Journal Pre-proof

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PII: S0223-5234(20)31000-X

DOI: https://doi.org/10.1016/j.ejmech.2020.113028

Reference: EJMECH 113028

To appear in: *European Journal of Medicinal Chemistry*

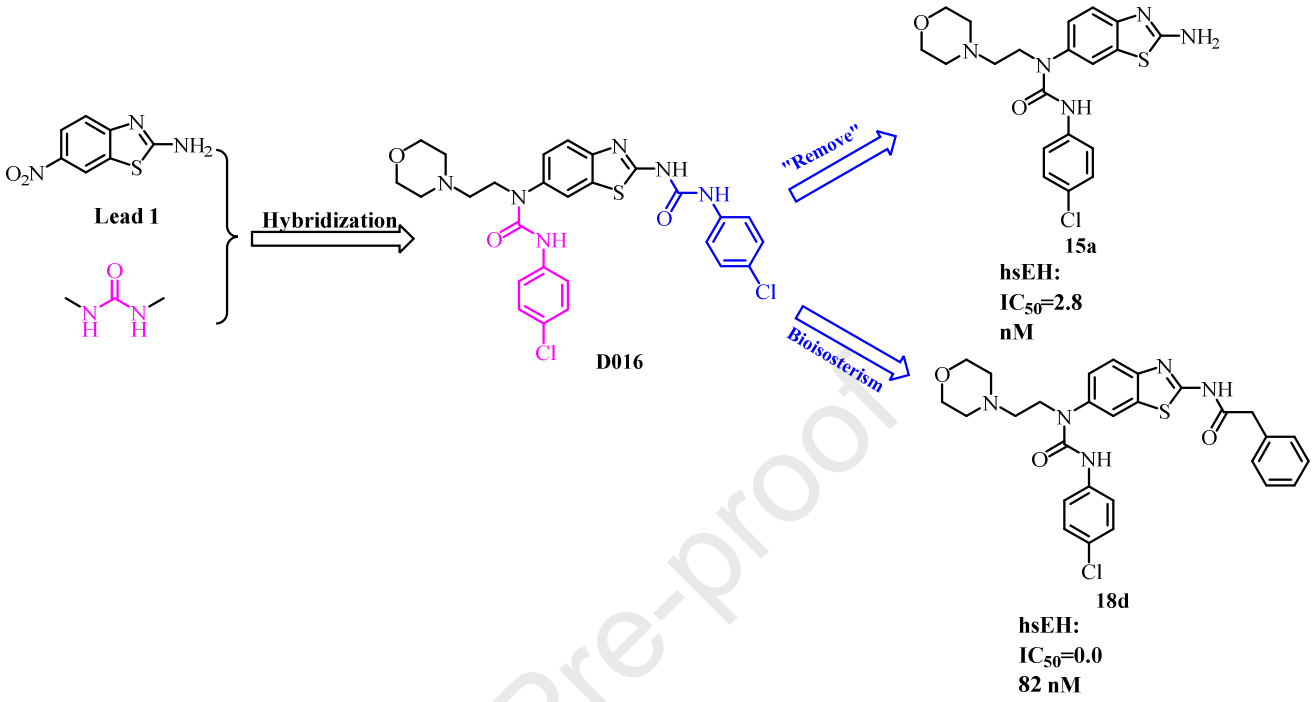
Received Date: 6 October 2020

Revised Date: 11 November 2020

Accepted Date: 13 November 2020

Please cite this article as: Y. Han, D. Huang, S. Xu, L. Li, Y. Tian, S. Li, C. Chen, Y. Li, Y. Sun, Y. Hou, Y. Sun, M. Qin, P. Gong, Z. Gao, Y. Zhao, Ligand-based optimization to identify novel 2-aminobenzo[*d*]thiazole derivatives as potent sEH inhibitors with anti-inflammatory effects, *European Journal of Medicinal Chemistry*, https://doi.org/10.1016/j.ejmech.2020.113028.

Graphical Abstract



Three series of novel 2-aminobenzo[*d*]thiazole derivatives were designed, synthesized and evaluated for their biological activities.

Ligand-based optimization to identify novel 2-aminobenzo[*d*]thiazole derivatives as potent sEH inhibitors with anti-inflammatory effects

Yufei Han [a], Desheng Huang [b], Sicong Xu [a], Lingling Li [b], Ye Tian [a], Shuo Li [b], Cong Chen [a], Yingxiu Li [a], Yanping Sun [b], Yunlei Hou [a], Yongjun Sun [b], Mingze Qin [a, c], Ping Gong [a], Zibin Gao [b1*], Yanfang Zhao [a1*]

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Abstract:

Inhibition of the soluble epoxide hydrolase (sEH) is a promising new therapeutic approach in the treatment of inflammation. Driven by the in-house database product lead **1**, a hybridization strategy was utilized for the design of a series of novel benzo[*d*]thiazol derivatives. To our delight, **D016**, a byproduct of compound **9**, was obtained with an extraordinarily low IC_{50} value of 0.1 nM but poor physical and chemical properties. After removal of a non-essential urea moiety or replacement of the urea group by an amide group, compounds **15a**, **17p,** and **18d** were identified as promising sEH inhibitors, and their molecular binding modes to sEH were constructed. Furthermore, compounds **15a** and **18d** exhibited more effective in *vivo* anti-inflammatory effect than ***t*-AUCB** in carrageenan-induced mouse paw edema. Compound **15a** also showed moderate metabolic stability with a half-time of 34.7 min. Although **18d** was unstable in rat liver microsomes, it might be a "prodrug". In conclusion, this study could provide valuable insights into discovery of new sEH inhibitors, and compounds **15a** and **18d** were worthy of further development as potential drug candidates to treat inflammation.

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Keywords: sEH, 2-aminobenzo[*d*]thiazole, structure-based design, anti-inflammation

1. Introduction

Inflammation is a complex pathophysiological process, involving a myriad of enzymes, mediators, and receptors [1]. The typical physiological characteristics of inflammation are redness, swelling, fever, pain, and even loss of organ function [2]. In the past decades, several studies have suggested that the arachidonic acid (AA) pathway generates bioactive lipid mediators, which regulate a diverse range of inflammatory processes. There are three major enzymatic pathways involved in regulating eicosanoid synthesis, namely cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome P450 proteins. Recently, the cytochrome P450 pathway has garnered great attention. Epoxyeicosatrienoic acids (EETs) are cytochrome P450-derived eicosanoids that can produce vasodilation and inhibit the cytokine-induced inflammatory response in the vasculature, heart, and kidney [3, 4]. EETs are known as important signal components in an organism and produce biological effects such as vasodilation, blood pressure, and anti-inflammation. EETs were reported to inhibit the activity of IκB kinase (IKK) [5], thus inhibiting the cytokine-induced activation of nuclear factor κB (NF-κB), an essential regulatory factor for endothelium activation, and playing an anti-inflammation role [6]. Moreover, EETs can transcriptionally downregulate cyclooxygenase 2 (COX-2), resulting in reduction of inflammatory metabolites [7, 8]. Studies have shown that inhibition of soluble epoxide hydrolase (sEH) in humans could increase the concentration of EETs [9]. Thus, inhibition of sEH is considered a promising therapeutic approach for resolving inflammation.

sEH in humans is a bifunctional homodimeric enzyme in the cytosol and peroxisomes with both epoxide hydrolase and phosphatase activities [10]. Notably, the C-terminal domain of sEH transforms EETs to dihydroxyeicosatrienoic acids (DHETs), thus exhibiting an epoxide hydrolase activity [11]. sEH inhibitors were shown to increase the concentration of EETs in plasma and tissues [12-14] and exhibit more potent anti-inflammation effect in *vivo* than NSAIDs and COXIBs, in a variety of inflammation-based assays [15-17]. These results indicate that maintaining the in *vivo* concentration of EETs through inhibiting sEH enzyme is a promising therapeutic

approach for inflammation. Thus, there is increasing interest in the development and preclinical evaluation of novel sEH inhibitors.

Although several inhibitors of sEH have been identified, none is available on the market to date. Only a few inhibitors have reached clinical trials. As shown in **Fig.1**, di-substituted ureas and amides were found to be the most popular class of sEH inhibitors [18-22]. Among these inhibitors, EC5026, a first-in-class and orally active sEH inhibitor shows efficacy for inflammatory and neuropathic pain and for use as a non-addictive opioid alternative [23]. GSK2256924 shows a protective effect against cigarette smoke-induced pulmonary inflammation in mice [24]. Furthermore, X-ray crystallographies of most sEH inhibitors, such as t-AUCB and TPPU, have demonstrated that ureas or amides could form a strong net like hydrogen bonds with residues Asp335, Tyr466, and Tyr383 in the catalytic domain of sEH [25-28].

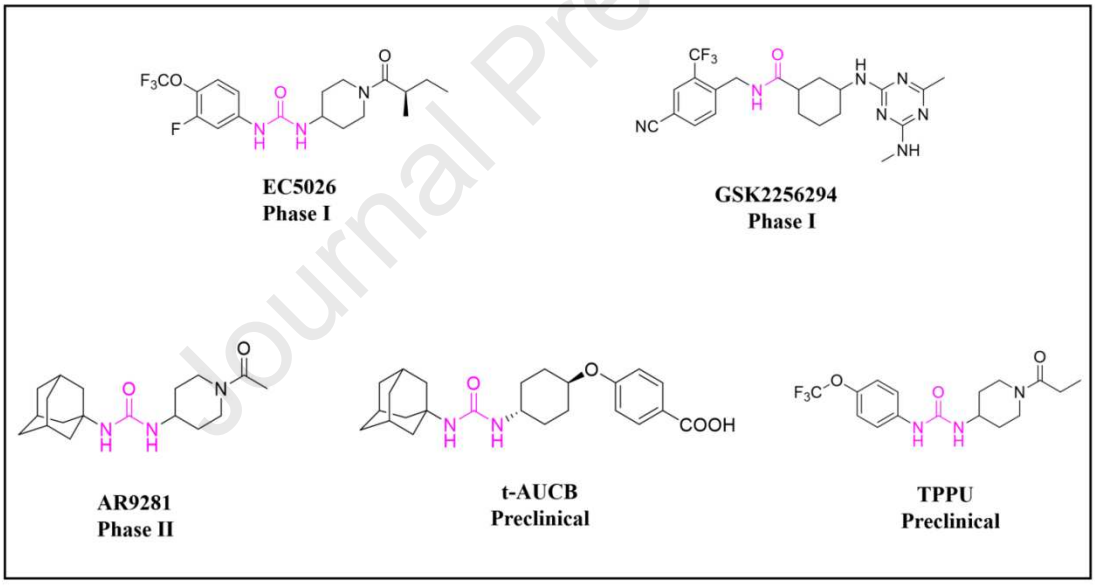


Fig.1. Representative examples of sEH inhibitors.

Through a screening of the in-house database, lead compound **1**, 2-aminobenzo[*d*]thiazole, was identified with sEH inhibitory activity of 0.8 μM. To identify novel scaffold entities as potent sEH inhibitors, a molecular hybridization strategy between the urea group and lead **1** was applied. Thus, a series of 1-(benzo[*d*]thiazol-2-yl)-3-phenylurea derivatives were designed and synthesized. Interestingly, during the synthesis of compound **9**, **D016**, a byproduct, exerted with 67-fold potency against sEH than t-AUCB (IC_{50} = 0.1 nM). Further modification

efforts were made through a structure-activity relationships (SARs) study of **D016**; three other series of 1-(2-aminobenzo[*d*]thiazol-6-yl)urea derivatives (compounds **15a-h**, **17a-v**, **and 18a-r**) were obtained. Of these, compounds **15a**, **17p,** and **18d exhibited** significant enzymatic activities, and they were assayed using carrageenan-induced mice models.

2. Results and discussion

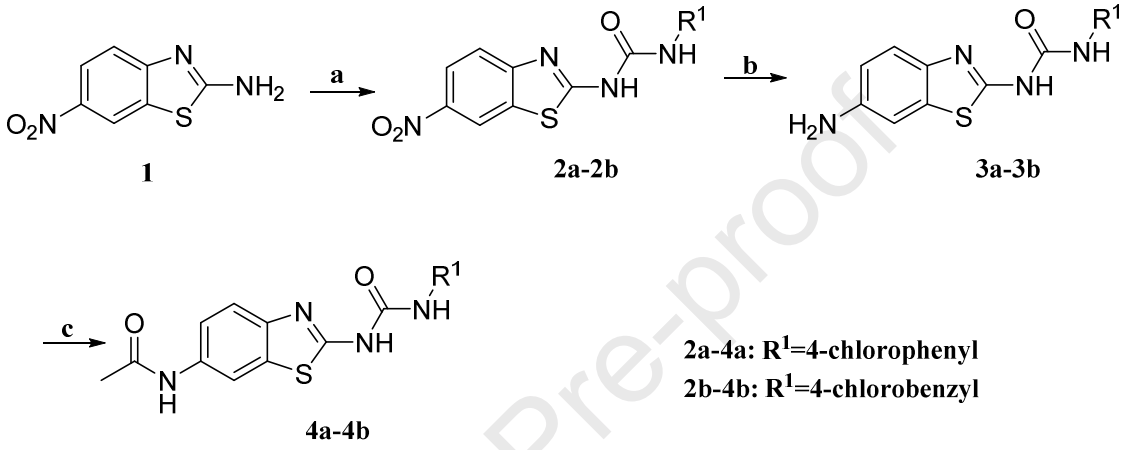
2.1 Chemistry

The general synthetic routes of title compounds are illustrated in **Scheme 1-5**. Commercially available starting material 2-amino-6-nitrobenzothiazole (**1**) was reacted with phenyl (4-chlorophenyl) carbamate or phenyl (4-chlorobenzyl) carbamate in a DBU/1,4-dioxane system to produce a satisfactory yield of **2a-2b**, followed by reduction of **2a-2b** with 80% hydrazine hydrate and $FeCl_3{\cdot}6H_2O$ to yield **3a-3b**, and then a typical *N*-acetylation reaction to produce **4a-4b**. The synthetic routes of **9** and **D016** are outlined in **Scheme 2**. 2-amino-6-nitrobenzothiazole (**1**) was reacted with di-*tert*-butyl dicarbonate, then reduced with 80% hydrazine hydrate and $FeCl_3{\cdot}6H_2O$ to yield **6**. Reaction of intermediate **6** with 4-(2-chloroethyl) morpholine followed by deprotection resulted in **8**. During the preparation of compound **9**, the yield depended highly on the content of phenyl (4-chlorophenyl) carbamate. Lower content of phenyl (4-chlorophenyl) carbamate led to a mixture of compound **9** and **D016**. After optimization of the coupling conditions, **D016** could be conveniently prepared with 80% yield.

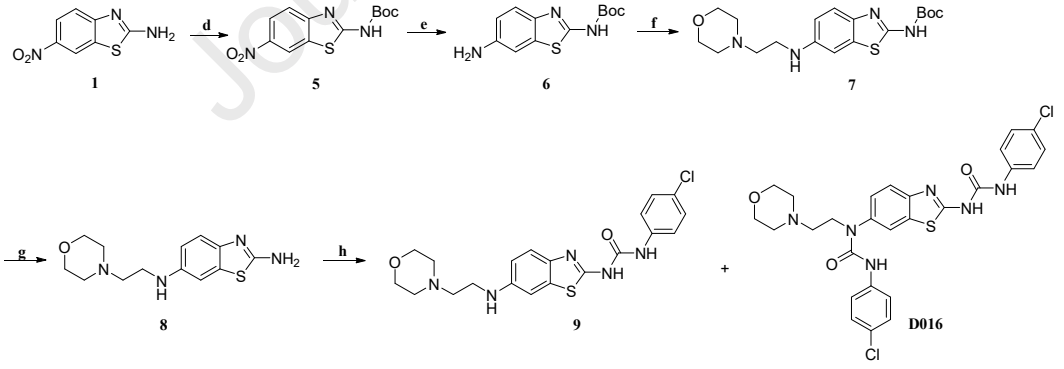
The synthetic route of compounds **12a-j** is depicted in **Scheme 3**. Intermediate **6** was reacted with phenyl chloroformate in acetone at room temperature to generate **10**, which was further condensed with different anilines to produce the corresponding compounds **11a-j**. After treating intermediates **11a-j** with trifluoroacetic acid in dichloromethane, target compounds **12a-j** were obtained in high yields. The syntheses of target compounds **15a-h** are depicted in **Scheme 4**. The substitution reaction of key intermediate **6** with appropriate alkyl halide in the presence of K_2CO_3 in 1,4-dioxane at 90 °C afforded the corresponding compounds **13a-h**. Target compounds **15a-h** were generated through substitution of **13a-h** with phenyl (4-chlorophenyl) carbamate

and then deprotection with trifluoroacetic acid.

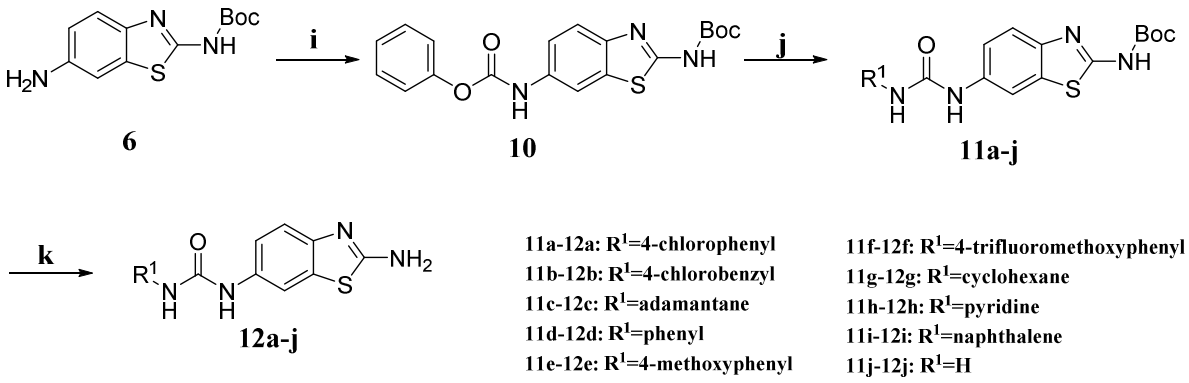
As shown in **Scheme 5**, intermediate **7** was condensed with different substituent carbamates, followed by deprotection with trifluoroacetic acid at room temperature to produce target compounds **17a-v.** Compounds **18a-v** were then obtained in moderate yields through condensation reactions of **15a**, **17e,** and **17p** with different acyl chlorides.



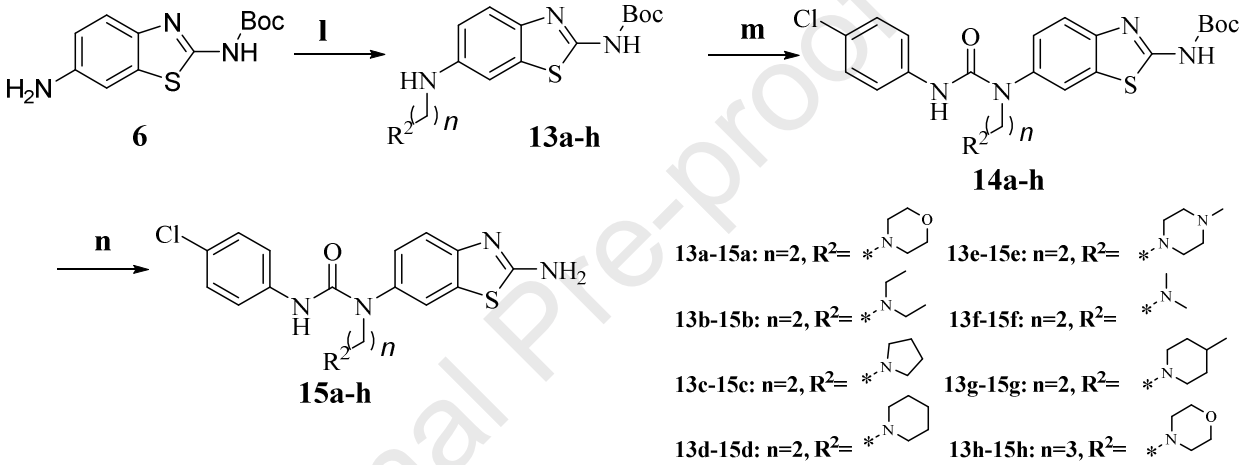
Scheme 1 Reagents and conditions: (a) phenyl (4-chlorophenyl)carbamate or phenyl (4-chlorobenzyl)carbamate, DBU, 1,4-dioxane, 80 ^{o}C, 12 h; (b) 80% $N_2H_4{\cdot}H_2O$, $FeCl_3{\cdot}6H_2O$, active carbon, 1,4-dioxane, 80 ^{o}C, 12 h; (c) acetyl chloride, Et_3N, DCM, 0 ^{o}C, 2 h.



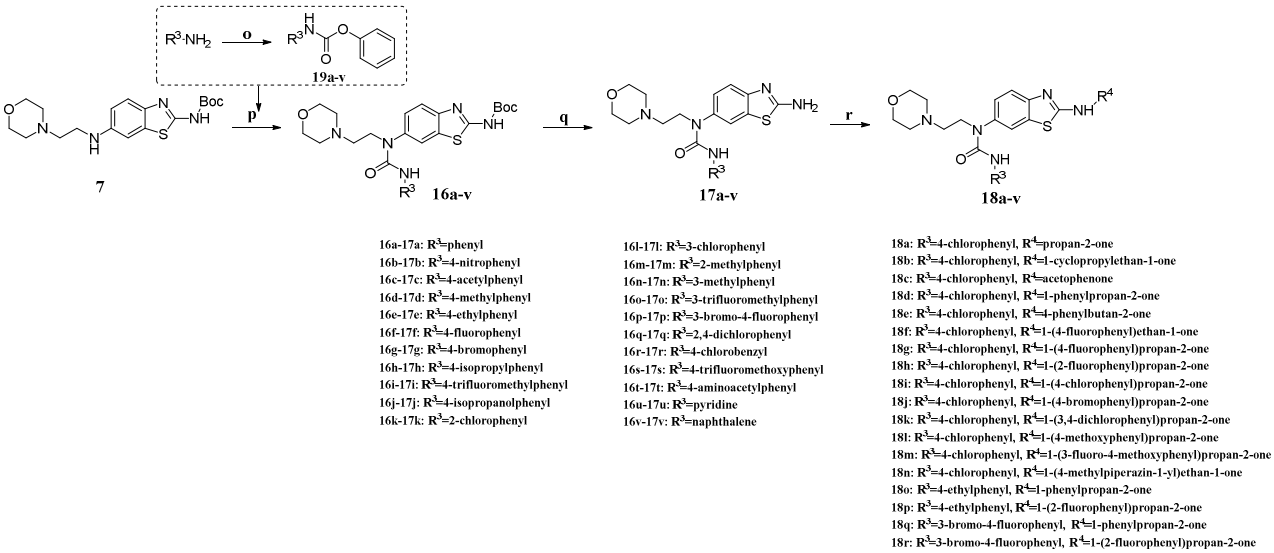
Scheme 2 Reagents and conditions: (d) $(Boc)_2O$, DMAP, DMF, 120 ^{o}C, 12 h; (e) 80% $N_2H_4{\cdot}H_2O$, $FeCl_3{\cdot}6H_2O$, active carbon, 1,4-dioxane, 80 ^{o}C, 12 h; (f) 4-(2-chloroethyl)morpholine, K_2CO_3, 1,4-dioxane, 90 ^{o}C, 12 h; (g) CF_3COOH, DCM, rt, 3 h; (h) phenyl (4-chlorophenyl) carbamate, DBU, 1,4-dioxane, 80 ^{o}C, 12 h.



