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Discovery of IACS-9439, a potent, exquisitely selective and orally bioavailable inhibitor of CSF1R

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3 Keywords: CSF1, CSF1R, CSF1R inhibitor, colony-stimulating factor 1, immune-oncology,
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5 macrophage, tumor associated macrophage, TAM
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13 ABSTRACT

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18 Tumor-associated macrophages (TAMs) have a significant presence in the tumor stroma
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21 across multiple human malignancies and are believed to be beneficial to tumor growth.
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25 Targeting CSF1R has been proposed as a potential therapy to reduce TAMs, especially
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28 the pro-tumor, immune-suppressive M2 TAMs. Additionally, high expression of CSF1R
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32 on tumor cells has been associated with poor survival in certain cancers, suggesting
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35 tumor dependency and therefore a potential therapeutic target. The CSF1–CSF1R
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39 signaling pathway modulates the production, differentiation and function of TAMs;
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42 however, the discovery of selective CSF1R inhibitors devoid of Type III kinase activity
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46 has proven to be challenging. We discovered a potent, highly selective and orally
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49 bioavailable CSF1R inhibitor, IACS-9439 (1). Treatment with 1 led to a dose dependent
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3 reduction in macrophages, promoted macrophage polarization toward the M1 phenotype,
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7 and led to tumor growth inhibition in MC38 and PANC02 syngeneic tumor models.
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15 Strategies to enhance anti-tumor immunity by reactivating the adaptive and innate
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19 immune compartments through checkpoint inhibitors against programmed cell death 1
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22 (PD1), PD1 ligand (PDL1) and cytotoxic T lymphocyte antigen 4 (CTLA4) have shown
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26 favorable clinical responses, yet, only a fraction of patients show durable responses.
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29 While tumor-intrinsic resistance mechanisms may exist, clinical and preclinical evidences
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32 highlight the abundance of tumor-associated macrophages (TAMs) as critical regulatory
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37 immune cells that promote tumor progression. Macrophages exist primarily in two main
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40 polarization states with the alternatively activated M2 TAM responsible for promoting
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43 tumor progression by secreting anti-inflammatory cytokines such as IL-10 and TGF β ,
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47 whereas the classically activated M1 promote immune-mediated tumor killing through the
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50 production of pro-inflammatory cytokines such as IL-1 β , IL-6 and tumor necrosis factor α
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53 (TNF- α).¹⁻³ To overcome the immunosuppressive and pro-tumoral functions of TAMs,
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3 therapeutic strategies have focused on TAM depletion in the tumor microenvironment as
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7 well as TAM reprogramming to favor anti-tumoral functions (polarization from M2 to M1).⁴
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14 Colony-stimulating factor 1 receptor (also known as CSF1R, or macrophage colony-
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17 stimulating factor receptor, M-CSFR) is a membrane-associated tyrosine kinase. It acts
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21 as the receptor for colony-stimulating factor 1 (CSF1), a cytokine which controls the
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24 production, differentiation and function of macrophages. Ligand binding activates CSF1R
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27 through a process of oligomerization within the membrane, transphosphorylation of the
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31 intracellular domain, and subsequent signaling.⁶
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35 In several preclinical studies using murine breast cancer and glioblastoma models, it
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38 was demonstrated that CSF1–CSF1R signaling blockade slowed primary tumor growth,
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42 reduced metastatic potential and improved the long-term survival of tumor-bearing mice.⁷
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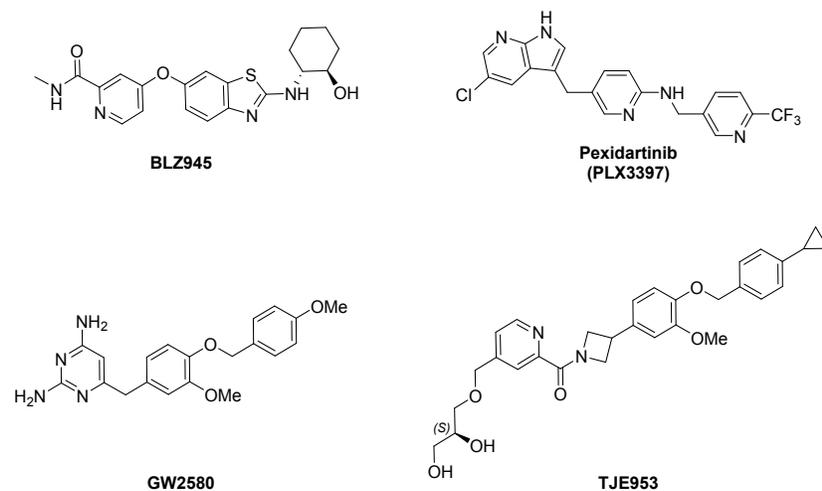
45 ⁸ Treatment with BLZ945, a potent CSF1R inhibitor, attenuated tumor growth, correlated
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48 with decreased TAM presence, and enriched CD8⁺ T cells in tumor stroma in a murine
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52 K14-HPV-16 transgenic mouse model of cervical carcinogenesis.⁹ These findings have
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56 been translated into a first-in-human Phase I/II study of BLZ945 alone or in combination
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3 with PDR001, a monoclonal antibody against PD-1 in advanced solid tumors
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7 (NCT02829723).^{10,11} Pexidartinib (PLX3397), an unselective CSF1R inhibitor that also
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9
10 inhibits cKIT, FLT3, PDGFR α and PDGFR β with low nanomolar activity, recently received
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13 FDA approval.¹² Pexidaritinib demonstrated reduction of TAMs, efficient target
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16 engagement, and clinical efficacy as a monotherapy, with a 38% overall response rate in
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19 pigmented villonodular synovitis (PVNS), an orphan disease characterized by
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22 overexpression of CSF1R.¹²⁻¹⁶ Additionally, emactuzumab (RG7155), a neutralizing
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25 antibody against CSF1R not only reduced macrophage infiltration in mouse models but
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28 also demonstrated similar therapeutic effects against diffuse-type giant cell tumors in
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31 patients.¹⁷ Two other neutralizing antibodies against CSF1R, AMG 820 (NCT01444404),
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34 and IMC-CS4 (NCT01346358) are also currently in Phase I clinical trials for the treatment
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37 of advanced solid tumors.^{18, 19} While there are several clinical opportunities for single
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40 agent activity by suppressing CSF1R biology in dependent tumor cells, full clinical benefit
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43 is likely to come from combining CSF1R inhibition in macrophages with immune
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46 modulating agents targeting T-cell biology, such as anti-CTLA4, anti-PD-1 and anti-PD-
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49 L1 therapeutics. These combinations have the potential to promote tumor suppression
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3 through modulation of multiple arms of the immune system. These combinations
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7 currently are being explored in numerous clinical trials (i.e. NCT02777710, NCT04301778
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10 and NCT03927105).²⁰⁻²²

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14 Several small molecule CSF1R inhibitors have been described in the literature,
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17 however, only a few were reported to possess high levels of selectivity (Figure 1).^{7-16, 23-}
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21 ³¹ BLZ945 was shown to inhibit CSF1R enzymatic activity with an $IC_{50} = 1$ nM, and was
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24 reported to have a >3200-fold selectivity for CSF1R over other related kinases.⁸ GW2580
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27 inhibited CSF1R enzymatic activity with an $IC_{50} = 30$ nM, showing 22-fold and 75-fold
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31 selectivity towards TrkB and C, respectively, and over 150-fold selectivity against other
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35 kinases.^{29,32} GW2580 displayed an unfavorable in vivo PK profile with low exposures and
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38 rapid clearance, and has not been further developed.²⁹ A structurally related, highly
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42 selective inhibitor with an azetidone scaffold, JTE-952 was also reported in the literature.
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45 In a panel consisting of 51 kinases, JTE-952 was reported to have a 20-fold selectivity for
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48 CSF1R over TrkA, however, selectivity for TrkB and TrkC was not reported.^{30, 31} Analysis
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52 of the crystal structure of PLX3397 bound to CSF1R (PDB 4R7H)¹⁵ and protein-ligand
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56 binding models of other CSF1R inhibitors (using PDB 3LCO) suggested that these
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4 inhibitors bind in the ATP binding pocket in the inactive, DFG-out conformation of the
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7 kinase.

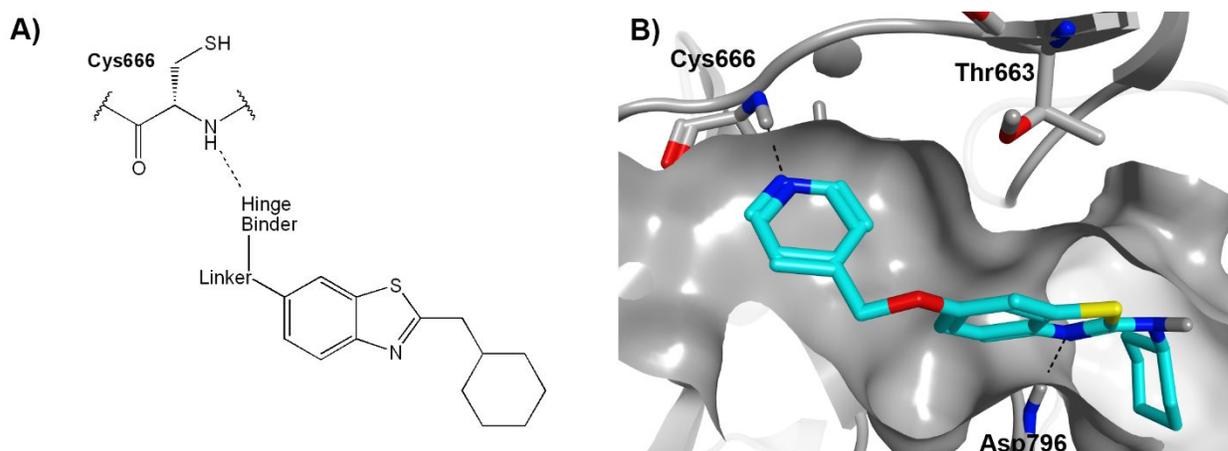


29 **Figure 1.** Representative examples of small molecule CSF1R inhibitors.

30 31 32 33 34 35 36 RESULTS AND DISCUSSION

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40 The objective of our program was to discover a highly selective small molecule CSF1R
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43 inhibitor with excellent physicochemical and pharmacokinetic properties that potentially
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46 could be used in combination with other immuno-oncology therapies. Herein, we describe
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49 our efforts to identify a potent, orally bioavailable tool compound that we transformed into
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54 a highly selective and efficacious lead candidate.

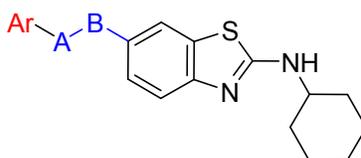
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4 At the outset of our program, we performed a scaffold hopping exercise using
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7 benzothiazole as a template, with the search directed towards finding novel combinations
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10 of hinge binding and linker groups (Figure 2A); BREED³³, as implemented in MOE³⁴, was
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13 used for this purpose. A set of 1,945 pre-aligned kinase-ligand complexes from the PDB
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16 were compared with the benzothiazole in a molecular model of BLZ945 bound to CSF1R,
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19 resulting in novel compounds that maintained both the position of the benzothiazole core
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22 and the interactions with the kinase hinge region. Examination of these results suggested
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24
25 and the interactions with the kinase hinge region. Examination of these results suggested
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28 that the kinase hinge residue, Cys666, could be engaged efficiently using two-atom
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31 linkers placed between the benzothiazole core and suitable hinge-binding moieties
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35 (Figure 2B).

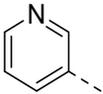
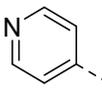


1
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3 **Figure 2.** A) Schematic showing of scaffold hopping template and kinase hinge target
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7 interactions B) Molecular model of 4-pyridyl hinge binding motif with -C-O- linker engaging
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10 the backbone NH of Cys666 (PDB 3LCO).

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17 To test this hypothesis, a focused SAR study utilizing a variety of two-atom linkers was
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20 performed. This study revealed that the -C-O- linker was preferred when combined with
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23 a 4-pyridyl hinge binding motif resulting in compound **3**, which exhibited an IC₅₀ of 303
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26 nM in a CSF1R FRET-based displacement binding assay (Table 1). There was less
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29 preference among the two-atom linkers when combined with the 3-pyridyl hinge-binding
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32 moiety; none of these compounds matched the enzymatic activity of compound **3**.

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41 **Table 1.** Enzymatic activity of compounds containing a two-atom linker between the
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44 hinge-binding pyridine and the benzothiazole core.



A-B \ Ar		
-C-O- CSF1R IC ₅₀ (nM)	2 781	3 303
-O-C- CSF1R IC ₅₀ (nM)	4 450	5 4,413
-NH-C- CSF1R IC ₅₀ (nM)	6 655	7 27,712

In an effort to improve potency, an amine substituent was incorporated onto the pyridine of compound **3**, which was predicted to form a second hinge interaction with the backbone carbonyl of Cys666. Indeed, this modification led to a six-fold improvement in enzymatic potency as demonstrated by compound **8**. Incorporating a chloro group at the three position of the pyridine led to similar improvement in potency as shown by compound **9**. Combining these two substituents had an additive effect with a 75-fold improvement in potency relative to compound **3**, with compound **10** exhibiting an IC₅₀ of 4 nM in the CSF1R FRET-based displacement binding assay. Compound **10** also inhibited mCSF1-

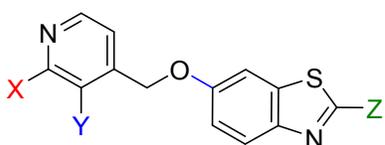
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3 mediated proliferation of MNFS-60 myelogenous leukemia cells with an IC_{50} of 228 nM,
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7 while it did not show significant activity against mCSF1-independent NS0 cell proliferation
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10 (Table 2).

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14 Our molecular models predicted that the cyclohexyl ring extended into the deep pocket
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17 of the DFG-out conformation of CSF1R, a region that provided additional opportunities to
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20 improve potency and selectivity, given the modest differences in sequence among type
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23 III kinases in this area (ie. Met637 is a Leu in cKIT, G795 is a Cys in other type III kinases).
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27 To engage the side chain of Glu633 in the deep pocket region, a hydroxyl substituent was
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30 incorporated on the cyclohexyl ring. To maximize the probability of engaging this
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33 interaction, we examined all four stereoisomers of the 2-amino cyclohexan-1-ol group.
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37 However, incorporating the hydroxyl group did not make a significant contribution to
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40 enzymatic or cellular potency.
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45 To evaluate whether incorporation of the hydroxyl group on the cyclohexane ring offered
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48 any selectivity advantage, and whether the orientation of the hydroxyl group affected
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51 selectivity, we evaluated these inhibitors in a binding assay against a representative set
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55 of type III kinases that are related to CSF1R, including FLT3, cKIT, PDGFR α and
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3 PDGFR β . Overall, compound **12** exhibited a favorable selectivity profile. The cellular
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7 activity of compound **12** was measured by monitoring inhibition of CSF1R
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10 autophosphorylation after CSF1 stimulation of THP-1 cells, a human monocytic cell line.
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14 In order to assess the compound's selectivity against PDGFR β , we measured agonist
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17 (PDGF-DD) induced PDGFR β autophosphorylation in a HEK293 cell line expressing
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20 human recombinant PDGFR β (HEK293/PDGFR β cells). BLZ945 was also tested in this
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24 assay as a competitor with reported selectivity against PDGFR β . Our results confirmed
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28 that both BLZ945 and **12** exhibited minimal selectivity against PDGFR β in a cellular
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31 setting, with **12** inhibiting CSF1 stimulated CSF1R autophosphorylation in THP-1 cells
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35 with an IC₅₀ of 183 nM, while inhibiting PDGFR β autophosphorylation in
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38 HEK293/PDGFR β cells with an IC₅₀ of 446 nM. BLZ945 displayed an IC₅₀ of 155 nM in
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41 the cellular phospho-CSF1R (THP) assay and IC₅₀ of 579 nM in the phospho-PDGFR β
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45 (HEK293/ PDGFR β) assay.
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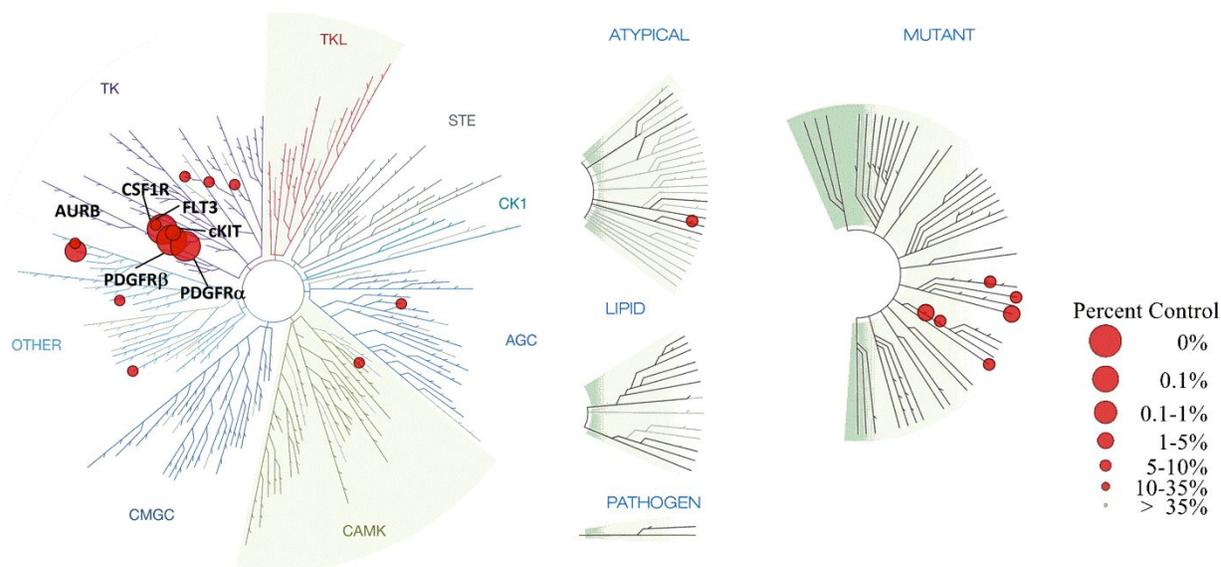
52 **Table 2.** SAR studies in the hinge region and deep pocket area of the 6-(pyridin-4-
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56 ylmethoxy)benzo[d]thiazole scaffold lead to significant improvement in potency.
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Compound	8	9	10	11	12	13	14
X	-NH ₂	-H	-NH ₂				
Y	-H	-Cl	-Cl	-Cl	-Cl	-Cl	-Cl
Z							
CSF1R IC ₅₀ [nM]	52	30	4	2	1.4	1.1	2.4
CSF1R Kd [nM] (fold)	ND	ND	2.5	1.1	0.6	1.2	1.9
cKIT Kd [nM] (fold)	ND	ND	79 (31)	44 (40)	180 (300)	170 (141)	1000 (526)
FLT3 Kd [nM] (fold)	ND	ND	170 (68)	65 (59)	74 (86)	400 (333)	320 (168)
PDGFR α Kd [nM] (fold)	ND	ND	46 (18)	1.3 (1.2)	12 (20)	28 (23)	94 (49)
PDGFR β Kd [nM] (fold)	ND	ND	3.8 (1.5)	0.84 (0.76)	19 (32)	1.2 (1)	5.6 (3)
Phospho- CSF1R (THP- 1) IC ₅₀ [nM]	ND	ND	ND	ND	183	ND	ND

Phospho-PDGFR β (HEK293) IC ₅₀ [nM] (Fold X)	ND	ND	ND	ND	446 (2.4)	ND	ND
MNSF60 IC ₅₀ [nM]	2269	1204	228	50	133	98	189
NS0 IC ₅₀ [nM]	18,809	14,989	41,666	27,500	47,945	ND	ND

To assess broad kinase selectivity, compound **12** was evaluated against a panel of 468 kinases (Figure 3). It exhibited an acceptable selectivity profile, with significant activity against only a handful of related type III kinases at 1 μ M including c-KIT, FLT3, PDGFR α and PDGFR β (Table S1).



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3 **Figure 3.** Selectivity profile of compound **12** against a panel of 468 kinases at 1 μ M test
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7 concentration. The larger the red circle, the greater the inhibition. Six kinases with percent
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10 of control < 10: CSF1R, cKIT, FLT3, AURB, PDGFR α and PDGFR β . See also Table S1,
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14 Supporting information.

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18 Compound **12** displayed low to moderate microsomal and hepatic clearance in *in vitro*
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21 microsomal and hepatocyte preparations and high plasma protein binding. It exhibited a
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24 suitable *in vivo* PK profile across species, with low clearance, reasonable half-life and
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28 good oral bioavailability (Table 3).

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35 **Table 3.** Microsomal and hepatic stability, plasma protein binding and *in vivo* PK profile of
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39 compound **12**.

Specie	<i>In vitro</i> ADME properties of	<i>In vivo</i> pharmacokinetic data of compound 12
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s	Plasma protein binding (%) bound)	Hepato cytes CLint,s caled [ml /min	Microso mes CLint,sc aled [ml /min	Dose IV/PO [mg/kg]	CL [mL/min/kg] (IV)	Vss [L/kg]	T _{1/2} [h] (IV)	Oral F [%]
Mouse	>99	11	55	0.3/10	23	1.8	1.6	>90
Rat	>99	30	68	0.3/3	3.2	0.75	3	67
Dog	>99	155	293	0.3/3	12	1.3	4.8	34
Monke	>99	58	95	0.3/1	1.2	0.6	7.0	72
Human	>99	10	25					

Given its *in vitro* and *in vivo* profiles, compound **12** was used to probe CSF1R biology, with BLZ945 serving as a comparitor in these experiements. An immunohistochemistry (IHC) assay was used to measure the well-established macrophage marker F4/80 in PANC02 tumors from mice that had been treated with daily oral doses of 200 mg/kg compound **12** or BLZ945 for 7 days (n=4 mice/group). Treatment with each compound resulted in similar extent of reduction of F4/80 positive cells within the tumors (Figures 4A-D), consistent with macrophage depletion. As an orthogonal measure of compound activity, CSF1R protein levels were measured by ELISA in tumors from mice similarly treated, and were found to be depleted to a similar extent by both compounds (Figure 4E) (n=4 mice/group). Furthermore, as an additional measure of anti-macrophage activity, mRNA transcripts of well known macrophage expressed genes were measured, and

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3 similar to the IHC and ELISA results, 200 mg/kg of compound **12** or BLZ945 (n=3
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7 mice/group) reduced the expression of the panel by 62-93% (Figure 4F). Similar
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10 depletion of macrophages was observed for MC38 and EMT6 tumors treated with
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14 compound **12** or BLZ945 (data not shown). Taken together, treatment of PANC02 tumors
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17 with compound **12** reduced tumor macrophages to a similar extent as BLZ945.
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21 Treatment of mice bearing MC38 allograft tumors with 200 mg/kg compound **12** (n=10
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24 mice/group) resulted in 62% reduction in tumor growth (Figure 4G), consistent with the notion
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27 that inhibition of CSF1R has therapeutic potential, possibly through macrophage depletion.
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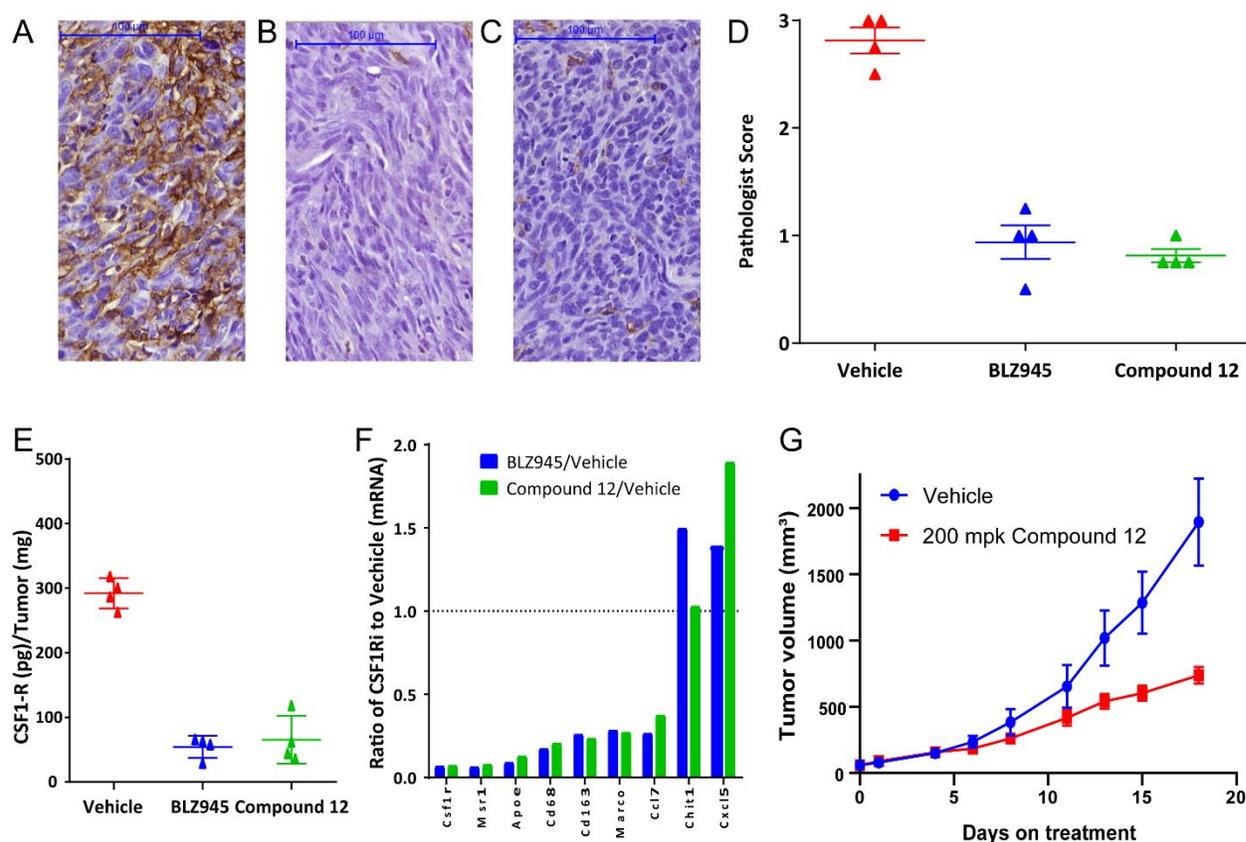


Figure 4. CSF1R inhibitors depletes tumor macrophages and reduces tumor growth. IHC for F4/80 for PANC02 tumors in mice that were treated daily for 7 days with (A) vehicle, (B) 200 mg/kg BLZ945 or (C) 200 mg/kg compound 12 (n=4 mice/group). (D) Pathologist scored quantification of F4/80 IHC signal in PANC02 tumors from mice treated daily for 7 days with vehicle, 200 mg/kg BLZ945 or 200 mg/kg compound 12 (n=4 mice/group). (E) Relative CSF1R protein expression in PANC02 tumors from mice treated daily for 7 days with vehicle, 200 mg/kg BLZ945, or 200 mg/kg compound 12 as measured using ELISA

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4 (n = 4 mice/group). (F) Relative mRNA expression of known macrophage genes in
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7 PANC02 tumors from mice treated daily for 7 days with vehicle, 200 mg/kg BLZ945, or
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10 200 mg/kg compound **12** (n=3 mice/group) as measured using a nanostring code set. (G)
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14 Growth of MC38 tumors implanted subcutaneously in mice and treated daily with vehicle
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17 or 200 mg/kg compound **12** (n=10 mice/group).
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22 While the anti-tumor impact of compound **12** was encouraging, we were not satisfied
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25 with the overall potency, physicochemical properties and the level of selectivity that this
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28 inhibitor exhibited toward related type III RTKs, especially toward PDGFR β . Therefore,
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31 we continued our quest to identify inhibitors with significantly improved potency,
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34 properties and selectivity. In order to improve the selectivity profile of the benzothiazole
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37 scaffold, we used a combination of X-ray structures from the PDB and the patent
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40 literature. Sequence and structural comparison of the CSF1R active site with c-KIT, FLT3,
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43 PDGFR α and PDGFR β ³⁵ revealed that CSF1R is unique among these kinases; the
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46 CSF1R Gly795 residue aligns with a cysteine in the other related kinases (Figures 5A
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49 and 5B). Substitution at the 4-position of the benzothiazole core would fill the space
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adjacent to Gly795 in CSF1R, while this substitution pattern would not be tolerated in c-KIT, FLT3, PDGFR α or PDGFR β as it would lead to a steric clash with the cysteine residue (Figure 5C). Furthermore, overlaying models of compound **12** and GW2580, (Figure 5D), an inhibitor that showed high selectivity toward CSF1R over other related kinases, but not TrkA, led to the same conclusion.

