4, 131.4, 96.7, 94.4, 60.0, 55.3, 41.5, 36.4, 36.0, 27.8; ESI-MS (*m*/*z*) = 401.3 (100.0%) $[M+H]^+$; Anal. Calcd for $C_{22}H_{28}N_2O_3S$: C (65.97) H (7.05) N (6.99) S (8.01); found: C (65.82) H (6.58) N (6.57) S (7.81); mp = 214.0 °C.

5.1.4.28. 4-Adamantyl-*N*-(2-methoxyphenyl)thiazol-2-amine (28). General procedure 5.1.4 was used to couple 1-adamantylbromomethylketone (0.30 g, 1.17 mmol) and 1-(2-methoxyphenyl)thiourea (0.21 g, 1.17 mmol) in 3 ml ethanol to yield the title compound. Pale yellow powdered solid, yield: 241 mg (61%). ^{1}H NMR (250 MHz, DMSO-d_6) δ = 9.25 (s, 1H, –N*H*–), 8.36–8.32 (m, 1H, 3*H*-Ph), 7.02–6.90 (m, 3H, 4*H*,5*H*,6*H*-Ph), 6.30 (s, 1H, 5*H*-thiazole) 3.84 (s, 3H, –OCH_3), 2.02 (m, 3H, –C*H*–(adamantyl)), 1.89 (m, 6H, –CH_2–(adamantyl)), 1.72 (m, 6H, –CH_2–(adamantyl)); ^{13}C NMR (63 MHz, DMSO-d_6) δ = 162.9, 161.5, 147.7, 130.5, 121.4, 120.5, 117.6, 110.7, 99.5, 55.5, 41.5, 36.3, 35.9, 27.8; ESI-MS (*m*/*z*) = 341.3 (100.0%) $[M+H]^+$; Anal. Calcd for $C_{20}H_{24}N_2OS$: C (70.55) H (7.10) N (8.23) S (9.42); found: C (70.26) H (6.70) N (7.61) S (9.62); mp = 107.0 °C.

5.1.4.29. 4-Adamantyl-*N*-(4-(methylthio)phenyl)thiazol-2-amine hydrobromide (29). General procedure 5.1.4 was used to couple 1-adamantylbromomethylketone (0.30 g, 1.17 mmol) and 1-(2-methoxyphenyl)thiourea (0.23 g, 1.17 mmol) in 3 ml ethanol to yield the title compound. White crystalline solid, yield: 469 mg (92%). ^{1}H NMR (250 MHz, DMSO-d_6) δ = 10.33 (s, 1H, –N*H*–), 7.54 (d, *J* = 8.7 Hz, 2H, 3*H*,5*H*-Ph), 7.28 (d, *J* = 8.7 Hz, 2H, 2*H*,6*H*-Ph), 6.41 (s, 1H, 5*H*-thiazole), 2.45 (s, 3H, –SCH_3), 2.03 (m, 3H, –C*H*–(adamantyl)), 1.89 (m, 6H, –CH_2–(adamantyl)), 1.72 (m, 6H, –CH_2–(adamantyl)); ^{13}C NMR (63 MHz, DMSO-d_6) δ = 160.5, 155.0, 138.5, 127.9, 118.2, 99.3, 86.2, 41.2, 36.2, 36.0, 27.8, 15.9; ESI-MS (*m*/*z*) = 358.1 (100.0%) $[M+H]^+$; Anal. Calcd for $C_{20}H_{24}N_2S_2$·HBr: C (54.91) H (5.76) N (6.40) S (14.66); found: C (54.79) H (5.59) N (6.41) S (15.16); mp = 264.0 °C.