Scheme 3 Reagents and conditions: (i) phenyl chloroformate, acetone, rt, 1 h; (j) R^1-NH_2, Et_3N, 1,4-dioxane, 60 ^oC, 2 h; (k) CF_3COOH, DCM, rt, 3 h.



Scheme 4 Reagents and conditions: (l) $R^2(CH_2)_nCl$, alkyl halide, K_2CO_3, 1,4-dioxane, 90 ^oC, 12 h; (m) phenyl (4-chlorophenyl) carbamate, Et_3N, 1,4-dioxane, 60 ^oC, 12 h; (n) CF_3COOH, DCM, rt, 3 h.



Scheme 5 Reagents and conditions: (o) phenyl ester, sodium carbonate, THF/EA/H_2O, rt, 12 h; (p)

Et_3N, 1,4-dioxane, 60 ^oC, 12h; (q) CF_3COOH, DCM, rt, 3 h. (r) R^4-COCl, K_2CO_3, EA, rt, 5 h.

2.2 Biological activity and discussion

2.2.1 In vitro sEH activity and SARs study

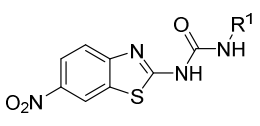
*2.2.1.1 Target compounds for hybridization strategy and **D016***

The urea group was reported as the active essential moiety of sEH inhibitors. With the discovered lead compound **1**, molecular hybridization strategy was employed to introduce the 4-chlorophenyl or 4-chlorobenzyl urea group in the 2-position or 6-position of benzo[*d*]thiazole. Therefore, nine different biaryl urea compounds were synthesized (**2a-2b, 3a-3b, 4a-4b**, **9,** and **12a-b**).

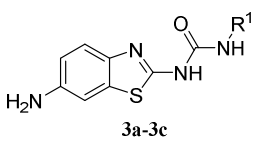
As shown in **Table 1**, compounds with the urea group at 2-position of benzo[*d*]thiazole (**2a-2b, 3a-3b, 4a-4b** and **9**) exerted weak sEH inhibitory activities (inhibition activity values < 20%). However, the 6-ureido derivatives showed higher activity, of which, compound **12a** displayed potent sEH inhibitory activity with an IC_{50} value of 10.1 nM. Subsequently, compounds **12c-j** with different R^1 substituents were prepared. Among them, compound **12c** with 1-adamantyl group exhibited more potent activity than other substituents but was still weaker than **12a**.

Interestingly, during the preparation of compound **9** according to **Scheme 2**, a byproduct (compound **D016)** was separated and obtained with 67-fold potency against sEH than t-AUCB (IC_{50} = 0.1 nM). Then, by increasing the content of phenyl (4-chlorophenyl) carbamate in the synthetic process, **D016** could be obtained with 80% yield. However, **D016** showed poor physical and chemical properties (high ClogP value and low solubility), which prompted us to conduct further modification.

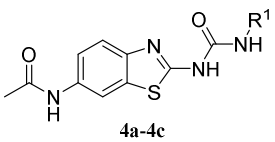
Table 1. In *vitro* sEH activities assay of series A compounds.



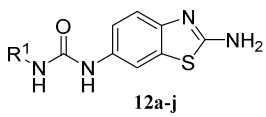
2a-2c



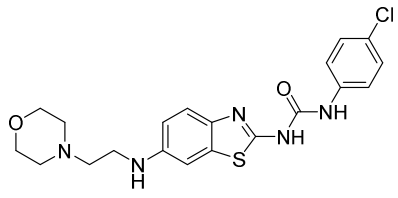
3a-3c



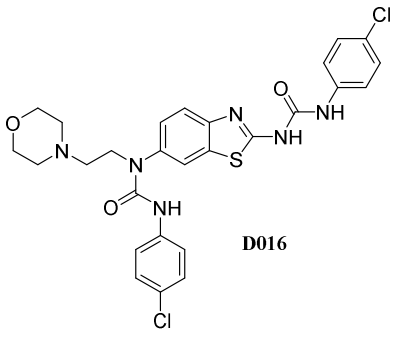
4a-4c



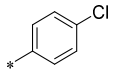
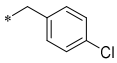
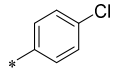
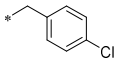
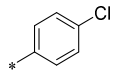
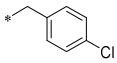
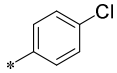
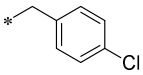
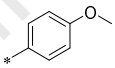
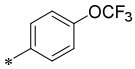
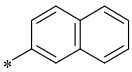
12a-j



9



D016

Compd.	R^1	sEH inhibitory activity [a]	
		Inhibition @100nM	IC_{50} (nM)
2a		**7.2%**	**ND** [b]
2b		**10.9%**	**ND**
3a		**6.7%**	**ND**
3b		**14.2%**	**ND**
4a		**12.5%**	**ND**
4b		**14.5%**	**ND**
9		**16.2%**	**ND**
12a		**76.1%**	**10.1**
12b		**47%**	**ND**
12c		**77.3%**	**822**
12d		**40.1%**	**ND**
12e		**39.3%**	**ND**
12f		**60.2%**	**ND**
12g		**59.9%**	**4.1**
12h		**20.5%**	**ND**
12i		**34.1%**	**ND**
12j	H	**25.3%**	**ND**
D016		**90.2%**	**0.1**
t-AUCB	-	**87.8%**	**6.7**

[a] All compounds were assayed at least twice, and the inhibitory values were averaged.

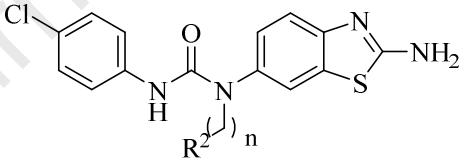
[b] Not determined.

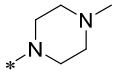
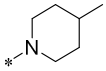
2.2.1.2 Examination of the effect of morpholino moiety

Based on previous SARs, it was speculated that the urea group at 2-position of benzo[*d*]thiazole is not essential for sEH inhibitory activities. Thus, to reduce ClogP

of **D016**, the urea group at the 2-position was removed to afford compound **15a**. Expectedly, compound **15a** exerted good sEH inhibitory activity with an IC_{50} value of 5.8 nM. Consequently, further modification was planned. The morpholino moiety was first explored. Other hydrophilic substituents were introduced to obtain compounds **15a-h**. As shown in **Table 2**, substituting the morpholino group for a 4-methylpiperidinyl group (**15 g**) decreased the activity against sEH (%inhibition range from 89.1% to 77.5%). Compounds **15b-f** were synthesized to investigate the effects of other hydrophilic substituents, such as *N*, *N*-diethylamino group (**15b**), pyrrolidinyl group (**15c**), and piperidinyl group (**15d**). However, these compounds showed weaker sEH inhibition than compound **15a**. When the length of R^2 was increased, the sEH inhibition rate of **15h** were decreased from 89.1% to 9.44%. Regrettably, the expected results of modification in the morpholino group were not obtained. However, a gap remains between the promising results with **D016** and ***t*-AUCB**. Further pharmacological evaluation shed more light on the importance of the 4-chlorophenyl moiety.

Table 2. In *vitro* sEH enzymatic assay of target compounds(15a-15h).



Compd.	n	R^2	sEH inhibitory activity [a]	
			Inhibition @100nM	IC_{50} (nM)
15a	**2**		**89.1%**	**2.8**
15b	**2**		**86.3%**	**24.5**
15c	**2**		**48.3%**	**ND** [b]
15d	**2**		**84.6%**	**6.5**
15e	**2**		**45.9%**	**ND**
15f	**2**		**39.1%**	**ND**
15g	**2**		**77.5%**	**20.2**

15h	**3**		**9.44%**	**ND**
t-AUCB	**-**	**-**	**87.8%**	**6.7**

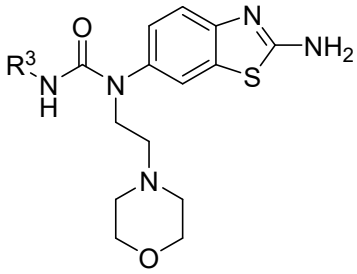
[a] All compounds were assayed at least twice, and the inhibitory values were averaged.

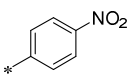
[b] Not determined.

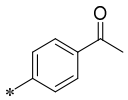
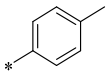
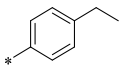
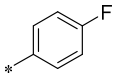
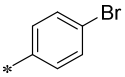
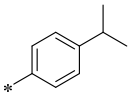
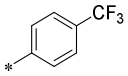
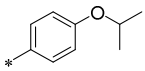
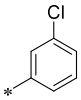
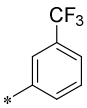
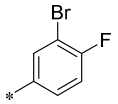
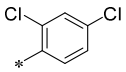
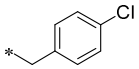
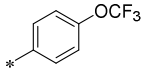
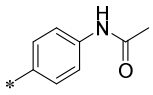
2.2.1.3 Examination of the effect of 4-chlorophenyl group

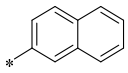
Based on the analysis described above, compounds **17a-17v** were designed and synthesized to investigate the influence of the phenyl group on potency. As shown in **Table 3**, compounds containing electron-donating groups on the phenyl ring showed more potent inhibitory activities against sEH than those with electron-withdrawing groups (**17b** vs **17d** and **17c** vs **17j**). Moreover, compounds **17f** and **17g** with 4-fluorophenyl and 4-bromophenyl motifs, respectively, showed superior activity to compound **17d**. The halogen atom was essential for improved activity. In addition, the phenyl ring terminal substituents exhibited a vital influence on sEH inhibition. As expected, introducing 4-fluorophenyl and 4-methylphenyl groups yielded compounds **17f** and **17d,** respectively, which retained a high potency against sEH (%inhibition activity values ranging from 73.1% to 74.3%). The sterically larger 4-ethylphenyl (**17e**) group as R_3 moiety resulted in a pronounced increase in inhibitory activity against sEH (%inhibition range from 84.7% to 85.8%). However, when larger substituents were introduced, the inhibitory activities of related compounds **17h**, **17i,** and **17j** against sEH were significantly reduced. These phenomena indicated that the size of the R_3 group plays an essential role in the activity against sEH.

Table 3. In *vitro* sEH enzymatic assay of target compounds (17a-17v).



Cpd.	R^3	sEH inhibitory activity [a]	
		Inhibition @100nM	IC_{50} (nM)
17a		**40.4%**	**ND** [b]
17b		**19.2%**	**ND**

17c		**28.2%**	**ND**
17d		**73.1%**	**8.3**
17e		**84.7%**	**1.9**
17f		**74.3%**	**ND**
17g		**83.8%**	**16.2**
17h		**75.2%**	**11.2**
17i		**62%**	**ND**
17j		**56.6%**	**122**
17k		**59.7%**	**ND**
17l		**36.5%**	**ND**
17m		**26.3%**	**ND**
17n		**61.7%**	**101.3**
17o		**6.18%**	**ND**
17p		**90.5%**	**0.25**
17q		**58.4%**	**ND**
17r		**14.5%**	**ND**
17s		**61.3%**	**ND**
17t		**59.4%**	**ND**

17u		**16.2%**	**ND**
17v		**39.0%**	**150.8**
D016	-	**90.2%**	**0.1**
t-AUCB	-	**87.8%**	**6.7**

[a] All compounds were assayed at least twice, and the inhibitory values were averaged.

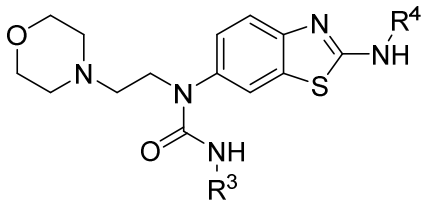
[b] Not determined.

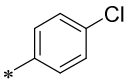
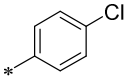
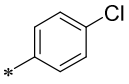
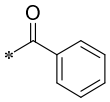
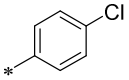
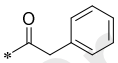
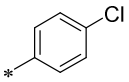
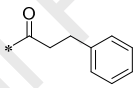
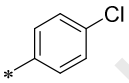
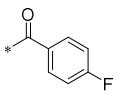
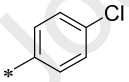
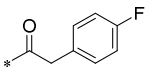
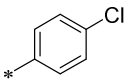
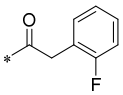
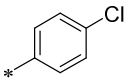
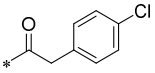
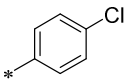
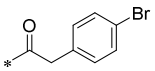
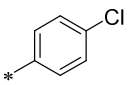
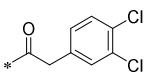
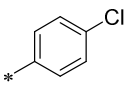
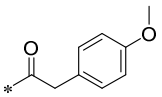
To investigate the influence of substituent positions at the phenyl ring, compounds **17k-o** were synthesized. Compound **15a** with the *para*-substituent at the phenyl ring showed the greatest potency against sEH, and a similar trend was found among other analogues with their parent compounds. Then, di-substituted compounds (**17p** and **17q**) were synthesized. However, replacement of the phenyl ring with heterocycle motifs (**17u** and **17v**) reduced sEH potency. Surprisingly, compound **17p** with 3-fluro-4-bromo at the phenyl ring showed remarkably increased activity (IC_{50} = 0.25 nM). We still have not found a compound with superior sEH inhibitory activity than **D016**, indicating a certain cavity at the 2-position of benzo[*d*]thiazole.

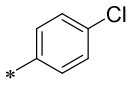
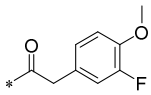
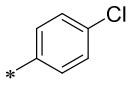
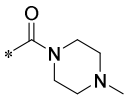
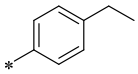
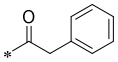
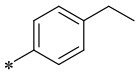
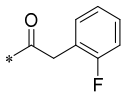
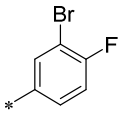
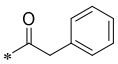
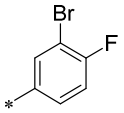
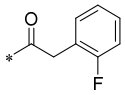
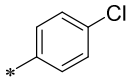
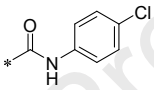
2.2.1.4 Further modification of the 2-position of benzo[d]thiazole

To improve the physical properties of **D016**, we employed the principle of bioisosterism in substituting the urea group for an amide group to obtain compounds **18a-n**. First, the effect of the R_4 substituent was considered (**Table 4**); for **18a-e**, compound **18d** bearing phenylacetic acid motif showed the best potency. Next, effects of various substituents with different electrostatic properties on the phenyl ring were investigated. Unexpectedly, the introduction of substituents on the phenyl ring seemed to be unfavorable to activity. However, compound **18h** with an *ortho*-fluoro phenyl group exerted comparable potency relative to ***t*-AUCB**. Preliminary conclusions indicate that compound **18d** bearing arylacetyl and compound **18h** bearing *ortho*-fluoroarylacetyl group at the 2-position of benzo[d]thiazole showed more potent activities than the other substituents.

Table 4. In *vitro* sEH enzymatic assay of target compounds (18a-18r).



Cpd.	R^3	R^4	sEH inhibitory activity [a]	
			Inhibition @100nM	IC_{50} (nM)
18a			**68.1%**	**ND** [b]
18b			**32.5%**	**ND**
18c			**48.8%**	**ND**
18d			**84.7%**	**0.082**
18e			**77.7%**	**ND**
18f			**64%**	**ND**
18g			**74.9%**	**ND**
18h			**82.5%**	**4.08**
18i			**71.9%**	**ND**
18j			**70.6%**	**ND**
18k			**71.8%**	**ND**
18l			**65.8%**	**ND**

18m			**58.1%**	**ND**
18n			**64%**	**ND**
18o			**76.8%**	**ND**
18p			**85.2%**	**5.4**
18q			**74.2%**	**ND**
18r			**73.8%**	**ND**
D016			**90.2%**	**0.1 nM**
t-AUCB	-	-	**87.8%**	**6.7 nM**

[a] All compounds were assayed at least twice, and the inhibitory values were averaged.

[b] Not determined.

Subsequently, the well-tolerated *para*-ethylphenyl and 3-bromo-4-fluorophenyl groups (compounds **18o**-**18r**) were explored according to the SARs above. However, they all showed lower sEH inhibition than **18d**. Therefore, compound **18d** was selected as the most potent sEH inhibitor with the IC_{50} value of 0.082 nM.

2.2.2 Enzyme inhibitory kinetics

To explore the mode of action of the representative compound **18d**, enzyme kinetic studies were conducted as shown in **Fig. 2**. The results showed that increasing concentrations of **18d** decreased V_{max} but increased K_m, indicating that **18d** is a mixed-type inhibitor of sEH. Moreover, the values of K_i and K_{is} were calculated to be 4.77 nM and 23.15 nM, respectively, which confirmed that **18d** preferentially binds to free enzyme rather than to the enzyme-substrate complex.

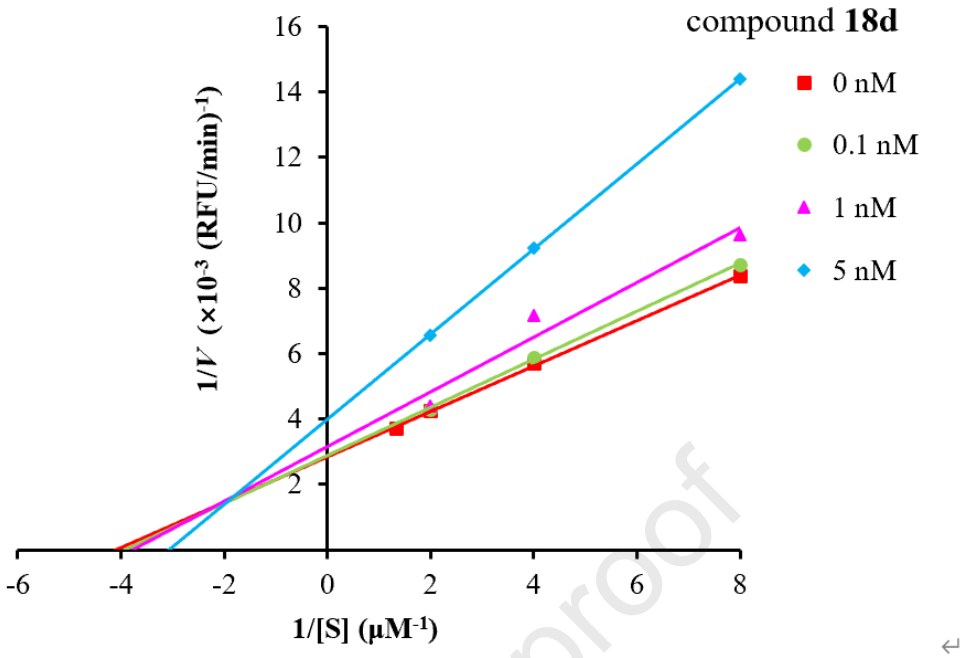


Fig 2. Lineweaver-Burk plot of fluorogenic substrate (PHOME) in the presence of compound **18d**. **[S]**: the concentrations of PHOME (μM); ***V*:** initial reaction velocity (RFU/min).

2.2.3 Evaluation of water solubility of potent compounds

Compound **D016** showed potent sEH activity but poor solubility (30 μg/mL) and high cLogP (5.96), possibly caused by the two urea groups. Therefore, optimal compounds were selected to evaluate for solubility in water, and the results are shown in **Table 5**. Compared with **D016**, compounds **15a** and **17p**, which were obtained by removing the urea group at the 2- position of benzo[*d*]thiazole exhibited a decreased cLogP; however, only compound **17p** had an improved solubility (68 μg/mL). Moreover, compound **18d** obtained by bioisosterism exhibited an increased solubility (61 μg/mL); this is considered a successful optimization strategy.

Table 5. Water solubility and cLogP of selected potent compounds

Compounds	sEH IC_{50} (nM)	Solubility [a] (μg/mL)	cLogP [b]
15a	2.8	39	3.38
17p	0.25	68	3.69
18d	0.082	61	5.28
D016	0.1	30	5.96

[a] Tested using UV Spectrophotometer (UV-2600).

[b] Calculated using instant JChem.

2.2.4 Common properties of target compounds

Based on the in *vitro* data, compounds **15a**, **17p**, **18d,** and **D016** were chosen for further studies. First, we focused on evaluating the drug-likeness score through an empirical model [29]. With ***t*-AUCB** (drug-likeness score 0.38) and **EC-5026** (drug-likeness score 0.54) as the reference points, we present the prediction details of these inhibitors in **Fig. 3**. Overall, all compounds were superior to ***t*-AUCB** (scored 0.38) and **EC-5026** (scored 0.54). Among them, compound **15a** and **18d** scored 1.13 and 1.17, respectively, indicating that they have promising druggability.