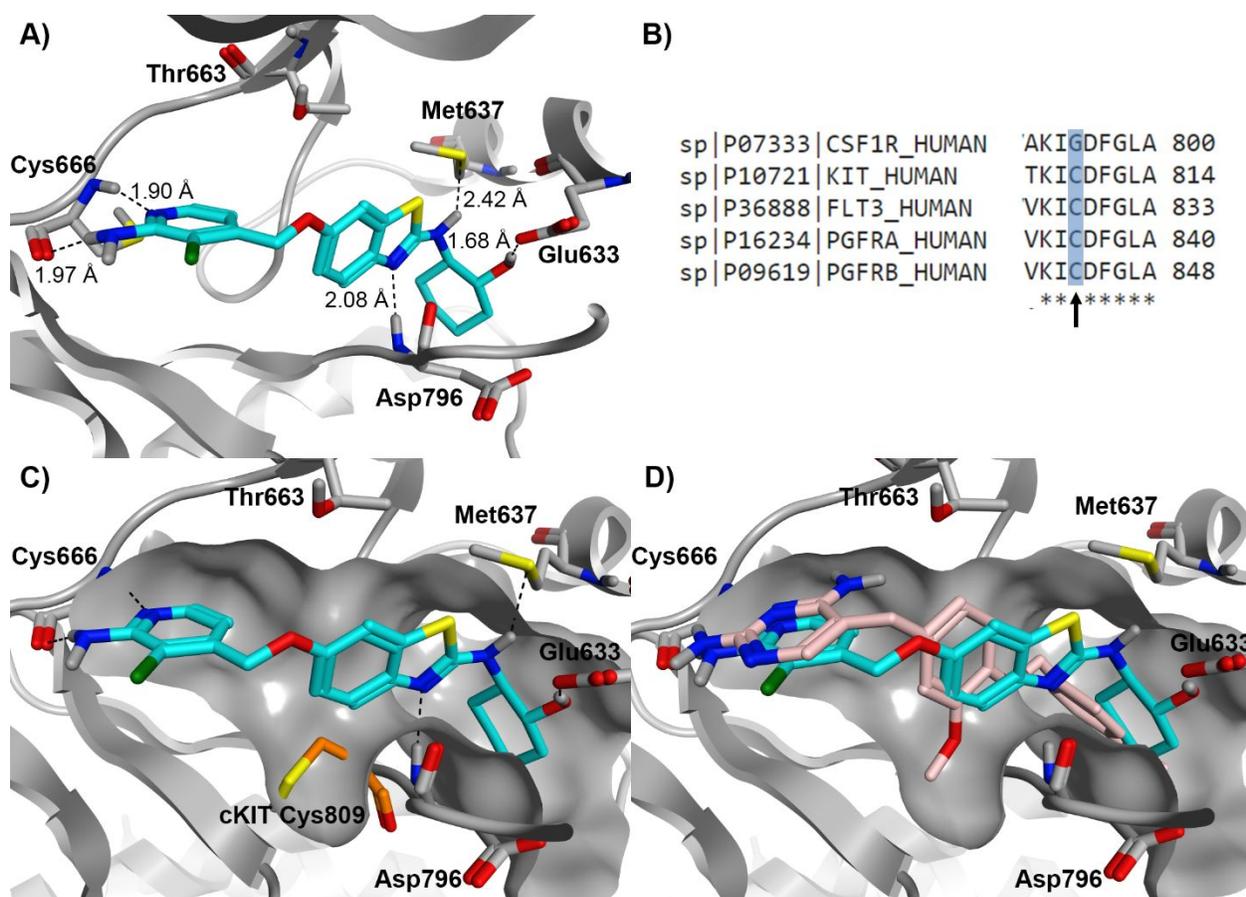


Figure 5. A) Model of compound **12** bound to CSF1R (PDB 3LCO). B) Sequence alignment of class III receptor tyrosine kinases highlighting a key difference in the xDFG

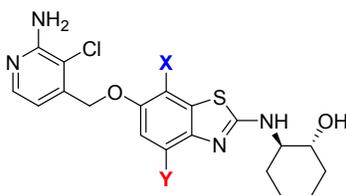
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3 motif. C) Overlay of cKIT Cys809 (orange; PDB 4HVS) with model of compound **12** bound
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7 to CSF1R shows a small pocket that is present in CSF1R that is filled by a cysteine side
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10 chain in other class III RTKs. D) Overlay of GW2580 (pink) with model of compound **12**
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14 shows a methoxy group filling this pocket.
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21 To test this hypothesis, we synthesized the 4-methoxy substituted derivative of **12**,
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24 compound **15**. Indeed, this modification maintained high affinity towards CSF1R in
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27 binding and cellular activity assays, and led to a dramatic improvement in selectivity
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31 against related kinases cKIT, FLT3, PDGFR α and PDGFR β . The same high level of
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35 selectivity was observed in the cellular phospho-PDGFR β target engagement assay
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38 (Table 4).
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41 To understand whether introducing other substituents into the 4-position would lead to
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45 the same level of selectivity increase, we performed a focused SAR study. We found that
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48 introduction of a trifluoromethoxy substituent into the 4-position (**16**) leads to decreased
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51 cellular potency. Incorporation of a fluoro- (**17**) or chloro (**18**) substituent was tolerated
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55 for potency, however, only lower levels of selectivity was achieved compared to that of
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3 the methoxy-substituted analog **15**. Further structural analysis revealed that a hydrophobic
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7 pocket formed by the sidechains of Thr663 and Met637 could potentially accommodate
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10 a substituent in the 7-position of the benzothiazole core and that filling this pocket could
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14 lead to a potency improvement. To test this hypothesis, the 7-chloro- (**19**) and 7-fluoro
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17 benzothiazole (**20**) analogs were prepared. These inhibitors revealed that while a
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21 substituent in the 7-position was indeed tolerated for potency, this modification was
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24 detrimental to selectivity.

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32 **Table 4.** SAR study of substituted benzothiazole analogs leads to dramatic improvement
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35 of selectivity.
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Compound #	15	16	17	18	19	20
X	-H	-H	-H	-H	-Cl	-F
Y	-OMe	-OCF ₃	-Cl	-F	-H	-H

CSF1R IC50 [nM]	4	17	1	1	1.2	1.1
CSF1R Kd [nM] (Fold X)	0.6	ND	8.1	2.5	3.4	1.3
FLT3 Kd [nM] (Fold X)	30,000 (>1875)	ND	590 (72)	1300 (520)	5.4 (1.58)	23 (17)
cKit Kd [nM] (Fold X)	30,000 (>1875)	ND	>30,000 (>3703)	2100 (840)	530 (98)	790 (607)
PDGFR α Kd [nM] (Fold X)	30,000 (>1875)	ND	>30,000 (>3703)	370 (148)	12 (3.5)	32 (24)
PDGFR β Kd [nM] (Fold X)	30,000 (>1875)	ND	>30,000 (>3703)	230 (92)	4.1 (1.2)	6.7 (5.1)
MNSF60 IC50 [nM]	345	930	288	125	104	194

Phospho- CSF1R (THP- 1) [nM]	243	ND	191	127	176	ND
Phospho- PDGFR β (HEK293) [nM] (Fold X)	>50,000 (>200)	ND	4114 (20)	1339 (10)	250 (1.4)	ND
cLogP	3.5	5.2	4.32	3.8	4.3	3.8
PPB [% bound], (r/h)	>99 / 98.7	ND	>99 / >99	>99 / 98.6	ND	ND
CL _{int,scaled} [mL/min/kg] (r/h)	99/38	ND	78/32	91/19	ND	ND

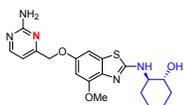
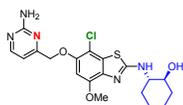
Introducing a substituent into the 4-position led to the desired selectivity improvement, however, these inhibitors possessed suboptimal *in vitro* DMPK properties, particularly unfavorable metabolic turnover in rat and human microsomes (Table 4).

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3 Analyzing the correlation between liver microsomal intrinsic clearance and cLogP for
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7 nearly one hundred analogs that were synthesized in the 6-methoxy benzothiazole series
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10 revealed that inhibitors with a cLogP > 3.5 tended to have high clearance in human liver
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13 microsomes (Figure S1). Based on this observation we targeted the design and synthesis
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16 of inhibitors with a cLogP of less than 3.5 to improve metabolic stability, while retaining
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19 favorable enzymatic and cellular potency.
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24 To increase polarity and modulate physicochemical properties, we devised SAR studies
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27 in the hinge as well as deep pocket area. Decreasing the cLogP by structural
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30 modifications in the hinge region quickly led to improved microsomal stability, while
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33 selectivity was preserved as demonstrated by compound **21** (Table 5). Reversing the
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36 stereochemistry of the amino- and hydroxyl- substituents on the cyclohexyl ring led to
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39 improved potency, while maintaining selectivity and favorable *in vitro* DMPK properties
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42 as shown by compound **22**. Introduction of a 7-chloro substituent onto the benzothiazole
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45 core led to further improvement in potency, as exemplified by compound **23**, without a
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48 significant deleterious effect on selectivity or physicochemical properties.
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Since compounds **22** and **23** exhibited acceptable enzymatic and cellular potency, as well as a promising *in vitro* ADME profile, we decided to evaluate these inhibitors *in vivo*. Both inhibitors had a suitable *in vivo* PK profiles across species with low to moderate clearance, reasonable half-life and excellent oral bioavailability in rat and mouse, and with moderate oral bioavailability in dog (Table S2).

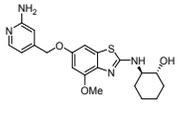
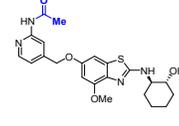
Table 5. Property optimization of benzothiazole analogs.

			
Compound #	21	22	23
CSF1R IC ₅₀ [nM]	21	7	2
CSF1R Kd [nM]	6.7	4.1	2.6
FLT3 [nM]	>30,000	>30,000	>30,000
(Fold X)	(>4,477)	(7,317)	(>11,538)
cKit [nM]	4,800	>30,000	>30,000
(Fold X)	(750)	(7,317)	(>11,538)
PDGFR α [nM]	4,100	>30,000	24,000

(Fold X)	(611)	(7,317)	(9,230)
PDGFR β [nM]	4,900	>30,000	27,000
(Fold X)	(731)	(7,317)	(10,384)
MNSF60 IC ₅₀ [nM]	504	178	60
Phospho-CSF1R [nM] (THP-1)	486	215	63
Phospho-PDGFR β [nM] (HEK293)	41000	>50000	34254
(Fold X)	(84)	(>23)	(543)
cLogP	2.41	2.41	3.02
PPB [% bound], (r/h)	90.4/77.1	92.2/77.6	98.3/97.2
CLint,scaled [mL/min/kg] (r/h)	24/10	20/9.5	43/33

These compounds exhibited excellent overall properties, however, we were not satisfied with the cellular potency of these inhibitors. SAR studies performed earlier on aminopyridine derivative **24** revealed that introduction of an acetamide onto the aminopyridine moiety of **24** resulted in a significant boost in potency (Table 6).

Table 6. Potency improvement by introducing an acetamide onto compound **24**.

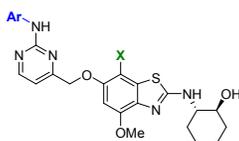
		
Compound #	24	25
CSF1R IC ₅₀ [nM]	76	4
MNSF60 IC ₅₀ [nM]	1455	82

Although compound **25** possessed excellent potency, it suffered from significant non-NADPH-dependant microsomal metabolism; incubation with human and mouse liver microsomes for 45 min led to 80% and 54% parent remaining, respectively, even in the absence of NADPH (Table S4.) Metabolite identification studies in human and mouse liver microsomes confirmed that the major metabolite is the hydrolysis product of the amide of compound **25** (Table S5).

Based on this information we hypothesized that we would be able to further boost the potency, while maintaining optimal properties, if we introduced a substituent onto the amino pyrimidine moiety of inhibitor **22**. However, instead of introducing a hydrolytically unstable amide, we decided to incorporate a urea, as well as a series of heterocyclic

groups including pyrazole, triazole and pyridine. Assessing the enzymatic and cellular potency as well as microsomal stability of these inhibitors clearly indicated that the methylpyrazole analog IACS-9439 (**1**) stands out. IACS-9439 (**1**) displayed the predicted potency boost, with single-digit nanomolar potency in the MNSF-60 cell phenotypical assay and possessed reasonable metabolic stability when incubated with human and rat microsomal preparations. To test whether we could further improve the potency, we also prepared the 7-chloro analog of IACS-9439. While this analog displayed excellent potency, it had high metabolic turnover in rat and human microsomes.

Table 7. SAR studies to improve potency of selective benzothiazole analogs.



	R = 	R = 	R = 	R = 	R = 	R = 	R = 	R = 	R = 
	X = H	X = H	X = H	X = H	X = H	X = H	X = H	X = H	X = Cl
Compound #	26	1	27	28	29	30	31	32	33

CSF1R IC50 [nM]	11	1.7	9	0.81	48	2.6	5.7	626	1.2
Cell. IC50 [nM] (MNFS- 60)	270	7	105	7	1304	26	48	2511	14
cLogP	2.37	3.11	3.7	2.98	3.07	3.05	3.32	3.46	3.71
CLint,scaled [mL/min/kg] (r/h)	19/23	38/14	46.2/1 7	120/32	ND	87/61	25/43	ND	124/65

Based on the excellent potency and selectivity profile, we decided to further evaluate IACS-9439 (1). To assess the selectivity, the inhibitor was tested against a panel of kinases (ScanEdge, 97-member kinase panel, DiscoverX) at 1 μ M (Figure 6). Profiling revealed that IACS-9439 exhibits a high level of selectivity for CSF1R with no significant inhibition of other kinases; the outcome was also in agreement with the results of binding affinity determination that confirmed the high selectivity of IACS-9439 for CSF1R vs FLT3 (9,500-fold), cKIT (>17,000-fold), PDGFR α (1,900-fold) and PDGFR β (450-fold). Notably, IACS-9439 (1) displayed superior selectivity when compared to BLZ945 in the above kinase panel. These results were also confirmed in a cellular context, where IACS-9439

not only showed improved potency in the pCSF1R cellular target engagement assay in comparison with BLZ945 (IC₅₀ of 17 nM vs 155 nM), but also significantly higher selectivity for CSF1R against PDGFRβ (150-fold vs 3.7-fold) (Table 8).

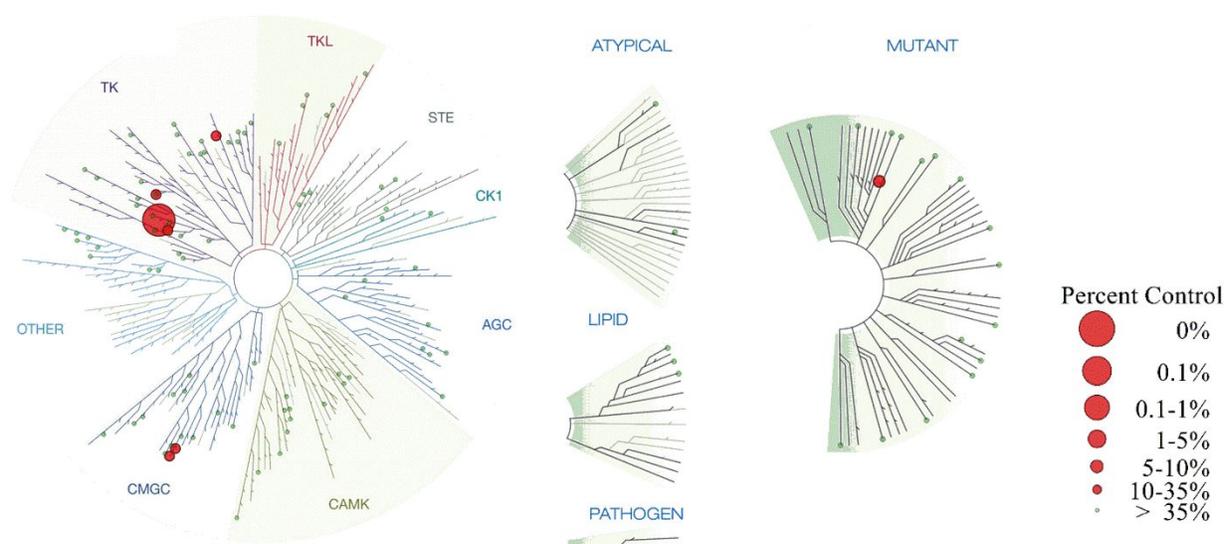


Figure 6. Selectivity profile of IACS-9439 (**1**) against a panel of 97 kinases (DiscoverX) at 1 uM test concentration. The larger the red circle, the greater the inhibition. One kinase with percent of control < 10: CSF1R. See also Table S3, Supporting information.

Table 8. Selectivity profile of IACS-9439 (1) and BLZ945 in binding affinity assays against type III kinases CSF1R, FLT3, cKIT, PDGFR α and PDGFR β and in cellular pCSFR1 and pPDGFR β target engagement assays.

Compound #	BLZ945	IACS-9439 (1)
Kd [nM]		1
(Fold X)	3.4	
CSF1R		
FLT3 [nM]	8700	9,500
(Fold X)	(2552)	(9,500)
cKIT [nM]	3000	17,000
(Fold X)	(882)	(17,000)
PDGFR α [nM]	210	1,900
(Fold X)	(61)	(1,900)
PDGFR β [nM]	46	450
(Fold X)	(13)	(450)
Phospho-CSF1R [nM] (THP-1)	155	17

Phospho- PDGFR β (HEK293) [nM] (Fold X)	579 (3.7)	3560 (150)
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IACS-9439 (1) had low to moderate microsomal stability in rat and human microsomes and high microsomal turnover in mouse, dog and monkey microsomes. It had an acceptable free plasma concentration across species, with a free fraction (fu) of 6.5% in human (Table 9). IACS-9439 (1) possessed an suitable *in vivo* PK profile in rat and mouse, with moderate clearance, acceptable half-life and excellent oral bioavailability. In dog and monkey, the compound had higher clearance and shorter half-life, as well as lower oral bioavailability.

Table 9. Microsomal and hepatic stability, plasma protein binding and *in vivo* PK profile of IACS-9439.

Species	<i>In vitro</i> ADME properties of IACS-9439			<i>In vivo</i> pharmacokinetic data of IACS-9439				
	Plasma protein binding (%)	Hepato cytes CL _{int,s} calculated [mL/min]	Microsomes CL _{int,sc} aled [mL/min]	Dose IV/PO [mg/kg]	CL [mL/min/kg] (IV)	V _{ss} [L/kg]	T _{1/2} [h] (IV)	Oral F [%]
Mouse	>99	21	91	0.3/10	14	1	2.2	100
Rat	97.9	15	38	0.3/3	13	3.4	1.7	81
Dog	95.2	64	293	0.3/3	30	3	1.37	37
Monkey	98.4	37	208	0.3/1	7.2	0.45	0.96	21
Human	93.5	32	14					

To confirm the biological activity of IACS-9439 (1) and compare it to compound 12 and BLZ945, we performed a series of *in vivo* experiments utilizing PANC02 and MC38 syngeneic tumor models. MC38 tumor bearing mice were treated with 20, 60 or 200 mg/kg BLZ945, compound 12 or IACS-9439 (1) for 10 days (n=3 mice/group) and similar dose dependent reductions in macrophages were observed by IHC and mRNAs using nanostring (Figure 7A) for each of the compounds.

To better understand the PK/PD relationship, mice bearing PANC02 tumors were treated with 10, 25, 50, 100 or 200 mg/kg IACS-9439 (1) or BLZ945 for 5 days (n=4 mice/group) before harvesting tumor and plasma to measure changes in EMR1 (F4/80)

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3 mRNA, as a measure of macrophages, and CD4 mRNA, as a measure of CD4 T-cells
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7 and compare these to the concentration of IACS-9439 (1) or BLZ945 in the plasma. For
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10 both compounds and both mRNAs, there was a clear PK/PD relationship with IACS-9439
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13 (1) working at lower plasma concentrations (Figure 7B-C), a $\sim 3 \mu\text{M}$ IC_{50} for IACS-9439
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16 (1) compared to $\sim 18 \mu\text{M}$ IC_{50} for BLZ945 for EMR1 mRNA and an $\sim 18 \mu\text{M}$ IC_{50} for IACS-
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19 9439 (1) compared to an $\sim 56 \mu\text{M}$ IC_{50} for BLZ945 for CD4 mRNA.
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24 CSF1R inhibitors have been reported to promote macrophage polarization toward the
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27 M1 phenotype.⁸ Consistent with these reports, we found, using flow cytometry, that
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30 treatment of mice bearing MC38 tumors with 10, 50 or 200 mg/kg IACS-9439 (1) or
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33 BLZ945 for 10 days (n=10 mice/group) resulted in a dose dependent increase in the
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36 percentage of macrophages of the M1 class, with a concomitant dose dependent
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39 decrease in the percentage of macrophages in the tumors that were of the M2 phenotype
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42 (Figure 7D). This data is consistent with treatment of tumors with IACS-9439 (1) and the
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45 other CSF1R inhibitors shifting the tumor macrophage balance from a pro-tumor M2
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49 phenotype to an anti-tumor M1 phenotype.
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The functional consequence of this shift in macrophage biology, reduction of M2 macrophages, while increasing the percentage of M1 macrophages is highlighted by the observed identical decreases in the growth of MC38 tumors in mice treated with 200 mg/kg of IACS-9439 (1) or BLZ945 (Figure 7E) (n=10 mice/group). Taken together, the data indicate that IACS-9439 (1) is a potent selective inhibitor of CSF1R which impacts macrophage biology and the growth of tumors in immune competent environments.

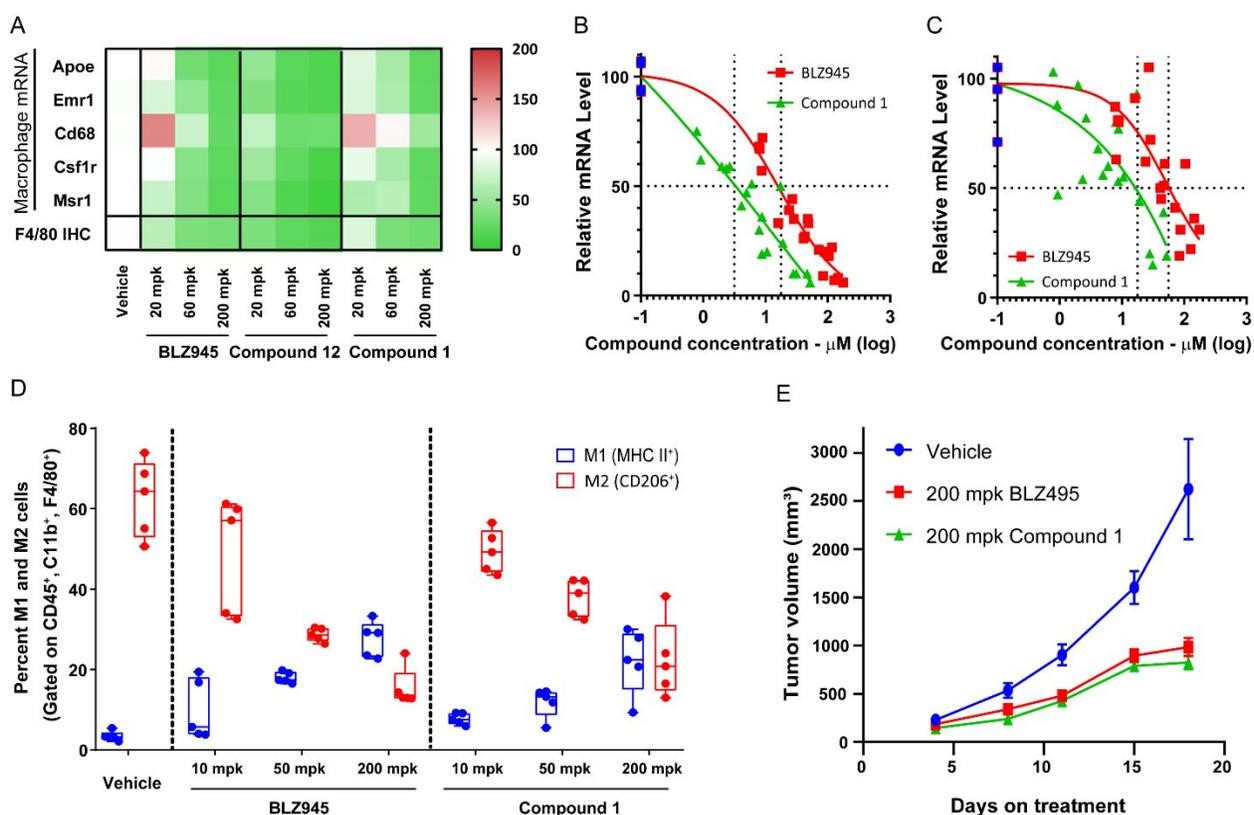


Figure 7. IACS-9439 (1) depletes tumor macrophages, promotes M1 macrophage and inhibits tumor growth. (A) Heat map of nanostring and IHC measuring relative

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3 macrophage mRNAs or F4/80 protein levels from MC38 tumors treated daily for 10 days
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7 with vehicle or 20, 60 or 200 mg/kg of BLZ945, compound **12** or IACS-9439 (**1**) (n=3
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10 mice/group). Note that 100 = no change (white) compared to vehicle treated mice. (B)
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13 Relative EMR1 (F4/80) or (C) CD4 mRNA from PANC02 tumors treated daily for 10 days
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16 with 10, 25, 50, 100 or 200 mg/kg BLZ945 or IACS-9439 (**1**) (n=4 mice/group). Tumor
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19 samples from CSF1Ri treated mice were normalized to vehicle treated mice and plotted
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22 in relation to the concentration of compound in the plasma. Note that vehicle was
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27 artificially set as 0.1 μ M to enable curve fitting. (D) Flow cytometry depicting the percent
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30 of M1 (MHC II+) and M2 (CD206+) macrophages (CD45+, C11b+, F4/80+) in MC38
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33 tumors from mice treated daily for 10 days with vehicle, 10, 50 or 200 mg/kg BLZ945 or
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36 IACS-9439 (**1**) (n=10 mice/group). (E) Growth of MC38 tumors implanted subcutaneously
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39 in mice and treated daily with vehicle, BLZ945 or 200 mg/kg IACS-9439 (**1**) (n=10
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45 mice/group).