5.1.4.30. 4-Adamantyl-*N*-(4-isopropylphenyl)thiazol-2-amine hydrobromide (30). General procedure 5.1.4 was used to couple 1-adamantylbromomethylketone (0.33 g, 1.29 mmol) and 1-(4-isopropylphenyl)-2-thiourea (0.25 g, 1.29 mmol) in 3 ml ethanol to yield the title compound. White solid, yield: 370 mg (66%). ^{1}H NMR (250 MHz, DMSO-d_6) δ = 10.45 (s, 1H, –N*H*–), 7.45 (d, *J* = 8.5 Hz, 2H, 2*H*,6*H*-Ph), 7.26 (d, *J* = 8.5 Hz, 2H, 3*H*,5*H*-Ph), 6.44 (s, 1H, 5*H*-thiazole), 2.88 (septett, 1H, –C*H*(CH_3)$_2$), 2.03 (m, 3H, -C*H*–(adamantyl)), 1.89 (m, 6H, –CH_2–(adamantyl)), 1.71 (m, 6H, –CH_2–(adamantyl)), 1.19 (d, *J* = 6.9 Hz, 6H, –CH(CH_3)$_2$); ^{13}C NMR (63 MHz, DMSO-d_6) δ = 160.9, 155.1, 141.5, 138.7, 127.0, 118.7, 99.5, 41.1, 37.7, 36.1, 32.8, 28.4, 23.8; ESI-MS (*m*/*z*) = 353.9 (100.0%) $[M+H]^+$; Anal. Calcd for $C_{22}H_{28}N_2S$·HBr: C (60.96) H (6.74) N (6.46) S (7.40); found: C (61.04) H (6.43) N (6.39) S (7.65); mp = 276.0 °C.

5.1.4.31. N^1-(4-Adamantyl-thiazol-2-yl)-N^4,N^4-dimethylbenzene-1,4-diamine hydrobromide (31). General procedure 5.1.4 was used to couple 1-adamantylbromomethylketone (0.40 g, 1.54 mmol) and 1-(4-(dimethylamino)phenyl)-2-thiourea (0.30 g, 1.54 mmol) in 4 ml ethanol to yield the title compound. Yellow solid, yield: 530 mg (79%). ^{1}H NMR (250 MHz, DMSO-d_6) δ = 10.35 (s, 1H, –N*H*–), 7.74–7.52 (m, (2+2)H, 2*H*,6*H*-Ph, 3*H*,5*H*-Ph), 6.43 (s, 1H, 5*H*-thiazole), 3.12 (m, 6H, –N(CH_3)$_2$), 2.03 (m, 3H, –CH_2–(adamantyl)), 1.90 (m, 6H, –CH_2–(adamantyl)), 1.72 (m, 6H, –CH_2–(adamantyl)); ^{13}C NMR (63 MHz, DMSO-d_6) δ = 166.3, 157.4, 140.9, 124.0, 118.0, 115.6, 99.4, 41.3, 40.4, 36.2, 35.9, 27.8; ESI-MS (*m*/*z*) = 354.9 (100.0%) $[M+H]^+$; Anal. Calcd for $C_{21}H_{27}N_3S$·HBr: C (58.06) H (6.50) N (9.67) S (7.38); found: C (58.15) H (6.44) N (9.60) S (7.58); mp = 256.0 °C.

5.1.4.32. 4-Adamantyl-*N*-(benzo[*d*][1,3]dioxol-5-yl)thiazol-2-amine (32). General procedure 5.1.4 was used to couple 1-adamantylbromomethylketone (0.15 g, 0.58 mmol) and 1-(benzo[*d*][1,3]dioxol-5-yl)thiourea (0.11 g, 0.58 mmol) in 2.5 ml ethanol to yield the title compound. White solid, yield: 141 mg (68%). ^{1}H NMR (250 MHz, DMSO-d_6) δ = 9.92 (s, 1H, –N*H*–), 7.42 (d, *J* = 2.0 Hz, 6*H*-benzo[*d*][1,3]dioxol), 6.93 (dd, ^4J = 2.0 Hz, ^3J = 8.4 Hz, 1H, 7*H*-benzo[*d*][1,3]dioxol), 6.84 (d, *J* = 8.4 Hz, 1H, 4*H*-benzo[*d*][1,3]dioxol), 6.29 (s, 1H, 5*H*-thiazole), 5.96 (s, 2H, –OCH_2O–), 2.02 (m, 3H, –CH_2–(adamantyl)), 1.88 (m, 6H, –CH_2–(adamantyl)), 1.72 (m, 6H, –CH_2–(adamantyl)); ^{13}C NMR (63 MHz, DMSO-d_6) δ = 162.8, 161.9, 147.2, 141.1, 136.3, 109.0, 108.1, 100.6, 99.2, 98.4, 41.5, 36.3, 35.9, 27.8; ESI-MS (*m*/*z*) = 354.8 (100.0%) $[M+H]^+$; Anal. Calcd for $C_{20}H_{22}N_2O_2S$: C (67.77) H (6.26) N (7.90) S (9.05); found: C (67.85) H (6.25) N (7.86) S (9.18); mp = 173.0 °C.