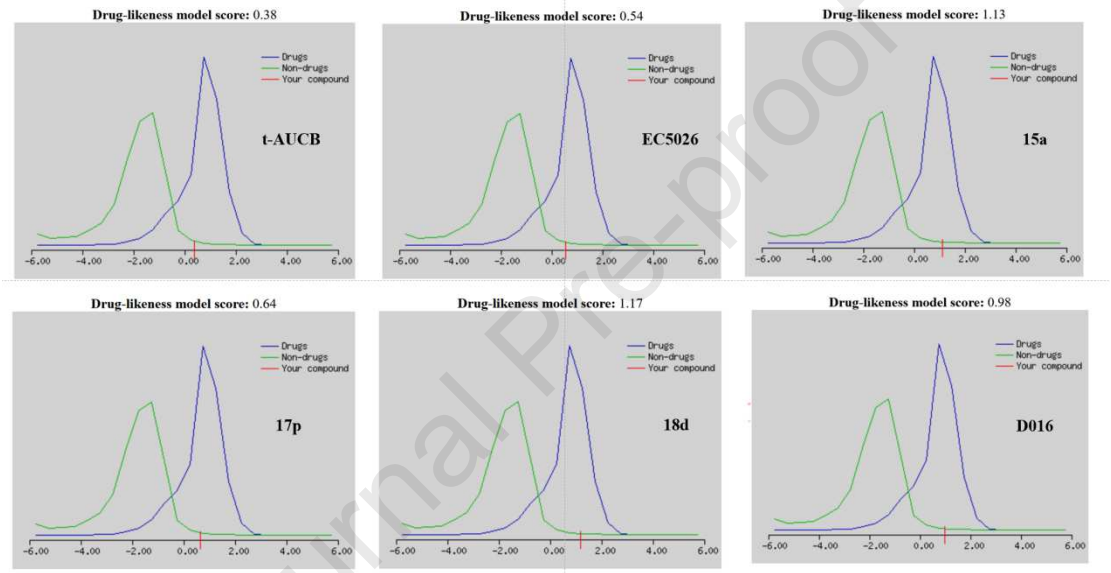


Fig 3. Representative drug-likeness model score results of **t-AUCB**, **EC5026**, **15a**, **17p**, **18d** and **D016**. (The green curve indicates that a compound is possibly not a drug, while the blue curve suggests the compound may possess drug-likeness properties.). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2.2.5 In silico study

The inhibition profiles of the 2-aminobenzo[*d*]thiazole derivatives were rationalized by studying the binding modes of compounds **12a**, **15a,** and **18d** to sEH (PDB code: 5ALW). As shown in **Fig. 4A**, the oxygen atom of urea in compound **12a** formed an H-bond with Tyr 466, and the sulfur atom of 2-aminobenzo[*d*]thiazole formed an H-bond with residue Trp 525.

The concrete binding mode of compound **15a** to sEH is presented in **Fig. 4B**. Except for the existing interactions between **12a** and sEH, the newly introduced 2-ethylmorpholine moiety in compound **15a** forms an H-bond with Asp 335 and thus may increase the inhibitory activity against sEH. The 2-aminobenzo[*d*]thiazole was

oriented towards a wide open and hydrophobic pocket, which may explain why incorporating a benzoyl or phenylacetyl group into the 2-position of benzothiazole was well-tolerated (see compounds **D016** and **18b-r**).

The binding pose of the most potent compound **18d** with sEH is depicted in **Fig. 4C**. As mentioned above, the terminal phenylacetyl group of **18d** dovetailed in the hydrophobic pocket, and an additional H-bond between the oxygen atom of amide and Ser 415 was formed.

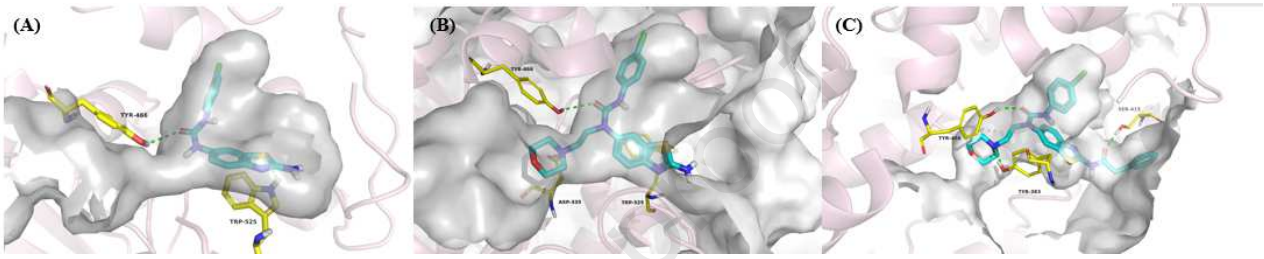


Fig. 4. The proposed binding mode of compound **12a**, **15a** and **18d** to sEH hydrolase catalytic domain (PDB code: 5ALW), hydrogen bonds were shown as green dashes.

2.3 Assessment of in vitro cytotoxicity and metabolism

Based on their in *vitro* sEH inhibitory activities, **15a, 17p,** and **18d** were selected for in *vitro* cytotoxicity and metabolic stability, and the results are outlined in **Table 5.** All three compounds exhibited low cytotoxicity (IC_{50} values >25 μM) against HepG-2 cells, indicating their low toxicity against hepatic cells. Rat liver microsome studies revealed that compound **17p** exhibited an intrinsic liver clearance of 55.8 μL/min/mg and was more stable than **15a** with an intrinsic liver clearance of 71.9 μL/min/mg. However, compound **18d** had a half-life of 1.7 min and intrinsic liver clearance of 1476.7 μL/min/mg, which indicated that compound **18d** was quite unstable in rat liver microsomes. Compound **18d** which had an amide group introduced at the 2-position of compound **15a** cleared more rapidly than **15a**, indicating amide as a potential metabolic site for **18d**. Besides, **17p** exhibited acceptable stability with a half-life of 44.7 min and showed moderate metabolism in rat liver microsomes.

Table 6. In *vitro* cytotoxicity and parameters of Rat Liver Microsomes Stability

Cpd	HepG-2 cytotoxicity $IC_{50}(\mu M)$	$T_{1/2}$ (min)	$CL_{int(mic)}$ (μL/min/mg)[a]	$CL_{int(liver)}$ (μL/min/mg)[b]	Remaining (T=60min)	Remaining (NCF=60min)[c]
15a	>25	34.7	40.0	71.9	30.1%	99.5%
17p	>25	44.7	31.0	55.8	36.2%	85.0%

18d	>25	1.7	820.4	1476.7	0%	115.5%
Propafenone	ND [d]	1.3	1047.3	1885.1	0.4%	98%
Diclofenac	ND [d]	23.4	59.3	106.7	16.9%	96.3%

[a] $CL_{int(mic)}$: intrinsic clearance; $CL_{int(mic)}=0.693/T_{1/2}$/mg microsomal protein per mL

[b] $CL_{int(liver)}$: $CL_{int(mic)}$ × mg microsomal protein/g liver weight × g liver weight/kg body weight

[c] NCF (no co-factor): No NADPH regenerating system is added to NCF samples during the 60 min incubation.

[d] Not determined.

2.4 In vivo anti-inflammatory activity: carrageenan-induced mice paw edema test

Anti-inflammatory activity of **15a**, **17p,** and **18d** were analyzed in *vivo* using the carrageenan-induced mouse paw edema method reported by Sasso *et al.* [30]. **t-AUCB** served as the positive control at a dose of 100 mg/kg. The compounds showed a rapid onset of action and sustained duration until the fourth hour after administration. Compounds **15a** and **18d** exhibited more potent anti-inflammatory activity than **t-AUCB,** and compound **17p** exhibited equivalent in *vivo* activity to **t-AUCB** (**Table 6** and **Fig. 5**). However, compound **18d** was unstable in rat liver microsomes, so we speculated that it was metabolized to **15a**. Meanwhile, **18d** exhibited a high drug-likeness score, though it was unstable in rat liver microsomes and this warrants further research.

Table 7. In *vivo* anti-inflammatory activities of compounds 15a, 17d, 18d and *t*-AUCB (100mg/kg) in BALB/c mice (n=6).

	0h	1h		2h		3h		4h	
	paw *thickness* [a,b] (mm)	paw *thickness* (mm)	Edema%	paw *thickness* (mm)	Edema%	paw *thickness* (mm)	Edema%	paw *thickness* (mm)	Edema%
Control	2.64±0.09	3.51±0.19	32.82	3.56±0.29	34.58	3.61±0.36	36.49	3.60±0.12	36.24
15a	2.57±0.08	3.11±0.17	20.99*[c]	3.06±0.09	19.06*	3.13±0.21	21.64*	3.17±0.12	23.39*
17p	2.40±0.06	3.15±0.04	30.86	3.16±0.16	31.49	3.06±0.07	27.39*	3.06±0.10	27.12*
18d	2.74±0.09	3.33±0.16	21.56*	3.22±0.21	17.58*	3.19±0.19	16.48*	3.30±0.11	20.32*
***t*-AUCB**	2.64±0.13	3.30±0.15	25.01*	3.23±0.15	22.61*	3.29±0.22	24.98*	3.28±0.15	24.71*

[a] Paw thickness were measured before (0 h) or 1, 2, 3, 4 h after injection of 50 μL of carrageenan in mice right hind paw and were significantly different compared to vehicle-treated group.

[b] Data are expressed as mean ± SEM. The data were compared using two-way analysis of variance (ANOVA) followed by Bonferroni's test for multiple comparisons.

[c] *$P < 0.05$ *vs*. vehicle.

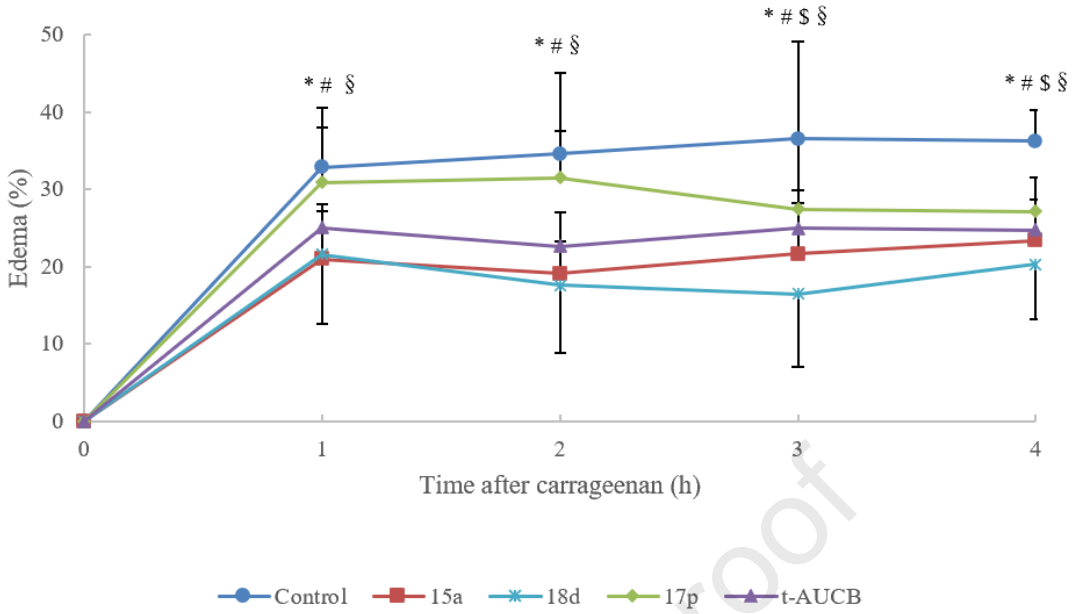


Fig.5. Graphical representation of in *vivo* anti-inflammatory activities of the selected compounds in carrageenan-induced mice paws edema. *P **<** 0.05 *vs*.**15a**, #P **<** 0.05 *vs*.**18d**, $ P **<** 0.05 *vs*.**17p**, § P **<** 0.05 *vs*.**t-AUCB**.

3. Conclusion

4. Driven by the in-house database product lead **1**, we employed a hybridization strategy for the design of a series of novel benzo[*d*]thiazol derivatives as sEH inhibitors. To our delight, **D016**, a by-product of compound **9**, was obtained with extraordinarily low IC_{50} value of 0.1 nM but poor physical and chemical properties. Then, ligand-based optimization was implemented to design and synthesize three series of compounds (**15a-h**, **17a-v,** and **18a-r)**. Among them, compounds **15a**, **17p,** and **18d** were identified as potent sEH inhibitors with IC_{50} values of 2.8 nM, 0.25 nM, and 0.082 nM, respectively. Moreover, compounds **15a** and **17p** exhibited acceptably moderate metabolic stability in a metabolism assay of rat liver microsomes but not compound **18d.**. Compared to **t-AUCB**, compound **15a** and **18d** demonstrated a more effective *in vivo* anti-inflammatory effect in carrageenan-induced mouse paw edema models. Molecular binding models identified the critical interactions between **15a** and **18d** with sEH, which coincided with the SARs analysis. Altogether, **15a** and **18d** warrant further study, to eventually confirm their potential application.

4. Experimental section

4.1 Chemistry

All melting points were acquired on a Mettler Melting Point MP70 apparatus (Mettler, Toledo, Switzerland) without calibration. Mass spectra (MS) was recorded in electrospray ionization (ESI) mode on Agilent 1100 LC-MS (Agilent, Palo Alto, CA, USA) with positive switching. Samples were analyzed from a 100 mM MeCN/H2O (6:4) solution with a 3 mL injection volume. The reverse phase HPLC was conducted on an Agilent 1260 Infinity chromatograph, which was equipped with ZORBAX SB-C18 column (250 mm × 4.6 mm). The mobile phase A was methanol, and mobile phase B was 30 mM NaH_2PO_4 in water (pH 2.5). The gradient of 5−95% A was run at a flow rate of 1.0 mL/min over 30 min. Reactions were monitored by thin-layer chromatography (TLC) on silica plates (F-254) and visualized under UV light. ^{1}H NMR and ^{13}C NMR spectra were performed using Bruker spectrometers (Bruker Bioscience, respectively, Billerica, MA, USA) with TMS as an internal standard. Column chromatography was run on silica gel (200-300 mesh) from Qingdao Ocean Chemicals (Qingdao, Shandong, China). Unless otherwise noted, all materials were obtained from commercially available sources and used without further purification.

4.1.1 General procedure for compounds **2a-4a** and **2b-4b**

*4.1.1.1 1-(4-chlorophenyl)-3-(6-nitrobenzo[d]thiazol-2-yl)urea (**2a**)*

To a solution of 2-amino-6-nitrobenzothiazole (**1**) (10 g, 51.2 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (15.6 g, 102.4 mmol) in DMF (100 mL) was added phenyl (4-chlorophenyl)carbamate (15.2 g, 61.4 mmol). The resulting solution was allowed to stir 18 h at 120 ^{o}C. The resulting mixture was poured into stirring 2M HCl (500 mL), and then the yellow solid was filtered and dried under reduced pressure. The crude product was further purified by flash column chromatography using PE/EtOAc (5:1) as eluent to afford 14.5 g of **2a** as a yellow solid; m.p.:156.2 – 156.6 °C. Yield: 82%; HPLC purity: 96.58%, retention time = 26.823 min. MS (ESI) *m/z*: 349.3 $[M+H]^{+}$. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.98 (d, *J* = 2.4 Hz, 1H), 8.25 (dd, *J* = 8.9, 2.4 Hz, 1H), 7.80 (d, *J* = 8.9 Hz, 1H), 7.57 (d, *J* = 8.8 Hz, 2H), 7.41 (d, *J* = 8.8 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 164.96, 142.89, 137.65, 132.44, 129.25, 129.01, 127.18, 122.20, 120.74, 119.74, 119.09.

*4.1.1.2 1-(4-chlorobenzyl)-3-(6-nitrobenzo[d]thiazol-2-yl)urea (**2b**)*

Synthesized using the procedure for **2a,** 10 g (51.2 mmol) of **1** and 15,6 g (102.4 mmol) 1,8-Diazabicyclo[5.4.0]undec-7-ene in 100 mL DMF and 16.1 g (61.4 mmol) of phenyl (4-chlorobenzyl)carbamate, and 13.3 g of **2b** was obtained as yellow solid; m.p.:146.1 – 146.3 °C. Yield: 72%; HPLC purity: 99.70%, retention time = 26.207min. MS (ESI) *m/z*: 363.4 [M+H]. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.22 (d, *J* = 7.8 Hz, 1H), 7.76 (d, *J* = 7.8 Hz, 1H), 7.41 (s, 2H), 7.37 (s, 2H), 7.28 (d, *J* = 7.0 Hz, 1H), 4.40 (s, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 165.67, 142.68, 140.41, 138.66, 132.63, 131.95, 131.42, 129.50, 129.18, 128.72, 128.49, 42.80.

*4.1.1.3 1-(6-aminobenzo[d]thiazol-2-yl)-3-(4-chlorophenyl)urea (**3a**)*

To a suspension of intermediate **2a** (12.0 g, 34.5 mmol) in 120 mL of 1,4-dioxane was added $FeCl_3{\cdot}H_2O$ (1.4 g, 5.2 mmol), carbon (0.12 g, 10.4 mmol) and 80% hydrazine hydrate (32 mL, 517.5 mmol) in batches. The mixture was heated for 12 h at 90 ^{o}C. After completion of the reaction as indicated by TLC, the mixture was filtered through a celite bed and concentrated in a vacuum to afford 9.0 g of **3a** as a pale solid, m.p.: 158.2 – 160.0 °C. Yield: 73%; HPLC purity: 95.47%, retention time = 20.800 min. MS (ESI) *m/z*: 319.2 $[M+H]^{+}$. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.54 (d, *J* = 8.6 Hz, 2H), 7.37 (d, *J* = 8.6 Hz, 2H), 7.33 (d, *J* = 8.2 Hz, 1H), 6.97 (s, 1H), 6.67 (dd, *J* = 8.2, 2.0 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 168.55, 158.83, 152.57, 138.04, 135.37, 131.75, 129.18, 126.74, 120.48, 119.53, 118.63, 111.76.

*4.1.1.4 1-(6-aminobenzo[d]thiazol-2-yl)-3-(4-chlorobenzyl)urea (**3b**)*

Synthesized using the procedure for **3a,** 10g (27.7 mmol) of **2b** and 1.1 g (4.2 mmol) of $FeCl_3{\cdot}H_2O$ and 0.1 g (8.3 mmol) of carbon in 100 mL 1,4-dioxane, 28 mL (415.5 mmol) of 80% hydrazine hydrate was added, and 7 g of **3b** was obtained as a pale solid, m.p.: 165.6 – 167.1 °C. Yield: 76%; HPLC purity: 97.25%, retention time = 23.304 min. MS (ESI) *m/z*: 333.5 $[M+H]^{+}$. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.40 (d, *J* = 8.1 Hz, 1H), 7.37 (d, *J* = 8.0 Hz, 2H), 7.33 (d, *J* = 8.1 Hz, 1H), 7.30 (s, 1H), 7.26 (d, *J* = 8.0 Hz, 2H), 4.21 (d, *J* = 5.8 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 158.39, 145.42, 140.42, 139.14, 131.81, 131.41, 129.43, 129.19, 128.70, 128.50, 114.24, 104.78, 42.68.

*4.1.1.5 N-(2-(3-(4-chlorophenyl)ureido)benzo[d]thiazol-6-yl)acetamide (**4a**)*

Compound **3a** (6g, 18.9 mmol) was diluted in 60 mL DCM together with Et_3N (2.1 g, 20.8 mmol). Acetyl chloride (1.6 g, 20.8 mmol) was added slowly dropwise at room temperature. After completion of the reaction (thin-layer chromatography, TLC). the reaction mixture was diluted with water (30 mL) and extracted with dichloromethane (3 x 30 mL). The combined dichloromethane extracts were washed successively with 10% sodium bicarbonate and H_2O, dried (Na_2SO_4), and concentrated under reduced pressure. The crude product was further purified by flash column chromatography using PE/EtOAc (1:1) as eluent to afford 4.8 g of **4a** as a white solid; m.p.:165.2 – 166.8 °C. Yield: 71%; HPLC purity: 95.74%, retention time = 23.658 min. MS (ESI) *m/z*: 361.4 $[M+H]^+$. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.12 (s, 1H), 9.99 (s, 1H), 8.26 (d, *J* = 1.1 Hz, 1H), 7.58 (d, *J* = 8.6 Hz, 1H), 7.56 (d, *J* = 8.7 Hz, 2H), 7.47 (dd, *J* = 8.6, 1.1 Hz, 1H), 7.38 (d, *J* = 8.7 Hz, 2H), 2.07 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 168.55, 158.83, 152.57, 138.04, 135.37, 131.75, 129.18, 126.74, 120.48, 119.53, 118.63, 111.76, 24.33.

*4.1.1.6 N-(2-(3-(4-chlorobenzyl)ureido)benzo[d]thiazol-6-yl)acetamide (**4b**)*

Synthesized using the procedure for **4a,** 5 g (15.1 mmol) of **3b** and 1.7 g (16.6 mmol) Et_3N in 50 mL DCM and 1.3 g (16.6 mmol) acetyl chloride, and 3g of **4b** was obtained as a white solid; m.p.:156.2 – 158.3 °C. Yield: 71%; HPLC purity: 99.27%, retention time = 22.382 min. MS (ESI) *m/z*: 375.1 $[M+H]^+$. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.22 (s, 1H), 7.54 (d, *J* = 8.6 Hz, 1H), 7.44 (dd, *J* = 8.7, 1.3 Hz, 1H), 7.41 (d, *J* = 8.4 Hz, 2H), 7.35 (d, *J* = 8.4 Hz, 2H), 7.27 (d, *J* = 8.7 Hz, 1H), 4.37 (d, *J* = 5.9 Hz, 2H), 4.22 (t, *J* = 6.1 Hz, 1H), 2.07 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 168.49, 162.69, 140.42, 138.98, 135.07, 131.84, 131.40, 129.44, 129.18, 128.70, 128.49, 118.41, 111.62, 42.63, 24.31.

*4.1.2 Procedure for preparation of compound **9***

*4.1.2.1 tert-butyl (6-nitrobenzo[d]thiazol-2-yl)carbamate (**5**)*

To a solution of 2-amino-6-nitrobenzothiazole (**1**) (10 g, 51.2 mmol) and 4-dimethylaminopyridine (3.2 g, 26.2 mmol) in DMF (100 mL) was added di-*tert*-butyldicarbonate (16.8 g, 77.0 mmol) and the resulting solution was allowed to

stir 18 h at 90 ^{o}C. The resulting mixture was poured into stirring ice-water (500 mL), and then the yellow solid was filtered and dried under reduced pressure. The crude product was further purified by flash column chromatography using PE/EtOAc (3:1) as eluent to afford 15.3 g of **5** as a yellow solid; m.p.:125.2 – 126.6 °C. Yield: 85%; HPLC purity: 96.20%, retention time = 25.703 min.MS (ESI) *m/z*: 296.3 $[M+H]^+$. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.25 (s, 1H), 9.00 (d, *J* = 2.3 Hz, 1H), 8.24 (dd, *J* = 8.9, 2.3 Hz, 1H), 7.82 (d, *J* = 8.9 Hz, 1H), 1.54 (s, 9H).