51 CHEMISTRY

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4 Compounds **2**, **3** and **8-20** were synthesized as described in Scheme 1. Compound **2**
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7 was prepared starting from 2-bromo-6-methoxybenzo[d]thiazole **34** which was reacted with
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10 cyclohexylamine in the presence of K_2CO_3 . Subsequent demethylation with BBr_3 afforded
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12 intermediate **35**, which was alkylated with 3-(chloromethyl)pyridine to afford compound **2**.
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14 Compound **3** was prepared in a similar manner, utilizing 4-(chloromethyl)pyridine as the
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16 alkylating agent in the last step. Compound **8** was obtained following a similar route, by alkylating
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18 intermediate **35** with tert-butyl 4-(chloromethyl)pyridin-2-ylcarbamate, followed by removal of
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20 the Boc group by TFA to provide final compound **8**. Alkylation of intermediate **35** with 3-chloro-
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22 4-(chloromethyl)pyridine afforded compound **9**. To obtain compound **10**, intermediate **35**
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25 was reacted with 3-chloro-4-(chloromethyl)-N-(4-methoxybenzyl)pyridin-2-amine **44**,
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27 which was prepared in three steps from 2,3-dichloroisonicotinic acid **43**. Final
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29 deprotection with TFA provided the desired compound **10**. Compounds **11** and **12** were
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31 synthesized in a similar fashion. 2-Bromo-6-methoxybenzo[d]thiazole **34** was reacted
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33 with (1S,2S)-2-aminocyclohexan-1-ol or (1R,2R)-2-aminocyclohexan-1-ol, respectively,
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35 and the resulting product was demethylated using BBr_3 to provide intermediate **35**.
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37 Subsequent alkylation with 3-chloro-4-(chloromethyl)-N-(4-methoxybenzyl)pyridin-2-
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39 amine **44** and deprotection with TFA provided the final products **11** and **12**. To synthesize
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3 compounds **13** and **14**, 2-chloro-6-methoxybenzo[d]thiazole **34** was demethylated and
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7 alkylated with 3-chloro-4-(chloromethyl)-N-(4-methoxybenzyl)pyridin-2-amine **44**. S_NAr
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10 reaction with (1R,2S)-2-aminocyclohexan-1-ol or (1S,2R)-2-aminocyclohexan-1-ol and
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13 subsequent deprotection provided the desired products **13** and **14**. Compound **15** was
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16 synthesized starting from 4-fluoro-2-methoxy-1-nitrobenzene **38**, which was reacted with
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21 BnOH under S_NAr conditions and subsequently reduced to the corresponding aniline
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24 derivative **39**. The benzothiazole ring was formed by treating compound **39** with KSCN
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27 and CuSO₄ in MeOH. Subsequent reaction with *t*-BuONO and CuBr₂ led to intermediate
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31 **40**. S_NAr reaction of **40** with (1R,2R)-2-aminocyclohexan-1-ol followed by deprotection
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33
34 with TFA provided the intermediate **35**. Alkylation of **35** with 3-chloro-4-(chloromethyl)-
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36
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38 N-(4-methoxybenzyl)pyridin-2-amine **44** and subsequent deprotection led to analog **15**.
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42 Compound **16** was synthesized in a slightly different manner. Thiazole derivative **42** was
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45 prepared by reacting 4-bromo-2-(trifluoromethoxy)aniline **41** with KSCN in the presence
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48 of Br₂ and AcOH and subsequent treatment of the product with *t*-BuONO and CuBr₂. S_NAr
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51 reaction of **42** with (1R,2R)-2-aminocyclohexan-1-ol followed by introduction of the
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54
55 4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl functionality by Pd(dppf)Cl₂ catalyzed
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3 coupling with B_2Pin_2 and subsequent treatment with H_2O_2 led to phenol intermediate **35**.
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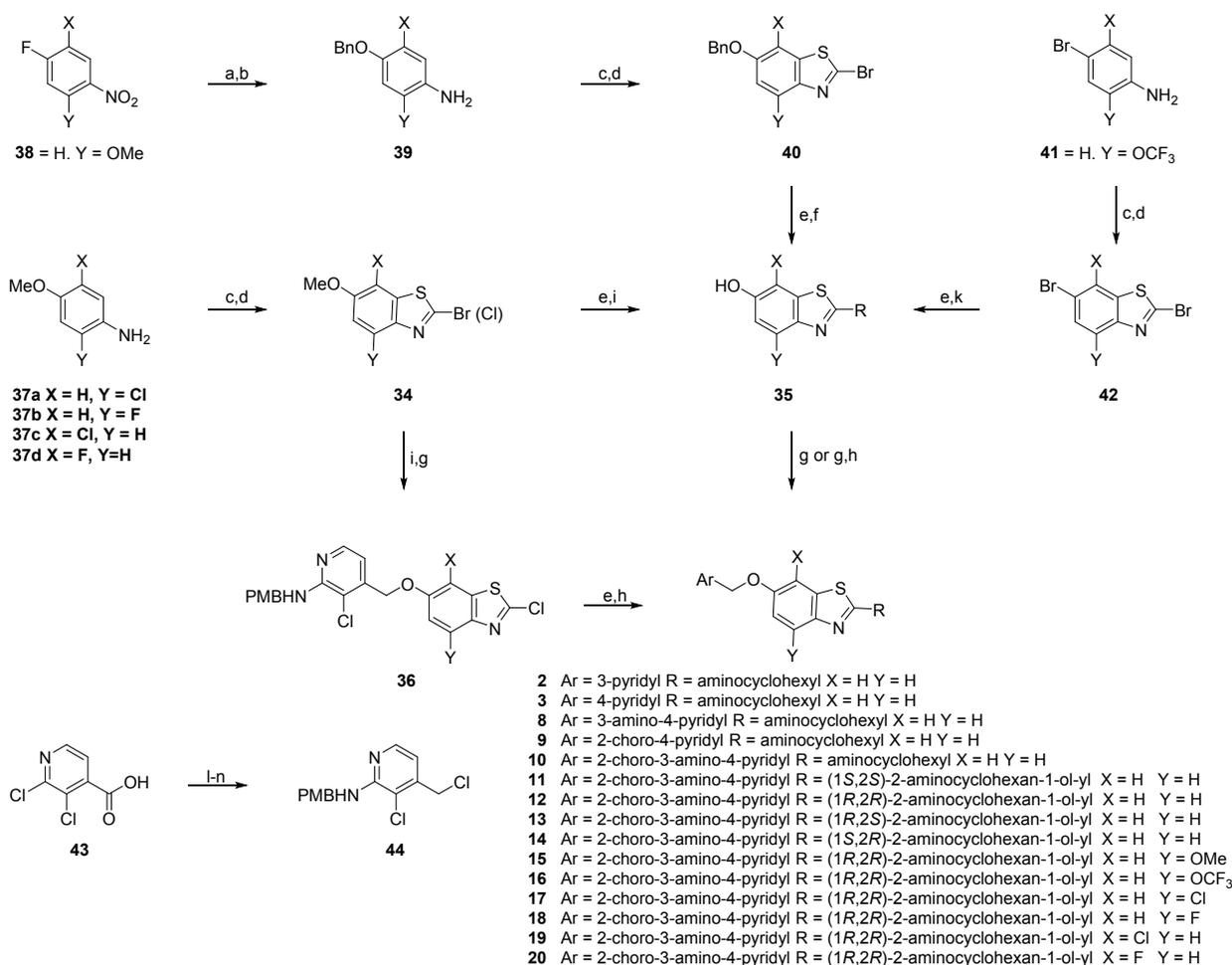
6
7 Alkylation and subsequent deprotection provided the desired product **16**. Compounds **17-**
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10 **20** were prepared according to the sequence as it was described for compound **12**,
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13 utilizing the appropriately substituted 2-bromo-6-methoxybenzo[d]thiazole intermediates
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17 **34**, which in turn could be obtained from substituted anilines **37a-d** in two steps.
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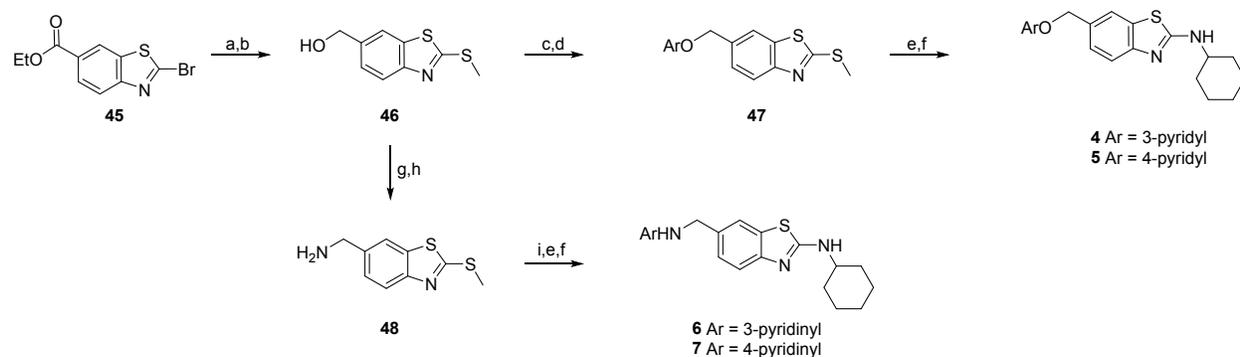
20
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24 **Scheme 1**. Chemical synthesis of compounds **2**, **3** and **8-20**.
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Reagents and conditions: a) BnOH, NaH, 0°C, DMF, 99%; b) N₂H₄-H₂O, Raney-Ni, rt, MeOH, 88%; c) KSCN, CuSO₄, 90°C, MeOH, 37%; or KSCN, Br₂, HOAc, 0°C to rt, 4h, AcOH, 12.5-78%; d) t-BuONO, CuBr₂, 0°C to rt, MeCN, 30-53%; e) amine, K₂CO₃, DMSO, 100°C, 81%; or amine, DIPEA, DMF, 110°C, 78-100%; or amine, 110°C, 79%; or amine, Na₂CO₃, NMP, 125°C, 66-70%; f) TFA, 110°C, 100%; g) ArCH₂Cl, K₂CO₃, DMF, 80°C, 42-88%; or ArCH₂Cl, Cs₂CO₃, DMF, 80°C, 27-80%; h) TFA, rt, quant.; i) BBr₃, 0°C to rt, DCM, 78-90%; j) B₂Pin₂, KOAc, Pd(dppf)Cl₂, 100°C, 1,4-dioxane, quant.; k) H₂O₂, rt, 1,4-dioxane, 60%; l) BH₃-THF; 60°C, 45%; m) 4-methoxybenzyl amine, 150°C, 64%; n) SOCl₂, rt, 2h, DCM, 94%.

Compounds **4-7** were synthesized as described in Scheme 2. 2-Bromobenzo[d]thiazole-6-carboxylate **45** was reduced with DIBAL-H and subsequently reacted with sodium thiomethoxide to provide intermediate **46**. Mesylate formation and reaction with pyridin-3-ylmethanol or pyridin-4-ylmethanol, respectively, led to intermediate **47**, which, upon oxidation with *m*CPBA, and subsequent S_NAr reaction provided compounds **4** and **5**. To obtain compounds **6** and **7**, intermediate **46** was treated with DPPA and the resulting azide was reduced to the corresponding amine **48** using Lindlar's catalyst. Amine **48** was reacted with 3-iodopyridine or 4-iodopyridine, respectively. Oxidation of intermediate **48** followed by S_NAr reaction led to compounds **6** and **7**.

Scheme 2. Chemical synthesis of compounds **4-7**.

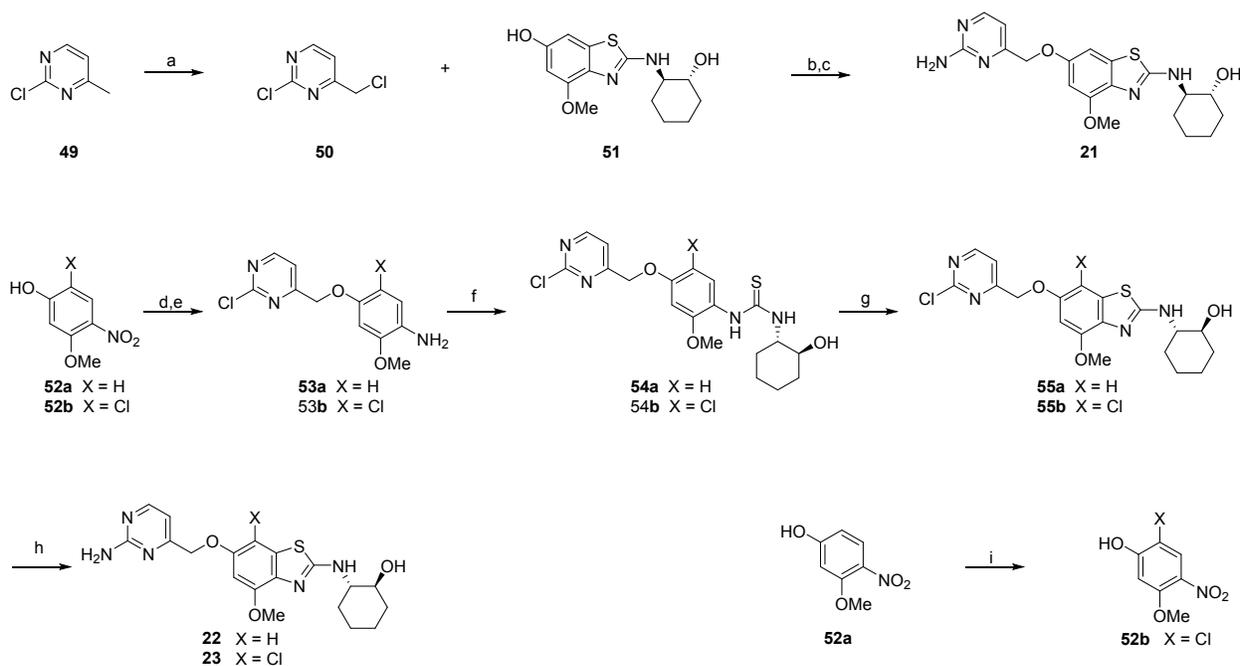


Reagents and conditions: a) DIBAL-H, THF, 0°C-rt 4h, 98%; b) MeSNa, DMF, 12h, 71%; c) MsCl, DIPEA, DMF, DCM, 0°C-rt, 12h, quant.; d) pyridin-3-ylmethanol or pyridin-4-ylmethanol, *t*BuOK, DMA, 100°C, 2h, quant.; e) *m*-CPBA, DCM, 2h, quant.; f) cyclohexanamine, DIPEA, DMA, 120°C 2h, 18%; g) DPPA, DBU, THF, rt, 3h, 92%; h)

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3 Lindlar Cat., H₂, EtOH, 2h; i) 3-iodopyridine or 4-iodopyridine, Cu, CsOAc, DMSO, 90°C,
4 2h, 78%.
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11 Compounds **21-23** were synthesized as described in Scheme 3. 2-Chloro-4-
12 methylpyrimidine **49** was treated with NCS in the presence of AIBN in CCl₄. The resulting
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14 chloromethyl pyridine derivative **50** was reacted with benzothiazole derivative **51**.
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19 Subsequent S_NAr reaction with NH₃-H₂O provided the desired product **21**. To synthesize
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Scheme 3. Chemical synthesis of compounds 21-23.

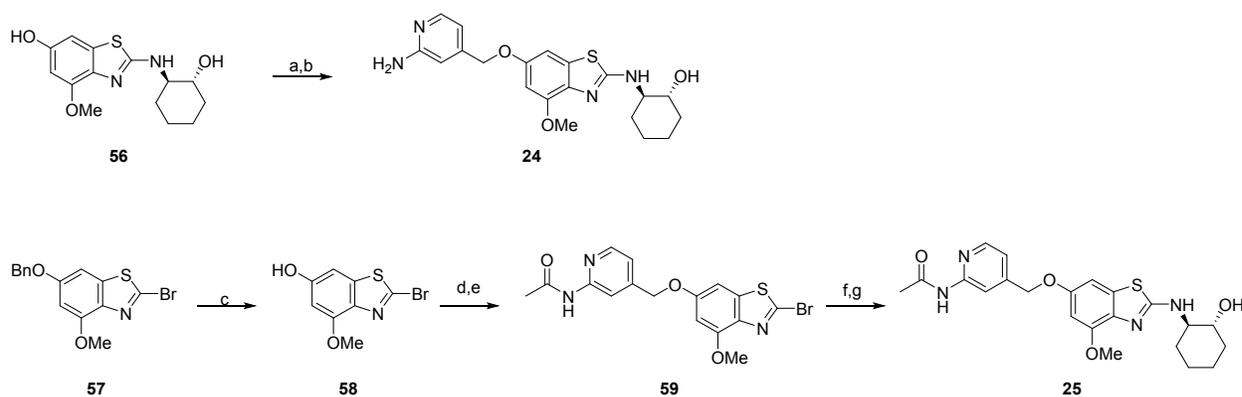


Reagents and conditions: a) NH₃, H₂O 90°C, 3h, 49%; b) AIBN, NCS, CCl₄, 18h, 67%; c) 50, Cs₂CO₃, rt, 4h, DMF, 8%; d) 50, K₂CO₃, rt, 20h, DMF, 93%; e) 10% Pt-C, rt, 2h, THF-MeOH, (5:1), 97%; f) di(1H-imidazol-1-yl)methanethione, (1S,2S)-2-aminocyclohexan-1-ol, rt, 4h, DCM, 96%; g) BnMe₃NBr₃, BSTFA, rt, 20 min, DCM, 95%; h) NH₃, (7M, in MeOH), 120°C, 4h, 64%; i) NCS, 50°C, 2h, DCM, quant.

Compounds 24 and 25 were synthesized as described in Scheme 4. Intermediate 56 was alkylated with tert-butyl (4-(chloromethyl)pyridin-2-yl)carbamate and was subsequently deprotected to provide the desired product 24. Compound 25 was prepared

1
2
3 following a modified sequence, starting with intermediate **57** that was deprotected to
4
5
6 afford benzothiazole derivative **58**. Mitsunobu reaction with tert-butyl (4-
7
8 (hydroxymethyl)pyridin-2-yl)carbamate yielded intermediate **59**. Removal of the Boc
9
10 (hydroxymethyl)pyridin-2-yl)carbamate yielded intermediate **59**. Removal of the Boc
11
12 group by TFA, followed by acylation with Ac₂O in the presence of pyridine, and
13
14 subsequent S_NAr reaction with (1R,2R)-2-aminocyclohexan-1-ol provided the desired
15
16
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18 product **25**.
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28 **Scheme 4. Chemical synthesis of compounds **24** and **25**.**

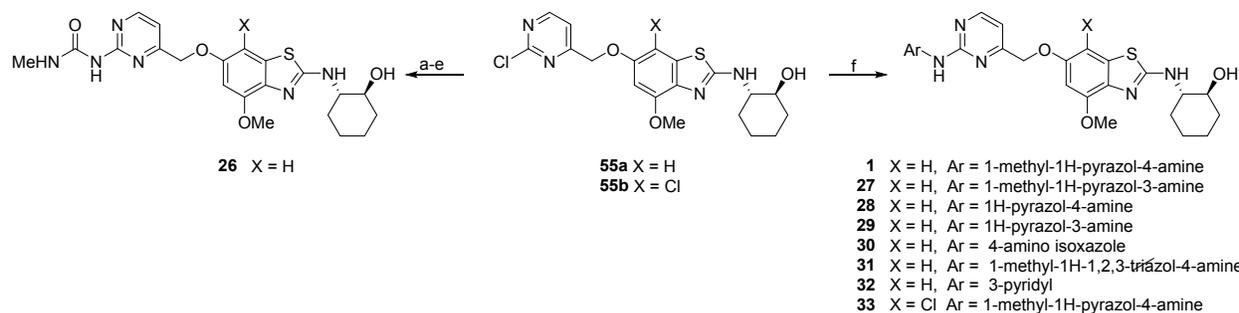


Reagents and conditions: a) tert-butyl (4-(chloromethyl)pyridin-2-yl)carbamate, Cs₂CO₃, 80°C, 2h, DMF, 17%; b) TFA, rt, 16h, 35%; c) TFA, 65°C, 24h 40h, 24%; d) tert-butyl (4-(hydroxymethyl)pyridin-2-yl)carbamate, Ph₃P, DtBAD, rt, 16h, THF, 93%; e) TFA, rt, 3h, DCM, 60%; f) Ac₂O, pyr, 60°C, 1h, DMF, 88%; g) (1R,2R)-2-aminocyclohexan-1-ol DIPEA, DMA, 100°C, 12h, 38%.

Compounds **26-33** were prepared as shown in Scheme 5. Compound **26** was prepared from intermediate **55a** in five steps. First, the secondary alcohol was protected with a TBS group. Subsequent S_NAr reaction with ammonia, followed by treatment with phenylchloroformate and methylamine, and final deprotection yielded compound **26**.

Compounds **1** and **26-31** were prepared from intermediate **55a** in an S_NAr reaction with the appropriate aromatic amine. Compound **32** was prepared from intermediate **55b**, in a similar manner, via an S_NAr reaction with 1-methyl-1H-pyrazol-4-amine to provide the desired product.

Scheme 5. Chemical synthesis of compounds **26-33**.



1
2
3
4 Reagents and conditions: a) TBSCl, imid., DMF, 12h, rt, 91%; b) NH₃ in *i*-PrOH
5 (2M), 120°C, MW, 8h, 72%; c) phenylchloroformate, Py, DMAP (Cat.), 3h, rt; d) MeNH₂,
6 12h, rt, 51% for two steps; e) TBAF, 12h, rt, 40%; f) i. 1-methyl-1H-pyrazol-4-amine
7 DIPEA, 120°C, 10h, DMA, 46% (**1**); ii. 1-methyl-1H-pyrazol-3-amine, DIPEA, 120°C, 5h,
8 2-Propanol, MW, 15%; (**27**); iii. 1H-pyrazol-4-amine, TEA, 120°C, 2h, DMSO, MW, 44%
9 (**28**); iv. 1H-pyrazol-3-amine, Pd₂(dba)₃, Xantphos, Cs₂CO₃, dioxane, 110°C, 2h, 14%
10 (**29**); v. 4-amino isoxazole hydrochloride, TEA, 120°C, 2h, DMSO, MW, 63% (**30**); vi. 1-
11 methyl-1H-1,2,3-triazol-4-amine, TsOH, dioxan, 110°C, 12h, 10% (**31**); vii. 1-methyl-1H-
12 pyrazol-3-amine, DIPEA, 110°C, 18h, , 5% (**32**).
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23 CONCLUSIONS

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27 Rational design and iterative, hypothesis driven potency and properties optimization
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29
30 allowed us to develop a highly potent, exquisitely selective and orally bioavailable CSF1R
31
32
33 inhibitor, IACS-9439 (**1**). Deep analysis of the active site in the DGF-out conformation of
34
35
36 kinases CSF1R, cKit, FLT3, PDGFR α and PDGFR β enabled us to rapidly design analogs
37
38
39
40 of the initial lead, compound **12**, with drastically improved selectivity. Comprehensive
41
42
43 evaluation of the selectivity of these inhibitors was enabled by utilizing a suite of assays
44
45
46 including kinase binding as well as enzymatic assays for the above kinases. To evaluate
47
48
49 selectivity in a cellular context, CSF1R and PDGFR β cellular target engagement assays
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51
52
53 were implemented. Subsequent hypothesis-driven properties optimization of the highly
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3 selective CSF1R inhibitor **15** led to analogs with improved DMPK properties. Finally, a
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6
7 significant boost in potency was achieved by introducing a substituent onto the hinge
8
9
10 binding motif, resulting in the identification of IACS-9439 (**1**). The excellent potency,
11
12
13 exquisite CSF1R selectivity and *in vivo* PK profile of IACS-9439 (**1**) allowed us to utilize
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15
16
17 it as an *in vivo* tool to probe CSF1R-mediated biology. We demonstrated that treatment
18
19
20
21 with IACS-9439 (**1**) led to dose-dependent reduction of macrophages and promoted
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23
24 macrophage polarization toward the M1 phenotype in the syngeneic tumor model, and
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28 led to tumor growth inhibition in MC38 and PANC01 tumor models. We also developed a
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30
31 novel, highly efficient and scalable synthetic route for the preparation of 4-methoxy
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35 substituted benzothiazole analogs and IACS-9439 (**1**).
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42 EXPERIMENTAL SECTION

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44
45 **Synthetic methods:** The inhibitors described were synthesized by employing standard
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47
48 chemical transformations. Starting materials and reagents were purchased from
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50
51 commercial suppliers such as Sigma-Aldrich, Alfa Aesar, TCI, or Acros and will be used
52
53
54
55 without further purification unless otherwise indicated. Anhydrous solvents (e.g., THF,
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1
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3 DMF, DMA, DMSO, MeOH, DCM, toluene) were purchased from Sigma-Aldrich and used
4
5
6 directly. Purification of inhibitors were performed by column chromatography utilizing a
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8
9 Biotage system applying Biotage SNAP columns with Biotage KP-Sil silica or Biotage Zip
10
11
12 Si columns with Biotage KP-Sil silica or a Teledyne ISCO system with RediSep Rf normal
13
14
15 phase silica cartridges. Other inhibitors were purified by preparative HPLC using a Waters
16
17
18 Autopurify system with a Waters Xbridge Prep C18 5 μm OBD, 19 mm \times 150 mm or 50
19
20
21 mm \times 100 mm column and SQ detector mass spectrometer with ESI ionization. The
22
23
24 identity of all compounds with reported biological activity was confirmed by NMR
25
26
27 spectroscopy and Low Resolution Mass Spectrometry, and for selected analogs, High
28
29
30 Resolution Mass Spectrometry. Purity of all compounds with reported biological activity
31
32
33 was $\geq 95\%$ and was determined by Ultra Performance Liquid Chromatography (UPLC).
34
35
36 NMR spectra were recorded on Bruker instruments operating at 300, 500, or 600 MHz.
37
38
39 NMR spectra were obtained as CDCl_3 , CD_3OD , D_2O , $(\text{CD}_3)_2\text{SO}$, $(\text{CD}_3)_2\text{CO}$, C_6D_6 , or
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41
42 CD_3CN solutions (reported in ppm), using tetramethylsilane (0.00 ppm) or residual solvent
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3 mass spectral were obtained on either a Waters H class UPLC with a Waters Acquity
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5
6 UPLC BEH C18 1.7 μm , 2.1 mm \times 50 mm column, UV detection between 200 and 400
7
8
9
10 nm, evaporating light scattering detection, and a SQ detector mass spectrometer with ESI
11
12
13 ionization or a Water I class UPLC with a Waters Acquity UPLC CSH C18 1.7 μm , 2.1
14
15
16 mm \times 50 mm column, UV detection at 254 and 290 nm, evaporating light scattering
17
18
19 detection, and a SQ detector 2 mass spectrometer with ESI ionization. High-resolution
20
21
22 mass spectra were obtained on a Waters Acquity I-Class UPLC coupled to a LTQ-
23
24
25 Orbitrap Elite mass spectrometer. The injection volume was 5 μL . Chromatographic
26
27
28 separation was performed on a Waters Acquity UPLC BEH C18 1.7 μm , 2.1 mm \times 50 mm
29
30
31 column, at a flow rate of 0.5 mL/min. The mobile phases were 0.1% Acetic Acid in Water
32
33
34 (solvent A) and 0.1% Acetic acid in acetonitrile (solvent B). The gradient had a total run
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36
37 time of 18 minutes and was as follows: 0-2 minutes 5% B; 2-12 minutes from 5% to 65%B;
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12-14 minutes from 65% to 95% B; 14-16 minutes at 95%B; 16-16.1 minutes from 95%
to 5%B and 16.1-18 minutes at 5% B. The column temperature was kept at 40 $^{\circ}\text{C}$. The
samples were analyzed using the positive electrospray ionization (ESI) mode. The ESI
source temperature was set at 375 $^{\circ}\text{C}$, the capillary temperature at 320 $^{\circ}\text{C}$ and the

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electrospray voltage at 4.1 kV. Sheath and auxiliary gas were 45 arbitrary unit and 10 arbitrary unit, respectively.