5.1.4.33. 4-Adamantyl-*N*-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)thiazol-2-amine (33). General procedure 5.1.4 was used to couple 1-adamantylbromomethylketone (0.25 g, 0.95 mmol) and 1-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)thiourea (0.20 g, 0.95 mmol) in 3 ml ethanol to yield the title compound. Pale brown solid, yield: 132 mg (38%). ^{1}H NMR (250 MHz, DMSO-d_6) δ = 9.83 (s, 1H, –N*H*–), 7.36 (s, 1H, 5*H*-(2,3-dihydrobenzo[*b*][1,4]dioxin)), 6.88 (dd, ^4J = 2.5 Hz, ^3J = 8.8 Hz, 1H, 7*H*-(2,3-dihydrobenzo[*b*][1,4]dioxin)), 6.77 (d, *J* = 8.7 Hz, 1H, 8*H*-(2,3-dihydrobenzo[*b*][1,4]dioxin)), 6.27 (s, 1H, 5*H*-thiazole), 4.20 (m, 4H, 2*H*,3*H*-(2,3-dihydrobenzo[*b*][1,4]dioxin)), 2.02 (m, 3H, –CH_2–(adamantyl)), 1.87 (m, 6H, –CH_2–(adamantyl)), 1.72 (m, 6H, –CH_2–(adamantyl)); ^{13}C NMR (63 MHz, DMSO-d_6) δ = 162.8, 161.9, 143.0, 137.6, 135.5, 116.9, 110.0, 105.7, 98.2, 64.1, 41.5, 36.3, 35.9, 27.8;

ESI-MS (m/z) = 369.6 (100.0%) $[M+H]^+$; Anal. Calcd for $C_{21}H_{24}N_2O_2S$: C (68.45) H (6.56) N (7.60) S (8.70); found: C (68.15) H (6.49) N (7.53) S (8.49); mp = 151.0 °C.

5.1.4.34. 4-Adamantyl-5-methyl-*N*-(4-methylthiazol-2-yl)thiazol-2-amine hydrobromide (34). General procedure 5.1.4 was used to couple 1-(1-adamantyl)-2-bromopropan-1-on (0.24 g, 0.87 mmol) and 1-(4-methylthiazol-2-yl)thiourea (0.15 g, 0.87 mmol) in 2 ml ethanol to yield the title compound. White solid, yield: 214 mg (58%). ^{1}H NMR (250 MHz, DMSO-d_6) δ = 12.98 (s, 1H, –N*H*–), 6.87 (s, 1H, 5*H*-(4-methylthiazol-2-yl)), 2.48 (s, 3H, 5-C*H*$_3$-thiazole), 2.28 (s, 3H, 4-C*H*$_3$-(4-methylthiazol-yl), 2.06 (m, (3+6)H, -C*H*–(adamantyl), –C*H*$_2$–(adamantyl)), 1.72 (m, 6H, –C*H*$_2$–(adamantyl)); ^{13}C NMR (63 MHz, DMSO-d_6) δ = 156.8, 155.3, 147.8, 146.8, 115.2, 104.7, 38.4, 37.5, 35.7, 27.7, 14.2, 12.9; ESI-MS (m/z) = 346.2 (100.0%) $[M+H]^+$; Anal. Calcd for $C_{18}H_{23}N_3S_2$·HBr: C (50.70) H (5.67) N (9.85) S (15.04); found: C (50.46) H (5.68) N (9.91) S (15.40); mp = 300.0 °C (sublimation).