*4.1.2.2 tert-butyl (6-aminobenzo[d]thiazol-2-yl)carbamate (**6**)*

To a suspension of intermediate **2** (12.0 g, 40.5 mmol) in 120 mL of 1,4-dioxane was added $FeCl_3{\cdot}H_2O$ (1.0 g, 6.2 mmol), active carbon (0.15 g, 12.5 mmol) and 80% hydrazine hydrate (35 mL, 600 mmol) in batches. The mixture was heated for 12 h at 90 ^{o}C. After completion of the reaction as indicated by TLC, the mixture was filtered through a celite bed and concentrated in a vacuum to afford 9.3 g of **6** as a pale solid, m.p.: 128.2 – 130.0 °C. Yield: 73.3%; HPLC purity: 90.17%, retention time = 17.117 min. MS (ESI) *m/z*: 266.2 $[M+H]^+$.

*4.1.2.3 tert-butyl (6-((2-morpholinoethyl)amino)benzo[d]thiazol-2-yl)carbamate (**7**)*

2-chloro-*N*, *N*-diethylethan-1-amine (2.1 g, 21.3 mmol) was added to a mixture of tert-butyl (6-aminobenzo[*d*]thiazol-2-yl)carbamate (**6**) (1.3 g, 7.5 mmol) and K_2CO_3 (2.1 g, 22.4 mmol) in 1,4-dioxane (20 mL). The mixture was stirred at reflux for 8 h. After the reaction was cooled to room temperature, it was poured into 80 mL water and extracted with ethyl acetate (2×80 mL). The combined organic layers were washed with brine (60 mL), dried over sodium sulfate, concentrated in *vacuo*, then dried under vacuum to obtain 1.0 g of **7** as brown solid; m.p.: 131.2 – 133.5 °C. Yield: 56%; HPLC purity: 88.35%, retention time = 11.666 min. MS (ESI) *m/z*: 379.6 $[M+H]^+$.

*4.1.2.4 N^6-(2-morpholinoethyl)benzo[d]thiazole-2,6-diamine (**8**)*

The crude product (**7**) from the previous step was dissolved in 10 mL of dichloromethane; 10 mL of trifluoroacetate was then slowly added. After stirring overnight at room temperature, the mixture was evaporated under reduced pressure and the resultant was added to 15 mL water, adjusting pH to 8 with saturated sodium

hydrogen carbonate, the precipitate was filtered off and washed with water, then the crude product was dried in vacuum desiccator and further purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **8** as a pale yellow solid; yield: 77%; m.p.: 124.3 – 125.7 °C; HPLC purity: 99.42%, retention time = 9.070 min. MS (ESI) m/z: 279.4 $[M+H]^+$.

*4.1.2.5 1-(4-chlorophenyl)-3-(6-((2-morpholinoethyl)amino)benzo[d]thiazol-2-yl)urea (**9**)*

To a mixture of **8** (0.3 g, 1.1 mmol) and phenyl (4-chlorophenyl)carbamate (0.29 g, 1.2 mmol) in 5 mL DMF, triethylamine (0.13 g, 1.3 mmol) was slowly added, after stirring at 60 °C for 12 h, the mixture was poured into 10 mL water, the solution was neutralized with 1N hydrochloric acid, then the precipitate was filtered and washed with water, the crude product was dried in vacuum desiccator and further purified by column chromatography on silica using mobile phase dichloromethane−methanol (50:1, v/v) to furnish **9** as a pale yellow solid; m.p.: 133.1 – 135.6 °C Yield: 28%. HPLC purity: 98.05%, retention time = 17.513 min. MS (ESI) *m/z*: 432.1 $[M+H]^+$. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.71 (d, *J* = 8.1 Hz, 2H), 7.30 (d, *J* = 8.1 Hz, 2H), 7.22 (d, *J* = 8.7 Hz, 1H), 6.89 (d, *J* = 2.0 Hz, 1H), 6.69 (dd, *J* = 8.7, 2.0 Hz, 1H), 4.36 (t, *J* = 6.7 Hz, 2H), 3.52 (s, 4H), 2.63 (t, *J* = 6.8 Hz, 2H), 2.50 (s, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 160.42, 145.83, 139.97, 128.72, 127.70, 126.92, 125.48, 120.06, 113.83, 112.63, 106.61, 66.58, 55.40, 53.89, 42.33.

*4.1.2.6 3-(4-chlorophenyl)-1-(2-(3-(4-chlorophenyl)ureido)benzo[d]thiazol-6-yl)-1-(2-morpholinoethyl)urea (**D016**)*

To a mixture of **8** (0.3 g, 1.1 mmol) and phenyl (4-chlorophenyl)carbamate (0.5 g, 2.2 mmol) in 5 mL DMF, triethylamine (0.13 g, 1.3 mmol) was slowly added, after stirring at 60 °C for 12 h, the mixture was poured into 10 mL water, the solution was neutralized with 1N hydrochloric acid, then the precipitate was filtered and washed with water, the crude product was dried in vacuum desiccator and further purified by column chromatography on silica using mobile phase dichloromethane−methanol (50:1, v/v) to furnish **9** as a pale yellow solid; m.p.: 133.1 – 135.6 °C Yield: 80%. HPLC purity: 96.87%, retention time = 27.044 min. MS (ESI) *m/z*: 432.1 $[M+H]^+$. ^{1}H

NMR (600 MHz, DMSO-d_6) δ 10.67 (s, 1H), 8.86 (s, 2H), 8.82 (s, 1H), 8.10 (s, 1H), 7.65 (d, *J* = 8.0 Hz, 1H), 7.57 (d, *J* = 7.4 Hz, 2H), 7.50 (d, *J* = 7.4 Hz, 2H), 7.45 (d, *J* = 7.5 Hz, 2H), 7.36 (d, *J* = 8.0 Hz, 1H), 7.33 (d, *J* = 7.5 Hz, 2H), 4.44 (s, 2H), 3.54 (s, 4H), 2.77 (s, 2H), 2.56 (s, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 160.21, 154.16, 152.93, 144.19, 139.13, 137.82, 135.73, 133.72, 129.97, 129.12, 129.01, 127.82, 125.69, 123.00, 120.84, 120.09, 118.13, 110.54, 66.42, 57.59, 55.30, 53.97.

*4.1.3 General procedure for preparation of compounds **12a-j**.*

*4.1.3.1 tert-butyl phenyl benzo[d]thiazole-2,6-diyldicarbamate (**10**)*

To a stirred solution of intermediate **3** (10 g, 37.7 mmol) in acetone (100 mL) was dropped phenyl chloroformate (9.0 g, 57.7 mmol). The mixture was stirred at room temperature for 2 h. After completion of the reaction as indicated by TLC, the mixture was filtered to obtain 9.4 g of **10** as a white solid, m.p.: 210.1 – 212.2 °C. Yield: 83.6%; MS (ESI) *m/z*: 386.1 $[M+H]^+$.

*4.1.3.2 General procedure for preparation of intermediates **11a-j***

*4.1.3.2.1 tert-butyl (6-(3-(3-chlorophenyl)ureido)benzo[d]thiazol-2-yl)carbamate (**11a**)*

tert-Butyl phenyl benzo[*d*]thiazole-2,6-diyldicarbamate (**10**) (0.5 g, 1.3 mmol) in 5 mL 1,4-dioxane was added cyclohexylamine (0.15 g, 1.5 mmol) followed by triethylamine (0.13 g, 0.1 mL, 1.3 mmol). The mixture was stirred at 60 ^{o}C for 3 h. The resulting precipitate was filtered off, washed with diethyl ether, and dried to afford 0.3 g of **5b** as a white solid, yield: 85.6%; MS (ESI) *m/z*: 419.3 $[M+H]^+$.

*4.1.3.2.2 tert-butyl (6-(3-(4-chlorobenzyl)ureido)benzo[d]thiazol-2-yl)carbamate (**11b**)*

Synthesized using the procedure for **11a**, 0.5 g (1.3 mmol) of **10** and 0.2 g (1.5 mmol) of 4-chlorobenzylamine in 5 mL of 1,4-dioxane and 0.1 mL of trimethylamine, then 0.3 g of **11b** was obtained as yellow solid; yield: 67.9%; MS (ESI) *m/z*: 433.1 $[M+H]^+$

4.1.3.2.3

*tert-butyl(6-(3-((3s,5s,7s)-adamantane-1-yl)ureido)benzo[d]thiazol-2-yl)carbamate (**11c**)*

Synthesized using the procedure for **11a**, 0.5 g (1.3 mmol) of **10** and 0.2 g (1.5 mmol) of amantadine in 5 mL 1,4-dioxane and 0.1 mL of trimethylamine, then 0.3 g of **11c** was obtained as yellow solid; yield: 89%; MS (ESI) *m/z*: 443.4 $[M+H]^+$.

*4.1.3.2.4 tert-butyl (6-(3-phenylureido)benzo[d]thiazol-2-yl)carbamate (**11d**)*

Synthesized using the procedure for **11a**, 0.5 g (1.3 mmol) of **10** and 0.15 g (1.5 mmol) of aniline in 5 mL of 1,4-dioxane and 0.1 mL of trimethylamine, then 0.3 g of **11d** was obtained as yellow solid; yield: 88.3%; MS (ESI) *m/z*: 385.1 $[M+H]^+$.

*4.1.3.2.5 tert-butyl (6-(3-(3-methoxyphenyl)ureido)benzo[d]thiazol-2-yl)carbamate (**11e**)*

Synthesized using the procedure for **11a**, 0.5 g (1.3 mmol) of **10** and 0.2 g (1.5 mmol) of para-anisidine in 5 mL of 1,4-dioxane and 0.1 mL of trimethylamine, then 0.3 g of **11e** was obtained as yellow solid; yield: 85.4%; MS (ESI) *m/z*: 415.6 $[M+H]^+$

*4.1.3.2.6 tert-butyl-(6-(3-(3-(trifluoromethoxy)phenyl)ureido)benzo[d]thiazol-2-yl)carbamate (**11f**)*

Synthesized using the procedure for **11a**, 0.5 g (1.3 mmol) of **10** and 0.2 g (1.5 mmol) of 4-(trifluoromethoxy)benzeneaime in 5 mL of 1,4-dioxane and 0.1 mL of trimethylamine, then 0.3 g of **11f** was obtained as yellow solid; yield: 78.5%; MS (ESI) *m/z*: 469.4 $[M+H]^+$

*4.1.3.2.7 tert-butyl (6-(3-cyclohexylureido)benzo[d]thiazol-2-yl)carbamate (**11g**)*

Synthesized using the procedure for **11a**, 0.5 g (1.3 mmol) of **10** and 0.2 g (1.5 mmol) of 4-(trifluoromethoxy)benzeneaime in 5 mL of 1,4-dioxane and 0.1 mL of trimethylamine, then 0.3 g of **11g** was obtained as yellow solid; yield: 86.1%; MS (ESI) *m/z*: 391.2 $[M+H]^+$

*4.1.3.2.8 tert-butyl (6-(3-(pyridin-2-yl)ureido)benzo[d]thiazol-2-yl)carbamate (**11h**)*

Synthesized using the procedure for **11a**, 0.5 g (1.3 mmol) of **10** and 0.15 g (1.5 mmol) of 2-aminopyridine in 5 mL of 1,4-dioxane and 0.1 mL of trimethylamine, then 0.3 g of **11h** was obtained as yellow solid; yield: 50.2%; MS (ESI) *m/z*: 386.4 $[M+H]^+$

*4.1.3.2.9 tert-butyl (6-(3-(naphthalen-2-yl)ureido)benzo[d]thiazol-2-yl)carbamate (**11i**)*

Synthesized using the procedure for **11a**, 0.5 g (1.3 mmol) of **10** and 0.2 g (1.5 mmol) of 2-naphthylamine in 5 mL of 1,4-dioxane and 0.1 mL of trimethylamine, then 0.3 g of **11i** was obtained as yellow solid; yield: 50.2%; MS (ESI) *m/z*: 435.2 $[M+H]^+$

*4.1.3.2.10 tert-butyl (6-ureidobenzo[d]thiazol-2-yl)carbamate (**11j**)*

To a solution of intermediate **10** (0.5 g, 1.9 mmol) in acetic acid (5 mL) and water (2.5 mL), the sodium cyanate (0.25 g, 3.8 mmol) in water (2.5 mL) was added slowly at room temperature. The mixture was stirred at room temperature for 2 h and monitored by TLC, and then was poured into water and adjusted to pH=7. After stirring for 30 min, the precipitate was collected by filtration, and dried to provide compound 0.3 g of compound **11j** as white solid, yield: 83.6%; MS (ESI) *m/z*: 309.2 $[M+H]^+$.

*4.1.3.3 General procedure for preparation of compounds **12a-j***

*4.1.3.3.1 1-(2-aminobenzo[d]thiazol-6-yl)-3-(4-chlorophenyl)urea (**12a**)*

The crude product (**11a**) from the previous step was dissolved in 3 mL of dichloromethane; 3 mL of trifluoroacetate was then slowly added. After stirring overnight at room temperature, the mixture was evaporated under reduced pressure and the resultant was added to 5 mL water, adjusting pH to 8 with saturated sodium hydrogen carbonate, the precipitate was filtered off and washed with water, then the crude product was dried in vacuum desiccator and further purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **12a** as a pale yellow solid; yield: 66.8%; m.p.: 195.5 – 197.0 °C; HPLC purity: 96.56%, retention time = 20.379 min. MS (ESI) m/z: 319.3 $[M+H]^+$; ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.88 (s, 1H), 7.55 (d, *J* = 8.8 Hz, 2H), 7.27 (d, *J* = 8.9 Hz, 2H), 7.25 (s, 2H), 7.21 (d, *J* = 8.6 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 165.29, 153.56, 148.06, 140.20, 134.60), 131.60, 128.76, 124.79, 119.78, 117.86, 117.38, 111.04.

*4.1.3.3.2 1-(2-aminobenzo[d]thiazol-6-yl)-3-(4-chlorobenzyl)urea (**12b**)*

Synthesized using the procedure for **12a**, crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v)

to furnish **12b** as a pale yellow solid; yield: 50.7%; m.p.: 173.4 – 177.7 °C; HPLC purity: 99.28%, retention time = 19.193 min. MS (ESI) m/z: 333.2 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.69 (s, 1H), 7.83 (s, 1H), 7.38 (d, *J* = 8.4 Hz, 2H), 7.32 (d, *J* = 8.4 Hz, 2H), 7.24 (s, 2H), 7.19 (d, *J* = 8.6 Hz, 1H), 7.10 (d, *J* = 8.6 Hz, 1H), 6.83 (s, 1H), 4.27 (d, *J* = 5.8 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 165.30, 155.99, 147.97, 140.22, 134.78, 131.75, 131.57, 129.42, 128.64, 117.95, 117.14, 110.90, 42.55.

*4.1.3.3.3 1-((3s,5s,7s)-adamantan-1-yl)-3-(2-aminobenzo[d]thiazol-6-yl)urea (**12c**)*

Synthesized using the procedure for **12a**, crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **12c** as a pale yellow solid; yield: 89.2%; m.p.: 200.3 – 202.5 °C; HPLC purity: 98.71%, retention time = 22.534 min. MS (ESI) m/z: 343.4 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.45 (s, 1H), 7.82 (d, *J* = 2.1 Hz, 1H), 7.23 (s, 2H), 7.18 (d, *J* = 8.6 Hz, 1H), 7.06 (dd, *J* = 8.6, 2.2 Hz, 1H), 2.09 (s, 1H), 1.97 (s, 2H), 1.62 (d, *J* = 14.8 Hz, 2H), 1.57 (s, 3H), 1.53 (s, 2H), 1.50 (s, 1H), 1.48 (d, *J* = 2.3 Hz, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.28, 156.66, 147.93, 134.88, 131.73, 117.94, 117.07, 110.84, 46.43, 45.72, 36.49, 31.60, 29.69.

*4.1.3.3.4 1-(2-aminobenzo[d]thiazol-6-yl)-3-phenylurea (**12d**)*

Synthesized using the procedure for **12a**, crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **12d** as a pale yellow solid; yield: 75.3%; m.p.: 189.5 – 191.7 °C; HPLC purity: 99.81%, retention time = 16.940 min. MS (ESI) m/z: 285.5 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.04 (s, 1H), 8.99 (s, 1H), 7.88 (d, *J* = 1.6 Hz, 1H), 7.48 (d, *J* = 7.9 Hz, 2H), 7.29 (d, *J* = 1.9 Hz, 2H), 7.26 (s, 1H), 7.24 (d, *J* = 3.6 Hz, 1H), 7.19 (dd, *J* = 8.6, 1.7 Hz, 1H), 6.94 (t, *J* = 7.3 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.57, 153.32, 148.41, 140.59, 134.12, 131.82, 129.17, 121.95, 118.51, 118.02, 117.53, 111.35.

*4.1.3.3.5 1-(2-aminobenzo[d]thiazol-6-yl)-3-(4-methoxyphenyl)urea (**12e**)*

Synthesized using the procedure for **12a**, crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v)

to furnish **12e** as a pale yellow solid; yield: 80.3%; m.p.: 190.3 – 192.7 °C; HPLC purity: 97.44%, retention time = 16.456 min. MS (ESI) m/z: 315.3 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.60 (s, 1H), 8.55 (s, 1H), 7.85 (d, *J* = 1.8 Hz, 1H), 7.36 (d, *J* = 8.9 Hz, 2H), 7.27 (s, 2H), 7.23 (d, *J* = 8.6 Hz, 1H), 7.15 (dd, *J* = 8.6, 1.9 Hz, 1H), 6.86 (d, *J* = 8.9 Hz, 2H), 3.71 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.53, 154.77, 153.43, 148.36, 134.15, 133.47, 131.83, 120.31, 118.02, 117.49, 114.43, 111.3.

*4.1.3.3.6 1-(2-aminobenzo[d]thiazol-6-yl)-3-(4-(trifluoromethoxy)phenyl)urea (**12f**)*

Synthesized using the procedure for **12a**, crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **12f** as a pale yellow solid; yield: 57.8%; m.p.: 185.4 – 187.9 °C; HPLC purity: 97.50%, retention time = 21.948 min. MS (ESI) m/z: 369.5 $[M+H]^+$; ^{1}H NMR (400 MHz,) δ 8.93 (s, 1H), 8.70 (s, 1H), 7.85 (s, 1H), 7.56 (d, *J* = 8.7 Hz, 2H), 7.31 (s, 2H), 7.28 (s, 1H), 7.25 (d, *J* = 9.8 Hz, 2H), 7.18 (d, *J* = 8.4 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.74, 153.15, 148.69, 142.90, 139.72, 133.63, 131.85, 122.15, 119.69, 118.03, 117.78, 111.69.

*4.1.3.3.7 1-(2-aminobenzo[d]thiazol-6-yl)-3-cyclohexylurea (**12g**)*

Synthesized using the procedure for **12a**, crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **12g** as a pale yellow solid; yield: 87.1%; m.p.: 180.1 – 182.3 °C; HPLC purity: 99.07%, retention time = 18.994 min. MS (ESI) m/z: 291.3 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.19 (s, 1H), 7.79 (d, *J* = 2.0 Hz, 1H), 7.22 (s, 2H), 7.18 (d, *J* = 8.6 Hz, 1H), 7.05 (dd, *J* = 8.6, 6.5 Hz, 1H), 6.00 (d, *J* = 6.5 Hz, 1H), 3.45 (m, 1H), 1.80 (m, *J* = 8.4, 3.8 Hz, 2H), 1.66 (m, 2H), 1.53 (m, *J* = 8.7, 4.1 Hz, 2H), 1.30 (m, 2H), 1.16 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 165.19, 155.10, 147.84, 134.98, 131.81, 117.98, 116.84, 110.56, 48.06, 33.49, 25.73, 24.83.

*4.1.3.3.8 1-(2-aminobenzo[d]thiazol-6-yl)-3-(pyridin-2-yl)urea (**12h**)*

Synthesized using the procedure for **12a**, crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **12h** as a pale yellow solid; yield: 45.8%; m.p.: 181.2 – 183.6 °C; HPLC

purity: 98.56%, retention time = 14.145 min. MS (ESI) m/z: 286.7 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.49 (s, 1H), 9.38 (s, 1H), 8.53 (s, 1H), 7.36 (d, *J* = 7.1 Hz, 1H), 7.26 (s, 1H), 7.20 (s, 2H), 7.18 (d, *J* = 7.9 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 165.84, 153.44, 152.73, 148.88, 147.22, 138.85, 133.07, 131.80, 117.98, 117.67, 112.26, 111.98.

*4.1.3.3.9 1-(2-aminobenzo[d]thiazol-6-yl)-3-(naphthalen-1-yl)urea (**12i**)*

Synthesized using the procedure for **12a**, crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **12i** as a pale yellow solid; yield: 67.3%; m.p.: 188.2 – 190.5 °C; HPLC purity: 98.54%, retention time = 19.763 min. MS (ESI) m/z: 335.4 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.01 (s, 1H), 8.93 (s, 1H), 8.03 (s, 1H), 7.95 (s, 1H), 7.90 (s, 1H), 7.56 (dd, *J* = 59.8, 27.4 Hz, 2H), 7.25 (s, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) ^{13}C NMR (101 MHz, DMSO) δ 165.41, 153.44, 148.18, 134.27, 131.72, 128.76, 126.25, 123.10, 117.94, 117.36, 111.21.

*4.1.3.3.10 1-(2-aminobenzo[d]thiazol-6-yl)urea (**12j**)*

Synthesized using the procedure for **12a**, crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **12j** as a pale yellow solid; yield: 77%; m.p.: 183.3 – 185.5 °C; HPLC purity: 98.93%, retention time = 22.636 min. MS (ESI) m/z: 209.2 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.41 (s, 1H), 7.83 (d, *J* = 1.9 Hz, 1H), 7.24 (s, 2H), 7.19 (d, *J* = 8.6 Hz, 1H), 7.06 (dd, *J* = 8.6, 2.0 Hz, 1H), 5.78 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.32, 156.66, 147.96, 134.83, 131.74, 117.95, 117.11, 110.90.