Synthesis of N-cyclohexyl-6-(pyridin-3-ylmethoxy)benzo[d]thiazol-2-amine (2)

Step 1: N-cyclohexyl-6-methoxybenzo[d]thiazol-2-amine. A mixture of 2-bromo-6-methoxybenzo[d]thiazole (2.0 g, 8.2 mmol), cyclohexanamine (1.1 g, 11 mmol) and K_2CO_3 (2.3 g, 16 mmol) in DMSO (15 mL) was heated at 100°C for 22 h. The reaction mixture was cooled to rt, diluted with water (25 mL) and extracted with EtOAc (3 x 30 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography (10% to 30% EtOAc in Hexanes) to provide N-cyclohexyl-6-methoxybenzo[d]thiazol-2-amine (1.7 g, 81%) as a yellow solid. MS (ES+) $C_{14}H_{18}N_2OS$ requires: 262, found: 263[M+H]⁺.

Step 2: 2-(cyclohexylamino)benzo[d]thiazol-6-ol. To a solution of N-cyclohexyl-6-methoxybenzo[d]thiazol-2-amine (1.7 g, 6.7 mmol) in DCM (30 mL), at 0°C, BBr_3 (4.2 g, 17 mmol) was added slowly and the resulting mixture was stirred at rt for 3 h. The reaction mixture was slowly quenched with ice-water. $NaHCO_3$ (4.2 g, 51 mmol) was

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2
3 added. The precipitate was filtered to afford 2-(cyclohexylamino)benzo[d]thiazol-6-ol (1.3
4
5
6
7 g, 78%) as a beige solid. MS (ES+) C₁₃H₁₆N₂OS requires: 248, found: 249[M+H]⁺.
8
9

10 *Step 3: N-cyclohexyl-6-(pyridin-3-ylmethoxy)benzo[d]thiazol-2-amine*. A mixture of 2-
11
12 (cyclohexylamino)benzo[d]thiazol-6-ol (75 mg, 0.30 mmol), 3-(chloromethyl)pyridine
13
14 hydrochloride (59 mg, 0.36 mmol) and K₂CO₃ (83 mg, 0.60 mmol) in DMF (1 mL) was
15
16
17 heated to 80°C and was stirred at this temperature for 16 h. The mixture was purified by
18
19
20 preparative HPLC (Mobile phase: A = 0.1% TFA/H₂O, B = 0.1% TFA/MeCN to provide N-
21
22
23 cyclohexyl-6-(pyridin-3-ylmethoxy)benzo[d]thiazol-2-amine (89 mg, 88%) as a brown
24
25
26 solid. MS (ES+) C₁₉H₂₁N₃OS requires: 339, found: 340[M+H]⁺; ¹H NMR (500 MHz, *d*₆-
27
28 DMSO) δ 8.84 (s, 1H), 8.72 (d, *J* = 4.3 Hz, 1H), 8.63 (s, 1H), 8.22 (d, *J* = 7.7 Hz, 1H),
29
30
31 7.73 (dd, *J* = 7.3, 5.7 Hz, 1H), 7.50 (d, *J* = 1.9 Hz, 1H), 7.35 (d, *J* = 8.7 Hz, 1H), 7.01 (dd,
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41
42 *J* = 8.8, 2.0 Hz, 1H), 5.23 (s, 2H), 3.73 – 3.60 (m, 1H), 2.02 – 1.93 (m, 2H), 1.77 – 1.68
43
44
45 (m, 2H), 1.62 – 1.54 (m, 1H), 1.39 – 1.17 (m, 5H).
46
47
48

49 **Synthesis of N-cyclohexyl-6-(pyridin-4-ylmethoxy)benzo[d]thiazol-2-amine (3)**

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51

52 *Step 1: N-cyclohexyl-6-(pyridin-4-ylmethoxy)benzo[d]thiazol-2-amine*. A mixture of 2-
53
54 (cyclohexylamino)benzo[d]thiazol-6-ol (75 mg, 0.30 mmol), 4-(chloromethyl)pyridine
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3 hydrochloride (59 mg, 0.36 mmol) and K_2CO_3 (83 mg, 0.60 mmol) in DMF was heated at
4
5
6
7 80°C for 2 h. The residue was purified by preparative HPLC (Mobile phase: A = 0.1%
8
9
10 TFA/ H_2O , B = 0.1% TFA/MeCN to give N-cyclohexyl-6-(pyridin-4-
11
12
13 ylmethoxy)benzo[d]thiazol-2-amine (79 mg, 78%) as a beige solid. MS (ES+) $C_{19}H_{21}N_3OS$
14
15
16
17 requires: 339, found: 340[M+H]⁺; ¹H NMR (500 MHz, d_6 -DMSO) δ 8.81 (s, 1H), 7.81 (d,
18
19
20 J = 2.5 Hz, 2H), 7.44 (d, J = 2.6 Hz, 1H), 7.33 (d, J = 8.8 Hz, 1H), 6.97 (dd, J = 8.8, 2.6
21
22 Hz, 1H), 5.34 (s, 2H), 3.70 – 3.61 (m, 1H), 2.02 – 1.93 (m, 2H), 1.77 – 1.68 (m, 2H), 1.62
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24
25 – 1.55 (m, 1H), 1.38 – 1.18 (m, 5H).
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31 **Synthesis of 6-((2-aminopyridin-4-yl)methoxy)-N-cyclohexylbenzo[d]thiazol-2-amine**
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35 **(8):**
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38 *Step 1 : tert-butyl 4-(chloromethyl)pyridin-2-ylcarbamate.* To a solution of tert-butyl 4-
39
40 (hydroxymethyl)pyridin-2-ylcarbamate (67 mg, 0.30 mmol) in DCM (2 mL) was added
41
42 $SOCl_2$ (10 drops). The reaction mixture was stirred at rt for 2 h. The mixture was
43
44 concentrated to provide tert-butyl 4-(chloromethyl)pyridin-2-ylcarbamate as a yellow solid
45
46
47
48
49 which was used directly in the next step without further purification. MS (ES+)
50
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55
56 $C_{11}H_{15}ClN_2O_2$ requires: 242, found: 243[M+H]⁺.
57
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4 *Step 2: 6-((2-aminopyridin-4-yl)methoxy)-N-cyclohexylbenzo[d]thiazol-2-amine.* A
5
6
7 mixture of 2-(cyclohexylamino)benzo[d]thiazol-6-ol (50 mg, 0.20 mmol) and Cs₂CO₃ (163
8
9
10 mg, 0.50 mmol) in DMF (2 mL) was stirred at rt for 2 h. *Tert*-butyl 4-(chloromethyl)pyridin-
11
12
13 2-ylcarbamate (73 mg, 0.30 mmol) was added. The reaction mixture was stirred at 120°C
14
15
16 for 4 h. The residue was purified by preparative-HPLC (Mobile phase: A = 0.1%
17
18 NH₄HCO₃/H₂O, B = MeCN; Gradient: B = 5 - 95%; 12 min; Column: C18 to provide 6-((2-
19
20
21 aminopyridin-4-yl)methoxy)-N-cyclohexylbenzo[d]thiazol-2-amine (29 mg, 28%) as a
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beige solid. MS (ES+) C₁₉H₂₂N₄OS requires: 354, found: 355 [M+H]⁺; ¹H NMR (400 MHz,
*d*₄-Methanol) δ 7.88 (dd, *J* = 5.4, 0.7 Hz, 1H), 7.33 (d, *J* = 8.8 Hz, 1H), 7.24 (d, *J* = 2.5
Hz, 1H), 6.94 (dd, *J* = 8.8, 2.6 Hz, 1H), 6.72 – 6.65 (m, 2H), 5.02 (s, 2H), 3.69-3.66 (m,
1H), 2.10-2.08 (m, 2H), 1.86 – 1.77 (m, 2H), 1.70-1.68 (m, 1H), 1.47-1.42 (m, 2H), 1.37 –
1.25 (m, 3H).

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Synthesis of 6-((3-chloropyridin-4-yl)methoxy)-N-cyclohexylbenzo[d]thiazol-2-amine
(9)

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2
3 *Step 1: Synthesis of (3-chloropyridin-4-yl)methanol.* To a solution of 3-
4 chloroisonicotinaldehyde (660 mg, 4.66 mmol) in MeOH (20 mL) was added NaBH₄ (530
5 mg, 143 mmol) 0°C. The reaction mixture was stirred at 0°C for 2 h. The reaction was
6
7 quenched with NH₄Cl (sat. aq.), diluted with water (80 mL) and extracted with DCM/MeOH
8
9 20:1 (3 x 30 mL). The organic layer was dried over MgSO₄, and concentrated under
10
11 reduced pressure to provide (3-chloropyridin-4-yl)methanol as a light yellow solid (520
12
13 mg, 78%), which was used in the next step without further purification. MS (ES+)
14
15 C₆H₆ClNO requires: 143, found: 144[M+H]⁺.
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31 *Step 2: Synthesis of 3-chloro-4-(chloromethyl)pyridine.* To a solution of (3-chloropyridin-
32 4-yl)methanol (520 mg, 3.63 mmol) in DCM (4 mL) was added SOCl₂ (2.0 mL, 27 mmol).
33
34 The reaction mixture was stirred at rt for 2 h and concentrated under reduced pressure to
35
36 provide 3-chloro-4-(chloromethyl)pyridine as a tan solid, which was used in the next step
37
38 without further purification. MS (ES+) C₆H₅Cl₂N requires: 161, found: 162[M+H]⁺.
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49 *Step 3: Synthesis of 6-((3-chloropyridin-4-yl)methoxy)-N-cyclohexylbenzo[d]thiazol-2-*
50 *amine.* A mixture of 2-(cyclohexylamino)benzo[d]thiazol-6-ol (40 mg, 0.16 mmol) and
51
52 K₂CO₃ (110 mg, 0.797 mmol) in DMF (2 mL) was stirred at rt for 1 h. 3-Chloro-4-
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(chloromethyl)pyridine (78 mg, 0.48 mmol) was added. The reaction mixture was stirred at rt for 48 h, purified by prep-HPLC (Mobile phase: A = 0.1% NH₄HCO₃/H₂O, B = MeCN; Gradient: B = 5 - 95%; 12 min; Column: C18) to provide 6-((3-chloropyridin-4-yl)methoxy)-N-cyclohexylbenzo[d]thiazol-2-amine (25 mg, 42%) as a yellow solid. MS (ES+) C₁₉H₂₀ClN₃OS requires: 373, found: 374 [M+H]⁺; ¹H NMR (500 MHz, *d*₄-MeOD) δ 8.59 (s, 1H), 8.51 (d, *J* = 5.0 Hz, 1H), 7.70 (d, *J* = 5.0 Hz, 1H), 7.34 (dd, *J* = 23.0, 5.7 Hz, 2H), 7.00 (dd, *J* = 8.8, 2.6 Hz, 1H), 5.25 (s, 2H), 3.84 – 3.52 (m, 1H), 2.13 – 2.04 (m, 2H), 1.87 – 1.75 (m, 2H), 1.69 (dd, *J* = 9.3, 3.8 Hz, 1H), 1.53 – 1.22 (m, 5H).

Synthesis of 6-((2-amino-3-chloropyridin-4-yl)methoxy)-N-cyclohexylbenzo[d]thiazol-2-amine (10)

Step 1: (2,3-dichloropyridin-4-yl)methanol. A mixture of 2,3-dichloroisonicotinic acid (960 mg, 5.00 mmol) and BH₃•THF (1.0 M, 25 mL, 25 eq) was heated at 60°C for 4 h. The reaction mixture was cooled to rt, MeOH (5 mL) was added, and the volatiles were removed under reduced pressure. The mixture was diluted with water (50 mL) and extracted with DCM (3 x 50 mL). The combined organic phases were washed with brine

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4 (1 x 100 mL), dried over Na₂SO₄, filtered and concentrated to provide (2,3-dichloropyridin-
5
6
7 4-yl)methanol (400.5 mg, 45%) as a white solid. MS (ES+) C₆H₅Cl₂NO requires: 178,
8
9
10 found: 179 [M+H]⁺.

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13
14 *Step 2: (3-chloro-2-(4-methoxybenzylamino)pyridin-4-yl)methanol.* A mixture of (2,3-
15
16
17 dichloropyridin-4-yl)methanol (200 mg, 1.12 mmol) and (4-methoxyphenyl)methanamine
18
19
20 (1.0 mL, 7.6 mmol) was heated at 150°C for 4 h. The residue was purified by mass-
21
22
23 triggered preparative HPLC (Mobile phase: A = 0.1% TFA/H₂O, B = 0.1% TFA/MeCN;
24
25
26
27 Gradient: B = 10 - 90%; 12 min; Column: C18) to provide (3-chloro-2-(4-
28
29
30 methoxybenzylamino)pyridin-4-yl)methanol (201 mg, 64%) as a white solid. MS (ES+)
31
32
33 C₁₄H₁₅ClN₂O₂ requires: 278, found: 279 [M+H]⁺. ¹H NMR (500 MHz, DMSO-*d*₆) δ 7.94 (d,
34
35
36 *J* = 5.3 Hz, 1H), 7.24 (d, *J* = 8.7 Hz, 2H), 6.91 – 6.72 (m, 3H), 4.52 (d, *J* = 12.7 Hz, 4H),
37
38
39 3.71 (s, 3H).

40
41
42
43
44
45 *Step 3: Synthesis of 3-chloro-4-(chloromethyl)-N-(4-methoxybenzyl)pyridin-2-amine.* A
46
47
48 mixture of (3-chloro-2-(4-methoxybenzylamino)pyridin-4-yl)methanol (50 mg, 0.18 mmol)
49
50
51 and SOCl₂ (2.0 mL, 27 mmol) in DCM (2 mL) was stirred at rt for 2 h. The volatiles were
52
53
54
55 removed under reduced pressure to provide 3-chloro-4-(chloromethyl)-N-(4-
56
57
58

1
2
3 methoxybenzyl)pyridin-2-amine (51 mg, 94%) as a yellow solid. MS (ES+) $C_{14}H_{14}Cl_2N_2O$
4
5
6
7 requires: 297, found: 298 $[M+H]^+$. 1H NMR (500 MHz, $DMSO-d_6$) δ 7.96 (d, J = 5.5 Hz,
8
9
10 1H), 7.25 (t, J = 31.7 Hz, 2H), 6.86 (d, J = 8.7 Hz, 3H), 4.76 (s, 2H), 4.60 (d, J = 15.5 Hz,
11
12
13 2H), 3.77 – 3.68 (m, 3H).
14
15

16
17 *Step 4: 6-((3-chloro-2-(4-methoxybenzylamino)pyridin-4-yl)methoxy)-N-*
18
19
20 *cyclohexylbenzo[d]thiazol-2-amine.* A mixture of 3-chloro-4-(chloromethyl)-N-(4-
21
22 methoxybenzyl)pyridin-2-amine (51 mg, 0.17 mmol), 2-
23
24 (cyclohexylamino)benzo[d]thiazol-6-ol (42 mg, 0.17 mmol) and Cs_2CO_3 (110 mg, 0.338
25
26 mmol) in DMF (2 mL) was stirred at 80°C for 3 h. The volatiles were removed under
27
28 reduced pressure. The residue was purified by preparative HPLC (Mobile phase: A =
29
30 0.1% TFA/ H_2O , B = 0.1% TFA/MeCN; Gradient: B = 5 - 95%; 12 min; Column: C18) to
31
32 provide 6-((3-chloro-2-(4-methoxybenzylamino)pyridin-4-yl)methoxy)-N-
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51 $C_{27}H_{29}ClN_4O_3S$ requires: 524, found: 525 $[M+H]^+$.

52
53 *Step 5: 6-((2-amino-3-chloropyridin-4-yl)methoxy)-N-cyclohexylbenzo[d]thiazol-2-*
54
55
56
57
58
59
60 *amine.* A mixture of 6-((3-chloro-2-(4-methoxybenzylamino)pyridin-4-yl)methoxy)-N-

1
2
3 cyclohexylbenzo[d]thiazol-2-amine (31 mg, 0.057 mmol) in TFA (2 mL) was stirred at rt
4
5
6
7 for 8 h. The volatiles were removed under reduced pressure. The residue was purified by
8
9
10 preparative HPLC (Mobile phase: A = 0.1% NH₄HCO₃/H₂O, B = MeCN; Gradient: B = 5 -
11
12
13 95%; 12 min; Column: C18) to provide 6-((2-amino-3-chloropyridin-4-yl)methoxy)-N-
14
15
16 cyclohexylbenzo[d]thiazol-2-amine (7 mg, 18%) as a white solid. MS (ES+)
17
18
19
20
21 C₁₉H₂₁ClN₄OS requires: 388, found: 389 [M+H]⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.66
22
23
24 (s, 1H), 7.97 (d, *J* = 5.7 Hz, 1H), 7.49 (d, *J* = 2.5 Hz, 1H), 7.36 (d, *J* = 8.8 Hz, 1H), 7.00
25
26
27 (d, *J* = 8.8 Hz, 1H), 6.86 (d, *J* = 5.7 Hz, 1H), 5.18 (s, 2H), 3.68 (dt, *J* = 13.5, 8.7 Hz, 1H),
28
29
30
31 2.06 – 1.91 (m, 2H), 1.80 – 1.65 (m, 2H), 1.58 (dd, *J* = 16.3, 10.0 Hz, 1H), 1.29 (qd, *J* =
32
33
34
35 25.5, 12.8 Hz, 5H).

41
42 **Synthesis of (1*S*,2*S*)-2-(6-((2-amino-3-chloropyridin-4-yl)methoxy)benzo[d]thiazol-2-**
43
44
45 **ylamino)cyclohexanol (11)**

46
47
48
49 *Step 1: (1*S*,2*S*)-2-((6-methoxybenzo[d]thiazol-2-yl)amino)cyclohexanol.* To a solution
50
51
52 of 2-bromo-6-methoxybenzo[d]thiazole (200 mg, 0.82 mmol) and (1*S*,2*S*)-2-
53
54
55 aminocyclohexanol (145mg, 1.23 mmol) in DMA (1 mL) was added DIPEA (0.28 mL,
56
57
58
59
60

1
2
3 1.64 mmol) and the resulting mixture was stirred at 100°C for 12 h. The reaction mixture
4
5
6
7 was diluted with EtOAc (15 mL) and washed with water (10 mL). The layers were
8
9
10 separated, and the organic layer was washed with brine (10 mL), dried over MgSO₄,
11
12
13 filtered and concentrated under reduced pressure. The residue was purified via silica gel
14
15
16 chromatography (40 to 80 % EtOAc in hexanes to provide (1S,2S)-2-((6-
17
18 methoxybenzo[d]thiazol-2-yl)amino)cyclohexanol (180 mg, 0.647 mmol, 79 % yield) as a
19
20
21 white solid. MS (ES+) C₁₄H₁₈N₂O₂S requires: 278, found: 279 [M+H] +.
22
23
24
25
26
27

28 *Step 2: 2-(((1S,2S)-2-hydroxycyclohexyl)amino)benzo[d]thiazol-6-ol.* To a solution of
29
30
31 (1S,2S)-2-((6-methoxybenzo[d]thiazol-2-yl)amino)cyclohexanol (150mg, 0.54 mmol) in
32
33
34 DCM (5 mL) at 0 °C, was added BBr₃ (51 μL, 0.54mmol) slowly. The resulting mixture
35
36
37 was stirred at rt for 3 h. The reaction mixture was then quenched slowly with ice-water (5
38
39
40 mL) followed by sat. NaHCO₃ (5 mL).The reaction mixture was diluted with EtOAc (20
41
42
43 ml).The layers were separated, and the organic layer was washed with NaHCO₃ (5 mL),
44
45
46 dried over MgSO₄, filtered and concentrated under reduced pressure to give 2-(((1S,2S)-
47
48
49 2-hydroxycyclohexyl)amino)benzo[d]thiazol-6-ol (120 mg, 84% yield) as white powder.
50
51
52
53
54
55
56 MS (ES+) C₁₃H₁₆N₂O₂S requires: 264, found: 265 [M+H] +.
57
58
59
60

1
2
3
4 *Step 3:* *(1S,2S)-2-(6-((3-chloro-2-(4-methoxybenzylamino)pyridin-4-*
5
6
7 *yl)methoxy)benzo[d]thiazol-2-ylamino)cyclohexanol.* A mixture of 3-chloro-4-
8
9
10 (chloromethyl)-N-(4-methoxybenzyl)pyridin-2-amine (50 mg, 0.17 mmol), 2-((1S,2S)-2-
11
12 hydroxycyclohexylamino)benzo[d]thiazol-6-ol (45 mg, 0.17 mmol) and Cs₂CO₃ (110 mg,
13
14 0.34 mmol) in DMF (2 mL) was stirred at 80°C for 3 h. The volatiles were removed under
15
16
17 reduced pressure. The residue was purified by preparative HPLC (Mobile phase: A =
18
19
20 0.1% TFA/H₂O, B = 0.1% TFA/MeCN; Gradient: B = 5 - 95%; 12 min; Column: C18) to
21
22
23 give the title compound (30 mg, 34%) as a white solid. MS (ES+) C₂₇H₂₉ClN₄O₃S requires:
24
25
26
27
28
29
30
31 524, found: 525 [M+H]⁺.

32
33
34
35 *Step 4:* *(1S,2S)-2-(6-((2-amino-3-chloropyridin-4-yl)methoxy)benzo[d]thiazol-2-*
36
37
38 *ylamino)cyclohexanol.* A mixture of (1S,2S)-2-(6-((3-chloro-2-(4-
39
40
41 methoxybenzylamino)pyridin-4-yl)methoxy)benzo[d]thiazol-2-ylamino)cyclohexanol (30
42
43
44 mg, 0.06 mmol) in TFA (2 mL) was stirred at rt for 8 h. The volatiles were removed under
45
46
47 reduced pressure. The residue was purified by preparative HPLC (Mobile phase: A =
48
49
50 0.1% NH₄HCO₃/H₂O, B = MeCN; Gradient: B = 5 - 95%; 12 min; Column: C18) to provide
51
52
53
54
55
56 the title compound (17 mg, 73%) as a white solid. MS (ES+) C₁₉H₂₁ClN₄O₂S requires:
57
58
59
60

1
2
3 404, found: 405 [M+H]⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.90 (d, *J* = 5.0 Hz, 1H), 7.74
4
5
6
7 (d, *J* = 7.5 Hz, 1H), 7.37 (d, *J* = 2.6 Hz, 1H), 7.27 (d, *J* = 8.7 Hz, 1H), 6.88 (dd, *J* = 8.7,
8
9
10 2.6 Hz, 1H), 6.72 (d, *J* = 5.0 Hz, 1H), 6.34 (s, 2H), 5.07 (s, 2H), 4.75 (d, *J* = 5.1 Hz, 1H),
11
12
13 3.56 – 3.47 (m, 1H), 3.41 – 3.35 (m, 1H), 2.07 (dd, *J* = 13.4, 7.7 Hz, 1H), 1.93 – 1.85 (m,
14
15
16 1H), 1.68 – 1.57 (m, 2H), 1.32 – 1.14 (m, 4H).
17
18
19
20
21
22
23

24 **Synthesis of (1R,2R)-2-((6-((2-Amino-3-chloropyridin-4-yl)methoxy)benzo[d]thiazol-2-**
25
26
27 **yl)amino)cyclohexan-1-ol (12)**
28
29
30

31 *Step 1: (1R,2R)-2-((6-Methoxybenzo[d]thiazol-2-yl)amino)cyclohexan-1-ol.* A mixture of
32
33
34 2-bromo-6-methoxybenzo[d]thiazole (3.00 g, 12.3 mmol) and (1R,2R)-2-
35
36
37 aminocyclohexanol (4.25 g, 36.9 mmol) was heated at 110 °C for 6 h. The reaction was
38
39
40 cooled to rt, diluted with water (30 mL), and extracted with EtOAc (3 x 30 mL). The
41
42
43 combined organic extracts were dried over Na₂SO₄ and concentrated to provide (1R,2R)-
44
45
46 2-((6-methoxybenzo[d]thiazol-2-yl)amino)cyclohexan-1-ol (3.01 g, 88%) as a brown solid.
47
48
49
50
51
52 MS (ES⁺) C₁₄H₁₈N₂O₂S requires: 278, found: 279 [M+H]⁺. ¹H NMR (500 MHz, CDCl₃) δ
53
54
55 7.37 (d, *J* = 11 Hz, 1H), 7.03 (d, *J* = 3.5 Hz, 1H), 6.84 (dd, *J* = 11, 3.5 Hz, 1H), 6.27 (bs,
56
57
58
59
60

1
2
3
4 1H), 3.78 (s, 3H), 3.4-3.35 (m, 2H), 2.12-2.01 (m, 2H), 1.70-1.65 (m, 2H), 1.38-1.16 (m,
5
6
7 4H).

8
9
10 *Step 2: 2-(((1R,2R)-2-Hydroxycyclohexyl)amino)benzo[d]thiazol-6-ol.* To a solution of
11
12
13
14 2-(6-methoxybenzo[d]thiazol-2-ylamino)cyclohexanol (3.00 g, 10.8 mmol) in DCM (30
15
16
17 mL) at 0 °C, was added boron tribromide (5.4 g, 21 mmol) slowly. The resulting mixture
18
19
20
21 was stirred at rt for 3 h. The reaction mixture was then diluted slowly with ice-water (20
22
23
24 mL) followed by sat. NaHCO₃ (10 mL). The resulting precipitate was filtered and collected
25
26
27
28 to provide 2-(((1R,2R)-2-hydroxycyclohexyl)amino)benzo[d]thiazol-6-ol (2.6 g, 90%) as a
29
30
31 tan solid. MS (ES+) C₁₃H₁₆N₂O₂S requires: 264, found: 265 [M+H]⁺. ¹H NMR (500 MHz,
32
33
34 *d*₄-MeOD) δ 7.12 (d, *J* = 9 Hz, 1H), 6.89 (d, *J* = 2 Hz, 1H), 6.62 (dd, *J* = 9, 2 Hz, 1H), 3.50-
35
36
37 3.41 (m, 1H), 3.31-3.36 (m, 1H), 2.10-2.03 (m, 1H), 1.98-1.93 (m, 1H), 1.70-1.59 (m, 2H),
38
39
40
41 1.36-1.17 (m, 4H).