5.1.4.35. 4-Adamantyl-5-methyl-*N*-(2-methoxyphenyl)thiazol-2-amine hydrobromide (35). General procedure 5.1.4 was used to couple 1-(1-adamantyl)-2-bromopropan-1-on (0.25 g, 0.92 mmol) and 1-(2-methoxyphenyl)thiourea (0.17 g, 0.92 mmol) in 2 ml ethanol to yield the title compound. White crystalline solid, yield: 212 mg (53%). ^{1}H NMR (250 MHz, DMSO-d_6) δ = 10.04 (s, 1H, –N*H*–), 7.66 (m, 1H, 3*H*-Ph), 7.27–7.15 (m, 1H, 4*H*,5*H*-Ph), 7.06–7.00 (m, 1H, 6*H*-Ph), 3.86 (s, 3H, –OC*H*$_3$), 2.36 (s, 3H, –C*H*$_3$-thiazole), 2.03 (m, (3+6)H, –C*H*–(adamantyl), –C*H*$_2$–(adamantyl)), 1.73 (m, 6H, –C*H*$_2$–(adamantyl)); ^{13}C NMR (63 MHz, DMSO-d_6) δ = 163.7, 152.1, 150.7, 130.5, 127.1, 126.8, 126.7, 112.8, 112.1, 55.8, 40.4, 37.0, 35.6, 27.7, 12.2; ESI-MS (m/z) = 355.3 (100.0%) $[M+H]^+$; Anal. Calcd for $C_{21}H_{26}N_2OS$·HBr: C (57.93) H (6.25) N (6.43) S (7.36); found: C (57.79) H (6.14) N (6.29) S (7.31); mp = 222.0 °C.

5.1.4.36. 4-Adamantyl-5-methyl-*N*-(3,4,5-trimethoxyphenyl)thiazol-2-amine (36). General procedure 5.1.4 was used to couple 1-(1-adamantyl)-2-bromopropan-1-on (0.25 g, 0.92 mmol) and 1-(3,4,5-trimethoxyphenyl)thiourea (0.22 g, 0.92 mmol) in 3 ml ethanol to yield the title compound. Pale brown solid, yield: 194 mg (51%). ^{1}H NMR (250 MHz, DMSO-d_6) δ = 9.73 (s, 1H, –N*H*–), 7.07 (s, 2H, 2*H*,6*H*-Ph), 3.75 (s, 6H, 3-OC*H*$_3$, 5-OC*H*$_3$), 3.59 (s, 3H, 4-OC*H*$_3$), 2.33 (s, 3H, –C*H*$_3$-thiazole), 2.05–2.00 (m, (3+6)H, –C*H*–(adamantyl), –C*H*$_2$–(adamantyl)), 1.71 (m, 6H, –C*H*$_2$–(adamantyl)); ^{13}C NMR (63 MHz, DMSO-d_6) δ = 157.2, 152.8, 137.8, 131.2, 112.5, 99.5, 94.1, 60.1, 55.4, 41.7, 38.5, 36.4, 28.2, 11.7; ESI-MS (m/z) = 416.1 (100.0%) $[M+H]^+$; Anal. Calcd for $C_{23}H_{30}N_2O_3S$: C (66.64) H (7.29) N (6.76) S (7.73); found: C (66.77) H (7.36) N (6.41) S (7.34); mp = 221.0 °C.

5.1.4.37. 4-Adamantyl-5-methyl-*N*-(4-(methylthio)phenyl)thiazol-2-amine (37). General procedure 5.1.4 was used to couple 1-(1-adamantyl)-2-bromopropan-1-on (0.25 g, 0.92 mmol) and 1-(2-methoxyphenyl)thiourea (0.18 g, 0.92 mmol) in 3 ml ethanol to yield the title compound. Pale yellow powdered solid, yield: 180 mg (53%). ^{1}H NMR (250 MHz, DMSO-d_6) δ = 9.78 (s, 1H, –N*H*–), 7.54 (d, J = 8.7 Hz, 2H, 3*H*,5*H*-Ph), 7.22 (d, J = 8.7 Hz, 2H, 2*H*,6*H*-Ph), 2.42 (s, 3H, –SC*H*$_3$), 2.34 (s, 3H, 5-C*H*$_3$-thiazole), 2.03 (m, (3+6)H, –C*H*–(adamantyl), C*H*$_2$–(adamantyl)), 1.72 (m, 6H, –C*H*$_2$–(adamantyl)); ^{13}C NMR (63 MHz, DMSO-d_6) δ = 157.0, 153.0, 139.5, 128.2, 127.9, 116.9, 112.6, 41.5, 37.6, 36.2, 28.1, 16.3, 11.8; ESI-MS (m/z) = 371.8 (100.0%) $[M+H]^+$; Anal. Calcd for $C_{21}H_{26}N_2S_2$: C (68.06) H (7.07) N (7.56) S (17.31); found: C (68.34) H (6.94) N (7.60) S (17.19); mp = 137.0 °C.