*4.1.4 General procedure for preparation of compounds **15a-h***

*4.1.4.1 General procedure for preparation of intermediates **13a-h***

*4.1.4.1.1 tert-butyl (6-((2-morpholinoethyl)amino)benzo[d]thiazol-2-yl)carbamate (**13a**)*

4-(2-chloroethyl)morpholine (2.1 g, 21.3 mmol) was added to a mixture of tert-butyl (6-aminobenzo[*d*]thiazol-2-yl)carbamate (**6**) (2.0 g, 7.5 mmol) and K_2CO_3 (3.1 g, 22.4 mmol) in 1,4-dioxane (20 mL). The mixture was stirred at reflux for 8 h. After the reaction was cooled to room temperature, it was poured into 80 mL water

and extracted with ethyl acetate (2×80 mL). The combined organic layers were washed with brine (60 mL), dried over sodium sulfate, concentrated in *vacuo*, then dried under vacuum to obtain 1.2 g of **13a** as brown solid; yield: 78.3%; HPLC purity: 81.56%, retention time = 12.276 min. **13a** was used without further purification.

*4.1.4.1.2 tert-butyl (6-((2-(diethylamino)ethyl)amino)benzo[d]thiazol-2-yl)carbamate **(13b)***

Synthesized using the procedure for **13a**, 2.0 g (7.5 mmol) of **3** and 2.1 g (21.3 mmol) of 1-(2-chloroethyl)pyrrolidine in 20 mL of 1,4-dioxane and K_2CO_3 (3.1 g, 22.4 mmol), then 1.8 g of **13b** was obtained as yellow solid; yield: 95%. HPLC purity: 81.89%, retention time = 12.049 min.

*4.1.4.1.3 tert-butyl (6-((2-(pyrrolidin-1-yl)ethyl)amino)benzo[d]thiazol-2-yl)carbamate (**13c**)*

Synthesized using the procedure for **13a**, 2.0 g (7.5 mmol) of **6** and 2.1 g (21.3 mmol) of 1-(2-chloroethyl)pyrrolidine in 20 mL of 1,4-dioxane and K_2CO_3 (3.1 g, 22.4 mmol), then 0.9 g of **13c** was obtained as yellow solid; yield: 46.6%. HPLC purity: 87.65%, retention time = 13.690 min.

*4.1.4.1.3 tert-butyl (6-((2-(piperidin-1-yl)ethyl)amino)benzo[d]thiazol-2-yl)carbamate **(13d)***

Synthesized using the procedure for **13a**, 2.0 g (7.5 mmol) of **6** and 2.1 g (21.3 mmol) of 1-(2-chloroethyl)piperidine in 20 mL of 1,4-dioxane and K_2CO_3 (3.1 g, 22.4 mmol), then 1.1 g of **13d** was obtained as yellow solid; yield: 57.1%. HPLC purity: 84.64%, retention time = 11.816 min.

*4.1.4.1.4 tert-butyl-(6-((2-(4-methylpiperazin-1-yl)ethyl)amino)benzo[d]thiazol-2-yl)carbamate **(13e)***

Synthesized using the procedure for **13a**, 2.0 g (7.5 mmol) of **6** and 2.1 g (21.3 mmol) of 1-(2-chloroethyl)-4-methylpiperazine in 20 mL of 1,4-dioxane and K_2CO_3 (3.1 g, 22.4 mmol), then 1.4 g of **13e** was obtained as yellow solid; yield: 67.4%. HPLC purity: 85.66%, retention time = 10.507 min.

4.1.4.1.5 tert-butyl

*(6-((2-(dimethylamino)ethyl)amino)benzo[d]thiazol-2-yl)carbamate (**13f**)*

Synthesized using the procedure for **13a**, 2.0 g (7.5 mmol) of **6** and 2.1 g (21.3 mmol) of 1-(2-chloroethyl)-4-methylpiperazine in 20 mL of 1,4-dioxane and K_2CO_3 (3.1 g, 22.4 mmol), then 1.5 g of **13e** was obtained as yellow solid; yield: 75%. HPLC purity: 85.80%, retention time = 13.294 min.

4.1.4.1.6

*tert-butyl-(6-((2-(4-methylpiperidin-1-yl)ethyl)amino)benzo[d]thiazol-2-yl)carbamate (**13g**)*

Synthesized using the procedure for **13a**, 2.0 g (7.5 mmol) of **6** and 2.1 g (21.3 mmol) of 1-(2-chloroethyl)-4-methylpiperidine in 20 mL of 1,4-dioxane and K_2CO_3 (3.1 g, 22.4 mmol), then 1.5 g of **13g** was obtained as yellow solid; yield: 85.2%. HPLC purity: 86.99%, retention time = 12.333 min.

*4.1.4.1.7 tert-butyl (6-((3-morpholinopropyl)amino)benzo[d]thiazol-2-yl)carbamate (**13h**)*

Synthesized using the procedure for **13a**, 2.0 g (7.5 mmol) of **6** and 2.1 g (21.3 mmol) of 4-(3-chloropropyl)morpholine in 20 mL of 1,4-dioxane and K_2CO_3 (3.1 g, 22.4 mmol), then 1.5 g of **13h** was obtained as yellow solid; yield: 89%. HPLC purity: 80.35%, retention time = 14.142 min.

*4.1.4.2 General procedure for preparation of intermediates **14a-h***

4.1.4.2.1

*tert-butyl-(6-(3-(4-chlorophenyl)-1-(2-morpholinoethyl)ureido)benzo[d]thiazol-2-yl) carbamate (**14a**)*

To a mixture of **13a** (0.3 g, 0.8 mmol) and phenyl (4-chlorophenyl)carbamate (0.25 g, 1.0 mmol) in 5 mL DMF, triethylamine (0.08 g, 0.8 mmol) was slowly added, after stirring at 60 ^{o}C for 12 h, the mixture was poured into 10 mL water, the solution was neutralized with 1N hydrochloric acid, then the precipitate was filtered and washed with water, the crude product was dried in vacuum desiccator and further purified by column chromatography on silica using mobile phase dichloromethane−methanol (50:1, v/v) to furnish **14a** as a pale yellow solid. Yield: 73.2%. MS (ESI) *m/z*: 532.6 $[M+H]^+$.

4.1.4.2.2

*tert-butyl-(6-(3-(4-chlorophenyl)-1-(2-(diethylamino)ethyl)ureido)benzo[d]thiazol-2-yl)carbamate (**14b**)*

Synthesized using the procedure for **14a**, 0.5 g (1.3 mmol) of **13b** and 0.25 g (1.0 mmol) of phenyl (4-chlorophenyl)carbamate and 0.08 g (0.8 mmol) of triethylamine were mixed and heated in 5 mL of DMF. The crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (50:1, v/v) to furnish **14b** as a pale yellow solid. Yield: 70.1%. MS (ESI) *m/z*: 518.3 $[M+H]^+$.

4.1.4.2.3

*tert-butyl-(6-(3-(4-chlorophenyl)-1-(2-(pyrrolidin-1-yl)ethyl)ureido)benzo[d]thiazol-2-yl)carbamate (**14c**)*

Synthesized using the procedure for **14a**, 0.3 g (0.8 mmol) of **13c** and 0.25 g (1.0 mmol) of phenyl (4-chlorophenyl)carbamate and 0.08 g (0.8 mmol) of triethylamine were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (50:1, v/v) to furnish **14c** as a pale yellow solid. Yield: 75.3%. MS (ESI) *m/z*: 516.2 $[M+H]^+$.

4.1.4.2.4

*tert-butyl-(6-(3-(4-chlorophenyl)-1-(2-(piperidin-1-yl)ethyl)ureido)benzo[d]thiazol-2-yl)carbamate (**14d**)*

Synthesized using the procedure for **14a**, 0.3 g (0.8 mmol) of **13d** and 0.25 g (1.0 mmol) of phenyl (4-chlorophenyl)carbamate and 0.08 g (0.8 mmol) of triethylamine were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (50:1, v/v) to furnish **14d** as a pale yellow solid. Yield: 60.3%. MS (ESI) *m/z*: 530.4 $[M+H]^+$.

4.1.4.2.5

*tert-butyl-(6-(3-(4-chlorophenyl)-1-(2-(4-methylpiperazin-1-yl)ethyl)ureido)benzo[d]thiazol-2-yl)carbamate (**14e**)*

Synthesized using the procedure for **14a**, 0.3 g (0.8 mmol) of **13e** and 0.25 g (1.0 mmol) of phenyl (4-chlorophenyl)carbamate and 0.08 g (0.8 mmol) of triethylamine were mixed and heated in 5 mL of DMF. Crude product was purified by column

chromatography on silica using mobile phase dichloromethane−methanol (50:1, v/v) to furnish **14e** as a pale yellow solid. Yield: 54.5%. MS (ESI) *m/z*: 545.6 $[M+H]^+$.

4.1.4.2.6

*tert-butyl-(6-(3-(4-chlorophenyl)-1-(2-(dimethylamino)ethyl)ureido)benzo[d]thiazol-2-yl)carbamate (**14f**)*

Synthesized using the procedure for **14a**, 0.3 g (0.8 mmol) of **13f** and 0.39 g (1.6 mmol) of phenyl (4-chlorophenyl)carbamate and 0.13 g (1.3 mmol) of triethylamine were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (50:1, v/v) to furnish **14f** as a pale yellow solid. Yield: 68.3%. MS (ESI) *m/z*: 490.1 $[M+H]^+$.

4.1.4.2.7

*tert-butyl-(6-(3-(4-chlorophenyl)-1-(2-(4-methylpiperidin-1-yl)ethyl)ureido)benzo[d]thiazol-2-yl)carbamate (**14g**)*

Synthesized using the procedure for **14a**, 0.3 g (0.8 mmol) of **13g** and 0.25 g (1.0 mmol) of phenyl (4-chlorophenyl)carbamate and 0.08 g (0.8 mmol) of triethylamine were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (50:1, v/v) to furnish **14g** as a pale yellow solid. Yield: 65.4%. MS (ESI) *m/z*: 544.5 $[M+H]^+$.

4.1.4.2.8

*tert-butyl-(6-(3-(4-chlorophenyl)-1-(3-morpholinopropyl)ureido)benzo[d]thiazol-2-yl)carbamate (**14h**)*

Synthesized using the procedure for **14a**, 0.3 g (0.8 mmol) of **13h** and 0.25 g (1.0 mmol) of phenyl (4-chlorophenyl)carbamate and 0.08 g (0.8 mmol) of triethylamine were mixed and heated in 5 mL of DMF. The crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (50:1, v/v) to furnish **14h** as a pale yellow solid. Yield: 61.9%. MS (ESI) *m/z*: 546.2 $[M+H]^+$.

*4.1.4.3 General procedure for preparation of compounds **15a-h***

4.1.4.3.1

*1-(2-aminobenzo[d]thiazol-6-yl)-3-(4-chlorophenyl)-1-(2-morpholinoethyl)urea (**15a**)*

The product (**14a**) from the previous step was dissolved in 3 mL of

dichloromethane; 3 mL of trifluoroacetate was then slowly added. After stirring overnight at room temperature, the mixture was evaporated under reduced pressure and the resultant was added to 5 mL water, adjusting pH to 8 with saturated sodium hydrogen carbonate, the precipitate was filtered off and washed with water, then the crude product was dried in vacuum desiccator and further purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **15a** as a pale yellow solid; yield: 74.3%; m.p.: 196.3 – 198.4 °C; HPLC purity: 98.65%, retention time = 18.968 min. MS (ESI) m/z: 432.3 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.02 (s, 1H), 8.85 (s, 1H), 7.86 (d, *J* = 1.9 Hz, 1H), 7.80 (s, 1H), 7.49 (d, *J* = 8.7 Hz, 2H), 7.31 (d, *J* = 8.7 Hz, 2H), 7.28 (d, *J* = 8.6 Hz, 1H), 7.18 (dd, *J* = 8.5, 1.9 Hz, 1H), 3.58 (t, *J* = 4.5 Hz, 4H), 3.46 (t, *J* = 6.4 Hz, 2H), 2.53 (d, *J* = 6.6 Hz, 2H), 2.42 (s, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.42, 153.27, 148.27, 139.66, 133.97, 131.16, 128.99, 125.39, 120.01, 118.21, 117.70, 111.48, 66.63, 57.55, 53.80, 41.54.

4.1.4.3.2

1-(2-aminobenzo[d]thiazol-6-yl)-3-(4-chlorophenyl)-1-(2-(diethylamino)ethyl)urea ***(15b)***

Synthesized using the procedure for **15a**, crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **15b** as a pale yellow solid; yield: 78.2%; m.p.: 195.3 – 197.1 °C; HPLC purity: 99.70%, retention time = 19.560 min. MS (ESI) m/z: 390.2 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.12 (s, 1H), 8.95 (s, 1H), 7.80 (d, *J* = 1.9 Hz, 1H), 7.73 (t, *J* = 5.2 Hz, 1H), 7.44 (d, *J* = 8.8 Hz, 2H), 7.25 (d, *J* = 8.8 Hz, 2H), 7.12 (dd, *J* = 8.4, 1.9 Hz, 1H), 2.39 (t, *J* = 6.5 Hz, 2H), 2.12 (s, 6H), 2.11 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 158.30, 158.09, 133.75, 131.12, 128.93, 119.90, 118.75, 118.10, 117.57, 116.76,58.24, 45.62, 42.32.

4.1.4.3.3

1-(2-aminobenzo[d]thiazol-6-yl)-3-(4-chlorophenyl)-1-(2-(pyrrolidin-1-yl)ethyl)urea ***(15c)***

Synthesized using the procedure for **15a**, crude product was purified by column

chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **15c** as a pale yellow solid; yield: 89.2%; m.p.: 195.3 – 198.6 °C; HPLC purity: 95.17%, retention time = 19.035 min. MS (ESI) m/z: 416.2 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.57 (s, 1H), 9.39 (s, 1H), 7.88 (s, 1H), 7.83 (d, *J* = 5.3 Hz, 1H), 7.53 (d, *J* = 8.6 Hz, 2H), 7.30 (d, *J* = 8.6 Hz, 2H), 7.22 (d, *J* = 8.5 Hz, 1H), 3.45 (d, *J* = 5.2 Hz, 2H), 2.63 (t, *J* = 6.6 Hz, 2H), 1.69 (s, 4H), 1.23 (s, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 153.29, 148.11, 139.80, 134.09, 131.05, 129.97, 128.86, 125.12, 119.86, 118.07, 117.51, 111.25, 54.97, 54.00, 43.51, 23.54.

4.1.4.3.4

*1-(2-aminobenzo[d]thiazol-6-yl)-3-(4-chlorophenyl)-1-(2-(piperidin-1-yl)ethyl)urea (**15d**)*

Synthesized using the procedure for **15a**, crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **15d** as a pale yellow solid; yield: 78.2%; m.p.: 199.3 – 202.8 °C; HPLC purity: 99.15%, retention time = 19.756 min. MS (ESI) m/z: 430.2 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.37 (s, 1H), 9.21 (s, 1H), 7.90 (d, *J* = 2.0 Hz, 1H), 7.52 (d, *J* = 8.9 Hz, 2H), 7.31 (d, *J* = 8.9 Hz, 2H), 7.22 (dd, *J* = 8.6, 2.1 Hz, 1H), 3.55 (s, 4H), 2.73 (s, 4H), 1.59 (s, 4H), 1.44 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.50, 159.29, 158.98, 153.25, 147.95, 139.66, 134.22, 131.20, 129.00, 125.45, 119.99, 118.33, 117.69, 111.39, 57.04, 53.96, 24.86, 23.40.

4.1.4.3.5

*1-(2-aminobenzo[d]thiazol-6-yl)-3-(4-chlorophenyl)-1-(2-(4-methylpiperazin-1-yl) ethyl)urea (**15e**)*

Synthesized using the procedure for **15a**, crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **15e** as a pale yellow solid; yield: 65.7%; m.p.: 188.4 – 190.8 °C; HPLC purity: 97.23%, retention time = 18.054 min. MS (ESI) m/z: 445.6 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.85 (s, 1H), 9.67 (s, 1H), 7.89 (d, *J* = 2.1 Hz, 1H), 7.76 (t, *J* = 5.2 Hz, 1H), 7.54 (d, *J* = 8.9 Hz, 2H), 7.29 (d, *J* = 8.9 Hz, 2H), 7.23 (dd, *J* = 8.5, 2.1 Hz, 1H), 3.44 (dd, *J* = 12.0, 6.4 Hz, 2H), 2.52 (s, 2H), 2.41 (s, 4H), 2.31 (s, 4H), 2.14

(s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.27, 153.48, 148.06, 140.06, 134.41, 131.09, 128.90, 125.09, 119.92, 118.16, 117.55, 111.22, 57.16, 55.17, 53.22, 46.25, 41.89.

4.1.4.3.6

*1-(2-aminobenzo[d]thiazol-6-yl)-3-(4-chlorophenyl)-1-(2-(dimethylamino)ethyl)urea (**15f**)*

Synthesized using the procedure for **15a**, crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **15f** as a pale yellow solid; yield: 77%; m.p.: 192.4 – 194.5 °C; HPLC purity: 99.33%, retention time = 23.484 min. MS (ESI) m/z: 418.3 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.82 (s, 1H), 8.64 (s, 1H), 7.85 (d, *J* = 2.2 Hz, 1H), 7.76 (t, *J* = 5.5 Hz, 1H), 7.48 (d, *J* = 8.9 Hz, 2H), 7.31 (d, *J* = 8.9 Hz, 2H), 7.28 (d, *J* = 8.6 Hz, 1H), 7.16 (dd, *J* = 8.6, 2.2 Hz, 1H), 3.39 (dd, *J* = 12.8, 6.3 Hz, 2H), 2.61 (t, *J* = 6.8 Hz, 2H), 2.53 (d, *J* = 7.1 Hz, 2H), 2.50 – 2.49 (m, 2H), 0.97 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.54, 153.10, 148.49, 139.41, 133.61, 131.20, 129.04, 125.56, 120.06, 118.20, 117.78, 111.66, 51.74, 47.13, 42.71, 12.38.

4.1.4.3.7

*1-(2-aminobenzo[d]thiazol-6-yl)-3-(4-chlorophenyl)-1-(2-(4-methylpiperidin-1-yl) ethyl)urea (**15g**)*

Synthesized using the procedure for **15a**, crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **15g** as a pale yellow solid; yield: 80.3%; m.p.: 210.2 – 212.8 °C; HPLC purity: 95.12%, retention time = 20.204 min. MS (ESI) m/z: 444.6 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.76 (s, 1H), 9.58 (s, 1H), 7.89 (s, 1H), 7.75 (s, 1H), 7.54 (d, *J* = 8.7 Hz, 2H), 7.29 (d, *J* = 8.7 Hz, 2H), 7.26 (s, 1H), 7.23 (d, *J* = 9.8 Hz, 1H), 3.43 (d, *J* = 4.9 Hz, 2H), 3.34 (s, 4H), 2.82 (dd, *J* = 22.5, 11.2 Hz, 4H), 1.93 (t, *J* = 10.7 Hz, 3H), 0.87 (t, *J* = 6.7 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.32, 153.42, 148.16, 139.96, 134.27, 131.12, 128.93, 125.17, 119.95, 118.16, 117.59, 111.29, 62.84, 57.54, 54.00, 34.44, 30.80, 22.34.

4.1.4.3.8

*1-(2-aminobenzo[d]thiazol-6-yl)-3-(4-chlorophenyl)-1-(3-morpholinopropyl)urea (**15h**)*

Synthesized using the procedure for **15a**, crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **15h** as a pale yellow solid; yield: 81.4%; m.p.: 189.4 – 191.6 °C; HPLC purity: 98.47%, retention time = 18.981 min. MS (ESI) m/z: 446.5 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.85 (s, 1H), 7.47 (d, *J* = 8.6 Hz, 2H), 7.22 (d, *J* = 8.6 Hz, 2H), 7.17 (d, *J* = 8.5 Hz, 1H), 7.13 (d, *J* = 8.3 Hz, 1H), 3.56 (s, 4H), 3.32 (t, *J* = 6.8 Hz, 2H), 2.35 (d, *J* = 7.0 Hz, 2H), 2.33 (s, 4H), 1.75 – 1.71 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.37, 153.32, 148.33, 139.82, 133.99, 131.06, 128.96, 125.26, 120.02, 118.11, 117.67, 111.46, 66.69, 56.20, 53.68, 42.70.

*4.1.5 General procedure for preparation of compounds **17a-v***

*4.1.5.1 General procedure for preparation of intermediates **19a-v***

*4.1.5.1.1 Phenyl phenylcarbamate (**19a**)*

Into a stirring mixture of ethyl acetate (20 mL), tetrahydrofuran (4 mL), water (4 mL), sodium carbonate (1.4 g, 12.9 mmol), and aniline (2.0 g, 21.5 mmol), phenyl chloroformate (3.7 g, 23.7 mmol) was added dropwise at 0 °C. The reaction was stirred at room temperature overnight, then it was evaporated under reduced pressure, and the resultant was added into 20 mL water, the precipitate was filtered and washed with water, then dried under vacuum to afford 4.3 g of **19a** as a white solid; yield: 94.3%; MS (ESI) m/z: 214.1 $[M+H]^+$, 236.1 $[M+Na]^+$.

*4.1.5.1.2 Phenyl (4-nitrophenyl)carbamate (**19b**)*

Synthesized using the procedure for **19a**, using 2.0 g (14.5 mmol) of 4-nitroaniline and 0.9 g (8.7 mmol) of sodium carbonate and 2.5 g (15.9 mmol) of phenyl chloroformate in the mixture of ethyl acetate (20 mL), tetrahydrofuran (4 mL) and water (4 mL), 3.4 g of **19b** was obtained; yield: 91%; MS (ESI) m/z: 258.1 $[M+H]^+$, 280.1 $[M+Na]^+$.

*4.1.5.1.3 Phenyl-(4-acetylphenyl)carbamate (**19c**).*

Synthesized using the procedure for **13a**, using 2.0 g (14.8 mmol) of

1-(4-aminophenyl)ethan-1-one and 0.9 g (8.9 mmol) of sodium carbonate and 2.6 g (16.3 mmol) of phenyl chloroformate in the mixture of ethyl acetate (20 mL), tetrahydrofuran (4 mL) and water (4 mL), 3.3 g of **19c** was obtained; yield: 88%; MS (ESI) m/z: 256.1 $[M+H]^+$,278.1 $[M+Na]^+$.