42
43
44
45 *Step 3: (1R,2R)-2-(6-((3-Chloro-2-(4-methoxybenzylamino)pyridin-4-yl)methoxy)*
46
47
48 *benzo[d]thiazol-2-ylamino)cyclohexanol.* A mixture of 3-chloro-4-(chloromethyl)-N-(4-
49
50
51 methoxybenzyl)pyridin-2-amine (50 mg, 0.17 mmol), 2-(((1R,2R)-2-
52
53
54 hydroxycyclohexylamino)benzo[d]thiazol-6-ol (45 mg, 0.17 mmol) and Cs₂CO₃ (110 mg,
55
56
57
58
59
60

1
2
3
4 0.34 mmol) in DMF (2 mL) was stirred at 80 °C for 3 h. The volatiles were removed under
5
6
7 reduced pressure. The residue was purified by preparative HPLC (Mobile phase: A =
8
9
10 0.1% TFA/H₂O, B = 0.1% TFA/MeCN; Gradient: B = 5 - 95%; 12 min; Column: C18) to
11
12
13 provide (1R,2R)-2-(6-((3-chloro-2-(4-methoxybenzylamino)pyridin-4-yl)methoxy)
14
15
16
17 benzo[d]thiazol-2-ylamino)cyclohexanol (30 mg, 34%) as a white solid. MS (ES+)
18
19
20
21 C₂₇H₂₉ClN₄O₃S requires: 524, found: 525 [M+H]⁺.
22
23

24 *Step 4: (1R,2R)-2-(6-((2-Amino-3-chloropyridin-4-yl)methoxy)benzo[d]thiazol-2-*
25
26
27 *ylamino)cyclohexanol.* A mixture of (1R,2R)-2-(6-((3-chloro-2-(4-
28
29
30
31 methoxybenzylamino)pyridin-4-yl)methoxy) benzo[d]thiazol-2-ylamino)cyclohexanol (30
32
33
34
35 mg, 0.06 mmol) in TFA (2 mL) was stirred at RT for 8 h. The volatiles were removed under
36
37
38 reduced pressure. The residue was purified by preparative HPLC (Mobile phase: A =
39
40
41 0.1% NH₄HCO₃/H₂O, B = MeCN; Gradient: B = 5 - 95%; 12 min; Column: C18) to give
42
43
44
45 (1R,2R)-2-(6-((2-amino-3-chloropyridin-4-yl)methoxy)benzo[d]thiazol-2-
46
47
48
49 ylamino)cyclohexanol (5 mg, 21%) as a white solid. HRMS (ES+) C₁₉H₂₂ClN₄O₂S⁺
50
51
52 requires: 405.1147, found: 405.1151 [M+H]⁺. ¹H NMR (500 MHz, *d*₆-DMSO-*d*₆) δ 7.90 (d,
53
54
55 *J* = 5.0 Hz, 1H), 7.72 (d, *J* = 7.5 Hz, 1H), 7.36 (d, *J* = 2.5 Hz, 1H), 7.26 (d, *J* = 8.5 Hz, 1H),
56
57
58
59
60

1
2
3
4 6.88 (dd, $J = 8.5, 2.5$ Hz, 1H), 6.72 (d, $J = 5.0$ Hz, 1H), 6.31 (s, 2H), 5.07 (s, 2H), 4.73 (d,
5
6
7 $J = 5.5$ Hz, 1H), 3.52~3.48 (m, 1H), 3.36~3.31 (m, 1H), 2.06~2.04 (m, 1H), 1.89~1.86 (m,
8
9
10 1H), 1.64 – 1.60 (m, 2H), 1.29 – 1.17 (m, 4H). ^{13}C NMR (600 MHz, $\text{DMSO-}d_6$) δ 165.00,
11
12
13 155.76, 152.61, 147.29, 145.97, 143.84, 131.20, 117.99, 113.45, 111.59, 111.18, 106.80,
14
15
16
17 71.38, 66.70, 59.48, 39.89, 39.75, 39.61, 39.47, 39.33, 39.19, 39.05, 33.98, 30.64, 23.95,
18
19
20
21 23.60.
22
23

24 **Synthesis of (1R,2S)-2-((6-((2-amino-3-chloropyridin-4-yl)methoxy)benzo[d]thiazol-2-**
25
26
27
28 **yl)amino)cyclohexanol (13)**
29

30
31 *Step 1: 2-chlorobenzo[d]thiazol-6-ol.* To a solution of 2-chloro-6-
32
33
34 methoxybenzo[d]thiazole (150 g, 751 mmol, 1.0 *eq*) in DCM (1.5 L) was added BBr_3 (470
35
36
37 g, 1.9 mol, 180 mL, 2.5 *eq*) drop-wise at $-10^\circ\text{C} - 0^\circ\text{C}$. During the addition, the solid was
38
39
40 precipitated. After the addition, the reaction mixture was stirred at 25°C for 16 hr. Three
41
42
43 additional batches of the same reaction were run. The four batches were combined and
44
45
46 the mixture was poured into cooled water (10 L) slowly, then NaHCO_3 (sat.) solution (6 L)
47
48
49 was added. The mixture was stirred at $0^\circ\text{C} - 5^\circ\text{C}$ for 0.5h, filtered and the filter cake was
50
51
52 washed with water (~10 L) until the pH of filtrate was 7. The filter cake was washed
53
54
55
56
57
58
59
60

1
2
3 with pet. ether (5 L) and dried under vacuum to afford 2-chlorobenzo[d]thiazol-6-ol (507
4
5
6
7 g, 2.59 mol, 86% yield) as the white solid. The product was used in the next step without
8
9
10 further purification. MS (ES+) C₇H₄ClNOS requires: 185, found: 186 [M+H]⁺.

11
12
13
14 *Step 2: 3-chloro-4-(((2-chlorobenzo[d]thiazol-6-yl)oxy)methyl)-N-(4-*
15
16
17 *methoxybenzyl)pyridin-2-amine.* To a solution of 2-chlorobenzo[d]thiazol-6-ol (143g, 733
18
19
20 mmol) in DMF (800 mL) was added 3-chloro-4-(chloromethyl)-N-(4-
21
22
23 methoxybenzyl)pyridin-2-amine (220 g, 733 mmol) and Cs₂CO₃ (477 g, 1.47) at 15°C.
24
25
26
27 The mixture was stirred at 25°C for 16 hr. Water (1L) was added drop-wise and the solid
28
29
30 was precipitated. The suspension was stirred at 25°C for 1 h. Three additional batches
31
32
33 of the same reaction were run. The three batches were combined, filtered, washed with
34
35
36 water (10 L) and the filter cake was collected. The cake was sub packaged slurried in pet
37
38
39 ether/EtOAc = 5/1 (2L, two times), filtered, collected and dried under vacuum to provide
40
41
42 3-chloro-4-(((2-chlorobenzo[d]thiazol-6-yl)oxy)methyl)-N-(4-methoxybenzyl)pyridin-2-
43
44
45
46
47
48 amine (800g, 1.76 mol, 80%) as the light yellow solid. MS (ES+) C₂₁H₁₇Cl₂N₃O₂S
49
50
51 requires: 445, found: 446 [M+H]⁺.

1
2
3
4 *Step 3: (1R,2S)-2-((6-((2-amino-3-chloropyridin-4-yl)methoxy)benzo[d]thiazol-2-*
5
6
7 *yl)amino)cyclohexanol.* To a solution of 3-chloro-4-(((2-chlorobenzo[d]thiazol-6-
8
9
10 *yl)oxy)methyl)-N-(4-methoxybenzyl)pyridin-2-amine* (2.0 g, 4.5 mmol) in NMP (20 mL)
11
12
13
14 was added Na_2CO_3 (2.4 g, 22 mmol) and (1R,2S)-2-aminocyclohexan-1-ol (0.81 mg, 5.4
15
16
17 mmol.). The mixture was stirred at 125°C for 16 hr. The mixture was poured into water
18
19
20
21 (100 mL), extracted with ethyl acetate (2 x 100 mL) and the residue was purified by pre-
22
23
24 HPLC (Mobile phase: A = 0.1% $\text{NH}_4\text{HCO}_3/\text{H}_2\text{O}$, B = MeCN; Gradient: B = 5 - 95%; 12
25
26
27 min; Column: C18) to provide the (1R,2S)-2-((6-((3-chloro-2-((4-
28
29
30 methoxybenzyl)amino)pyridin-4-yl)methoxy)benzo[d]thiazol-2-yl)amino)cyclohexan-1-ol
31
32
33
34 (1.58 g, 2.98 mmol, 66% yield) as a brown solid. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ : 7.94 (d,
35
36
37 $J= 4.8$ Hz, 1H), 7.62 (d, $J= 8.0$ Hz, 1H), 7.37 (d, $J= 2.4$ Hz, 1H), 7.23 - 7.28 (m, 3H), 7.09 (t, $J= 6.4$
38
39
40 Hz, 1H), 6.84 - 6.88 (m, 3H), 6.72 (d, $J= 4.8$ Hz, 1H), 5.08 (s, 2H), 4.65 (d, $J= 4.0$ Hz, 1H), 4.53 (d,
41
42
43 $J= 6.4$ Hz, 2H), 3.92 (s, 1H), 3.79 - 3.84 (m, 1H), 3.71 (s, 3H), 1.48 - 1.71 (m, 6H), 1.29 - 1.34 (m,
44
45
46
47 2H).

48
49
50 *Step 4: (1R,2S)-2-((6-((2-amino-3-chloropyridin-4-yl)methoxy)benzo[d]thiazol-2-*
51
52
53
54 *yl)amino)cyclohexanol:* A solution of (1R,2S)-2-((6-((3-chloro-2-((4-
55
56
57
58
59
60

1
2
3 methoxybenzyl)amino)pyridin-4-yl)methoxy)benzo[d]thiazol-2-yl)amino)cyclohexan-1-ol
4
5
6 (1.0 g, 1.9 mmol) in TFA (10 mL) was stirred at 25°C for 16 hr. The mixture was quenched with water
7 (100 mL) and the pH was adjusted to 9 with NH₄OH (aq., sat.). The solids were filtered and collected.
8
9 The solid was purified by slurring with EtOAc and Pet. Ether 1:3 (40 mL). The mixture was filtered
10 to collect the solids and provide the product (500 mg, 1.22 mmol, 64% yield) as a light brown solid.
11
12
13 ¹H NMR (400 MHz, DMSO-*d*₆) δ: 7.90 (d, *J* = 4.8 Hz, 1H), 7.61 (d, *J* = 7.6 Hz, 1H), 7.36 (d, *J* =
14 2.4 Hz, 1H), 7.27 (d, *J* = 8.8 Hz, 1H), 6.87 (dd, *J* = 2.4 Hz, 8.4 Hz, 1H), 6.72 (d, *J* = 5.2 Hz, 1H), 6.31
15 (s, 2H), 5.07 (s, 2H), 4.64 (d, *J* = 4.0 Hz, 1H), 3.92 (s, 1H), 3.79 - 3.84 (m, 1H), 1.48 - 1.71 (m, 6H),
16 1.29 - 1.34 (m, 2H).
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27
28 **Synthesis of (1S,2R)-2-((6-((2-amino-3-chloropyridin-4-yl)methoxy)benzo[d]thiazol-2-**
29 **yl)amino)cyclohexanol (14)**
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32

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34
35 *Step 1: Synthesis of (1S,2R)-2-((6-((2-amino-3-chloropyridin-4-*
36 *yl)methoxy)benzo[d]thiazol-2-yl)amino)cyclohexanol.* To a solution of 3-chloro-4-(((2-
37 chlorobenzo[d]thiazol-6-yl)oxy)methyl)-N-(4-methoxybenzyl)pyridin-2-amine (2.0 g, 3.8
38 mmol) in NMP (10 mL) were added (1S,2R)-2-aminocyclohexanol (685 mg, 4.52 mmol)
39 and Na₂CO₃ (2.0 g, 19 mmol). The reaction mixture was stirred at 120°C for 16 hr. Water
40 was added (100 mL), and the mixture was extracted with EtOAc (3 x 100 mL). The organic
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1
2
3 phases were washed with brine (2 x 100 mL), dried over Na₂SO₄, filtered and
4
5
6 concentrated under reduced pressure. The residue was purified by prep-HPLC (Mobile
7
8
9
10 phase: A = 0.1% NH₄HCO₃/H₂O, B = MeCN; Gradient: B = 5 - 95%; 12 min; Column:
11
12
13 C18) to provide (1S,2R)-2-((6-((3-chloro-2-((4-methoxybenzyl)amino)pyridin-4-
14
15
16
17
18 yl)methoxy)benzo[d]thiazol-2-yl)amino)cyclohexan-1-ol (2.9 g, 5.3 mmol, 70% yield) as
19
20 the gray solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 7.94 (d, *J* = 5.2 Hz, 1H), 7.61 (d, *J* = 7.6
21
22
23 Hz, 1H), 7.36 (d, *J* = 2.8 Hz, 1H), 7.27 - 7.23 (m, 3H), 7.07 (t, *J* = 6.0 Hz, 1H), 6.87 - 6.83
24
25
26
27 (m, 3H), 6.71 (d, *J* = 5.2 Hz, 1H), 5.07 (s, 2H), 4.64 (d, *J* = 4.0 Hz, 1H), 4.53 (d, *J* = 6.0
28
29
30 Hz, 2H), 3.91 (s, 1H), 3.81 (t, *J* = 8.0 Hz, 1H), 3.70 (s, 3H), 1.70 - 1.44 (m, 6H), 1.33 - 1.28
31
32
33
34 (m, 2H).

35
36
37
38 *Step 2: (1S,2R)-2-((6-((2-amino-3-chloropyridin-4-yl)methoxy)benzo[d]thiazol-2-*
39
40
41
42 *yl)amino)cyclohexanol.* (1S,2R)-2-((6-((3-Chloro-2-((4-methoxybenzyl)amino)pyridin-4-
43
44
45
46 yl)methoxy)benzo[d]thiazol-2-yl)amino)cyclohexan-1-ol (2.3 g, 4.2 mmol) in TFA (23 mL)
47
48
49 was stirred at rt for 16 h. Water (30 ml) was added and the pH was adjusted to 9 with
50
51
52 NH₄OH (aq., sat.). The solids were filtered and collected. The solid was purified by slurring with
53
54
55 EtOAc and Pet. Ether 1:3 (40 mL). The mixture was filtered and the solids were collected to provide
56
57
58
59
60

1
2
3 the product (804 mg, 1.92 mmol, 45%) as a yellow solid. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ :
4
5
6
7 7.89 (d, $J = 5.2$ Hz, 1H), 7.60 (d, $J = 8.0$ Hz, 1H), 7.36 (d, $J = 2.8$ Hz, 1H), 7.27 (d, $J =$
8
9
10 8.4 Hz, 1H), 6.87 (dd, $J = 2.4, 8.8$ Hz, 1H), 6.72 (d, $J = 4.8$ Hz, 1H), 6.31 (s, 2H), 5.06 (s,
11
12
13 2H), 4.64 (d, $J = 4.0$ Hz, 1H), 3.91 (s, 1H), 3.83 - 3.78 (m, 1H), 1.70 - 1.44 (m, 6H), 1.31
14
15
16
17 - 1.28 (m, 2H).
18
19

20
21 **Synthesis of (1R,2R)-2-({6-[(2-Amino-3-chloropyridin-4-yl)methoxy]-4-methoxy-1,3-
22
23
24 benzothiazol-2-yl}amino)cyclohexan-1-ol (15)**
25
26
27

28 *Step 1: 4-(benzyloxy)-2-methoxy-1-nitrobenzene.* To a solution of phenylmethanol
29
30
31 (41.0 g, 380 mmol) in DMF (500 ml) was added NaH (9.60 g, 399 mmol) at 0°C . The
32
33
34
35 reaction mixture was stirred for 0.5 h then 4-fluoro-2-methoxy-1-nitrobenzene (50.0 g, 292
36
37
38 mmol) was added. The mixture was stirred at RT for 1 h. The reaction was quenched with
39
40
41
42 water (500 mL), filtered and concentrated under reduced pressure to give 4-(benzyloxy)-
43
44
45 2-methoxy-1-nitrobenzene (75 g, 99%) as yellow solid. LC-MS (ES+) $\text{C}_{14}\text{H}_{13}\text{NO}_4$ requires:
46
47
48
49 259, found: 260 (M+H) $^+$.
50
51

52 *Step 2: 4-(Benzyloxy)-2-methoxybenzenamine.* To a solution of 4-(benzyloxy)-2-
53
54
55
56 methoxy-1-nitrobenzene (75.0 g, 290 mmol) in MeOH (800 mL) was added Raney-Ni (3.0
57
58
59
60

1
2
3 g) followed by the dropwise addition of $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$ (44.0 g, 870 mmol) at 0 °C. the mixture
4
5
6
7 was stirred at rt for 16h. The reaction mixture was quenched by sat. NH_4OH (200 mL) and
8
9
10 extracted with EtOAc (2 x 100 mL). The organic layers were dried, filtered and
11
12
13 concentrated under reduced pressure to give 4-(benzyloxy)-2-methoxybenzenamine (56
14
15
16 g, 84 %) as yellow liquid. LC-MS (ES+) $\text{C}_{14}\text{H}_{15}\text{NO}_2$ requires: 229, found: 230 (M+H)⁺.
17
18
19

20
21 *Step 3: 6-(Benzyloxy)-4-methoxybenzo[d]thiazol-2-amine.* To a solution of 4-
22
23
24 (benzyloxy)-2-methoxybenzenamine (28.0 g, 0.122 mol) in methanol (600 mL) was added
25
26
27 KSCN (59.3g, 0.611 mol), followed by the addition of anhydrous CuSO_4 (195g, 1.22 mol).
28
29
30
31 The mixture was stirred for 16h at 90 °C and concentrated under reduced pressure. The
32
33
34 residue was dissolved in DCM (500 mL), the precipitate was filtered and the filter cake
35
36
37 was washed with dichloromethane (2 x 100 mL). The combined organic layer was washed
38
39
40 with NH_4OH (aq., sat., 500 mL). The aqueous layer was extracted with dichloromethane.
41
42
43
44
45 The combined organic layers were concentrated under reduced pressure. The residue
46
47
48 was purified by silica gel chromatography (50% to 100% EtOAc in pet. ether) to give 6-
49
50
51 (benzyloxy)-4-methoxybenzo[d]thiazol-2-amine (13 g, 37%) as a black solid. LC-MS
52
53
54
55 (ES+) $\text{C}_{15}\text{H}_{14}\text{N}_2\text{O}_2\text{S}$ requires: 286, found: 287 (M+H)⁺.
56
57
58
59
60

1
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3
4 *Step 4: 6-(Benzyloxy)-2-bromo-4-methoxybenzo[d]thiazole.* To a suspension of 6-
5
6
7 (benzyloxy)-4-methoxybenzo[d]thiazol-2-amine (22.0 g, 0.077 mol) in MeCN (100 mL)
8
9
10 was added t-BuONO (11.8 g, 0.110 mol) at 0 °C. The mixture was stirred for 30 min, then
11
12
13
14 CuBr₂ (10.3 g, 0.046 mol) was added. The reaction was stirred for an additional 2 h at
15
16
17 room temperature. Water was added, the precipitate was filtered and the filtrate was
18
19
20
21 extracted with EtOAc. The combined organic layers were washed with water, diluted
22
23
24 NH₄OH (aq., sat.), brine and concentrated under reduced pressure. The residue was
25
26
27
28 purified by silica gel chromatography (10% EtOAc in Petroleum ether) to give 6-
29
30
31 (benzyloxy)-2-bromo-4-methoxybenzo[d]thiazole (8.0 g, 30%) as a grey-white solid. LC-
32
33
34
35 MS (ES+) C₁₅H₁₂BrNO₂S requires: 349, found: 350 (M+H)⁺. (M+H)⁺. ¹H NMR (500 MHz,
36
37
38 CDCl₃) δ 7.51 – 7.29 (m, 5H), 6.88 (d, *J* = 2.2 Hz, 1H), 6.61 (d, *J* = 2.2 Hz, 1H), 5.09 (s,
39
40
41 2H), 3.98 (s, 3H).

42
43
44
45 *Step 5: (1R,2R)-2-(6-(Benzyloxy)-4-methoxybenzo[d]thiazol-2-ylamino)cyclohexanol.* A
46
47
48 mixture of 6-(benzyloxy)-2-bromo-4-methoxybenzo[d]thiazole (3.50 g, 10.0 mmol), (1R,
49
50
51 2R)-2-aminocyclohexanol (3.45 g, 30.0 mmol) and DIPEA (5.16 g, 40.0 mmol) in DMF
52
53
54
55 (10 mmol) was heated at 110 °C for 48 h. The reaction was cooled to rt, diluted with water
56
57
58
59
60

1
2
3
4 (30 mL), and extracted with EtOAc (3 x 30 mL). The combined organic extracts were
5
6
7 washed by water (100 ml), brine (100 ml), dried over Na₂SO₄ and concentrated to give
8
9
10 (1R,2R)-2-(6-(benzyloxy)-4-methoxybenzo[d]thiazol-2-ylamino)cyclohexanol (3.8 g,
11
12
13 100%, crude) as a brown solid. MS (ES+) C₂₁H₂₄N₂O₃S requires: 384, found: 385 [M+H]⁺.
14
15
16
17
18
19
20

21 *Step 6: 2-((1R,2R)-2-Hydroxycyclohexylamino)-4-methoxybenzo[d]thiazol-6-ol.* A
22
23
24 solution of (1R,2R)-2-(6-(benzyloxy)-4-methoxybenzo[d]thiazol-2-ylamino)cyclohexanol
25
26
27 (1152 mg, 3.0 mmol) in TFA (10 mL) was heated at 65 °C for 48 h. The solvent was
28
29
30 removed, the reaction mixture was diluted slowly by sat. NaHCO₃ and extracted with
31
32
33 EtOAc (3 x 50 mL). The combined organic extracts were washed with water (100 ml) and
34
35
36 brine (100 ml), dried over Na₂SO₄ and concentrated to give 2-((1R,2R)-2-
37
38
39 hydroxycyclohexylamino)-4-methoxybenzo[d]thiazol-6-ol (882 mg, 100%, crude) as a
40
41
42 brown solid. MS (ES+) C₁₄H₁₈N₂O₃S requires: 294, found: 295 [M+H]⁺.
43
44
45
46
47
48

49 *Step 7: 2-((1R,2R)-2-Hydroxycyclohexylamino)-4-methoxybenzo[d]thiazol-6-ol.* A
50
51
52 mixture of 3-chloro-4-(chloromethyl)-N-(4-methoxybenzyl)pyridin-2-amine (Example 1,
53
54
55
56 Step 3; 202 mg, 0.68 mmol), 2-((1R,2R)-2-hydroxycyclohexylamino)-4-
57
58
59
60

1
2
3 methoxybenzo[d]thiazol-6-ol (200 mg, 0.68 mmol) and Cs₂CO₃ (443 mg, 1.36 mmol) in
4
5
6
7 DMF (2 mL) was stirred at 80 °C for 3 h. The mixture was diluted by water (30 ml) and
8
9
10 extracted with EtOAc (3 × 30 mL). The combined organic phases were washed with water
11
12
13 (100 ml) and brine (100 ml), dried over Na₂SO₄ and concentrated to give (1R,2R)-2-(6-
14
15
16 ((3-chloro-2-(4-methoxybenzylamino)pyridin-4-yl)methoxy)-4-methoxybenzo[d]thiazol-2-
17
18
19
20
21 ylamino)cyclohexanol (300 mg, 79%, crude) as a brown solid. MS (ES+) C₂₈H₃₁ClN₄O₄S
22
23
24 requires: 554, found: 555 [M+H]⁺.
25
26

27
28 *Step 8: (1R,2R)-2-(6-((2-Amino-3-chloropyridin-4-yl)methoxy)-4-*
29
30
31 *methoxybenzo[d]thiazol-2-ylamino)cyclohexanol.* A mixture of (1R,2R)-2-(6-((3-chloro-2-
32
33
34 (4-methoxybenzylamino)pyridin-4-yl)methoxy)-4-methoxybenzo[d]thiazol-2-
35
36
37
38 ylamino)cyclohexanol (300 mg, 0.54 mmol) in TFA (2 mL) was stirred at rt for 8 h. The
39
40
41 volatiles were removed under reduced pressure. The residue was purified by preparative
42
43
44 HPLC (Mobile phase: A = 0.1% NH₄HCO₃/H₂O, B = MeCN; Gradient: B = 5 - 95%; 12
45
46
47 min; Column: C18) to give (1R,2R)-2-(6-((2-amino-3-chloropyridin-4-yl)methoxy)-4-
48
49
50
51
52 methoxybenzo[d]thiazol-2-ylamino)cyclohexanol (19 mg, 8%) as a white solid. MS (ES+)
53
54
55 C₂₀H₂₃ClN₄O₃S requires: 434, found: 435 [M+H]⁺. ¹H NMR (500 MHz, DMSO-*d*₆) δ 8.50
56
57
58
59
60

1
2
3
4 (s, 1H), 7.95 (d, $J = 5.4$ Hz, 1H), 7.04 (s, 1H), 6.85 (d, $J = 5.5$ Hz, 1H), 6.69 (s, 1H), 5.14
5
6
7 (s, 2H), 3.88 (s, 3H), 3.60 (d, $J = 11.0$ Hz, 1H), 3.32 (dd, $J = 17.5, 11.8$ Hz, 1H), 2.01 (d,
8
9
10 $J = 13.0$ Hz, 1H), 1.89 (d, $J = 8.9$ Hz, 1H), 1.70 – 1.56 (m, 2H), 1.42 – 0.92 (m, 4H).

11
12
13
14 **(1R,2R)-2-(6-((2-Amino-3-chloropyridin-4-yl)methoxy)-4-**
15
16
17 **(trifluoromethoxy)benzo[d]thiazol-2-ylamino)cyclohexanol (16)**
18

19
20
21 *Step 1: 6-Bromo-4-(trifluoromethoxy)benzo[d]thiazol-2-amine.* To a solution of 4-
22
23
24 bromo-2-(trifluoromethoxy)aniline (5.9 g, 23.1 mmol) in AcOH (100 ml) was added KSCN
25
26
27 (8.97 g, 92.5 mmol). After stirring for 30 min at rt, the reaction mixture was cooled to 0 °C,
28
29
30
31 and a solution of Br₂ (5.5 g, 34.6 mmol) in AcOH was added dropwise. The reaction
32
33
34
35 mixture was stirred for 4 h at rt. Subsequently, the pH of the reaction mixture was
36
37
38 adjusted to 7 with NH₃-H₂O. The mixture was extracted with EtOAc (3 × 80 mL), the
39
40
41
42 combined organic phases were dried over Na₂SO₄ and concentrated under reduced
43
44
45
46 pressure. The residue was purified by column chromatography (PE: THF = 10:1) to give
47
48
49 the title compound (0.9 g, 12.5%) MS (ES+) C₈H₄BrF₃N₂OS requires: 312, found: 313
50
51
52 [M+H]⁺.
53
54
55
56
57
58
59
60

1
2
3
4 *Step 2: 2,6-Dibromo-4-(trifluoromethoxy)benzo[d]thiazole.* A solution of 6-bromo-4-
5
6
7 (trifluoromethoxy)benzo[d]thiazol-2-amine (900 mg, 3 mmol) in MeCN (20 mL) was
8
9
10 cooled to -5°C and CuBr₂ (0.83 g, 3.6 mmol) and ^tBuONO (0.37 mg, 3.6 mmol) were
11
12
13 added dropwise. The reaction mixture was stirred at 0-5°C for 30 min, then it was heated
14
15
16 to 40°C and stirred for 6 h. The insoluble was filtered off and the filtrate was washed with
17
18
19 1 N HCl solution, the resultant solution was dried over Na₂SO₄, filtered and concentrated
20
21
22 to give the product (0.6 g, 53%). MS (ESI+) C₈H₂Br₂F₃NOS requires: 375, found: 376
23
24
25
26
27
28 [M+H]⁺.