5.1.4.38. (*E*)-*N*-(4-(4-Chlorophenyl)thiazol-2-yl)-3-(3,4-dimethoxyphenyl)acrylamide (38)[33,34]. 4-(4-Chlorphenyl)thiazol-2-amine (0.25 g, 0.12 mmol) was solved together with (*E*)-3-(3,4-dimethoxyphenyl)acryloyl chloride (0.27 g, 0.12 mmol) in 3 ml pyridine. The resulting mixture was heated to 60 °C under microwave irradiation for 30 min. The crude product was purified by column chromatography on silica gel (eluent: dichloromethane/methanol 9:1 (v/v)) that afforded the title compound. Brown solid, yield: 160 mg (33%). ^{1}H NMR (250 MHz, DMSO-d_6) δ = 12.38 (s, 1H, –N*H*–), 7.93 (d, J = 8.3 Hz, 2H, 2*H*,6*H*-Ph-Cl), 7.71 (s, 1H, 5*H*-thiazole), 7.66 (d, J = 15.7 Hz, 1H, —NH—CO—CH=C*H*—), 7.50 (d, J = 8.3 Hz, 2H, 3*H*,5*H*-Ph-Cl), 7.22 (m, 2H, 2*H*,6*H*-Ph-(OCH$_3$)$_2$), 7.04 (m, 1H, 5*H*-Ph-(OCH$_3$)$_2$), 6.82 (d, J = 15.7 Hz, 1H, —NH—CO—C*H*=CH—), 3.82 (m, 6H, 3-OC*H*$_3$, 4-OC*H*$_3$); ^{13}C NMR (63 MHz, DMSO-d_6) δ = 163.6, 158.2, 150.8, 148.8, 147.7, 142.5, 133.1, 132.1, 128.6, 127.3, 127.0, 121.9, 117.0, 111.8, 110.5, 109.0, 55.5; ESI-MS (m/z) = 401.1 (100.0%) $[M+H]^+$; HR-MS ($C_{20}H_{17}ClN_2O_3S$) calcd: 401.07212; found: 401.07192; mp = 184.2 °C.

5.1.4.39. (*E*)-*N*-(4-(4-Chlorophenyl)thiazol-2-yl)-3-(3,4,5-trimethoxyphenyl)acrylamide (39, ST-1577)[33]. (*E*)-3-(3,4,5-Trimethoxyphenyl)acrylic acid (0.30 g, 1.26 mmol) was solved in 2 ml DMF. *N*,*N*′-Diisopropylcarbodiimide (DIC, 0.15 g, 1.26 mmol) and 1-hydroxybenzotriazol (HOBt, 0.17 g, 1.26 mmol) were added and the solution was stirred for 10 min at room temperature. In a second reaction batch, 4-(4-chlorphenyl)thiazol-2-amine (0.24 g, 1.14 mmol) was suspended in 4 ml dichloromethane. *N*,*N*-Diisopropylethylamine (DIPEA, 0.22 g, 1.72 mmol) was added. Both reaction batches were combined and stirred under inert argon atmosphere at room temperature for 48 h. The crude product was purified by column chromatography on silica gel (eluent: hexane/EtOAc 1:2 (v/v)) that afforded the title compound. Yellow solid, yield: 147 mg (30%). ^{1}H NMR (250 MHz, DMSO-d_6) δ = 12.42 (s, 1H, –N*H*–), 7.94 (d, J = 8.6 Hz, 2H, 2*H*,6*H*-Ph-Cl), 7.73 (s, 1H, 5*H*-thiazole), 7.66 (d, J = 16.0 Hz, 1H, —NH—CO—C*H*=CH—), 7.51 (d, J = 8.6 Hz, 2H, 3*H*,5*H*-Ph-Cl), 6.99 (s, 2H, 2*H*,6*H*-Ph(OCH$_3$)$_3$), 6.91 (d, J = 15.8 Hz, 1H, —NH—CO—CH=C*H*—), 3.85 (s, 6H, 3-OC*H*$_3$, 5-OC*H*$_3$), 3.72 (s, 3H, 4-OC*H*$_3$); ^{13}C NMR (63 MHz, DMSO-d_6) δ = 163.4, 158.0, 153.0, 147.7, 142.4, 139.4, 133.0, 132.1, 129.8, 128.7, 127.3, 118.8, 109.1, 105.4, 60.0, 55.8; ESI-MS (m/z) = 431.3 (100.0%) $[M+H]^+$; HR-MS ($C_{21}H_{19}ClN_2O_4S$) calcd: 431.08268; found: 431.08214; mp = 210.0 °C.