*4.1.5.1.4 Phenyl p-tolylcarbamate (**19d**)*

Synthesized using the procedure for **13a**, using 2.0 g (18.7 mmol) of p-toluidine and 0.9 g (11.2 mmol) of sodium carbonate and 2.6 g (20.6 mmol) of phenyl chloroformate in the mixture of ethyl acetate (20 mL), tetrahydrofuran (4 mL) and water (4 mL), 3.3 g of **19d** was obtained; yield: 88%; MS (ESI) m/z: 228.1 $[M+H]^+$, 250.1 $[M+Na]^+$.

*4.1.5.1.5 Phenyl-(4-ethylphenyl)carbamate (**19e**).*

Synthesized using the procedure for **19a**, using 2.0 g (16.5 mmol) of 4-ethylaniline and 1.0 g (9.9 mmol) of sodium carbonate and 2.8 g (18.2 mmol) of phenyl chloroformate in the mixture of ethyl acetate (20 ml), tetrahydrofuran (4 mL) and water (4 mL), 3.4 g of **19e** was obtained; yield: 86%; MS (ESI) m/z: 242.1 $[M+H]^+$, 264.1 $[M+Na]^+$.

*4.1.5.1.6 Phenyl-(4-fluorophenyl)carbamate (**19f**).*

Synthesized using the procedure for **19a**, using 2.0 g (18.0 mmol) of 4-fluoroaniline and 1.1 g (10.8 mmol) of sodium carbonate and 3.1 g (19.8 mmol) of phenyl chloroformate in the mixture of ethyl acetate (20 mL), tetrahydrofuran (4 mL) and water (4 mL), 3.8 g of **19f** was obtained; yield: 92%; MS (ESI) m/z: 232.1 $[M+H]^+$, 254.1 $[M+Na]^+$.

*4.1.5.1.7 Phenyl (4-bromophenyl)carbamate (**19g**)*

Synthesized using the procedure for **19a**, using 2.0 g (11.6 mmol) of 4-bromoaniline and 1.1 g (6.96 mmol) of sodium carbonate and 3.1 g (12.8 mmol) of phenyl chloroformate in the mixture of ethyl acetate (20 mL), tetrahydrofuran (4 mL) and water (4 mL), 3.8 g of **19g** was obtained; yield: 89%; MS (ESI) m/z: 292.1 $[M+H]^+$, 314.1 $[M+Na]^+$.

*4.1.5.1.8 Phenyl (4-isopropylphenyl)carbamate (**19h**)*

Synthesized using the procedure for **19a**, using 2.0 g (14.8 mmol) of

4-isopropylaniline and 1.1 g (8.8 mmol) of sodium carbonate and 3.1 g (16.3 mmol) of phenyl chloroformate in the mixture of ethyl acetate (20 mL), tetrahydrofuran (4 mL) and water (4 mL), 3.8 g of **19h** was obtained; yield: 95%; MS (ESI) m/z: 256.1 $[M+H]^+$, 278.1 $[M+Na]^+$.

*4.1.5.1.9 Phenyl-(4-(trifluoromethyl)phenyl)carbamate (**19i**).*

Synthesized using the procedure for **19a**, using 2.0 g (12.4 mmol) of 4-(trifluoromethyl)aniline and 0.8 g (7.4 mmol) of sodium carbonate and 2.1 g (13.7 mmol) of phenyl chloroformate in the mixture of ethyl acetate (20 mL), tetrahydrofuran (4 mL) and water (4 mL), 3.3 g of **19i** was obtained; yield: 94%; MS (ESI) m/z: 282.1 $[M+H]^+$, 304.1 $[M+Na]^+$.

*4.1.5.1.10 Phenyl (4-isopropoxyphenyl)carbamate (**19j**)*

Synthesized using the procedure for **19a**, using 2.0 g (13.2 mmol) of 4-isopropoxyaniline and 0.8 g (7.9 mmol) of sodium carbonate and 2.0 g (13.2 mmol) of phenyl chloroformate in the mixture of ethyl acetate (20 mL), tetrahydrofuran (4 mL) and water (4 mL), 3.3 g of **19i** was obtained; yield: 90%; MS (ESI) m/z: 286.1 $[M+H]^+$, 308.1 $[M+Na]^+$.

*4.1.5.1.11 Phenyl (2-chlorophenyl)carbamate (**19k**)*

Synthesized using the procedure for **19a**, using 2.0 g (15.6 mmol) of 2-chloroaniline and 1.1 g (9.4 mmol) of sodium carbonate and 3.1 g (17.2 mmol) of phenyl chloroformate in the mixture of ethyl acetate (20 mL), tetrahydrofuran (4 mL) and water (4 mL), 3.8 g of **19k** was obtained; yield: 92%; MS (ESI) m/z: 248.1 $[M+H]^+$, 270.1 $[M+Na]^+$.

*4.1.5.1.12 Phenyl (3-chlorophenyl)carbamate (**19l**)*

Synthesized using the procedure for **19a**, using 2.0 g (15.6 mmol) of 3-chloroaniline and 1.1 g (9.4 mmol) of sodium carbonate and 3.1 g (17.2 mmol) of phenyl chloroformate in the mixture of ethyl acetate (20 mL), tetrahydrofuran (4 mL) and water (4 mL), 3.8 g of **19l** was obtained; yield: 94%; MS (ESI) m/z: 248.1 $[M+H]^+$, 270.1 $[M+Na]^+$.

*4.1.5.1.13 Phenyl o-tolylcarbamate (**19m**)*

Synthesized using the procedure for **19a**, using 2.0 g (18.7 mmol) of o-toluidine and 0.9 g (11.2 mmol) of sodium carbonate and 2.6 g (20.6 mmol) of phenyl chloroformate in the mixture of ethyl acetate (20 mL), tetrahydrofuran (4 mL) and water (4 mL), 3.3 g of **19m** was obtained; yield: 88%; MS (ESI) m/z: 228.1 $[M+H]^+$, 250.1 $[M+Na]^+$.

*4.1.5.1.14 Phenyl m-tolylcarbamate (**19n**)*

Synthesized using the procedure for **19a**, using 2.0 g (18.7 mmol) of o-toluidine and 0.9 g (11.2 mmol) of sodium carbonate and 2.6 g (20.6 mmol) of phenyl chloroformate in the mixture of ethyl acetate (20 mL), tetrahydrofuran (4 mL) and water (4 mL), 3.3 g of **19n** was obtained; yield: 92%; MS (ESI) m/z: 228.1 $[M+H]^+$, 250.1 $[M+Na]^+$.

*4.1.5.1.15 Phenyl (3-(trifluoromethyl)phenyl)carbamate (**19o**)*

Synthesized using the procedure for **19a**, using 2.0 g (12.4 mmol) of 3-(trifluoromethyl)aniline and 0.8 g (7.4 mmol) of sodium carbonate and 2.1 g (13.7 mmol) of phenyl chloroformate in the mixture of ethyl acetate (20 mL), tetrahydrofuran (4 mL) and water (4 mL), 3.3 g of **19o** was obtained; yield: 94%; MS (ESI) m/z: 282.1 $[M+H]^+$, 304.1 $[M+Na]^+$.

*4.1.5.1.16 Phenyl (3-bromo-4-fluorophenyl)carbamate (**19p**)*

Synthesized using the procedure for **19a**, using 2.0 g (10.5 mmol) of 3-bromo-4-fluoroaniline and 0.8 g (6.3 mmol) of sodium carbonate and 2.1 g (11.6 mmol) of phenyl chloroformate in the mixture of ethyl acetate (20 mL), tetrahydrofuran (4 mL) and water (4 mL), 3.3 g of **19p** was obtained; yield: 94%; MS (ESI) m/z: 310.1 $[M+H]^+$, 332.1 $[M+Na]^+$.

*4.1.5.1.17 Phenyl (2,4-dichlorophenyl)carbamate (**19q**)*

Synthesized using the procedure for **19a**, using 2.0 g (12.3 mmol) of 2,4-dichloroaniline and 0.8 g (7.4 mmol) of sodium carbonate and 2.1 g (13.5 mmol) of phenyl chloroformate in the mixture of ethyl acetate (20 mL), tetrahydrofuran (4 mL) and water (4 mL), 3.3 g of **19q** was obtained; yield: 94%; MS (ESI) m/z: 282.1 $[M+H]^+$, 304.1 $[M+Na]^+$.

*4.1.5.1.18 Phenyl (4-chlorobenzyl)carbamate (**19r**)*

Synthesized using the procedure for **19a**, using 2.0 g (14.0 mmol) of 4-chlorobenzylamine and 0.8 g (8.4 mmol) of sodium carbonate and 2.1 g (15.4 mmol) of phenyl chloroformate in the mixture of ethyl acetate (20 mL), tetrahydrofuran (4 mL) and water (4 mL), 3.3 g of **19r** was obtained; yield: 94%; MS (ESI) m/z: 262.1 $[M+H]^+$, 284.1 $[M+Na]^+$.

*4.1.5.1.19 Phenyl (4-(trifluoromethoxy)phenyl)carbamate (**19s**)*

Synthesized using the procedure for **19a**, using 2.0 g (11.3 mmol) of 4-(trifluoromethoxy)benzenaime and 0.8 g (6.8 mmol) of sodium carbonate and 2.1 g (12.4 mmol) of phenyl chloroformate in the mixture of ethyl acetate (20 mL), tetrahydrofuran (4 mL) and water (4 mL), 3.3 g of **19s** was obtained; yield: 90%; MS (ESI) m/z: 298.1 $[M+H]^+$, 320.1 $[M+Na]^+$.

*4.1.5.1.20 Phenyl (4-acetamidophenyl)carbamate (**19t**)*

Synthesized using the procedure for **19a**, using 2.0 g (13.3 mmol) of *N*-(4-aminophenyl)acetamide and 0.8 g (8.0 mmol) of sodium carbonate and 2.1 g (14.6 mmol) of phenyl chloroformate in the mixture of ethyl acetate (20 mL), tetrahydrofuran (4 mL) and water (4 mL), 3.3 g of **19t** was obtained; yield: 85%; MS (ESI) m/z: 271.1 $[M+H]^+$, 293.1 $[M+Na]^+$.

*4.1.5.1.21 Phenyl pyridin-2-ylcarbamate (**19u**)*

Synthesized using the procedure for **19a**, using 2.0 g (21.3 mmol) of 2-aminopyridine and 0.8 g (12.8 mmol) of sodium carbonate and 2.1 g (23.4 mmol) of phenyl chloroformate in the mixture of ethyl acetate (20 mL), tetrahydrofuran (4 mL) and water (4 mL), 3.3 g of **19u** was obtained; yield: 85%; MS (ESI) m/z: 215.1 $[M+H]^+$, 237.1 $[M+Na]^+$.

*4.1.5.1.22 Phenyl naphthalen-2-ylcarbamate (**19v**)*

Synthesized using the procedure for **19a**, using 2.0 g (14.0 mmol) of 2-naphthylamine and 0.8 g (8.4 mmol) of sodium carbonate and 2.1 g (15.4 mmol) of phenyl chloroformate in the mixture of ethyl acetate (20 mL), tetrahydrofuran (4 mL) and water (4 mL), 3.3 g of **19v** was obtained; yield: 90%; MS (ESI) m/z: 264.1 $[M+H]^+$, 286.1 $[M+Na]^+$.

*4.1.5.2 General procedure for preparation of **16a-v***

4.1.5.2.1

*tert-butyl-(6-(1-(2-morpholinoethyl)-3-phenylureido)benzo[d]thiazol-2-yl)carbamate (**16a**)*

To a mixture of tert-butyl-(6-((2-morpholinoethyl)amino)benzo[*d*]thiazol-2-yl)carbamate *(***7**) (0.5 g, 13.2 mmol, 1 equiv.) and **19a** (0.3 g, 14.1 mmol, 1.2 equiv.) in 5 mL DMF, triethylamine (0.13 g, 13.2 mmol, 1 equiv.) was slowly added, after stirring at 60 ^{o}C for 12 h, the mixture was poured into 10 mL water, the solution was neutralized with 1N hydrochloric acid, then the precipitate was filtered and washed with water, the crude product was dried in vacuum desiccator and further purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **16a** as a pale yellow solid; yield: 59.8%; MS (ESI) m/z: 498.1 $[M+H]^+$.

4.1.5.2.2

*tert-butyl-(6-(1-(2-morpholinoethyl)-3-(4-nitrophenyl)ureido)benzo[d]thiazol-2-yl)-carbamate (**16b**)*

Synthesized using the procedure for **16a**, 0.5 g (13.2 mmol) of **7** and 0.15 g (0.72 mmol) of **19b** and 0.22 g (1.8 mmol) of triethylamine were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **16b** as a pale yellow solid; yield: 90%; MS (ESI) m/z: 543.1 $[M+H]^+$.

4.1.5.2.3

*tert-butyl-(6-(3-(4-acetylphenyl)-1-(2-morpholinoethyl)ureido)benzo[d]thiazol-2-yl) carbamate (**16c**)*

Synthesized using the procedure for **16a**, 0.5 g (13.2 mmol) of **7** and 0.15 g (0.72 mmol) of **19c** and 0.22 g (1.8 mmol) of triethylamine were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **16c** as a pale yellow solid; yield: 90%; MS (ESI) m/z: 540.1 $[M+H]^+$.

4.1.5.2.4

*tert-butyl(6-(1-(2-morpholinoethyl)-3-(p-tolyl)ureido)benzo[d]thiazol-2-yl)carbamate (**16d**)*

Synthesized using the procedure for **16a**, 0.5 g (13.2 mmol) of **7** and 0.15 g (0.72 mmol) of **19d** and 0.22 g (1.8 mmol) of triethylamine were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **16d** as a pale yellow solid; yield: 90%; MS (ESI) m/z: 512.1 $[M+H]^+$.

4.1.5.2.5

*tert-butyl(6-(3-(4-ethylphenyl)-1-(2-morpholinoethyl)ureido)benzo[d]thiazol-2-yl)-carbamate (**16e**)*

Synthesized using the procedure for **16a**, 0.5 g (13.2 mmol) of **7** and 0.15 g (0.72 mmol) of **19e** and 0.22 g (1.8 mmol) of triethylamine were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **16e** as a pale yellow solid; yield: 90%; MS (ESI) m/z: 526.1 $[M+H]^+$.

4.1.5.2.6

*tert-butyl-(6-(3-(4-fluorophenyl)-1-(2-morpholinoethyl)ureido)benzo[d]thiazol-2-yl) carbamate (**16f**)*

Synthesized using the procedure for **16a**, 0.5 g (13.2 mmol) of **7** and 0.15 g (0.72 mmol) of **19f** and 0.22 g (1.8 mmol) of triethylamine were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **16f** as a pale yellow solid; yield: 90%; MS (ESI) m/z: 516.1 $[M+H]^+$.

4.1.5.2.7

*tert-butyl-(6-(3-(4-bromophenyl)-1-(2-morpholinoethyl)ureido)benzo[d]thiazol-2-yl) carbamate (**16g**)*

Synthesized using the procedure for **16a**, 0.5 g (13.2 mmol) of **7** and 0.15 g (0.72 mmol) of **19g** and 0.22 g (1.8 mmol) of triethylamine were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using

mobile phase dichloromethane−methanol (30:1, v/v) to furnish **16g** as a pale yellow solid; yield: 90%; MS (ESI) m/z: 576.1 $[M+H]^+$.

4.1.5.2.8

*tert-butyl-(6-(3-(4-isopropylphenyl)-1-(2-morpholinoethyl)ureido)benzo[d]thiazol-2-yl)carbamate (**16h**)*

Synthesized using the procedure for **16a**, 0.5 g (13.2 mmol) of **7g** and 0.15 g (0.72 mmol) of **19h** and 0.22 g (1.8 mmol) of triethylamine were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **16h** as a pale yellow solid; yield: 90%; MS (ESI) m/z: 540.1 $[M+H]^+$.

4.1.5.2.9

*tert-butyl-(6-(1-(2-morpholinoethyl)-3-(4-(trifluoromethyl)phenyl)ureido)benzo[d] thiazol-2-yl)carbamate (**16i**)*

Synthesized using the procedure for **16a**, 0.5 g (13.2 mmol) of **7** and 0.15 g (0.72 mmol) of **19i** and 0.22 g (1.8 mmol) of triethylamine were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **16i** as a pale yellow solid; yield: 90%; MS (ESI) m/z: 566.1 $[M+H]^+$.

4.1.5.2.10

*tert-butyl-(6-(3-(4-isopropoxyphenyl)-1-(2-morpholinoethyl)ureido)benzo[d]thiazol-2-yl)carbamate (**16j**)*

Synthesized using the procedure for **16a**, 0.5 g (13.2 mmol) of **7** and 0.15 g (0.72 mmol) of **19j** and 0.22 g (1.8 mmol) of triethylamine were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **16j** as a pale yellow solid; yield: 90%; MS (ESI) m/z: 556.1 $[M+H]^+$.

4.1.5.2.11

*tert-butyl-(6-(3-(2-chlorophenyl)-1-(2-morpholinoethyl)ureido)benzo[d]thiazol-2-yl) carbamate (**16k**)*

Synthesized using the procedure for **16a**, 0.5 g (13.2 mmol) of **7** and 0.15 g (0.72 mmol) of **19k** and 0.22 g (1.8 mmol) of triethylamine were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **16k** as a pale yellow solid; yield: 90%; MS (ESI) m/z: 532.1 $[M+H]^+$.

4.1.5.2.12

*tert-butyl-(6-(3-(3-chlorophenyl)-1-(2-morpholinoethyl)ureido)benzo[d]thiazol-2-yl) carbamate (**16l**)*

Synthesized using the procedure for **16a**, 0.5 g (13.2 mmol) of **7** and 0.15 g (0.72 mmol) of **19l** and 0.22 g (1.8 mmol) of triethylamine were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **16l** as a pale yellow solid; yield: 90%; MS (ESI) m/z: 532.1 $[M+H]^+$.

4.1.5.2.13

*tert-butyl(6-(1-(2-morpholinoethyl)-3-(o-tolyl)ureido)benzo[d]thiazol-2-yl)carbamate (**16m**)*

Synthesized using the procedure for **16a**, 0.5 g (13.2 mmol) of **7** and 0.15 g (0.72 mmol) of **19m** and 0.22 g (1.8 mmol) of triethylamine were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **16m** as a pale yellow solid; yield: 90%; MS (ESI) m/z: 512.1 $[M+H]^+$.

4.1.5.2.14

*tert-butyl(6-(1-(2-morpholinoethyl)-3-(m-tolyl)ureido)benzo[d]thiazol-2-yl)carbamate (**16n**)*

Synthesized using the procedure for **16a**, 0.5 g (13.2 mmol) of **7** and 0.15 g (0.72 mmol) of **19n** and 0.22 g (1.8 mmol) of triethylamine were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **16n** as a pale yellow solid; yield: 90%; MS (ESI) m/z: 512.1 $[M+H]^+$.

4.1.5.2.15

*tert-butyl-(6-(1-(2-morpholinoethyl)-3-(3-(trifluoromethyl)phenyl)ureido)benzo[d] thiazol-2-yl)carbamate (**16o**)*

Synthesized using the procedure for **16a**, 0.5 g (13.2 mmol) of **7** and 0.15 g (0.72 mmol) of **19o** and 0.22 g (1.8 mmol) of triethylamine were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **16o** as a pale yellow solid; yield: 90%; MS (ESI) m/z: 566.1 $[M+H]^+$.

4.1.5.2.16

*tert-butyl-(6-(3-(3-bromo-4-fluorophenyl)-1-(2-morpholinoethyl)ureido)benzo[d] thiazol-2-yl)carbamate (**16p**)*

Synthesized using the procedure for **16a**, 0.5 g (13.2 mmol) of **7** and 0.15 g (0.72 mmol) of **19p** and 0.22 g (1.8 mmol) of triethylamine were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **16p** as a pale yellow solid; yield: 90%; MS (ESI) m/z: 594.1 $[M+H]^+$.

4.1.5.2.17

*tert-butyl-(6-(3-(2,4-dichlorophenyl)-1-(2-morpholinoethyl)ureido)benzo[d]thiazol-2-yl)carbamate (**16q**)*

Synthesized using the procedure for **16a**, 0.5 g (13.2 mmol) of **7** and 0.15 g (0.72 mmol) of **19q** and 0.22 g (1.8 mmol) of triethylamine were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **16q** as a pale yellow solid; yield: 90%; MS (ESI) m/z: 566.1 $[M+H]^+$.

4.1.5.2.18

*tert-butyl-(6-(3-(4-chlorobenzyl)-1-(2-morpholinoethyl)ureido)benzo[d]thiazol-2-yl) carbamate (**16r**)*

Synthesized using the procedure for **16a**, 0.5 g (13.2 mmol) of **7** and 0.15 g (0.72 mmol) of **19r** and 0.22 g (1.8 mmol) of triethylamine were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using

mobile phase dichloromethane−methanol (30:1, v/v) to furnish **16r** as a pale yellow solid; yield: 90%; MS (ESI) m/z: 546.1 $[M+H]^+$.

4.1.5.2.19

*tert-butyl-(6-(1-(2-morpholinoethyl)-3-(4-(trifluoromethoxy)phenyl)ureido)benzo[d] thiazol-2-yl)carbamate (**16s**)*

Synthesized using the procedure for **16a**, 0.5 g (13.2 mmol) of **7** and 0.15 g (0.72 mmol) of **19s** and 0.22 g (1.8 mmol) of triethylamine were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **16s** as a pale yellow solid; yield: 90%; MS (ESI) m/z: 582.1 $[M+H]^+$.

4.1.5.2.20

*tert-butyl-(6-(3-(4-acetamidophenyl)-1-(2-morpholinoethyl)ureido)benzo[d]thiazol-2-yl)carbamate (**16t**)*

Synthesized using the procedure for **16a**, 0.5 g (13.2 mmol) of **7** and 0.15 g (0.72 mmol) of **19t** and 0.22 g (1.8 mmol) of triethylamine were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **16t** as a pale yellow solid; yield: 90%; MS (ESI) m/z: 555.1 $[M+H]^+$.

4.1.5.2.21

*tert-butyl-(6-(1-(2-morpholinoethyl)-3-(pyridin-2-yl)ureido)benzo[d]thiazol-2-yl)-carbamate (**16u**)*

Synthesized using the procedure for **16a**, 0.5 g (13.2 mmol) of **7** and 0.15 g (0.72 mmol) of **19u** and 0.22 g (1.8 mmol) of triethylamine were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **16u** as a pale yellow solid; yield: 90%; MS (ESI) m/z: 499.1 $[M+H]^+$.