31 *Step 3: (1R,2R)-2-(6-Bromo-4-(trifluoromethoxy)benzo[d]thiazol-2-*
32
33
34
35 *ylamino)cyclohexanol.*

36
37
38 A mixture of 2,6-dibromo-4-(trifluoromethoxy)benzo[d]thiazole (980 mg, 2.61 mmol),
39
40
41 (1R,2R)-2-aminocyclohexanol (901 mg, 7.83 mmol) and DIPEA (1 g, 7.83 mmol) in DMA
42
43
44 (10 ml) was heated to 100°C for 16 h. Saturated NH₄Cl solution (8 ml) was added and
45
46
47 the reaction mixture was extracted with EtOAc (3 x 5 mL). The combined organic phases
48
49
50 were dried over Na₂SO₄, filtered and concentrated. The residue was purified by column
51
52
53
54
55
56
57
58
59
60

1
2
3 chromatography (25% EtOAc in pet. ether) to give the product (836 mg, 78%) as a white
4
5
6
7 solid. MS (ESI+) $C_{14}H_{14}BrF_3N_2O_2S$ requires: 410, found: 411 [M+H]⁺.
8
9

10 *Step 4:* *(1R,2R)-2-(6-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)-4-*
11 *(trifluoromethoxy)benzo[d]thiazol-2-ylamino)cyclohexanol.* A mixture of (1R, 2R)-2-(6-
12
13
14 *(trifluoromethoxy)benzo[d]thiazol-2-ylamino)cyclohexanol.* A mixture of (1R, 2R)-2-(6-
15
16
17 bromo-4-(trifluoromethoxy)benzo[d]thiazol-2-ylamino)cyclohexanol (600 mg, 1.463
18
19
20 mmol), B_2Pin_2 (446 mg, 1.75 mmol), KOAc (286 mg, 2.92 mmol), $Pd(dppf)Cl_2$ (107 mg,
21
22
23 0.15 mmol) in 1,4-dioxane (20 ml) was heated to 100 °C and stirred for 16h. The mixture
24
25
26
27 was filtered and the filtrate was concentrated under reduced pressure to provide the
28
29
30 residue that was used in the next step without further purification (671 mg, 100%). MS
31
32
33
34
35 (ESI+) $C_{20}H_{26}BF_3N_2O_4S$ requires: 458, found: 459 [M+H]⁺.
36
37

38 *Step 5:* *2-((1R,2R)-2-Hydroxycyclohexylamino)-4-(trifluoromethoxy)benzo[d]thiazol-6-*
39
40
41 *ol.* To a solution of (1R,2R)-2-(6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-4-
42
43
44 (trifluoromethoxy)benzo[d]thiazol-2-ylamino)cyclohexanol (700 mg, 1.53 mmol) in
45
46
47 dioxane (5 ml) was added H_2O_2 (5 ml) and the reaction mixture was stirred for 2 h at rt.
48
49
50
51
52 The solvent was evaporated and the residue was taken up in EtOAc (5 ml)/water (4 ml).
53
54
55
56 The crude product was extracted with EtOAc (3 x 5 mL), the combined organic phases
57
58
59
60

1
2
3 were dried over Na₂SO₄, filtered and concentrated under reduced pressure. The residue
4
5
6
7 was purified by column chromatography (25% EtOAc in pet. ether) to provide the product
8
9
10 (320 mg, 60%) as an oil. MS (ESI+) C₁₄H₁₅F₃N₂O₃S requires: 348, found: 349 [M+H]⁺.

11
12
13
14 *Step 6: (1R,2R)-2-(6-((3-Chloro-2-(4-methoxybenzylamino)pyridin-4-yl)methoxy)-4-*
15
16
17 *(trifluoromethoxy)benzo[d]thiazol-2-ylamino)cyclohexanol.* A mixture of 2-((1R,2R)-2-
18
19
20 hydroxycyclohexylamino)-4-(trifluoromethoxy)benzo[d]thiazol-6-ol (150 mg, 0.43 mmol),
21
22
23
24 3-chloro-4-(chloromethyl)-N-(4-methoxybenzyl)pyridin-2-amine (153 mg, 0.517 mmol)
25
26
27 and Cs₂CO₃ (421 mg, 1.29 mmol) in DMF (3 ml) was stirred at 80°C for 16 h. The volatiles
28
29
30 were removed under reduced pressure and the residue was purified by column
31
32
33 chromatography (70% EtOAc in pet. ether) to provide the product (70 mg, 27%) as an oil.
34
35
36
37 MS (ESI+) C₂₈H₂₈ClF₃N₄O₄S requires: 608, found: 609 [M+H]⁺.

38
39
40
41
42 *Step 7: (1R,2R)-2-(6-((2-Amino-3-chloropyridin-4-yl)methoxy)-4-*
43
44
45 *(trifluoromethoxy)benzo[d]thiazol-2-ylamino)cyclohexanol.* To a solution of (1R, 2R)-2-(6-
46
47
48 ((3-chloro-2-(4-methoxybenzylamino)pyridin-4-yl)methoxy)-4-
49
50
51 (trifluoromethoxy)benzo[d]thiazol-2-ylamino)cyclohexanol (70 mg, 0.115 mmol) in DCM
52
53
54 (3 mL) was added TFA (3 ml) , then the reaction mixture was stirred at rt for 16 h. The
55
56
57
58
59
60

1
2
3 solvent was evaporated and 2N NaOH was added until the pH was adjusted to > 7. The
4
5
6
7 mixture was stirred for 1 h and the crude product was extracted with EtOAc (3 x 3 mL).
8
9
10 The combined organic phases were dried over Na₂SO₄, filtered and concentrated under
11
12
13 reduced pressure. The residue was purified by column chromatography (90% EtOAc in
14
15
16
17 pet. ether) to give the title compound (2.5 mg, 4.5%) as a white solid. MS (ES+)
18
19
20
21 C₂₀H₂₀ClF₃N₄O₃S requires: 488, found: 489.5 [M+H]⁺. ¹H NMR (500 MHz, DMSO-*d*₆) δ
22
23
24 8.14 (d, *J* = 6.5 Hz, 1H), 7.91 (d, *J* = 5 Hz, 1H), 7.49 (d, *J* = 2.5 Hz, 1H), 6.94 (s, 1H),
25
26
27 6.74 (d, *J* = 4.5 Hz, 1H), 6.35 (s, 2H), 5.11 (s, 2H), 4.80 (d, *J* = 4.5 Hz, 1H), 3.34-3.40 (m,
28
29
30
31 1H), 2.04-2.06 (m, 1H), 1.87-1.90 (m, 1H), 1.62-1.64 (m, 2H), 1.24-1.30 (m, 4H).
32
33

34
35 **Synthesis of (1R,2R)-2-(6-((2-Amino-3-chloropyridin-4-yl)methoxy)-4-**
36
37
38 **chlorobenzo[d]thiazol-2-ylamino)cyclohexanol (17)**
39
40

41
42 *Step 1: 4-Chloro-6-methoxybenzo[d]thiazol-2-amine.* To a solution of 2-chloro-4-
43
44
45 methoxyaniline (1.6 g, 10 mmol) in AcOH (20 ml) was added KSCN (3.95 g, 40.76 mmol)
46
47
48 and CuBr₂ (2.6, 11.9 mmol) the reaction mixture was cooled to 0°C. Br₂ (1.95 g, 12.2
49
50
51 mmol) was added dropwise and the reaction mixture was stirred for 4 h at rt. The pH of
52
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54
55 the mixture was adjusted to 7 with NH₄OH (aq., sat.) solution. The mixture was extracted
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3 with EtOAc (3 x 50 mL), the combined organic phases were dried over Na₂SO₄ and
4
5
6 concentrated under reduced pressure. The residue was purified by column
7
8 chromatography (30% EtOAc in pet. ether) to provide the 4-chloro-6-
9
10 methoxybenzo[d]thiazol-2-amine (1.7 g, 78%) MS (ES+) C₈H₇ClN₂OS requires: 214,
11
12
13 found: 215 [M+H]⁺. ¹H NMR (500 MHz, DMSO-*d*₆) δ 7.596 (s, 2H), 7.311(s, 1H), 6.925 (s,
14
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22
23 1H), 3.749 (s, 3H).

24
25 *Step 2: 2-Bromo-4-chloro-6-methoxybenzo[d]thiazole.* A solution of 4-chloro-6-
26
27 methoxybenzo[d]thiazol-2-amine (2.1 g, 7.9 mmol) in MeCN (40 mL) was cooled to -5°C
28
29 and ^tBuONO (1.23g, 11.9 mmol) was added dropwise. The mixture was stirred at 0°C for
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Steps 3-7: (1R,2R)-2-(6-((2-Amino-3-chloropyridin-4-yl)methoxy)-4-chlorobenzo[d]thiazol-2-ylamino)cyclohexanol. As described for compound **12**. (1R,2R)-
C₈H₅BrClNOS requires: 277, found: 278 [M+H]⁺.

1
2
3
4 2-(6-((2-Amino-3-chloropyridin-4-yl)methoxy)-4-chlorobenzo[d]thiazol-2-

5
6
7 ylamino)cyclohexanol MS (ES+) $C_{19}H_{20}Cl_2N_4O_2S$ requires: 438, found: 439 $[M+H]^+$. 1H

8
9
10 NMR (500 MHz, DMSO- d_6) δ 8.06 (d, $J = 8.0$ Hz, 1H), 7.91 (d, $J = 5$ Hz, 1H), 7.40 (d, $J =$

11
12
13 2.5 Hz, 1H), 7.02 (d, $J = 2.5$ Hz, 1H), 6.72 (d, $J = 5$ Hz, 1H), 6.53 (s, 2H), 5.10 (s, 2H),

14
15
16 4.81 (d, $J = 5$ Hz, 1H), 3.36-3.52 (m, 1H), 1.99-2.02 (m, 1H), 1.87-1.89 (m, 1H), 1.62-1.64

17
18
19 (m, 2H), 1.15-1.31 (m, 4H).

20
21
22
23
24 **Synthesis of (1R, 2R)-2-(6-((2-amino-3-chloropyridin-4-yl)methoxy)-4-**
25
26
27
28 **fluorobenzo[d]thiazol-2-ylamino)cyclohexanol (18)**

29
30
31 (1R, 2R)-2-(6-((2-Amino-3-chloropyridin-4-yl) methoxy)-4-fluorobenzo[d]thiazol-2-

32
33
34 ylamino)cyclohexanol was prepared as described for compound 17. MS (ES+)

35
36
37 $C_{19}H_{20}ClFN_4O_2S$ requires: 422, found: 423 $[M+H]^+$. 1H NMR (500 MHz, DMSO- d_6) δ 7.957

38
39
40 (d, $J = 7.5$ Hz, 1H), 7.910 (d, $J = 5$ Hz, 1H), 7.251 (d, $J = 2$ Hz, 1H), 7.864 (dd, $J_1 = 2$ Hz,

41
42
43 $J_2 = 12.5$ Hz, 1H), 6.725 (d, $J = 4.5$ Hz, 1H), 6.350 (s, 1H), 5.087 (s, 2H), 4.761 (d, $J = 5$

44
45
46 Hz, 1H), 3.514-3.526 (m, 1H), 2.041-2.065 (m, 1H), 1.874-1.897 (m, 1H), 1.616-1.657 (m,

47
48
49 2H), 1.182-1.309 (m, 4H).

1
2
3
4 **Synthesis** of **(1R,2R)-2-(6-((2-amino-3-chloropyridin-4-yl)methoxy)-7-**
5
6
7 **chlorobenzo[d]thiazol-2-ylamino)cyclohexanol (19)**

8
9
10 (1R,2R)-2-(6-((2-Amino-3-chloropyridin-4-yl)methoxy)-7-chlorobenzo[d]thiazol-2-
11
12
13
14 ylamino)cyclohexanol was prepared as described for compound 17. MS (ES+)
15
16
17 C₁₉H₂₀Cl₂N₄O₂S requires: 438, found: 439 [M+H]⁺. ¹H NMR (500 MHz, DMSO-*d*₆) δ 8.03
18
19
20
21 (d, *J* = 12.5 Hz, 1H), 7.93 (d, *J* = 5 Hz, 1H), 7.27 (d, *J* = 8.5 Hz, 1H), 7.07 (d, *J* = 8.5 Hz,
22
23
24 1H), 6.77 (d, *J* = 5 Hz, 1H), 6.37 (s, 2H), 5.18 (s, 2H), 4.77 (d, *J* = 5 Hz, 1H), 3.48-3.50
25
26
27 (m, 1H), 2.04-2.07 (m, 1H), 1.88-1.90 (m, 1H), 1.61-1.65 (m, 2H), 1.18-1.30 (m, 4H).
28
29
30

31 **Synthesis** of **(1R,2R)-2-(6-((2-amino-3-chloropyridin-4-yl)methoxy)-7-**
32
33
34 **fluorobenzo[d]thiazol-2-ylamino)cyclohexanol (20)**

35
36
37 (1R,2R)-2-(6-((2-Amino-3-chloropyridin-4-yl)methoxy)-7-fluorobenzo[d]thiazol-2-
38
39
40
41 ylamino)cyclohexanol was prepared as described for compound 17. MS (ES+)
42
43
44 C₁₉H₂₀ClFN₄O₂S requires: 422, found: 423.5 [M+H]⁺. ¹H NMR (500 MHz, DMSO-*d*₆) δ
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46
47
48 8.07 (d, *J* = 7.5 Hz, 1H), 7.91 (d, *J* = 5 Hz, 1H), 7.06-7.14 (m, 2H), 6.73 (d, *J* = 5 Hz, 1H),
49
50
51 6.36 (s, 2H), 5.16 (s, 2H), 4.77 (d, *J* = 5 Hz, 1H), 3.49-3.51 (m, 1H), 2.05-2.07 (m, 1H),
52
53
54 1.88-1.90 (m, 1H), 1.61-1.65 (m, 2H), 1.18-1.30 (m, 4H).
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Synthesis of N-cyclohexyl-6-((pyridin-3-yloxy)methyl)benzo[d]thiazol-2-amine (4)

Step 1: (2-bromobenzo[d]thiazol-6-yl)methanol: To a solution ethyl 2-bromobenzo[d]thiazole-6-carboxylate (1.2 g, 4.2 mmol) in THF (20 mL), at 0°C, DIBAL-H (10.5 mL, 10.5 mmol, 1M in toluene) was added slowly. The reaction mixture was stirred at rt for 4 h. The mixture was quenched with water (15 ml), filtered and the filtrate was extracted with EtOAc (3 x 30 mL). The combined organic layers were dried over MgSO₄ and concentrated under reduced pressure to provide (2-bromobenzo[d]thiazol-6-yl)methanol (1.0 g, 98%) as a yellow solid. MS (ES+) C₈H₆BrNOS requires: 244, found: 244, 246[M+H]⁺.

Step 2: (2-(methylthio)benzo[d]thiazol-6-yl)methanol. A mixture of (2-bromobenzo[d]thiazol-6-yl)methanol (1.0 g, 4.0 mmol) and CH₃SNa (560 mg, 8 mmol) in DMF (15 mL) was stirred at rt for 16h. The reaction mixture was diluted with water and extracted with EtOAs (3 x 30 mL). The combined organic layers were washed with brine, dried over MgSO₄ and concentrated under reduced pressure. The residue was purified by flash column chromatography (EtOAc in PE 20 to 45%) to afford (2-

(methylthio)benzo[d]thiazol-6-yl)methanol (598 mg, 71%) as a beige solid. MS (ES+)

$C_9H_9NOS_2$ requires: 211, found: 212 [M+H]⁺.

Step 3: 6-(chloromethyl)-2-(methylthio)benzo[d]thiazole. MsCl (572 mg, 5 mmol) was added to a solution of (2-(methylthio)benzo[d]thiazol-6-yl)methanol (350 mg, 1.66 mmol) and DIPEA (642 mg, 5 mmol) in DCM (10 mL) at 0°C. The mixture was treated with 3 drops of DMF and stirred at rt for 16h. NaHCO₃ (sat. aq. 15 mL) was added, and the aqueous phase was extracted with DCM (3 x 20 mL). The combined organic layers were washed with brine, dried over MgSO₄ and concentrated under reduced pressure to provide 6-(chloromethyl)-2-(methylthio)benzo[d]thiazole (380 mg, quant.) as a beige solid. MS (ES+) $C_9H_8ClNS_2$ requires: 229, found: 230[M+H]⁺.

Step 4: 2-(methylthio)-6-((pyridin-3-yloxy)methyl)benzo[d]thiazole. A solution of pyridine 3-ol (123 mg, 1.29 mmol) in DMA (1 mL) was added to a mixture of t-BuOK (171 mg, 1.5 mmol) in DMA (2 mL). It was stirred at rt for 1 h and subsequently heated to 100°C for 1 h. A solution of 6-(chloromethyl)-2-(methylthio)benzo[d]thiazole (229 mg, 1 mmol) in DMA (1 mL) was added. The mixture was stirred for 2 h. The reaction mixture was cooled to rt, it was diluted with water and extracted with EtOAc (3 × 15 mL). The

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2
3 combined organic phases were washed with brine, dried over MgSO₄ and concentrated
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5
6
7 under reduced pressure to provide 2-(methylthio)-6-((pyridin-3-
8
9
10 yloxy)methyl)benzo[d]thiazole (288 mg, quant). The product was used in the next step
11
12
13
14 without further purification. MS (ES+) C₁₄H₁₂N₂OS₂ requires: 288, found: 289[M+H]⁺.
15
16

17 *Step 5: 2-(methylsulfinyl)-6-((pyridin-3-yloxy)methyl)benzo[d]thiazole.*
18
19

20
21 To a solution of provide 2-(methylthio)-6-((pyridin-3-yloxy)methyl)benzo[d]thiazole
22
23
24 (288 mg, 1 mmol, crude) in DCM (3 mL) was added *m*-CPBA (202 mg, 1 mmol, 85%).
25
26
27
28 The reaction mixture was stirred at rt for 2 h. The reaction was quenched with Na₂CO₃
29
30
31 (sat. aq. 5 mL), extracted with DCM (3 x 15 mL) and the combined organic phases were
32
33
34
35 dried over MgSO₄ and concentrated under reduced pressure to provide 2-(methylsulfinyl)-
36
37
38 6-((pyridin-3-yloxy)methyl)benzo[d]thiazole (304 mg crude, 100 %). MS (ES+)
39
40
41 C₁₄H₁₂N₂O₂S₂ requires: 304, found: 305[M+H]⁺.
42
43
44

45 *Step 6: N-cyclohexyl-6-((pyridin-3-yloxy)methyl)benzo[d]thiazol-2-amine.*
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48
49 A mixture of 2-(methylsulfinyl)-6-((pyridin-3-yloxy)methyl)benzo[d]thiazole (50 mg, 0.16
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52 mmol) in cyclohexanamine (0.5 mL) was stirred at 100°C for 2 h. The residue was purified
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4 by preparative HPLC (Mobile phase: A = 0.1% NH₄HCO₃/H₂O, B = MeCN; Gradient: B =
5
6
7 5 - 95%; 12 min; Column: C18) to provide the product (10 mg, 18%).
8
9

10 MS (ES+) C₁₉H₂₁N₃OS requires: 339, found: 340[M+H]⁺; MS (ES+) C₁₉H₂₁N₃OS
11
12 requires: 339, found: 340[M+H]⁺; ¹H NMR (500 MHz, *d*₄-MeOD) δ 8.32 (d, *J* = 2.9 Hz,
13
14 1H), 8.14 (dd, *J* = 4.7, 0.6 Hz, 1H), 7.70 (d, *J* = 1.0 Hz, 1H), 7.52 (ddd, *J* = 8.5, 2.7, 0.8
15
16 Hz, 1H), 7.43 (d, *J* = 8.3 Hz, 1H), 7.40 – 7.34 (m, 2H), 5.20 (s, 2H), 3.78 – 3.71 (m, 1H),
17
18 2.14 – 2.07 (m, 2H), 1.86 – 1.80 (m, 2H), 1.73 – 1.67 (m, 1H), 1.52 – 1.42 (m, 2H), 1.39 –
19
20 1.26 (m, 3H).
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31 **Synthesis of N-cyclohexyl-6-((pyridin-4-yloxy)methyl)benzo[d]thiazol-2-amine (5)**

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33
34
35 N-cyclohexyl-6-((pyridin-4-yloxy)methyl)benzo[d]thiazol-2-amine (5) was prepared as
36
37
38 described for N-cyclohexyl-6-((pyridin-3-yloxy)methyl)benzo[d]thiazol-2-amine (4).
39
40
41

42 MS (ES+) C₁₉H₂₁N₃OS requires: 339, found: 340[M+H]⁺; ¹H NMR (500 MHz, DMSO-
43
44 *d*₆) δ 8.02 (d, *J* = 7.5 Hz, 1H), 7.79 – 7.71 (m, 2H), 7.65 (d, *J* = 1.6 Hz, 1H), 7.36 (d, *J* =
45
46 8.2 Hz, 1H), 7.19 (dd, *J* = 8.3, 1.8 Hz, 1H), 6.11 – 6.04 (m, 2H), 5.02 (s, 2H), 3.71 – 3.66
47
48 (m, 1H), 1.99 – 1.91 (m, 2H), 1.75 – 1.67 (m, 2H), 1.61 – 1.53 (m, 1H), 1.36 – 1.15 (m,
49
50 5H).
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Synthesis of N-cyclohexyl-6-((pyridin-3-ylamino)methyl)benzo[d]thiazol-2-amine (6)

Step 1: 6-(azidomethyl)-2-(methylthio)benzo[d]thiazole. To a solution of (2-(methylthio)benzo[d]thiazol-6-yl)methanol (100 mg, 0.473 mmol) in THF (1578 μ l) at 0 $^{\circ}$ C were added diphenyl phosphorazidate (122 μ l, 0.568 mmol) and DBU (86 μ l, 0.57 mmol) and the resulting mixture was stirred at rt for 3 h. The volatiles were removed under reduced pressure and the residue was purified via silica gel chromatography (0 to 100 % EtOAc in hexanes) to provide 6-(azidomethyl)-2-(methylthio)benzo[d]thiazole (103 mg, 92 % yield) as a colorless liquid that solidified overnight. MS (ES+) $C_9H_8N_4S_2$ requires: 236, found: NA.

Step 2: (2-(methylthio)benzo[d]thiazol-6-yl)methanamine. A reaction vessel was charged with 6-(azidomethyl)-2-(methylthio)benzo[d]thiazole (73 mg, 0.309 mmol), Lindlar's catalyst (66 mg, 0.031 mmol) and EtOH (3 mL) under an atmosphere of N_2 . The suspension was degassed with N_2 for 5 min. and purged with H_2 for 5 min. The reaction mixture was stirred under an atmosphere of H_2 at 1 atm for 2 h. The reaction mixture was purged with N_2 , filtered through Celite and concentrated under reduced pressure. The residue was purified via silica gel chromatography (0 - 10 % MeOH in DCM w/ 0.1%

1
2
3
4 NH₄OH) to provide (2-(methylthio)benzo[d]thiazol-6-yl)methanamine (70 mg, 81 % yield)
5
6
7 as a yellow liquid. MS (ES+) C₉H₁₀N₂S₂ requires: 210, found: 211 [M+H] +.
8
9

10
11 *Step 3: N-((2-(methylthio)benzo[d]thiazol-6-yl)methyl)pyridin-3-amine.* To a suspension
12
13
14 of 3-iodopyridine (49 mg, 0.24 mmol) and (2-(methylthio)benzo[d]thiazol-6-
15
16
17 yl)methanamine (50 mg, 0.24 mmol) in DMSO-*d*₆ (475 μl) were added Cu (1.5 mg, 0.024
18
19
20 mmol) and CsOAc (91 mg, 0.47 mmol) and the resulting mixture was stirred at 90 °C for
21
22
23
24 7 h. The reaction was diluted with MeOH (1 mL), and TFA was added. The mixture was
25
26
27
28 filtered, the solvent was removed under reduced pressure and the residue was purified
29
30
31 by mass-triggered preparative HPLC (Mobile phase: A = 0.1% TFA/H₂O, B = 0.1%
32
33
34 TFA/MeCN; Gradient: B = 10 - 90%; 12 min; Column: C18) to provide N-((2-
35
36
37 (methylthio)benzo[d]thiazol-6-yl)methyl)pyridin-3-amine (53 mg, 78% yield) as a brown
38
39
40
41 amorphous material. MS (ES+) C₁₄H₁₃N₃S₂ requires: 287, found: 288 [M+H] +.
42
43
44

45
46 *Step 4: N-((2-(methylsulfinyl)benzo[d]thiazol-6-yl)methyl)pyridin-3-amine.* To a
47
48
49 suspension of N-((2-(methylthio)benzo[d]thiazol-6-yl)methyl)pyridin-3-amine (50 mg,
50
51
52 0.17 mmol) in DCM (1.7 mL) at 0 °C was added *m*CPBA (43 mg, 0.17 mmol) in DCM (0.5
53
54
55 mL) in portions and the resulting mixture was stirred at 0 °C for 1 h. The solution was
56
57
58
59
60

1
2
3 diluted with NaHCO₃ (aq., sat., 2 mL) and extracted with DCM (3 x 1 mL). The volatiles
4
5
6
7 were removed under reduced pressure to provide N-((2-(methylsulfinyl)benzo[d]thiazol-
8
9
10 6-yl)methyl)pyridin-3-amine (53 mg, 100 % yield) as a brown amorphous material. MS
11
12
13
14 (ES+) C₁₄H₁₃N₃OS₂ requires: 303, found: 304 [M+H]⁺.
15
16

17 *Step 5: N-cyclohexyl-6-((pyridin-3-ylamino)methyl)benzo[d]thiazol-2-amine.* A
18
19
20 microwave vial was charged with a solution of N-((2-(methylsulfinyl)benzo[d]thiazol-6-
21
22
23 yl)methyl)pyridin-3-amine (21 mg, 0.069 mmol) in DMA (277 μl) and cyclohexanamine
24
25
26 (23 μl, 0.21 mmol) and Hunig'sBase (12 μl, 0.069 mmol) was added. The resulting mixture
27
28
29 was stirred at 120 °C for 24 h. The mixture was diluted with MeOH, acidified with TFA,
30
31
32 and the residue was purified by mass-triggered preparative HPLC (Mobile phase: A =
33
34
35 0.1% TFA/H₂O, B = 0.1% TFA/MeCN; Gradient: B = 10 - 90%; 12 min; Column: C18) to
36
37
38 provide N-cyclohexyl-6-((pyridin-3-ylamino)methyl)benzo[d]thiazol-2-amine (2 mg, 8.54
39
40
41 % yield) as a pale yellow amorphous material. MS (ES+) C₁₉H₂₂N₄S requires: 338, found:
42
43
44 339 [M+H]⁺. ¹H NMR (500 MHz, DMSO-*d*₆) δ 8.25 (brs, 1H), 8.06 (d, *J* = 5.0 Hz, 1H),
45
46
47 8.03 (d, *J* = 5.0 Hz, 1H), 7.92 (s, 1H), 7.70 (m, 3H), 7.37 (d, *J* = 10.0 Hz, 1H), 7.24 (d,
48
49
50
51
52
53
54
55
56
57
58
59
60 *J* = 5.0 Hz, 1H), 4.4 (s, 2H), 3.7 (m, 1H), 1.71-1.76 (m, 4H), 1.25-1.4 (m, 6H).