5.1.4.40. (*E*)-3-(3,4-Dimethoxyphenyl)-*N*-(thiazol-2-yl)acrylamide (40)[33]. (*E*)-3-(3,4-dimethoxyphenyl)acrylic acid (2.08 g, 0.99 mmol) was solved in 10 ml acetone. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, 1.71 g, 1.10 mmol) and 1-hydroxybenzotriazol (HOBt, 1.49 g, 1.10 mmol) were added and the mixture was stirred at room temperature for 30 min. 2-Aminothiazole (1.00 g, 0.99 mmol) was added and the mixture was heated to 60 °C under microwave irradiation for 10 min. The crude product was purified by column chromatography on silica gel (eluent: dichloromethane/methanol 98:2 (v/v)) that afforded the title compound. White solid, yield: 1.31 g (45%). ^{1}H NMR (250 MHz, DMSO-d_6) δ = 12.19 (s, 1H, –N*H*–), 7.66 (d, J = 15.8 Hz, 1H, —NH—CO—CH=C*H*—), 7.50 (d, J = 3.5 Hz, 1H, 4*H*-thiazole), 7.24–7.21 (m, (1–2)H, 5*H*-thiazole, 2*H*,6*H*-thiazole), 7.03 (d, J = 8.8 Hz, 1H, 5*H*-Ph), 6.80 (d, J = 15.8 Hz, 1H, —NH—CO—C*H*=CH—), 3.81 (d, 6H, 3-OC*H*$_3$, 4-OC*H*$_3$); ^{13}C NMR (63 MHz, DMSO-d_6) δ = 163.3, 158.0, 150.7, 148.8, 142.2, 137.7, 127.0, 121.9, 117.1, 113.5, 111.7, 110.4, 55.5; ESI-MS (m/z) = 289.1 (100.0%) $[M-H]^-$; Anal. Calcd for $C_{14}H_{14}N_2O_3S$: C (57.92) H (4.86) N (9.65) S (11.04); found: C (57.58) H (4.88) N (9.57) S (11.07); mp = 225.0 °C.

5.1.4.41. (*E*)-3-(3,4,5-Trimethoxyphenyl)-*N*-(thiazol-2-yl)acrylamide (41)[33]. (*E*)-3-(3,4,5-trimethoxyphenyl)acrylic acid (2.38 g, 0.99 mmol) was solved in 10 ml acetone. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, 1.71 g, 1.10 mmol) and 1-hydroxybenzotriazol (HOBt, 1.49 g, 1.10 mmol) were added and the mixture was stirred at room temperature for 30 min. 2-Aminothiazole (1.00 g, 0.99 mmol) was added and the mixture was heated to 60 °C under microwave irradiation for 10 min. The crude product was purified by column chromatography on silica gel (eluent: dichloromethane/methanol 99:1 (v/v)) that afforded the title compound. Beige crystalline solid, yield: 1.68 g (53%). ^{1}H NMR (250 MHz, DMSO-d_6) δ = 12.22 (s, 1H, –N*H*–), 7.66 (d, *J* = 15.7 Hz, 1H, —NH—CO—CH=C*H*—), 7.51 (d, *J* = 3.6 Hz, 1H, 4*H*-thiazole), 7.24 (d, *J* = 3.6 Hz, 1H, 5*H*-thiazole), 6.97 (s, 2H, 2*H*,6*H*-Ph), 6.88 (d, *J* = 15.8 Hz, 1H, —NH—CO—CH=C*H*—), 3.84 (s, 6H, 3-OCH_3, 5-OCH_3), 3.70 (s, 3H, 4-OCH_3); ^{13}C NMR (63 MHz, DMSO-d_6) δ = 163.1, 157.9, 153.0, 142.2, 139.3, 137.8, 129.8, 118.9, 113.6, 105.4, 60.0, 55.8; ESI-MS (*m*/*z*) = 321.0 (100.0%) [M−H]$^-$; Anal. Calcd for $C_{15}H_{16}N_2O_4S$: C (56.24) H (5.03) N (8.74) S (10.01); found: C (56.62) H (5.27) N (8.37) S (9.68); mp = 216.0 °C.