4.1.5.2.22

*tert-butyl-(6-(1-(2-morpholinoethyl)-3-(naphthalen-1-yl)ureido)benzo[d]thiazol-2-yl) carbamate (**16v**)*

Synthesized using the procedure for **16a**, 0.5 g (13.2 mmol) of **7** and 0.15 g (0.72 mmol) of **19v** and 0.22 g (1.8 mmol) of triethylamine were mixed and heated in 5 mL of DMF. Crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **16v** as a pale yellow solid; yield: 90%; MS (ESI) m/z: 548.1 $[M+H]^+$.

*4.1.5.3 General procedure for preparation of compounds **17a-v***

*4.1.5.3 1-(2-aminobenzo[d]thiazol-6-yl)-1-(2-morpholinoethyl)-3-phenylurea (**17a**)*

The product (**16a**) from the previous step was dissolved in 3 mL of dichloromethane; 3 mL of trifluoroacetate was then slowly added. After stirring overnight at room temperature, the mixture was evaporated under reduced pressure and the resultant was added to 5 mL water, adjusting pH to 8 with saturated sodium hydrogen carbonate, the precipitate was filtered off and washed with water, then the crude product was dried in vacuum desiccator and further purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **17a** as a pale yellow solid; yield: 77%; m.p.: 195.3 – 197.1 °C; HPLC purity: 99.36%, retention time = 16.023 min. MS (ESI) m/z: 390.2 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.30 (d, J = 15.7 Hz, 2H), 7.89 (d, J = 2.1 Hz, 1H), 7.80 (t, J = 5.3 Hz, 1H), 7.47 (d, J = 8.4 Hz, 2H), 7.32 – 7.21 (m, 3H), 7.19 (dd, J = 8.6, 2.2 Hz, 1H), 6.93 (t, J = 7.8 Hz, 1H), 3.58 (t, J = 4.6 Hz, 4H), 3.46 (dd, J = 12.1, 6.4 Hz, 2H), 2.52 (t, J = 6.6 Hz, 2H), 2.42 (s, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.31, 153.38, 148.12, 140.64, 134.21, 131.19, 129.16, 121.88, 118.43, 118.22, 117.46, 111.20, 66.64, 57.57, 53.82 , 41.54.

4.1.5.3.2

*1-(2-aminobenzo[d]thiazol-6-yl)-1-(2-morpholinoethyl)-3-(4-nitrophenyl)urea (**17b**)*

Synthesized using the procedure for **17a**, crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **17b** as a pale yellow solid; yield: 89.2%; m.p.: 195.3 – 198.6 °C; HPLC purity: 97.08%, retention time = 17.517 min. MS (ESI) m/z: 443.2 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.66 (s, 2H), 8.19 (d, J = 9.3 Hz, 2H), 7.91 (d, J = 2.1 Hz, 1H), 7.72 (d, J = 9.2 Hz, 2H), 7.31 (d, J = 8.6 Hz, 1H), 7.24 (dd, J = 8.6, 2.1 Hz, 1H),

3.61 – 3.54 (m, 4H), 3.47 (t, *J* = 6.6 Hz, 2H), 2.53 (t, *J* = 6.6 Hz, 2H), 2.42 (s, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.63, 152.76, 148.66, 147.47, 141.17, 133.44, 131.20, 125.59, 118.24, 117.92, 117.71, 111.76, 66.63, 57.54, 53.80, 41.54.

4.1.5.3.3

*3-(4-acetylphenyl)-1-(2-aminobenzo[d]thiazol-6-yl)-1-(2-morpholinoethyl)urea (**17c**)*

Synthesized using the procedure for **17a**, crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **17c** as a pale yellow solid; yield: 89.2%; m.p.: 195.3 – 198.6 °C; HPLC purity: 97.04%, retention time = 15.882 min. MS (ESI) m/z: 440.2 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.25 (s, 1H), 9.90 (s, 1H), 7.90 (s, 2H), 7.89 (s, 2H), 7.62 (s, 1H), 7.60 (s, 1H), 7.29 (d, *J* = 8.6 Hz, 1H), 7.22 (dd, *J* = 8.6, 2.0 Hz, 1H), 3.58 (t, *J* = 4.5 Hz, 4H), 3.47 (dd, *J* = 12.2, 6.4 Hz, 2H), 2.53 (d, *J* = 6.6 Hz, 2H), 2.51 (s, 3H), 2.42 (s, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 196.80, 165.46, 153.12, 148.27, 145.41, 133.84, 131.19, 130.43, 130.12, 118.22, 117.38, 117.16, 111.13, 66.61, 57.51, 53.78, 41.52, 26.76.

*4.1.5.3.4 1-(2-aminobenzo[d]thiazol-6-yl)-1-(2-morpholinoethyl)-3-(p-tolyl)urea (**17d**)*

Synthesized using the procedure for **17a**, crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **17d** as a pale yellow solid; yield: 89.2%; m.p.: 195.3 – 198.6 °C; HPLC purity: 96.13%, retention time = 17.969 min. MS (ESI) m/z: 412.2 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.60 (d, *J* = 23.3 Hz, 2H), 7.90 (d, *J* = 2.0 Hz, 1H), 7.78 (t, *J* = 5.4 Hz, 1H), 7.39 (d, *J* = 8.4 Hz, 2H), 7.26 (d, *J* = 8.6 Hz, 1H), 7.22 (dd, *J* = 8.6, 2.1 Hz, 1H), 7.05 (d, *J* = 8.3 Hz, 2H), 3.62 – 3.55 (m, 4H), 3.46 (dd, *J* = 12.1, 6.4 Hz, 2H), 2.55 – 2.51 (m, 2H), 2.42 (s, 4H), 2.23 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 165.18, 158.89, 158.69, 153.56, 147.87, 138.33, 134.66, 131.09, 130.42, 129.47, 118.52, 110.99, 66.63, 57.55, 53.80, 41.52, 20.79.

4.1.5.3.5

*1-(2-aminobenzo[d]thiazol-6-yl)-3-(4-ethylphenyl)-1-(2-morpholinoethyl)urea (**17e**)*

Synthesized using the procedure for **17a**, crude product was purified by column

chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **17e** as a pale yellow solid; yield: 89.2%; m.p.: 195.3 – 198.6 °C; HPLC purity: 99.18%, retention time = 19.715 min. MS (ESI) m/z: 426.2 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.59 (s, 2H), 7.88 (d, *J* = 2.0 Hz, 1H), 7.79 (s, 1H), 7.36 (d, *J* = 8.4 Hz, 2H), 7.29 (d, *J* = 8.6 Hz, 1H), 7.16 (dd, *J* = 8.6, 2.1 Hz, 1H), 7.10 (d, *J* = 8.4 Hz, 2H), 3.58 (t, *J* = 4.4 Hz, 4H), 3.48 – 3.45 (m, 2H), 2.54 (t, *J* = 6.2 Hz, 2H), 2.51 (dd, *J* = 5.9, 2.2 Hz, 2H), 2.42 (s, 4H), 1.15 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.41, 153.26, 148.22, 137.98, 137.46, 133.99, 131.22, 128.42, 118.73, 118.15, 117.56, 111.38 ,66.64, 57.56, 53.81, 41.54, 27.98, 16.25.

4.1.5.3.6

*1-(2-aminobenzo[d]thiazol-6-yl)-3-(4-fluorophenyl)-1-(2-morpholinoethyl)urea (**17f**)*

Synthesized using the procedure for **17a**, crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **17f** as a pale yellow solid; yield: 89.2%; m.p.: 195.3 – 198.6 °C; HPLC purity: 98.59%, retention time = 17.721 min. MS (ESI) m/z: 416.2 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.41 (s, 1H), 9.31 (s, 1H), 7.88 (d, *J* = 1.9 Hz, 1H), 7.78 (t, *J* = 4.9 Hz, 1H), 7.51 – 7.48 (m, 2H), 7.27 (d, *J* = 8.6 Hz, 1H), 7.21 (dd, *J* = 8.6, 1.9 Hz, 1H), 7.09 (t, *J* = 8.8 Hz, 2H), 3.59 – 3.57 (m, 4H), 3.48 – 3.44 (m, 2H), 2.55 – 2.52 (m, 2H), 2.42 (s, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.27, 153.60, 148.01, 137.24, 134.45, 131.10, 120.07, 118.18, 117.53, 115.66, 115.45, 111.21, 66.63, 57.56, 53.81, 41.54.

4.1.5.3.7

*1-(2-aminobenzo[d]thiazol-6-yl)-3-(4-bromophenyl)-1-(2-morpholinoethyl)urea (**17g**)*

Synthesized using the procedure for **17a**, crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **17g** as a pale yellow solid; yield: 89.2%; m.p.: 195.3 – 198.6 °C; HPLC purity: 98.82%, retention time = 19.560 min. MS (ESI) m/z: 476.2 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.62 (s, 1H), 9.53 (s, 1H), 7.99 (s, 1H), 7.50 (d, *J* = 8.4 Hz, 2H), 7.44 (d, *J* = 8.4 Hz, 2H), 7.39 (d, *J* = 8.5 Hz, 1H), 7.30 (d, *J* = 8.3 Hz, 1H), 3.86 (s, 4H), 3.80 (s, 2H), 3.42 (d, *J* = 5.2 Hz, 2H), 3.38 (s, 4H). ^{13}C NMR (101 MHz,

DMSO-d_6) δ 165.42, 153.27, 148.27, 139.66, 133.97, 131.16, 128.99, 125.39, 120.01, 118.21, 117.70, 111.48, 66.63, 57.55, 53.80, 41.54.

4.1.5.3.8

1-(2-aminobenzo[d]thiazol-6-yl)-3-(4-isopropylphenyl)-1-(2-morpholinoethyl)urea ***(17h)***

Synthesized using the procedure for **17a**, crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **17h** as a pale yellow solid; yield: 89.2%; m.p.: 195.3 – 198.6 °C; HPLC purity: 97.19%, retention time = 22.018 min. MS (ESI) m/z: 440.2 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.89 (d, *J* = 1.8 Hz, 1H), 7.39 (d, *J* = 8.4 Hz, 2H), 7.25 (d, *J* = 8.6 Hz, 1H), 7.19 (dd, *J* = 8.6, 1.7 Hz, 1H), 7.12 (d, *J* = 8.5 Hz, 2H), 3.59 – 3.57 (m, 4H), 3.45 (t, *J* = 6.6 Hz, 2H), 2.85 – 2.79 (m, 1H), 2.53 (d, *J* = 6.7 Hz, 2H), 2.42 (s, 4H), 1.19 (s, 3H), 1.17 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.27, 153.32, 148.07, 141.88, 138.27, 134.22, 131.15, 126.84, 118.58, 118.10, 117.33, 111.07, 66.64, 57.60, 53.82, 41.62, 33.22, 24.50.

4.1.5.3.9

1-(2-aminobenzo[d]thiazol-6-yl)-1-(2-morpholinoethyl)-3-(4-(trifluoromethyl)phenyl) urea ***(17i)***

Synthesized using the procedure for **17a**, crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **17i** as a pale yellow solid; yield: 89.2%; m.p.: 195.3 – 198.6 °C; HPLC purity: 98.71%, retention time = 22.534 min. MS (ESI) m/z: 440.2 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.89 (d, *J* = 1.8 Hz, 1H), 7.39 (d, *J* = 8.4 Hz, 2H), 7.25 (d, *J* = 8.6 Hz, 1H), 7.19 (dd, *J* = 8.6, 1.7 Hz, 1H), 7.12 (d, *J* = 8.5 Hz, 2H), 3.59 – 3.57 (m, 4H), 3.45 (t, *J* = 6.6 Hz, 2H), 2.85 – 2.79 (m, 1H), 2.53 (d, *J* = 6.7 Hz, 2H), 2.42 (s, 4H), 1.19 (s, 3H), 1.17 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 165.51, 158.74, 153.06, 148.47, 144.38, 133.66, 131.19, 126.49, 118.17, 117.81, 111.64, 66.63, 57.55, 53.81, 41.53.

4.1.5.3.10

*1-(2-aminobenzo[d]thiazol-6-yl)-3-(4-isopropoxyphenyl)-1-(2-morpholinoethyl)urea (**17j**)*

Synthesized using the procedure for **17a**, crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **17j** as a pale yellow solid; yield: 89.2%; m.p.: 195.3 – 198.6 °C; HPLC purity: 98.20%, retention time = 18.612 min. MS (ESI) m/z: 456.2 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.97 (s, 1H), 8.90 (s, 1H), 7.88 (s, 1H), 7.77 (s, 1H), 7.35 (d, *J* = 8.6 Hz, 2H), 7.27 (d, *J* = 8.5 Hz, 1H), 7.18 (d, *J* = 8.5 Hz, 1H), 6.83 (d, *J* = 8.6 Hz, 2H), 4.53 – 4.47 (m, 1H), 3.58 (s, 4H), 3.46 (d, *J* = 5.1 Hz, 2H), 3.33 (s, 2H), 2.42 (s, 4H), 1.24 (s, 3H), 1.23 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.24, 153.53, 152.75, 148.00, 134.43, 133.62, 131.16, 120.27, 118.21, 117.42, 116.56, 111.13, 69.91, 66.64, 57.57, 53.82, 41.54, 22.38.

4.1.5.3.11

*1-(2-aminobenzo[d]thiazol-6-yl)-3-(2-chlorophenyl)-1-(2-morpholinoethyl)urea (**17k**)*

Synthesized using the procedure for **17a**, crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **17k** as a pale yellow solid; yield: 89.2%; m.p.: 195.3 – 198.6 °C; HPLC purity: 98.54%, retention time = 18.815 min. MS (ESI) m/z: 432.2 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.15 (s, 1H), 8.07 (dd, *J* = 8.3, 1.4 Hz, 2H), 7.10 (d, *J* = 1.9 Hz, 2H), 6.99 (d, *J* = 8.6 Hz, 2H), 6.91 (m, 2H), 3.58 (m, 8H), 2.41 (s, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.04, 161.49, 159.52, 149.15, 145.43, 130.63, 128.70, 126.54, 124.35, 123.00, 117.21, 116.66, 116.31, 109.76, 66.64, 57.95, 53.87, 42.29.

4.1.5.3.12

*1-(2-aminobenzo[d]thiazol-6-yl)-3-(3-chlorophenyl)-1-(2-morpholinoethyl)urea (**17l**)*

Synthesized using the procedure for **17a**, crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **17l** as a pale yellow solid; yield: 89.2%; m.p.: 195.3 – 198.6 °C; HPLC purity: 98.71%, retention time = 22.534 min. MS (ESI) m/z: 432.2 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.88 (d, *J* = 2.2 Hz, 1H), 7.73 (d, *J* = 1.0 Hz, 1H), 7.28 (d, *J*

= 5.0 Hz, 1H), 7.27 (d, *J* = 2.1 Hz, 2H), 7.18 (dd, *J* = 8.6, 2.2 Hz, 1H), 6.98 (dd, *J* = 4.8, 1.9 Hz, 1H), 3.59 – 3.57 (m, 4H), 3.46 (s, 2H), 2.53 (d, *J* = 6.6 Hz, 2H), 2.42 (s, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.51, 153.09, 148.43, 142.06, 133.63, 131.20, 130.80, 121.60, 118.22, 117.84, 116.94, 111.67, 66.63, 57.55, 53.81, 41.52.

4.1.5.3.131-(2-aminobenzo[d]thiazol-6-yl)-1-(2-morpholinoethyl)-3-(o-tolyl)urea ***(17m)***

Synthesized using the procedure for **17a**, crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **17m** as a pale yellow solid; yield: 89.2%; m.p.: 195.3 – 198.6 °C; HPLC purity: 97.13%, retention time = 16.962 min. MS (ESI) m/z: 412.2 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.25 (s, 1H), 8.17 (s, 1H), 7.91 (d, *J* = 2.1 Hz, 1H), 7.80 (dd, *J* = 11.6, 6.5 Hz, 2H), 7.28 (d, *J* = 8.6 Hz, 1H), 7.20 – 7.17 (m, 1H), 7.13 (dd, *J* = 15.4, 7.6 Hz, 2H), 6.93 (t, *J* = 7.9 Hz, 1H), 3.58 (t, *J* = 4.5 Hz, 4H), 3.46 (dd, *J* = 12.1, 6.4 Hz, 2H), 2.52 (t, *J* = 6.6 Hz, 2H), 2.42 (s, 4H), 2.24 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.30, 153.47, 148.05, 138.16, 134.35, 131.23, 130.60, 128.18, 126.51, 122.95, 121.69, 118.26, 117.31, 111.06, 66.64, 57.57, 53.81, 41.54, 18.41.

4.1.5.3.14 1-(2-aminobenzo[d]thiazol-6-yl)-1-(2-morpholinoethyl)-3-(m-tolyl)urea ***(17n)***

Synthesized using the procedure for **17a**, crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **17n** as a pale yellow solid; yield: 89.2%; m.p.: 195.3 – 198.6 °C; HPLC purity: 95.50%, retention time = 18.073 min. MS (ESI) m/z: 412.2 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.07 (s, 1H), 9.05 (s, 1H), 7.90 (d, *J* = 2.2 Hz, 1H), 7.79 (t, *J* = 5.0 Hz, 1H), 7.32 (s, 1H), 7.26 (dd, *J* = 12.0, 8.5 Hz, 2H), 7.19 (dd, *J* = 8.6, 2.2 Hz, 1H), 7.13 (d, *J* = 7.8 Hz, 1H), 3.59 – 3.57 (m, 4H), 3.46 (d, *J* = 5.3 Hz, 2H), 2.53 (d, *J* = 6.6 Hz, 2H), 2.42 (s, 4H), 2.27 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.29, 153.40, 148.05, 140.64, 138.22, 134.35, 131.16, 128.97, 122.60, 119.03, 118.19, 117.47, 115.70, 111.20, 66.64, 57.57, 53.81, 41.54, 21.73.

4.1.5.3.15

*1-(2-aminobenzo[d]thiazol-6-yl)-1-(2-morpholinoethyl)-3-(3-(trifluoromethyl)phenyl) urea (**17o**)*

Synthesized using the procedure for **17a**, crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **17o** as a pale yellow solid; yield: 89.2%; m.p.: 195.3 – 198.6 °C; HPLC purity: 97.61%, retention time = 20.101 min. MS (ESI) m/z: 466.2 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.95 (s, 1H), 7.87 (s, 1H), 7.30 (s, 1H), 7.07 (d, *J* = 7.8 Hz, 1H), 7.03 – 7.00 (m, 1H), 6.85 (d, *J* = 8.5 Hz, 1H), 6.62 (d, *J* = 7.5 Hz, 1H), 3.58 (d, *J* = 4.5 Hz, 4H), 3.25 (t, *J* = 6.7 Hz, 2H), 2.47 (s, 2H), 2.41 (s, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.48, 160.84, 154.54, 130.35, 129.02, 128.58, 124.58, 116.78, 116.28, 116.00, 110.61, 109.70, 66.64, 58.71, 53.97, 45.57.

4.1.5.3.16

*1-(2-aminobenzo[d]thiazol-6-yl)-3-(3-bromo-4-fluorophenyl)-1-(2-morpholinoethyl) urea (**17p**)*

Synthesized using the procedure for **17a**, crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **17p** as a pale yellow solid; yield: 89.2%; m.p.: 195.3 – 198.6 °C; HPLC purity: 99.46%, retention time = 19.887 min. MS (ESI) m/z: 494.2 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.23 (s, 2H), 7.95 (dd, *J* = 6.3, 2.6 Hz, 1H), 7.88 (d, *J* = 2.1 Hz, 1H), 7.37 (dd, *J* = 8.9, 4.2, 2.7 Hz, 1H), 7.28 (dt, *J* = 8.8, 4.5 Hz, 2H), 7.19 (dd, *J* = 8.6, 2.1 Hz, 1H), 3.60 – 3.56 (m, 4H), 3.46 (t, *J* = 6.6 Hz, 2H), 2.53 (d, *J* = 6.6 Hz, 2H), 2.42 (s, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.48, 154.84, 153.30, 152.47, 148.36, 138.20, 133.80, 131.16, 122.51, 118.20, 117.83, 117.13, 116.90, 111.66, 66.63, 57.55, 53.81, 41.54.

4.1.5.3.17

*1-(2-aminobenzo[d]thiazol-6-yl)-3-(2,4-dichlorophenyl)-1-(2-morpholinoethyl)urea (**17q**)*

Synthesized using the procedure for **17a**, crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v)

to furnish **17q** as a pale yellow solid; yield: 89.2%; m.p.: 195.3 – 198.6 °C; HPLC purity: 95.05%, retention time = 21.875 min. MS (ESI) m/z: 466.2 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.06 (d, *J* = 9.0 Hz, 1H), 7.76 (s, 1H), 7.06 (d, *J* = 2.7 Hz, 1H), 6.91 (dd, *J* = 8.4, 2.1 Hz, 1H), 6.83 (dd, *J* = 9.0, 2.7 Hz, 1H), 6.57 (d, *J* = 8.4 Hz, 1H), 3.61 – 3.58 (m, 4H), 2.99 (s, 2H), 2.47 (d, *J* = 7.0 Hz, 2H), 2.39 (s, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 158.96, 158.65, 151.20, 127.62, 126.03, 125.38, 123.95, 122.18, 119.20, 116.13, 115.32, 113.24, 110.47, 66.54, 60.55, 54.20.

4.1.5.3.18

*1-(2-aminobenzo[d]thiazol-6-yl)-3-(4-chlorobenzyl)-1-(2-morpholinoethyl)urea (**17r**)*

Synthesized using the procedure for **17a**, crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **17r** as a pale yellow solid; yield: 89.2%; m.p.: 195.3 – 198.6 °C; HPLC purity: 96.89%, retention time = 18.137 min. MS (ESI) m/z: 446.2 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.56 (s, 2H), 7.86 (d, *J* = 1.7 Hz, 1H), 7.72 (t, *J* = 5.4 Hz, 1H), 7.37 (d, *J* = 8.5 Hz, 2H), 7.32 (d, *J* = 8.5 Hz, 2H), 7.21 (d, *J* = 8.6 Hz, 1H), 7.16 (d, *J* = 8.6 Hz, 1H), 4.25 (d, *J* = 6.0 Hz, 2H), 3.60 – 3.55 (m, 4H), 3.44 (dd, *J* = 12.1, 6.5 Hz, 2H), 2.56 – 2.51 (m, 2H), 2.41 (s, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.37, 158.84, 158.53, 156.85, 151.30, 140.78, 131.37, 129.39, 128.52, 117.29, 112.41, 112.13, 67.70, 66.51, 60.52, 54.12, 46.32.