Synthesis of N-cyclohexyl-6-((pyridin-4-ylamino)methyl)benzo[d]thiazol-2-amine (7)

N-cyclohexyl-6-((pyridin-4-ylamino)methyl)benzo[d]thiazol-2-amine was prepared as described for N-cyclohexyl-6-((pyridin-3-yloxy)methyl)benzo[d]thiazol-2-amine (6).

MS (ES+) $C_{19}H_{23}N_5O_3S$, requires:401, found: 402[M+H]⁺; ¹H NMR (400 MHz, *d*₄-MeOD) δ 8.27 (d, *J* = 5.2 Hz, 1H), 6.86 (d, *J* = 5.1 Hz, 1H), 6.83 (d, *J* = 2.3 Hz, 1H), 6.63 (d, *J* = 2.2 Hz, 1H), 4.97 (s, 2H), 3.94 (s, 3H), 3.66 – 3.54 (m, 1H), 3.46 – 3.37 (m, 1H), 2.20 – 2.10 (m, 1H), 2.07 – 2.00 (m, 1H), 1.80 – 1.71 (m, 2H), 1.44 – 1.31 (m, 4H).

Synthesis of (1R,2R)-2-(6-((2-Aminopyrimidin-4-yl)methoxy)-4-methoxybenzo[d]thiazol-2-ylamino)cyclohexanol (21)

Step 1: 2-Chloro-4-(chloromethyl)pyrimidine. To a solution of 2-chloro-4-methylpyrimidine (5.0 g, 39 mmol) in CCl₄ (100 ml) was added NCS (7.8 g, 58 mmol) and AIBN (0.64 g, 3.9 mmol) and the resulting mixture was heated at reflux for 18 h. The reaction mixture was allowed to cool to rt. The reaction mixture was filtered through a Celite pad, and the filtrate was concentrated under reduced pressure. The residue was purified via silica gel chromatography (10 to 40% EtOAc in hexanes) to give 2-chloro-4-

(chloromethyl)pyrimidine (3.1 g, 49%) as a clear oil. MS (ES+) $C_5H_4Cl_2N_2$ requires: 162, found: 163 [M+H]⁺.

Step 2: (1R,2R)-2-(6-((2-Chloropyrimidin-4-yl)methoxy)-4-methoxybenzo[d]thiazol-2-ylamino)cyclohexanol. A mixture of 2-((1R,2R)-2-hydroxycyclohexylamino)-4-methoxybenzo[d]thiazol-6-ol (40 mg, 0.14 mmol) and Cs_2CO_3 (116 mg, 0.36 mmol) in DMF (2 mL) was stirred at rt for 1 h. 2-Chloro-4-(chloromethyl)pyrimidine (30 mg, 0.19 mmol) was added and the mixture was stirred at rt for 4 h. The mixture was diluted with water, extracted with EtOAc (3 x 30 mL), the combined organic phases were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to provide the product (38 mg, 67%) as a brown solid. MS (ES+) $C_{19}H_{21}ClN_4O_3S$, requires: 421, found: 421, 423 [M+H]⁺.

Step 3: (1R,2R)-2-(6-((2-Aminopyrimidin-4-yl)methoxy)-4-methoxybenzo[d]thiazol-2-ylamino)cyclohexanol. A mixture of (1R,2R)-2-(6-((2-chloropyrimidin-4-yl)methoxy)-4-methoxybenzo[d]thiazol-2-ylamino)cyclohexanol (38 mg, 0.095 mmol) in NH_4OH (aq., sat.) solution was stirred at 90°C for 3 h in a sealed tube. The mixture was cooled to rt, concentrated and purified by prep-HPLC (NH_4HCO_3) to provide the product (3 mg, 8%)

1
2
3 as a beige solid. MS (ES+) $C_{19}H_{23}N_5O_3S$, requires: 401, found: 402 [M+H]⁺; ¹H NMR (400
4
5
6 MHz, *d*₄-MeOD) δ 8.27 (d, *J* = 5.2 Hz, 1H), 6.86 (d, *J* = 5.1 Hz, 1H), 6.83 (d, *J* = 2.3 Hz,
7
8
9 1H), 6.63 (d, *J* = 2.2 Hz, 1H), 4.97 (s, 2H), 3.94 (s, 3H), 3.66 – 3.54 (m, 1H), 3.46 – 3.37
10
11
12 (m, 1H), 2.20 – 2.10 (m, 1H), 2.07 – 2.00 (m, 1H), 1.80 – 1.71 (m, 2H), 1.44 – 1.31 (m,
13
14
15
16
17 4H).

18
19
20
21 **Synthesis of (1S,2S)-2-((6-((2-Aminopyrimidin-4-yl)methoxy)-4-**
22
23
24 **methoxybenzo[d]thiazol-2-yl)amino)cyclohexan-1-ol (22)**

25
26
27
28 *Step 1: 2-Chloro-4-((3-methoxy-4-nitrophenoxy)methyl)pyrimidine.* To a solution of 3-
29
30
31 methoxy-4-nitrophenol (2.83 g, 16.7 mmol) and 2-chloro-4-(chloromethyl)pyrimidine (3.0
32
33
34 g, 18 mmol) in DMF (16 ml) was added K_2CO_3 (2.8 g, 20 mmol) and the resulting mixture
35
36
37 was stirred at rt for 20 h. Ice cold water (30 ml) was added and the mixture was stirred for
38
39
40
41
42 10 min before filtering on a Buchner funnel. The solid was washed with cold water (10
43
44
45 mL) and dried under vacuum for 2 h to give 2-chloro-4-((3-methoxy-4-
46
47
48 nitrophenoxy)methyl)pyrimidine (4.6 g, 93%) as a light brown solid. MS (ES+)
49
50
51
52 $C_{12}H_{10}ClN_3O_4$ requires: 295, found: 296 [M+H]⁺.
53
54
55
56
57
58
59
60

1
2
3
4 *Step 2: 4-((2-Chloropyrimidin-4-yl)methoxy)-2-methoxyaniline.* A reaction vessel was
5
6
7 charged with 2-chloro-4-((3-methoxy-4-nitrophenoxy)methyl)pyrimidine (4.6 g, 16 mmol),
8
9
10 10% Pt-C (460 mg, 0.12 mmol), and THF-MeOH (5:1, 60 ml). The suspension was
11
12
13 degassed with N₂ for 3 min and purged with H₂ for 3 min. The reaction mixture was stirred
14
15
16 under an atmosphere of H₂ at 1 atm for 2 h. The reaction mixture was purged with N₂,
17
18
19 filtered through Celite, and concentrated under reduced pressure to give 4-((2-
20
21 chloropyrimidin-4-yl)methoxy)-2-methoxyaniline (4.0 g, 97%) as a light yellow powder.
22
23
24
25
26
27
28 MS (ES+) C₁₂H₁₂ClN₃O₂ requires: 265, found: 266 [M+H]⁺.
29
30

31 *Step 3: 1-(4-((2-Chloropyrimidin-4-yl)methoxy)-2-methoxyphenyl)-3-((1S,2S)-2-*
32
33
34 *hydroxycyclohexyl)thiourea.* To a solution of 4-((2-chloropyrimidin-4-yl)methoxy)-2-
35
36
37 methoxyaniline (4.0 g, 15 mmol) in DCM (200 ml) was added di(1H-imidazol-1-
38
39
40 yl)methanethione (3.2 g, 18 mmol) and the resulting mixture was stirred at rt for 4 h.
41
42
43 (1S,2S)-2-Aminocyclohexanol (3.5 g, 30 mmol) was added and the resulting mixture was
44
45
46 stirred at rt for 3 h. The volatiles were removed under reduced pressure. The residue was
47
48
49 purified via silica gel chromatography (25 - 100% EtOAc in hexanes) to give 1-(4-((2-
50
51
52 chloropyrimidin-4-yl)methoxy)-2-methoxyphenyl)-3-((1S,2S)-2-
53
54
55
56
57
58
59
60

1
2
3 hydroxycyclohexyl)thiourea (6.1 g, 96%) as a white solid. MS (ES+) C₁₉H₂₃N₄O₃S
4
5
6
7 requires: 422, found: 423 [M+H]⁺.
8
9

10 *Step 4: (1S,2S)-2-((6-((2-Chloropyrimidin-4-yl)methoxy)-4-methoxybenzo[d]thiazol-2-*
11
12
13
14 *yl)amino)cyclohexan-1-ol.* To a solution of 1-(4-((2-chloropyrimidin-4-yl)methoxy)-2-
15
16
17 methoxyphenyl)-3-((1S,2S)-2-hydroxycyclohexyl)thiourea (1.0 g, 2.3 mmol) in DCM (100
18
19
20 ml) was added BSTFA (1.3 ml, 4.7 mmol) and the resulting solution was stirred for 5 min.
21
22
23
24 Solid benzyltrimethylammonium tribromide (0.92 g, 2.3 mmol) was added into the
25
26
27 previous reaction mixture. After 20 min sat. NaHCO₃ (30 ml) was added and the reaction
28
29
30 mixture was stirred for 5 min. The biphasic mixture was transferred to a separatory funnel
31
32
33
34 with an additional 20 ml of DCM, and the layers were separated. The organic layers were
35
36
37 washed with saturated aq. NaHCO₃ (30 ml), followed by 10% aq. Na₂S₂O₃ (1 x 20 ml),
38
39
40
41 dried over MgSO₄, filtered, and concentrated under reduced pressure. The residue was
42
43
44 trituated with 1:2 Ether/Hexanes (30 ml) and the resulting solid was filtered through a
45
46
47
48 Buchner funnel. After rinsing with 1:1 Ether/hexanes (10 ml), (1S,2S)-2-((6-((2-
49
50
51 chloropyrimidin-4-yl)methoxy)-4-methoxybenzo[d]thiazol-2-yl)amino)cyclohexan-1-ol
52
53
54
55
56
57
58
59
60

1
2
3 was collected as off white powder (945 mg, 95%). MS (ES+) $C_{19}H_{21}ClN_4O_3S$ requires:
4
5
6
7 420, found: 421 [M+H]⁺.
8
9

10 *Step 5: (1S,2S)-2-((6-((2-Aminopyrimidin-4-yl)methoxy)-4-methoxybenzo[d]thiazol-2-*
11
12
13 *yl)amino)cyclohexan-1-ol.* A microwave vial was charged with (1S,2S)-2-((6-((2-
14
15
16
17
18 chloropyrimidin-4-yl)methoxy)-4-methoxybenzo[d]thiazol-2-yl)amino)cyclohexanol (300
19
20
21 mg, 0.71 mmol) and NH₃ (7M in MeOH, 2.0 mL). The vial was sealed and the reaction
22
23
24
25
26
27
28 mixture was heated to 120 °C in the microwave reactor for 4 h. The volatiles were
29
30
31 removed under reduced pressure. The residue was purified by reverse phase preparative
32
33
34 HPLC (Mobile phase: A = 0.1% TFA/H₂O, B = 0.1% TFA/MeCN; Gradient: B = 10 - 90%;
35
36
37 20 min; Column: C18) to give (1S,2S)-2-((6-((2-aminopyrimidin-4-yl)methoxy)-4-
38
39
40
41
42 methoxybenzo[d]thiazol-2-yl)amino)cyclohexan-1-ol as a TFA salt (182 mg, 64%) as an
43
44
45 off white powder. MS (ES+) $C_{19}H_{23}N_5O_3S$ requires: 401, found: 402 [M+H]⁺; ¹H NMR (600
46
47
48 MHz, DMSO-*d*₆) δ 8.27 (d, *J* = 6.5 Hz, 1H), 6.86 (d, *J* = 5.2 Hz, 1H), 6.83 (d, *J* = 2.4 Hz,
49
50
51 1H), 6.62 (d, *J* = 2.0 Hz, 1H), 4.90 (s, 2H), 3.93 (s, 3H), 3.59 (m, 1H), 3.40 (m, 1H), 2.14
52
53
54 (m, 1H), 2.02 (m, 1H), 1.76 (m, 2H), 1.43-1.28 (m, 4H).
55
56
57
58
59
60

1
2
3
4 **Synthesis** of **(1S,2S)-2-((6-((2-Aminopyrimidin-4-yl)methoxy)-7-chloro-4-**
5
6
7 **methoxybenzo[d]thiazol-2-yl)amino)cyclohexan-1-ol (23)**

8
9
10 *Step 1: 2-Chloro-5-methoxy-4-nitrophenol.* To a solution of 3-methoxy-4-nitrophenol
11
12 (1.0 g, 5.9 mmol) at 0 °C was added NCS (0.868g, 6.50 mmol), and the resulting mixture
13
14 was stirred at 50°C for 2 h. The reaction mixture was diluted with EtOAc (20 mL) and
15
16 was stirred at 50°C for 2 h. The reaction mixture was diluted with EtOAc (20 mL) and
17
18 washed with water (2 x 20 mL). The layers were separated, and the organic layer was
19
20 washed with water (2 x 20 mL). The layers were separated, and the organic layer was
21
22 washed with sat. NaCl (10 mL), dried over MgSO₄, filtered and concentrated under
23
24 reduced pressure to provide 2-chloro-5-methoxy-4-nitrophenol (1.2 g, ~100%) as a yellow
25
26 solid. MS (ES+) C₇H₆ClNO₄ requires: 203, found: 204 [M+H]⁺.
27
28
29
30
31
32
33

34
35 *Step 2-6: As described for compound 22.* MS (ES+) C₁₉H₂₂ClN₅O₃S requires: 435,
36
37 found: 436 [M+H]⁺; ¹H NMR (600 MHz, DMSO-*d*₆) δ 8.27 (d, *J* = 5.0 Hz, 1H), 7.89 (d, *J* =
38
39 7.7 Hz, 1H), 6.79 (s, 1H), 6.74 (d, *J* = 4.9 Hz, 1H), 6.67 (s, 2H), 5.04 (s, 2H), 4.75 (d, *J* =
40
41 5.1 Hz, 1H), 3.85 (s, 3H), 3.53 – 3.46 (m, 1H), 3.36 – 3.32 (m, 1H), 2.09 – 1.99 (m, 1H),
42
43 1.92 – 1.84 (m, 1H), 1.69 – 1.56 (m, 2H), 1.31 – 1.16 (m, 4H).
44
45
46
47
48
49
50
51

52 **Synthesis of (1R,2R)-2-(6-((2-aminopyridin-4-yl)methoxy)-4-methoxybenzo[d]thiazol-2-**
53
54 **ylamino)cyclohexanol (24)**

1
2
3
4 *Step 1: Tert-butyl 4-((2-((1R,2R)-2-hydroxycyclohexylamino)-4-*
5
6
7 *methoxybenzo[d]thiazol-6-yloxy)methyl)pyridin-2-ylcarbamate.* A solution of 2-((1R,2R)-
8
9
10 2-hydroxycyclohexylamino)-4-methoxybenzo[d]thiazol-6-ol (100 mg, 0.34 mmol) tert-
11
12
13 butyl 4-(chloromethyl)pyridin-2-ylcarbamate (83 mg, 0.34 mmol) and Cs₂CO₃ (333 mg,
14
15 1.02 mmol) in DMF (2 mL) was stirred at 80°C for 2 h. The reaction mixture was diluted
16
17
18 with water (5 mL) and extracted with EtOAc (3 x 50 mL). The combined organic phases
19
20
21 were washed with water (10 ml), brine (10 ml), dried over Na₂SO₄, filtered and
22
23
24 concentrated under reduced pressure to provide tert-butyl 4-((2-((1R,2R)-2-
25
26
27 hydroxycyclohexylamino)-4-methoxybenzo[d]thiazol-6-yloxy)methyl)pyridin-2-
28
29
30 ylcarbamate (30 mg, 17%) as a yellow solid. MS (ES+) C₂₅H₃₂N₄O₅S requires: 500, found:
31
32
33 501[M+H]⁺.
34
35
36
37
38
39
40
41

42 *Step 2: (1R,2R)-2-(6-((2-Aminopyridin-4-yl)methoxy)-4-methoxybenzo[d]thiazol-2-*
43
44
45 *ylamino)cyclohexanol.* A mixture of tert-butyl 4-((2-((1R,2R)-2-hydroxycyclohexylamino)-
46
47
48 4-methoxybenzo[d]thiazol-6-yloxy)methyl)pyridin-2-ylcarbamate (30 mg, 0.06 mmol) in
49
50
51 TFA (1mL) was stirred at rt for 16 h. The volatiles were removed under reduced pressure
52
53
54 and the residue was purified by prep-HPLC to provide (1R,2R)-2-(6-((2-aminopyridin-4-
55
56
57
58
59
60

1
2
3
4 yl)methoxy)-4-methoxybenzo[d]thiazol-2-ylamino)cyclohexanol (8.6 mg, 35%) as a white
5
6
7 solid. MS (ES+) $C_{21}H_{23}N_5O_2$ requires: 400, found: 401[M+H]⁺; ¹H NMR (500 MHz, DMSO-
8
9
10 *d*₆) δ 7.87 (d, *J* = 5.2 Hz, 1H), 7.62 (d, *J* = 7.5 Hz, 1H), 6.90 (d, *J* = 2.3 Hz, 1H), 6.54 –
11
12
13 6.48 (m, 3H), 5.97 (s, 2H), 4.95 (s, 2H), 4.78 (d, *J* = 5.0 Hz, 1H), 3.82 (s, 3H), 3.55 – 3.47
14
15
16
17 (m, 1H), 2.07 – 2.01 (m, 1H), 1.91 – 1.84 (m, 1H), 1.63 (t, *J* = 13.1 Hz, 2H), 1.32 – 1.16
18
19
20
21 (m, 4H).

22
23
24 **Synthesis** of **N-(4-(((2-(((1R,2R)-2-hydroxycyclohexyl)amino)-4-**
25
26
27 **methoxybenzo[d]thiazol-6-yl)oxy)methyl)pyridin-2-yl)acetamide (25)**

28
29
30
31 *Step 1: 2-bromo-4-methoxybenzo[d]thiazol-6-ol.* A vial was charged with 6-(benzyloxy)-
32
33
34 2-bromo-4-methoxybenzo[d]thiazole (0.80g, 2.28 mmol) and TFA (1.76 ml, 22.8 mmol)
35
36
37 and the suspension stirred at 65°C for 40h. The volatiles were removed under reduced
38
39
40
41
42 pressure. The residue was purified via silica gel chromatography (0 to 100 % EtOAc in
43
44
45 hexanes), however, the product was contaminated with starting material. The solid was
46
47
48 trituated with DCM (2 x 5 mL), centrifuged, and the liquid decanted. The remaining solid
49
50
51
52 was dried to provide 2-bromo-4-methoxybenzo[d]thiazol-6-ol (142 mg, 0.546 mmol, 24%
53
54
55
56 yield) as a white solid. MS (ES+) $C_8H_6BrNO_2S$ requires: 259 found: 259.9 [M+H]⁺.

1
2
3
4 *Step 2: Tert-butyl (4-(((2-bromo-4-methoxybenzo[d]thiazol-6-yl)oxy)methyl)pyridin-2-*
5
6
7 *yl)carbamate.* To a suspension of tert-butyl (4-(hydroxymethyl)pyridin-2-yl)carbamate
8
9
10 (133 mg, 0.592 mmol), 2-bromo-4-methoxybenzo[d]thiazol-6-ol (140 mg, 0.538 mmol),
11
12
13 and Ph₃P (198 mg, 0.754 mmol) in THF (2.6 mL) at 0 °C was added (E)-di-tert-butyl
14
15
16 diazene-1,2-dicarboxylate (174 mg, 0.754 mmol) and the resulting mixture was stirred at
17
18
19
20
21 rt for 16 h. The volatiles were removed under reduced pressure. The residue was purified
22
23
24 via silica gel chromatography (0 to 100 % EtOAc in hexanes) to provide tert-butyl (4-(((2-
25
26
27 bromo-4-methoxybenzo[d]thiazol-6-yl)oxy)methyl)pyridin-2-yl)carbamate (233 mg, 0.350
28
29
30 mmol, 93 % yield) as a pale yellow solid.

31
32
33
34
35 MS (ES+) C₁₉H₂₀BrN₃O₄S requires: 465 found: 466 [M+H]⁺.

36
37
38 *Step 3: N-(4-(((2-bromo-4-methoxybenzo[d]thiazol-6-yl)oxy)methyl)pyridin-2-*
39
40
41 *yl)acetamide.*

42
43
44
45 To a suspension of tert-butyl (4-(((2-bromo-4-methoxybenzo[d]thiazol-6-
46
47
48 yl)oxy)methyl)pyridin-2-yl)carbamate (233 mg, 0.50 mmol) in DCM (1 mL) at 0 °C was
49
50
51 added TFA (962 μl, 12.5 mmol) and the resulting mixture was stirred at rt for 3 h. The
52
53
54
55 residue was adsorbed onto Celite and purified via flash chromatography (0 - 10 % MeOH
56
57
58
59
60

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2
3
4 in DCM w/ 0.5% NH₄OH) to provide 4-(((2-bromo-4-methoxybenzo[d]thiazol-6-
5
6
7 yl)oxy)methyl)pyridin-2-amine (109 mg, 0.298 mmol, 60%) as a green amorphous
8
9
10 material. MS (ES+) C₁₄H₁₂BrN₃O₂S requires: 365 found: 366 [M+H]⁺.

11
12
13
14 *Step 4: N-(4-(((2-bromo-4-methoxybenzo[d]thiazol-6-yl)oxy)methyl)pyridin-2-*
15
16
17 *yl)acetamide.* To a suspension of 4-(((2-bromo-4-methoxybenzo[d]thiazol-6-
18
19
20 yl)oxy)methyl)pyridin-2-amine (69 mg, 0.19 mmol) and pyridine (76 μl, 0.942 mmol) in
21
22
23 DMF (377 μl) was added Ac₂O (89 μl, 0.94 mmol) and the resulting mixture was stirred at
24
25
26
27 60°C for 1 h. The volatiles were removed under reduced pressure. The residue was
28
29
30 purified via silica gel chromatography (0 - 10 % MeOH in DCM w/ 0.5% NH₄OH) to provide
31
32
33
34 N-(4-(((2-bromo-4-methoxybenzo[d]thiazol-6-yl)oxy)methyl)pyridin-2-yl)acetamide (68
35
36
37 mg, 88%) as a green foam solid.

38
39
40
41
42 MS (ES+) C₁₆H₁₄BrN₃O₃S requires: 407 found: 408 [M+H]⁺.

43
44
45 *Step 5: N-(4-(((2-(((1R,2R)-2-hydroxycyclohexyl)amino)-4-methoxybenzo[d]thiazol-6-*
46
47
48 *yl)oxy)methyl)pyridin-2-yl)acetamide.* To a solution of N-(4-(((2-bromo-4-
49
50
51 methoxybenzo[d]thiazol-6-yl)oxy)methyl)pyridin-2-yl)acetamide (68 mg, 0.17 mmol) and
52
53
54
55 (1R,2R)-2-aminocyclohexanol (57 mg, 0.50 mmol) in DMA (555 μl) was added DIPEA (32
56
57
58
59
60

1
2
3
4 μl , 0.183 mmol) and the resulting mixture was stirred at 100 °C for 12h. The volatiles were
5
6
7 removed under reduced pressure. The residue was purified via silica gel chromatography
8
9
10 (0 - 10 % MeOH in DCM w/ 0.5% NH_4OH) to provide N-(4-(((2-(((1R,2R)-2-
11
12 hydroxycyclohexyl)amino)-4-methoxybenzo[d]thiazol-6-yl)oxy)methyl)pyridin-2-
13
14 yl)acetamide (28 mg, 0.063 mmol, 38.0%) as a off-white solid. MS (ES+) $\text{C}_{22}\text{H}_{26}\text{N}_4\text{O}_4\text{S}$
15
16
17 requires: 442 found: 443 $[\text{M}+\text{H}]^+$. ^1H NMR (600 MHz, $\text{DMSO}-d_6$) δ 8.28 (d, $J = 6.0$ Hz,
18
19
20
21 1H), 7.63 (s, 1H), 7.14 (d, $J = 6.0$ Hz, 1H), 6.94 (d, $J = 6.0$ Hz, 1H), 6.56 (s, 1H), 5.13 (s,
22
23
24 2H), 4.78 (s, 1H), 3.83 (s, 3H), 3.5 (m, 1H), 2.09 (s, 3H), 2.03 (m, 1H), 1.86 (m, 1H), 1.63
25
26
27 (m, 2H), 1.23 (m, 4H).

28
29
30
31
32
33
34
35 **Synthesis** of **1-(4-(((2-(((1S,2S)-2-hydroxycyclohexyl)amino)-4-**
36
37 **methoxybenzo[d]thiazol-6-yl)oxy)methyl)pyrimidin-2-yl)-3-methylurea (26)**

38
39
40
41
42 *Step 1: N-((1S,2S)-2-((tert-butyldimethylsilyl)oxy)cyclohexyl)-6-((2-chloropyrimidin-4-*
43
44 *yl)methoxy)-4-methoxybenzo[d]thiazol-2-amine.* To a solution of (1S,2S)-2-(((2-
45
46 chloropyrimidin-4-yl)methoxy)-4-methoxybenzo[d]thiazol-2-yl)amino)cyclohexanol (300
47
48 mg, 0.72 mmol) in DMF (2 ml) were added TBDMSI (130 mg, 0.86 mmol) and imidazole
49
50
51 (150 mg, 2.2 mmol) and the resulting mixture was stirred at rt for 12 h. The residue was
52
53
54
55
56
57
58
59
60

1
2
3 purified via silica gel chromatography (10 to 50% EtOAc in hexanes to provide N-
4
5
6
7 ((1S,2S)-2-((tert-butyldimethylsilyl)oxy)cyclohexyl)-6-((2-chloropyrimidin-4-yl)methoxy)-
8
9
10 4-methoxybenzo[d]thiazol-2-amine (347 mg, 91 % yield) as an off-white solid. MS (ES+)
11
12
13
14 $C_{25}H_{35}ClN_4O_3SSi$ requires: 535, found: 536 [M+H] +.
15
16

17 *Step* 2: 6-((2-aminopyrimidin-4-yl)methoxy)-N-((1S,2S)-2-((tert-
18
19
20 *butyldimethylsilyl)oxy)cyclohexyl)-4-methoxybenzo[d]thiazol-2-amine*. A microwave vial
21
22
23
24 was charged with N-((1S,2S)-2-((tert-butyldimethylsilyl)oxy)cyclohexyl)-6-((2-
25
26
27 chloropyrimidin-4-yl)methoxy)-4-methoxybenzo[d]thiazol-2-amine (300 mg, 0.56 mmol),
28
29
30
31 and NH_4OH (aq., 2M) in 2-propanol (3 ml) was added. The vial was sealed and the
32
33
34
35 reaction mixture was heated to 120 °C in the microwave reactor for 8 h. The volatiles
36
37
38 were removed under reduced pressure. The residue was purified via silica gel
39
40
41
42 chromatography (20 to 70% EtOAc in hexanes to provide 6-((2-aminopyrimidin-4-
43
44
45 yl)methoxy)-N-((1S,2S)-2-((tert-butyldimethylsilyl)oxy)cyclohexyl)-4-
46
47
48 methoxybenzo[d]thiazol-2-amine (208 mg, 72%) as an off white solid. MS (ES+)
49
50
51
52 $C_{25}H_{37}N_5O_3SSi$ requires: 515, found: 516 [M+H] +.
53
54
55
56
57
58
59
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3
4 *Step 3: 1-(4-(((2-(((1S,2S)-2-((tert-butyldimethylsilyl)oxy)cyclohexyl)amino)-4-*
5
6
7 *methoxybenzo[d]thiazol-6-yl)oxy)methyl)pyrimidin-2-yl)-3-methylurea.* To a solution of 6-
8
9
10 *(((2-aminopyrimidin-4-yl)methoxy)-N-((1S,2S)-2-((tert-butyldimethylsilyl)oxy)cyclohexyl)-*
11
12
13
14 4-methoxybenzo[d]thiazol-2-amine (100 mg, 0.20 mmol) in DCM (5 ml) were added
15
16
17 pyridine (33 μ L, 0.39 mmol), DMAP (2 mg, 0.02 mmol) and phenyl chloroformate (40 mg,
18
19
20 0.25 mmol). The resulting mixture was stirred at rt for 3 h. The reaction was quenched
21
22
23
24 with MeNH₂ (0.2 ml, 1.4 mmol) (7N in MeOH) and stirred for 16 h. The volatiles were
25
26
27
28 removed under reduced pressure and the residue was diluted with EtOAc (15 mL),
29
30
31 NaHCO₃ (aq. 10w/w%, 5 mL) was added, and the layers were separated. The aqueous
32
33
34
35 phase was extracted with EtOAc (1 x 5 mL), the combined organic layers were washed
36
37
38 with NaHCO₃ (aq. 10w/w%, 5 mL), dried over MgSO₄, filtered and concentrated under
39
40
41
42 reduced pressure. The residue was purified via silica gel chromatography (20 to 80 %
43
44
45 EtOAc in hexane to provide 1-(4-(((2-(((1S,2S)-2-((tert-
46
47
48 butyldimethylsilyl)oxy)cyclohexyl)amino)-4-methoxybenzo[d]thiazol-6-
49
50
51
52 yl)oxy)methyl)pyrimidin-2-yl)-3-methylurea (57 mg, 51%) as a yellow oil. MS (ES+)
53
54
55
56 C₂₇H₄₀N₆O₄SSi requires: 572, found: 573 [M+H]⁺.