5.1.4.42. (*R*)-(1-(4-((3-Methyl-5-(phenylsulfonylmethyl)phenoxy)methyl)benzyl)pyrrolidin-2-yl)methanol (PF-543). Reference substance PF-543 (compound 42) was synthesized according to Schnute et al.[26] (cf. Suppl. mat.). ^{1}H NMR (250 MHz, DMSO-d_6) δ = 7.74–7.68 (m, 3H, 2H,6H-Ph, 4H-Ph), 7.61–7.55 (m, 2H, 3H,5H-Ph), 7.30 (s, 4H, 2H,6H-Ph-CH_2-prolinol, 3H,5H-Ph-CH_2-prolinol), 6.77 (s, 1H, 6H-Ph-CH_3), 6.54 (s, 1H, 2H-Ph-CH_3), 6.51 (s, 1H, 4H-Ph-CH_3), 4.88 (s, 2H, –SO_2–CH_2–), 4.55 (s, 2H, –O–CH_2–), 4.41 (br s, 1H, –OH), 4.04 (d, *J* = 13.3 Hz, 1H, –CH-prolinol), 3.50–3.40 (m, 1H, prolinol), 3.36–3.22 (m, 2H, prolinol), 2.83–2.69 (m, 1H, prolinol), 2.63–2.54 (m, 1H, prolinol), 2.17 (s, 3H, –CH_3), 2.14–2.04 (m, 1H, prolinol), 1.92–1.75 (m, 1H, prolinol), 1.67–1.48 (m, 3H prolinol); ^{13}H NMR (75 MHz, DMSO-d_6) δ = 158.0, 138.7, 138.4, 133.7, 129.6, 129.0, 128.7, 128.0, 127.4, 124.1, 115.2, 114.6, 68.9, 65.0, 60.6, 58.3, 58.2, 58.2, 53.9, 27.8, 22.3, 20.9; ESI-MS (*m*/*z*) = 466.2 (100.0%) [M+H]$^+$; Anal. Calcd For $C_{27}H_{31}NO_4S_2$·0.5 H_2O: C (68.33) H (6.80) N (2.95) S (6.76); found: C (68.35) H (6.84) N (2.71) S (6.55).

5.2. Biology

5.2.1. Sphingosine kinase assay

5.2.1.1. Materials. All general reagents were ordered from Sigma–Aldrich (Steinheim/Germany) and AppliChem (Darmstadt/Germany). Human recombinant SphK1 and SphK2 were supplied from Echelon (Salt Lake City/USA). SphK1 was formulated in 40 mM Tris–HCl, pH 8.0, 110 mM NaCl, 2.2 mM KCl, 200 mM imidazole, 3 mM DTT and 20% glycerol (enzyme concentration: 1.39 mg/mL). SphK2 was formulated in 45 mM Tris–HCl, pH 8.0, 124 mM NaCl, 2.4 mM KCl, 225 mM Imidazole, 3 mM DTT and 10% glycerol (enzyme concentration: 0.5 mg/mL). D-Erythro-sphingosine was supplied by Cayman/Biomol (Hamburg/Germany). Sphingosine kinase reaction buffer, DTT and BellBrook Transcreener® ADP^2 FI assay kit were provided by Echelon (Salt Lake City/USA) within the sphingosine kinase inhibitor screen assay kit K-4400. BellBrook Transcreener® ADP^2 FI Assay Kit contains Alexa Fluor® 594 labeled tracer (800 nM in 2 mM HEPES pH 7.5, 0.01% Brij-35), IRDye®-QC-1quencher-labeled monoclonal antibody (1.3 mg/mL in 100 mM K_2HPO_4 pH 8.5), stop and detect buffer (200 mM HEPES, 0.2% Brij-35, 400 mM EDTA, pH 7.5), ADP (5 mM in deionized water, pH 7.0) and ATP (5 mM in deionized water, pH 7.0). For fluorescence measurement, we used Infinite M200 plate reader from Tecan (Crailsheim/Germany). We utilized 384-well black low volume round bottom plates from Corning (Tewksbury/USA).