1
2
3
4 *Step 4: 1-(4-(((2-(((1S,2S)-2-hydroxycyclohexyl)amino)-4-methoxybenzo[d]thiazol-6-*
5
6
7 *yl)oxy)methyl)pyrimidin-2-yl)-3-methylurea.* To a solution of 1-(4-(((2-(((1S,2S)-2-((tert-
8
9
10 butyldimethylsilyl)oxy)cyclohexyl)amino)-4-methoxybenzo[d]thiazol-6-
11
12
13
14 *yl)oxy)methyl)pyrimidin-2-yl)-3-methylurea* (50 mg, 0.087 mmol) in DCM (2ml) was added
15
16
17 TBAF (0.3 mL, 0.3 mmol) and the resulting mixture was stirred at rt for 12 h. The volatiles
18
19
20
21 were removed under reduced pressure. The residue was purified by mass-triggered
22
23
24 preparative HPLC (Mobile phase: A = 0.1% TFA/H₂O, B = 0.1% TFA/MeCN; Gradient: B
25
26
27 = 10- 50%; 20 min; Column: C18) to provide 1-(4-(((2-(((1S,2S)-2-
28
29
30
31 hydroxycyclohexyl)amino)-4-methoxybenzo[d]thiazol-6-yl)oxy)methyl)pyrimidin-2-yl)-3-
32
33
34
35 methylurea (16 mg, 40%) as a white solid. MS (ES+) C₂₁H₂₆N₆O₄S requires: 458.5, found:
36
37
38 459.5 [M+H]⁺. ¹H NMR (600 MHz, DMSO-*d*₆) δ 10.49 (s, 1H), 8.64 (d, *J* = 5.4 Hz, 1H),
39
40
41 7.24 (d, *J* = 5.4 Hz, 1H), 7.18 (s, 1H), 7.1 (s, 1H), 7.02 (m, 1H), 6.68 (s, 1H), 5.09 (s, 2H),
42
43
44 3.87 (s, 3H), 3.66 (s, 3H), 3.56 (m, 1H), 3.31-3.35 (m, 1H), 2.01 (m, 1H), 1.88 (m, 1H),
45
46
47 1.64 (m, 2H), 1.2-1.32 (m, 4H).

51
52 **Synthesis of (1S,2S)-2-(7-chloro-4-methoxy-6-((2-(1-methyl-1H-pyrazol-4-**
53
54
55
56 **ylamino)pyrimidin-4-yl)methoxy)benzo[d]thiazol-2-ylamino)cyclohexanol IACS-9439 (1)**
57
58
59
60

1
2
3
4 *Step 1: – (1S,2S)-2-((4-methoxy-6-((2-((1-methyl-1H-pyrazol-4-yl)amino)pyrimidin-4-*
5
6
7 *yl)methoxy)benzo[d]thiazol-2-yl)amino)cyclohexan-1-ol.* A microwave vial was charged
8
9
10 with (1S,2S)-2-((6-((2-chloropyrimidin-4-yl)methoxy)-4-methoxybenzo[d]thiazol-2-
11
12
13
14
15
16
17
18
19
20
21
22
23
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yl)amino)cyclohexanol (550 mg, 1.31 mmol), 1-methyl-1H-pyrazol-4-amine (381 mg, 3.92 mmol) and DIPEA (456 μ l, 2.61 mmol) and 2-propanol (5.2 mL) was added. The vial was sealed and the reaction mixture was heated to 120 °C in the microwave reactor for 5 h. The volatiles were removed under reduced pressure. The residue was purified by reverse phase preparative HPLC (Mobile phase: A = 0.1% NH₄OH/H₂O, B = 0.1% NH₄OH/MeCN; Gradient: B = 10 - 90%; 20 min; Column: C18) to provide the title compound (290 mg, 46%) as a light tan foam solid. HRMS (ES+) C₂₃H₂₈N₇O₃S⁺ requires: 482.1969, found: 482.1971 [M+H]⁺. ¹H NMR (500 MHz, DMSO-*d*₆) δ 9.50 (s, 1H), 8.41 (d, *J* = 3.6 Hz, 1H), 7.88 (s, 1H), 7.61 (d, *J* = 6.0 Hz, 2H), 7.47 (s, 1 H), 6.95 (s, 1H), 6.82 - 6.89 (d, *J* = 4.0, 1H), 6.59 (s, 1H), 5.0.5 (s, 2H), 4.76 (d, *J* = 4.0 Hz, 1H), 3.83 (s, 3H), 3.78 (s, 3H), 3.5 (m, 1H), 2.05 (m, 1H), 1.86 (m, 1H), 1.62 (m, 2H), 1.29 - 1.16 (m, 4H). ¹³C NMR (151 MHz, DMSO-*d*₆) δ 166.6, 163.71, 159.18, 158.69, 153.44, 149.98, 136.38, 131.27,

1
2
3 129.77, 123.08, 120.45, 107.53, 98.11, 97.73, 71.49, 69.62, 59.26, 55.62, 39.92, 39.78,
4
5
6
7 39.64, 39.51, 39.37, 39.23, 39.09, 38.61, 30.59, 23.91, 23.51.
8
9

10 **Synthesis of (1S,2S)-2-((4-methoxy-6-((2-((1-methyl-1H-pyrazol-3-yl)amino)pyrimidin-**
11
12
13
14 **4-yl)methoxy)benzo[d]thiazol-2-yl)amino)cyclohexan-1-ol (27)**
15

16
17 *Step 1: (1S,2S)-2-((4-methoxy-6-((2-((1-methyl-1H-pyrazol-3-yl)amino)pyrimidin-4-*
18
19
20
21 *yl)methoxy)benzo[d]thiazol-2-yl)amino)cyclohexan-1-ol.* A solution of (1S,2S)-2-((6-((2-
22
23
24 chloropyrimidin-4-yl)methoxy)-4-methoxybenzo[d]thiazol-2-yl)amino)cyclohexanol (20
25
26
27 mg, 0.048 mmol), 1-methyl-1H-pyrazol-3-amine (46 mg, 0.47 mmol) and DIPEA (0.2 mL)
28
29
30
31 in DMA (0.5 ml) was stirred at 110°C for 18 h. The residue was purified by mass-triggered
32
33
34 preparative HPLC (Mobile phase: A = 0.1% TFA/H₂O, B = 0.1% TFA/MeCN; Gradient: B
35
36
37 = 10 - 50%; 20 min; Column: C18) to provide (1S,2S)-2-((4-methoxy-6-((2-((1-methyl-1H-
38
39
40
41 pyrazol-3-yl)amino)pyrimidin-4-yl)methoxy)benzo[d]thiazol-2-yl)amino)cyclohexanol (3.5
42
43
44 mg, 15% yield) as a brown solid. MS (ES⁺) C₂₃H₂₇N₇O₃S requires: 481, found: 482.5
45
46
47
48 [M+H]⁺. 1H NMR (600 MHz, *d*₄-MeOD) δ 8.4 (s, 1H), 7.48 (s, 1H), 7.02 (d, *J* = 5.4 Hz,
49
50
51
52 1H), 6.99 (s, 1H), 6.84 (s, 1H), 6.53 (s, 1H), 5.14 (s, 2H), 3.99 (s, 3H), 3.84 (s, 3H), 3.65
53
54
55
56 (m, 1H), 3.42-3.49 (m, 1H), 2.05 – 2.1 (m, 2H), 1.78 (m, 2H), 1.33-1.42 (m, 4H).
57
58
59
60

1
2
3
4 **Synthesis of (1S,2R)-2-((6-((2-((1H-pyrazol-4-yl)amino)pyrimidin-4-yl)methoxy)-4-**
5
6
7 **methoxybenzo[d]thiazol-2-yl)amino)cyclohexanol (28)**

8
9
10 *Step 1:* (1S,2R)-2-((6-((2-((1H-pyrazol-4-yl)amino)pyrimidin-4-yl)methoxy)-4-
11
12
13
14 *methoxybenzo[d]thiazol-2-yl)amino)cyclohexanol.* A microwave vial was charged with
15
16
17 (1S,2S)-2-((6-((2-chloropyrimidin-4-yl)methoxy)-4-methoxybenzo[d]thiazol-2-
18
19
20
21 yl)amino)cyclohexanol (25 mg, 0.06 mmol), 1*H*-pyrazol-4-amine (20 mg, 0.24 mmol), TEA
22
23
24 (0.025 mL, 0.18 mmol) and DMSO-*d*₆ (1.5 mL). The reaction mixture was heated to 120°C
25
26
27
28 in the microwave reactor for 2 h. The mixture was purified by mass-triggered preparative
29
30
31 HPLC (Mobile phase: A = 0.1% TFA/H₂O, B = 0.1% TFA/MeCN; Gradient: B = 20 - 50%;
32
33
34 12 min; Column: C18) to give (1S,2R)-2-((6-((2-((1H-pyrazol-4-yl)amino)pyrimidin-4-
35
36
37
38 yl)methoxy)-4-methoxybenzo[d]thiazol-2-yl)amino)cyclohexanol (12.2 mg, 0.026 mmol,
39
40
41 43.9 % yield) as a gray solid. MS (ES+) C₂₂H₂₅N₇O₃S requires: 467.5, found: 468.5 [M+H]
42
43
44
45 +. ¹H NMR (600 MHz, *d*₄-MeOD) δ 8.38 (brs, 1H), 8.15 (m, 2H), 7.0 (s, 1H), 6.92 (s, 1H),
46
47
48 6.86 (s, 1H), 5.13 (s, 2H), 3.99 (s, 3H), 3.60 (m, 1H), 3.47-3.50 (m, 1H), 2.05 – 2.09 (m,
49
50
51 2H), 1.79 (m, 2H), 1.32-1.48 (m, 4H).
52
53
54
55
56
57
58
59
60

1
2
3 **(1S,2S)-2-(6-((2-(1H-pyrazol-5-ylamino)pyrimidin-4-yl)methoxy)-4-methoxybenzo**
4
5
6
7 **[d]thiazol-2-ylamino)cyclohexanol (29)**
8
9

10 *Step 1: (1S,2S)-2-(6-((2-(1H-pyrazol-5-ylamino)pyrimidin-4-yl)methoxy)-4-*
11 *methoxybenzo [d]thiazol-2-ylamino)cyclohexanol.* A mixture of (1S,2S)-2-(6-((2-
12
13
14 chloropyrimidin-4-yl)methoxy)-4-methoxybenzo[d]thiazol -2-ylamino)cyclohexanol (45
15
16
17
18 mg, 0.11 mmol), 1H-pyrazol-3-amine (11 mg, 0.13 mmol), Pd₂(dba)₃ (10 mg, 0.011
19
20
21 mmol), Xantphos (6 mg, 0.011) and Cs₂CO₃ (72 mg, 0.22 mmol) in dioxane (2 ml) was
22
23
24
25 stirred at 110°C for 2 h under N₂ atmosphere. The solids were filtered and the filtrate was
26
27
28 concentrated under reduced pressure. The residue was purified by preparative HPLC
29
30
31 (NH₄HCO₃) to provide the title compound (6 mg, 14%) as a white solid. MS (ES+)
32
33
34
35 C₂₂H₂₅N₇O₃S requires: 467 found: 468 [M+H]⁺. ¹H NMR (500 MHz, *d*₄-MeOD) δ 8.68 (d,
36
37
38 J = 5 Hz, 1H), 8.41 (d, *J* = 3 Hz, 1H), 7.40 (d, *J* = 5 Hz, 1H), 6.90 (m, d, *J* = 2 Hz, 1H),
39
40
41
42 6.67 (d, *J* = 3 Hz, 1H), 6.00 (d, *J* = 2.5 Hz, 1H), 5.20 (s, 2H), 3.95 (s, 3H), 3.60-3.61 (m,
43
44
45
46 1H), 3.41-3.42 (m, 1H), 2.14-2.16 (m, 1H), 2.03-2.05 (m, 1H), 1.72-1.79 (m, 2H), 1.31-
47
48
49
50
51
52 1.43 (m, 4H).
53
54
55
56
57
58
59
60

1
2
3 **Synthesis of (1S,2S)-2-((6-((2-(isoxazol-4-ylamino)pyrimidin-4-yl)methoxy)-4-**
4
5
6
7 **methoxybenzo[d]thiazol-2-yl)amino)cyclohexan-1-ol (30)**

8
9
10 *Step 1:* (1S,2S)-2-((6-((2-(isoxazol-4-ylamino)pyrimidin-4-yl)methoxy)-4-
11
12
13
14 *methoxybenzo[d]thiazol-2-yl)amino)cyclohexan-1-ol.* A vial was charged with (1S,2S)-2-
15
16
17 ((6-((2-chloropyrimidin-4-yl)methoxy)-4-methoxybenzo[d]thiazol-2-
18
19
20
21 yl)amino)cyclohexanol (20 mg, 0.048 mmol), isoxazol-4-amine (18 mg, 0.14 mmol) (0.020
22
23
24 mL, 0.14 mmol) and MeOH (2mL) and the reaction was heated for 3 h at 100°C. The
25
26
27 residue was purified by mass-triggered preparative HPLC (Mobile phase: A = 0.1%
28
29
30 TFA/H₂O, B = 0.1% TFA/MeCN; Gradient: B = 20 - 50%; 12 min; Column: C18) to provide
31
32
33 (1S,2S)-2-((6-((2-(isoxazol-4-ylamino)pyrimidin-4-yl)methoxy)-4-
34
35
36
37
38 methoxybenzo[d]thiazol-2-yl)amino)cyclohexanol (14 mg, 0.030 mmol, 63%) as a light
39
40
41 brown solid. MS (ES+) C₂₂H₂₄N₆O₄S requires: 468.5, found: copy in m/z [M+H]⁺.

42
43
44
45 ¹H NMR (600 MHz, *d*₄-MeOD) δ 9.06 (s, 1H), 8.52 (s, 1H), 8.46 (d, *J* = 5.4 Hz, 1H), 6.99
46
47
48 (dd, *J* = 15.0 and 5.4 Hz, 2H), 6.87 (s, 1H), 5.16 (s, 2H), 3.99 (s, 3H), 3.60 (m, 1H), 3.47-
49
50
51 3.51 (m, 1H), 2.05 – 2.1 (m, 2H), 1.78 (m, 2H), 1.34-1.44 (m, 4H).
52
53
54
55
56
57
58
59
60

1
2
3 **(1S,2S)-2-(4-methoxy-6-((2-(1-methyl-1H-1,2,3-triazol-4-ylamino)pyrimidin-4-yl)**

4
5
6
7 **methoxy)benzo[d]thiazol-2-ylamino)cyclohexanol (31)**

8
9
10 *Step 1: (1S,2S)-2-(4-methoxy-6-((2-(1-methyl-1H-1,2,3-triazol-4-ylamino)pyrimidin-4-*
11 *yl) methoxy)benzo[d]thiazol-2-ylamino)cyclohexanol.* A mixture of (1S,2S)-2-(6-((2-
12
13
14
15
16
17 chloropyrimidin-4-yl)methoxy)-4-methoxybenzo [d]thiazol-2-ylamino)cyclohexanol (60
18
19
20 mg, 0.14 mmol), 1-methyl-1H-1,2,3-triazol-4-amine (17 mg, 0.17 mmol), TsOH (12 mg,
21
22 0.07 mmol) in dioxane (2 ml) was stirred at 110°C for 16 h. The mixture was diluted with
23
24 EtOAc, washed with NaHCO₃ (aq. Sat.) and dried over Na₂SO₄. The combined organic
25
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29
30
31 layer were concentrated under reduced pressure. The residue was purified by
32
33
34 preparative HPLC (Mobile phase: A = 0.1% NH₄HCO₃/H₂O, B = MeCN; Gradient: B = 5 -
35
36
37
38 95%; 12 min; Column: C18) to give the title compound (7.0 mg, 10%) as a white solid.
39
40
41 MS (ES+) C₂₂H₂₆N₈O₃S requires: 482 found: 483 [M+H]⁺. ¹H NMR (500 MHz, DMSO-*d*₆)
42
43
44
45 δ 10.25 (s, 1H), 8.47 (d, *J* = 5 Hz, 1H), 8.13 (s, 1H), 7.61 (d, *J* = 7.5 Hz, 2H), 6.94-6.96
46
47
48 (m, 2 H), 6.60 (s, 1 H), 5.10 (s, 2H), 4.76 (d, *J* = 5 Hz, 1H), 4.00 (s, 3H), 3.83 (s, 3H),
49
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52 2.02-2.05 (m, 1H), 1.86-1.88 (m, 1H), 1.60-1.65 (m, 2H), 1.18-1.30 (m, 4H).
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4 **Synthesis** of **(1S,2S)-2-((4-methoxy-6-((2-(pyridin-3-ylamino)pyrimidin-4-**
5
6
7 **yl)methoxy)benzo[d]thiazol-2-yl)amino)cyclohexan-1-ol (32)**
8

9
10 *Step* 1: *(1S,2S)-2-((4-methoxy-6-((2-(pyridin-3-ylamino)pyrimidin-4-*
11
12 *yl)methoxy)benzo[d]thiazol-2-yl)amino)cyclohexanol.* A microwave vial was charged with
13
14 *(1S,2S)-2-((6-((2-chloropyrimidin-4-yl)methoxy)-4-methoxybenzo[d]thiazol-2-*
15
16 *yl)amino)cyclohexanol* (20 mg, 0.48 mmol), pyridin-3-amine (14 mg, 0.14 mmol), one drop
17
18 of HCl (aq. 36.5%) and MeOH (1.5 mL). The vial was sealed and the reaction mixture
19
20
21 was heated to 110 °C in the microwave reactor for 3 h. The residue was purified by mass-
22
23 triggered preparative HPLC (Mobile phase: A = 0.1% TFA/H₂O, B = 0.1% TFA/MeCN;
24
25 Gradient: B = 20 - 50%; 12 min; Column: C18) to give *(1S,2S)-2-((4-methoxy-6-((2-*
26
27 *(pyridin-3-ylamino)pyrimidin-4-yl)methoxy)benzo[d]thiazol-2-yl)amino)cyclohexanol* (11
28
29 mg, 48%) as an off-white solid. MS (ES+) C₂₄H₂₆N₆O₃S requires: 478.6, found: 479.5
30
31 [M+H]. ¹H NMR (600 MHz, *d*₄-MeOD) δ 9.40 (s, 1H), 9.28 (m, 1H), 9.10 (d, *J* = 4.8 Hz,
32
33 1H), 8.02 (d, *J* = 4.8 Hz, 1H), 7.91 (m, 2H), 7.07 (d, *J* = 2.4 Hz, 1H), 6.93 (d, *J* = 1.8 Hz,
34
35 1H), 5.44 (s, 2H), 4.02 (s, 3H), 3.60 (m, 1H), 3.47-3.51 (m, 1H), 2.05 – 2.1 (m, 2H), 1.79
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37 (m, 2H), 1.34-1.46 (m, 4H).
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4 **Synthesis** of **(1S,2S)-2-(7-chloro-4-methoxy-6-((2-(1-methyl-1H-pyrazol-4-**
5
6
7 **ylamino)pyrimidin-4-yl)methoxy)benzo[d]thiazol-2-ylamino)cyclohexanol (33)**
8

9
10 *Step* 1: *(1S,2S)-2-(7-chloro-6-((2-chloropyrimidin-4-yl)methoxy)-4-*
11
12
13
14 *methoxybenzo[d]thiazol-2-ylamino)cyclohexanol:* A mixture of 7-chloro-2-((1S,2S)-2-
15
16
17 hydroxycyclohexylamino)-4-methoxybenzo[d]thiazol-6-ol (350 mg, 1.07 mmol), 2-chloro-
18
19
20 4-(chloromethyl)pyrimidine (208 mg, 1.28 mmol) and Cs₂CO₃ (698 mg, 2.14 mmol) in
21
22
23
24 DMF (8 ml) was stirred at 80°C for 3 h. The volatiles were removed under reduced
25
26
27
28 pressure to give the residue, which was purified by column chromatography (PE: EA =
29
30
31 1:9) to give *(1S,2S)-2-(7-chloro-6-((2-chloropyrimidin-4-yl)methoxy)-4-*
32
33
34 *methoxybenzo[d]thiazol-2-ylamino)cyclohexanol (3)* (185 mg, 35%) as a yellow solid. MS
35
36
37
38 (ES⁺) C₁₉H₂₀Cl₂N₄O₃S requires: 454 found: 455 [M+H]⁺.
39
40

41
42 *Step* 2: *(1S,2S)-2-(7-chloro-4-methoxy-6-((2-(1-methyl-1H-pyrazol-4-*
43
44
45 *ylamino)pyrimidin-4-yl)methoxy)benzo[d]thiazol-2-ylamino)cyclohexanol.* A mixture of
46
47
48
49 *(1S,2S)-2-(7-chloro-6-((2-chloropyrimidin-4-yl)methoxy)-4-methoxybenzo[d]thiazol-2-*
50
51
52 *ylamino)cyclohexanol (3)* (60 mg, 0.13 mmol), 1-methyl-1H-pyrazol-4-amine (21 mg, 0.15
53
54
55 mmol), TsOH (11 mg, 0.065 mmol) in dioxane (2 ml) was stirred at 110°C overnight. The
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3 mixture was diluted with EtOAc, washed with NaHCO₃ (aq. sat.) and dried over Na₂SO₄.
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7 The combined organic layer were concentrated under reduced pressure. The residue was
8
9

10 purified by preparative HPLC (Mobile phase: A = 0.1% NH₄HCO₃/H₂O, B = MeCN;
11
12

13 Gradient: B = 5 - 95%; 12 min; Column: C18) to give (1S,2S)-2-(7-chloro-4-methoxy-6-
14
15

16 ((2-(1-methyl-1H-pyrazol-4-ylamino)pyrimidin-4-yl)methoxy)benzo[d]thiazol-2-
17
18

19 ylamino)cyclohexanol (3.5 mg, 5%) as a white solid. MS (ES+) C₂₃H₂₆ClN₇O₃S requires:
20
21

22 515 found: 516 [M+H]⁺. ¹H NMR (500 MHz, DMSO-*d*₆) δ 9.51 (s, 1H), 8.44 (d, *J* = 4.5 Hz,
23
24

25 1H), 7.89 (d, *J* = 9.5 Hz, 2H), 7.48 (s, 1 H), 6.83-6.89 (m, 2 H), 5.17 (s, 2H), 4.17 (d, *J* =
26
27

28 5.5 Hz, 1H), 3.77 (s, 3H), 3.49 (s, 3H), 2.02-2.08 (m, 1H), 1.86-1.89 (m, 1H), 1.60-1.65
29
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31 (m, 2H), 1.19-1.30 (m, 4H).
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42 ASSOCIATED CONTENT

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46 Supporting information

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50 Assay conditions, materials and methods, kinase profiling data for compound **12** [Table
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54 S1] and for IACS-9439 (1) [Table S3] and a csv file containing molecular formula strings.
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3 The Supporting Information is available free of charge on the ACS Publication website at
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23 24 Author Contributions 25 26

27
28 The manuscript was written through contributions of all authors. All authors have given
29
30 approval to the final version of the manuscript.
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36 37 Notes 38 39

40 The authors declare no competing financial interest.
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18 ABBREVIATIONS

19
20
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22 °C, Celsius; ¹H-NMR, proton nuclear magnetic resonance; can, acetonitrile; AcOH,
23
24
25
26 acetic acid; BSA, bovine serum albumin; BSTFA, *N,O*-
27
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29 Bis(trimethylsilyl)trifluoroacetamide; *d*₄-MeOD, deuterated methanol; DMSO-*d*₆,
30
31
32 deuterated dimethyl sulfoxide; DBU, 1,8-diazabicycloundec-7-ene; DCM,
33
34
35
36 dichloromethane; DIBAL, diisobutylaluminum hydride; DIEA, N,N-diisopropylethylamine;
37
38
39
40 DMA, dimethyl acetamide; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; DPPA,
41
42
43
44 diphenylphosphoryl azide; DTT, dithiothreitol; ES⁺, electrospray positive ionization;
45
46
47
48 EGTA, ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid; ELISA,
49
50
51
52 enzyme-linked immunosorbent assay; Et₂O, diethyl ether; EtOAc, ethyl acetate; FRET,
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54
55
56 fluorescence resonance energy transfer; h, hour; HATU, 1-

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2
3 [bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide
4
5
6
7 hexafluorophosphate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HI
8
9
10 FBS, heat inactivated fetal bovine serum; HPLC, high pressure liquid chromatography;
11
12
13 HRP, horseradish peroxidase; Hz, hertz; IPA, isopropanol; M, molar; mCPBA, 3-
14
15
16 chloroperbenzoic acid; MeCN, acetonitrile; MHz, megahertz; min, minute; mL, milliliter;
17
18
19
20 MS, mass spectrometry; MW, microwave; PBS, phosphate buffer saline; PMB, p-
21
22
23 methoxybenzyl; qPCR, quantitative polymerase chain reaction; rt, room temperature;
24
25
26
27
28 Selectfluor, 1-Chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane
29
30
31 bis(tetrafluoroborate); tBuONO, tert-butyl nitrite; TFA, trifluoroacetic acid; THF,
32
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35 tetrahydrofuran.
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