5.2.1.2. Assay. The Transcreener® ADP^2 FI assay is a competitive fluorescence intensity assay based on the detection of ADP, flexible with regard to the ATP concentration (0.1–100 μM ATP). It is a one-step detection assay that provides signal evaluation at low substrate conversion. SphK1 and SphK2 use sphingosine and ATP to form S1P and ADP. We performed the assay according to the suppliers' protocol. We performed the experiments under conditions that ensured a 10–15% substrate conversion as proposed. We also considered the different activities of the isoenzymes that were relevant for incubation times to ensure that the reactions were linear over the time course of reactions at the concentrations of the enzymes used in the assay. Using this protocol and standard assay conditions, we generated an ATP/ADP standard curve and screened our test compounds including **SKI-II** (**1**) and **PF-543** (**42**) as reference compounds and compared to control (DMSO). Compounds and enzymes were pre-incubated for 15 min at room temperature. SphK1 reaction was incubated at room temperature for 30 min, the SphK2 reaction for 60 min. Readout of the assay is a percentage inhibition. An IC_{50} value could be determined for the most promising compound **ST-1803** (**24**). For the calculation of the IC_{50} value of **ST-1803** we measured eight increasing concentrations of the inhibitor (0.03–100 μM) in 3 independent experiments. IC_{50} values were calculated with GraphPad Prism 5.0 using a non-linear regression (95% confidence interval 4.89 to 10.81 μM for SphK1 and 4.45 to 9.42 for SphK2). For *c* log *P* calculation of **ST-1803** we used ChemOffice Ultra® 10.0 (CambridgeSoft/USA). TPSA was calculated with Molinspiration Property Engine v2013.09 (Molinspiration Cheminformatics/Slovakia).

5.3. Lactate dehydrogenase (LDH) cytotoxicity assay

The LDH assay (cytotoxicity detection 1 kit; Roche Diagnostics GmbH, Roche Applied Science, Mannheim, Germany) was used to determine cell death after treatment of U937 cells with test compound **ST-1803** (**24**). LDH leakage was measured as an index of loss of cell membrane integrity. U937 cells were seeded in 96-well plates at a density of 1.5×10^4 cells/well and incubated with increasing concentrations of the test compound or vehicle (DMSO) for 24 h. Plates were centrifuged (250 × g, 4 min) and an aliquot of the supernatant was transferred to a clean microplate. Cell toxicity was assessed according to the distributor's protocol using a microplate reader (infinite M200, Tecan Group Ltd, Crailsheim, Germany). A control detergent supplied by Sigma–Aldrich (Saint Louis, Mo, USA) was used for maximum LDH release and set to 100%. All experiments were done three times and mean ± SE were calculated.

5.4. Molecular modelling

The software package Molecular Operating Environment (MOE, 2013.08; Chemical Computing Group, Montreal/Canada) was used for all computational tasks carried out for this work. The structure of SphK1 co-crystallized with **SKI-II** (PDB code: 3VZD)[16] was used as a template for docking as well as for the homology modelling of SphK2. The receptor was arranged by means of the functions Protonate 3D and Energy Minimization (Amber 12:EHT force field). The Pharmacophore Query Editor was used to define the common pharmacophore of the docked structures and the template molecule **SKI-II**. Pharmacophore was furthermore used as docking placement algorithm which generated a maximum of 1000 docking poses for each molecule (time-out: 300 s). The 30 best poses where kept and energy minimized using Amber 12:EHT force field. For rescoring and final pose ranking the scoring function *GBVI*/*WSA dG* was applied. The sequence of human SphK2 was taken from Protein Knowledge Base UniProtKB[49] (ID: Q9NRA0) and aligned to the template sequence of SphK1 (PDB code: 3VZD.B).

The MOE Homology Model function was used with recommended settings.

6. Authorship

Conceived and designed the experiments: D.V., H.S. Performed the experiments: D.V., K.I., A.B. Analyzed the data: D.V., K.I. Computational modelling: J.W., E.P. Wrote the paper: D.V., J.W., H.S.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2014.07.044.

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