4.1.5.3.19

*1-(2-aminobenzo[d]thiazol-6-yl)-1-(2-morpholinoethyl)-3-(4-(trifluoromethoxy) -phenyl)urea (**17s**)*

Synthesized using the procedure for **17a**, crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **17s** as a pale yellow solid; yield: 89.2%; m.p.: 195.3 – 198.6 °C; HPLC purity: 97.90%, retention time = 21.267 min. MS (ESI) m/z: 446.2 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.48 (s, 1H), 9.21 (s, 1H), 7.87 (d, *J* = 40.0 Hz, 2H), 7.57 (s, 1H), 7.28 (s, 4H), 3.55 (s, 6H), 2.41 (s, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 153.20, 148.19, 139.91, 133.89, 131.09, 122.01, 119.50, 118.08, 117.54, 66.54, 53.68, 41.76.

4.1.5.3.20

*N-(4-(3-(2-aminobenzo[d]thiazol-6-yl)-3-(2-morpholinoethyl)ureido)phenyl) -acetamide (**17t**)*

Synthesized using the procedure for **17a**, crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **17t** as a pale yellow solid; yield: 89.2%; m.p.: 195.3 – 198.6 °C; HPLC purity: 95.32%, retention time = 17.535 min. MS (ESI) m/z: 455.2 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.77 (s, 1H), 9.30 (d, *J* = 5.4 Hz, 2H), 7.83 (d, *J* = 2.1 Hz, 1H), 7.72 (t, *J* = 5.4 Hz, 1H), 7.39 (d, *J* = 8.9 Hz, 2H), 7.32 (d, *J* = 8.9 Hz, 2H), 7.20 (d, *J* = 8.6 Hz, 1H), 7.13 (dd, *J* = 8.6, 2.1 Hz, 1H), 3.51 (t, *J* = 4.5 Hz, 4H), 3.39 (dd, *J* = 12.2, 6.4 Hz, 2H), 2.47 – 2.44 (m, 2H), 2.35 (s, 4H), 1.94 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.33, 165.30, 158.94, 158.64, 153.44, 147.99, 135.98, 134.39, 133.87, 131.12, 120.11, 118.84, 66.62, 57.54, 53.79, 41.52, 24.27.

4.1.5.3.21

*1-(2-aminobenzo[d]thiazol-6-yl)-1-(2-morpholinoethyl)-3-(pyridin-2-yl)urea (**17u**)*

Synthesized using the procedure for **17a**, crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v) to furnish **17u** as a pale yellow solid; yield: 89.2%; m.p.: 195.3 – 198.6 °C; HPLC purity: 95.83%, retention time = 13.572 min. MS (ESI) m/z: 399.2 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.58 (s, 1H), 9.45 (s, 1H), 8.28 (dd, *J* = 5.0, 1.1 Hz, 1H), 7.95 (d, *J* = 2.1 Hz, 1H), 7.74 (ddd, *J* = 8.9, 7.4, 1.9 Hz, 1H), 7.45 (d, *J* = 8.4 Hz, 1H), 7.31 (d, *J* = 8.6 Hz, 1H), 7.25 (dd, *J* = 8.6, 2.2 Hz, 1H), 7.02 – 6.98 (m, 1H), 3.58 (t, *J* = 4.6 Hz, 4H), 3.47 (t, *J* = 6.5 Hz, 2H), 2.53 (t, *J* = 6.6 Hz, 2H), 2.42 (s, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.70, 153.48, 152.79, 148.76, 147.29, 138.97, 133.11, 131.28, 118.22, 117.79, 112.33, 112.07, 66.63, 57.54, 53.80, 41.54.

4.1.5.3.22

*1-(2-aminobenzo[d]thiazol-6-yl)-1-(2-morpholinoethyl)-3-(naphthalen-1-yl)urea (**17v**)*

Synthesized using the procedure for **17a**, crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (30:1, v/v)

to furnish **17v** as a pale yellow solid; yield: 89.2%; m.p.: 195.3 – 198.6 °C; HPLC purity: 96.36%, retention time = 19.023 min. MS (ESI) m/z: 448.2 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.25 (dd, *J* = 5.7, 3.8 Hz, 1H), 8.00 (d, *J* = 8.6 Hz, 2H), 7.88 – 7.84 (m, 1H), 7.52 (d, *J* = 7.7 Hz, 2H), 7.50 – 7.47 (m, 1H), 7.42 (d, *J* = 7.8 Hz, 1H), 7.26 (d, *J* = 8.7 Hz, 2H), 3.60 – 3.57 (m, 4H), 3.46 (t, *J* = 6.6 Hz, 4H), 2.53 (d, *J* = 6.6 Hz, 2H), 2.42 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.13, 147.81, 134.87, 134.31, 131.19, 128.56, 127.00, 126.45, 126.07, 125.51, 122.58, 118.14, 117.27, 114.12, 110.86, 66.64, 57.62, 53.83, 41.62.

*4.1.6 General procedure for preparation of compounds **18a-r***

*4.1.6.1 N-(6-(3-(4-chlorophenyl)-1-(2-morpholinoethyl)ureido)benzo[d]thiazol-2-yl) acetamide (**18a**)*

To a stirred solution of compound **15a** (0.3g, 0.7 mmol) and Et_3N (0.3 mL, 0.8 mmol) in ethyl acetate. Acetyl chloride (0.08g, 1.0 mmol) was added dropwise and the temperature was kept below 20 °C. The resulting mixture was allowed to warm to room temperature and stirred overnight. The mixture was poured into water (20 mL) and extracted with ethyl acetate (3×30 mL). The organic layer was dried over Na_2SO_4 and filtered. The filtrate was concentrated in vacuo. The crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (100:1, v/v) to furnish **18a** as a pale yellow solid; yield: 69.2%; m.p.: 203.3 – 206.7 °C; HPLC purity: 95.21%, retention time = 20.595 min. MS (ESI) m/z: 474.3 $[M+H]^+$; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.24 (s, 2H), 9.23 (s, 1H), 8.13 (d, *J* = 2.1 Hz, 1H), 7.69 (d, *J* = 8.7 Hz, 1H), 7.52 (m, 2H), 7.43 (dd, *J* = 8.7, 2.1 Hz, 1H), 7.32 (m, 2H), 4.32 (t, *J* = 6.5 Hz, 2H), 3.55 (m, 4H), 2.68 (t, *J* = 6.6 Hz, 2H), 2.50 (dt, *J* = 3.6, 1.8 Hz, 4H), 2.47 (m, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 171.72, 153.04, 143.29, 139.22, 136.33, 133.68, 128.97, 125.63, 121.36, 120.11, 118.43, 110.43, 66.59, 56.41, 54.05, 45.97, 23.22.

*4.1.6.2 N-(6-(3-(4-chlorophenyl)-1-(2-morpholinoethyl)ureido)benzo[d]thiazol-2-yl)cyclo-propanecarboxamide (**18b**)*

Synthesized using the procedure for **18a**, cyclopropionyl chloride (0.09g, 1.0

mmol) crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (100:1, v/v) to furnish **18b** as a pale yellow solid; yield: 59%; m.p.: 204.3 – 207.5 °C; HPLC purity: 96.65%, retention time = 22.465 min. MS (ESI) m/z: 500.1 $[M+H]^+$; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.12 (s, 2H), 8.14 (s, 1H), 7.71 (s, 1H), 7.50 (s, 2H), 7.43 (s, 1H), 7.33 (s, 2H), 4.85 (d, *J* = 142.7 Hz, 2H), 3.97 (dd, *J* = 194.8, 133.9 Hz, 6H), 3.08 (s, 2H), 2.40 (s, 2H), 1.13 (d, *J* = 70.6 Hz, 7H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 174.10, 152.99, 143.26, 139.20, 136.29, 133.77, 128.99, 125.60, 121.37, 119.97, 118.24, 110.19, 66.59, 56.70, 54.04, 45.96, 10.03, 8.96, 8.05.

*4.1.6.3 N-(6-(3-(4-chlorophenyl)-1-(2-morpholinoethyl)ureido)benzo[d]thiazol-2-yl) benzamide (**18c**)*

Synthesized using the procedure for **18a**, benzyol chloride (0.1g, 1.0 mmol) crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (100:1, v/v) to furnish **18c** as a pale yellow solid; yield: 78%; m.p.: 210.3 – 212.8 °C; HPLC purity: 98.99%, retention time = 25.597 min. MS (ESI) m/z: 536.2 $[M+H]^+$; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.16 (s, 1H), 9.14 (s, 1H), 8.20 (s, 1H), 7.76 (d, *J* = 8.7 Hz, 1H), 7.68 (d, *J* = 6.4 Hz, 2H), 7.55 (d, *J* = 7.2 Hz, 3H), 7.52 (d, *J* = 8.7 Hz, 2H), 7.45 (d, *J* = 8.7 Hz, 1H), 7.34 (d, *J* = 8.7 Hz, 2H), 4.30 (s, 2H), 3.40 (s, 4H), 2.58 (s, 2H), 2.10 (s, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 171.37, 158.28, 153.07, 143.53, 139.25, 136.71, 135.04, 133.80, 133.31, 131.17, 129.72, 129.10, 129.02, 127.97, 125.76, 121.72, 120.10, 118.54, 110.45, 66.38, 56.55, 53.77.

*4.1.6.4 N-(6-(3-(4-chlorophenyl)-1-(2-morpholinoethyl)ureido)benzo[d]thiazol-2-yl)-2-phenylacetamide (**18d**)*

Synthesized using the procedure for **18a**, phenylacetyl chloride (0.13g, 1.0 mmol) crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (100:1, v/v) to furnish **18d** as a pale yellow solid; yield: 66%; m.p.: 215.3 – 218.6 °C; HPLC purity: 96.61%, retention time = 25.474 min. MS (ESI) m/z: 550.3 $[M+H]^+$; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.28 (d, *J* = 4.7 Hz, 2H),

8.15 (d, *J* = 2.0 Hz, 1H), 7.71 (d, *J* = 8.7 Hz, 1H), 7.52 (d, *J* = 8.8 Hz, 2H), 7.42 (dd, *J* = 8.7, 2.0 Hz, 1H), 7.38 (d, *J* = 8.7 Hz, 2H), 7.32 (m, 5H), 4.42 (t, *J* = 5.9 Hz, 2H), 4.27 (s, 2H), 3.58 (m, 4H), 2.71 (t, *J* = 6.4 Hz, 2H), 2.49 (m, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.36, 153.00, 143.29, 139.15, 136.35, 134.80, 130.13, 129.69, 128.98, 128.77, 128.53, 127.23, 125.68, 121.45, 120.11, 118.46, 110.38, 66.64, 56.49, 54.11, 45.49, 40.82.

4.1.6.5

*N-(6-(3-(4-chlorophenyl)-1-(2-morpholinoethyl)ureido)benzo[d]thiazol-2-yl)-3-phenylpropanamide (**18e**)*

Synthesized using the procedure for **18a**, phenylpropionyl chloride (0.17g, 1.0 mmol) crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (100:1, v/v) to furnish **18e** as a pale yellow solid; yield: 87%; m.p.: 221.1 – 224.9 °C; HPLC purity: 95.13%, retention time = 25.586 min. MS (ESI) m/z: 565.1 $[M+H]^+$; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.43 (s, 1H), 9.42 (s, 1H), 8.18 (d, *J* = 1.9 Hz, 1H), 7.73 (d, *J* = 8.7 Hz, 1H), 7.51 (d, *J* = 8.7 Hz, 2H), 7.44 (dd, *J* = 8.8, 1.9 Hz, 1H), 7.36 (m, 3H), 7.33 (s, 1H), 7.32 (d, *J* = 3.0 Hz, 2H), 7.22 (d, *J* = 7.2 Hz, 1H), 4.63 (s, 2H), 4.03 (d, *J* = 12.1 Hz, 2H), 3.77 (t, *J* = 11.9 Hz, 2H), 3.64 (d, *J* = 11.5 Hz, 2H), 3.53 (s, 2H), 3.20 (m, 4H), 2.99 (t, *J* = 7.5 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.00, 157.82, 153.14, 142.98, 141.01, 139.31, 136.71, 133.81, 129.10, 129.06, 128.77, 126.54, 125.65, 121.61, 119.92, 118.32, 110.22, 63.72, 52.00, 49.05, 35.51, 30.40.

4.1.6.6

*N-(6-(3-(4-chlorophenyl)-1-(2-morpholinoethyl)ureido)benzo[d]thiazol-2-yl)-4-fluoro benzamide (**18f**)*

Synthesized using the procedure for **18a**, 4-fluorobenzyl chloride (0.11g, 1.0 mmol) crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (100:1, v/v) to furnish **18f** as a pale yellow solid; yield: 85%; m.p.: 219.1 – 222.5 °C; HPLC purity: 98.19%, retention time = 21.654 min. MS (ESI) m/z: 555.2 $[M+H]^+$; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.51 (s, 1H), 9.47 (s, 1H), 8.21 (d, *J* = 1.5 Hz, 1H), 7.80 (d, *J* = 8.6 Hz, 2H), 7.78 (d, *J* = 8.6 Hz,

2H), 7.51 (d, *J* = 8.8 Hz, 2H), 7.48 (m, 1H), 7.40 (s, 1H), 7.33 (d, *J* = 8.8 Hz, 2H), 4.48 (s, 2H), 3.96 (d, *J* = 11.3 Hz, 2H), 3.71 (t, *J* = 11.4 Hz, 2H), 3.52 (s, 4H), 3.43 (d, *J* = 10.2 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 170.03, 164.57, 162.91, 158.17, 153.05, 143.10, 139.20, 137.06, 133.91, 130.51, 129.03, 125.60, 121.95, 119.84, 118.40, 116.39, 110.16, 63.55, 51.88, 48.97, 44.07.

4.1.6.7

*N-(6-(3-(4-chlorophenyl)-1-(2-morpholinoethyl)ureido)benzo[d]thiazol-2-yl)-2-(4-fluorophenyl)acetamide (**18g**)*

Synthesized using the procedure for **18a**, 4-fluorophenylacetyl chloride (0.13g, 1.0 mmol) crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (100:1, v/v) to furnish **18g** as a pale yellow solid; yield: 75%; m.p.: 221.1 – 224.9 °C; HPLC purity: 98.88%, retention time = 24.048 min. MS (ESI) m/z: 568.1 $[M+H]^+$; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.51 (s, 1H), 9.47 (s, 1H), 8.21 (d, *J* = 1.5 Hz, 1H), 7.80 (d, *J* = 8.6 Hz, 2H), 7.78 (d, *J* = 8.6 Hz, 2H), 7.51 (d, *J* = 8.8 Hz, 2H), 7.48 (m, 1H), 7.40 (s, 1H), 7.33 (d, *J* = 8.8 Hz, 2H), 4.48 (s, 2H), 3.96 (d, *J* = 11.3 Hz, 2H), 3.71 (t, *J* = 11.4 Hz, 2H), 3.52 (s, 4H), 3.43 (d, *J* = 10.2 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 170.03, 164.57, 162.91, 158.17, 153.05, 143.10, 139.20, 137.06, 133.91, 130.51, 129.03, 125.60, 121.95, 119.84, 118.40, 116.39, 110.16, 63.55, 51.88, 48.97, 44.07.

4.1.6.8

*N-(6-(3-(4-chlorophenyl)-1-(2-morpholinoethyl)ureido)benzo[d]thiazol-2-yl)-2-(2-fluorophenyl)acetamide (**18h**)*

Synthesized using the procedure for **18a**, 2-fluorophenylacetyl chloride (0.13g, 1.0 mmol) crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (100:1, v/v) to furnish **18h** as a pale yellow solid; yield: 64%; m.p.: 218.7 – 220.7 °C; HPLC purity: 97.35%, retention time = 24.834 min. MS (ESI) m/z: 568.1 $[M+H]^+$; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.15 (s, 1H), 9.14 (s, 1H), 8.14 (d, *J* = 1.8 Hz, 1H), 7.72 (d, *J* = 8.8 Hz, 1H), 7.50 (d, *J* = 8.8 Hz, 2H), 7.40 (d, *J* = 7.0 Hz, 1H), 7.37 (d, *J* = 7.6 Hz, 2H), 7.33 (s, 2H), 7.22 (dd, *J* = 13.3, 7.0 Hz, 2H), 4.46 (s, 2H), 4.36 (s, 2H), 3.57 (d, *J* = 3.7 Hz, 4H), 3.32 (m, 4H),

2.76 (t, *J* = 5.9 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 171.29, 160.52, 152.98, 143.26, 139.18, 136.39, 133.68, 132.72, 129.58, 128.99, 125.62, 124.70, 122.27, 121.49, 120.00, 118.34, 115.53, 110.25, 66.62, 56.75, 54.22, 45.57, 34.97.

4.1.6.9

*2-(4-chlorophenyl)-N-(6-(3-(4-chlorophenyl)-1-(2-morpholinoethyl)ureido)benzo[d] thiazol-2-yl)acetamide (**18i**)*

Synthesized using the procedure for **18a**, 4-chlorophenylacetyl chloride (0.15g, 1.0 mmol) crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (100:1, v/v) to furnish **18i** as a pale yellow solid; yield: 87%; m.p.: 235.3 – 237.8 °C; HPLC purity: 97.39%, retention time = 24.837 min. MS (ESI) m/z: 584.3 $[M+H]^+$; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.07 (s, 1H), 9.06 (s, 1H), 8.13 (s, 1H), 7.71 (d, *J* = 8.7 Hz, 1H), 7.50 (d, *J* = 8.7 Hz, 2H), 7.43 (d, *J* = 8.2 Hz, 2H), 7.40 (d, *J* = 8.2 Hz, 1H), 7.34 (s, 2H), 7.32 (s, 2H), 4.42 (s, 2H), 4.28 (s, 2H), 3.57 (s, 4H), 3.30 (s, 4H), 2.73 (t, *J* = 6.1 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.10, 152.96, 143.27, 139.15, 136.35, 133.98, 133.71, 132.25, 131.92, 131.71, 129.01, 128.61, 125.64, 121.48, 120.01, 118.36, 110.30, 66.66, 56.51, 54.13, 45.44, 40.44.

4.1.6.10

*2-(4-bromophenyl)-N-(6-(3-(4-chlorophenyl)-1-(2-morpholinoethyl)ureido)benzo[d] thiazol-2-yl)acetamide (**18j**)*

Synthesized using the procedure for **18a**, 4-bromophenylacetyl chloride (0.17g, 1.0 mmol) crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (100:1, v/v) to furnish **18j** as a pale yellow solid; yield: 70%; m.p.: 246.3 – 248.1 °C; HPLC purity: 98.51%, retention time = 27.661 min. MS (ESI) m/z: 628.1 $[M+H]^+$; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.50 (s, 1H), 9.49 (s, 1H), 8.15 (d, *J* = 1.6 Hz, 1H), 7.70 (d, *J* = 8.7 Hz, 1H), 7.56 (d, *J* = 8.8 Hz, 2H), 7.51 (d, *J* = 8.8 Hz, 2H), 7.41 (dd, *J* = 8.7, 1.6 Hz, 1H), 7.31 (d, *J* = 8.7 Hz, 2H), 7.28 (d, *J* = 8.2 Hz, 2H), 4.41 (s, 2H), 4.27 (s, 2H), 3.57 (s, 4H), 3.33 (s, 4H), 2.73 (d, *J* = 12.5 Hz, 2H).^{13}C NMR (151 MHz, DMSO-d_6) δ 172.00, 153.11, 143.15,

139.38, 136.61, 134.41, 132.63, 132.00, 131.54, 131.22, 128.95, 125.47, 121.45, 120.43, 119.93, 118.30, 110.12, 66.67, 56.51, 54.13, 45.44.

4.1.6.11

*N-(6-(3-(4-chlorophenyl)-1-(2-morpholinoethyl)ureido)benzo[d]thiazol-2-yl)-2-(2,4-dichlorophenyl)acetamide (**18k**)*

Synthesized using the procedure for **18a**, 2,4-dichlorophenylacetyl chloride (0.17g, 1.0 mmol) crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (100:1, v/v) to furnish **18k** as a pale yellow solid; yield: 66%; m.p.: 235.1 – 237.5 °C; HPLC purity: 95.65%, retention time = 24.310 min. MS (ESI) m/z: 618.2 $[M+H]^+$; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.66 (s, 1H), 9.65 (s, 1H), 8.16 (d, *J* = 4.4 Hz, 1H), 7.72 (s, 1H), 7.64 (d, *J* = 5.7 Hz, 2H), 7.51 (d, *J* = 5.7 Hz, 2H), 7.40 (dd, *J* = 6.7, 4.3 Hz, 1H), 7.33 (m, 3H), 4.32 (s, 2H), 3.97 (s, 4H), 3.70 (s, 2H), 3.58 (s, 4H), 2.76 (s, 2H).

4.1.6.12

*N-(6-(3-(4-chlorophenyl)-1-(2-morpholinoethyl)ureido)benzo[d]thiazol-2-yl)-2-(4-methoxyphenyl)acetamide (**18l**)*

Synthesized using the procedure for **18a**, 4-methoxyphenylacetyl chloride (0.13g, 1.0 mmol) crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (100:1, v/v) to furnish **18l** as a pale yellow solid; yield: 90%; m.p.: 240.1 – 242.9 °C; HPLC purity: 99.60%, retention time = 22.568 min. MS (ESI) m/z: 580.3 $[M+H]^+$; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.83 (s, 1H), 9.81 (s, 1H), 8.16 (d, *J* = 1.2 Hz, 1H), 7.70 (d, *J* = 8.7 Hz, 1H), 7.52 (d, *J* = 8.7 Hz, 2H), 7.40 (dd, *J* = 8.7, 1.5 Hz, 1H), 7.31 (d, *J* = 8.7 Hz, 2H), 7.22 (d, *J* = 8.4 Hz, 2H), 6.93 (d, *J* = 8.5 Hz, 2H), 4.39 (s, 2H), 4.18 (s, 2H), 3.75 (s, 3H), 3.72 (s, 2H), 3.57 (s, 4H), 3.36 (s, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.16, 158.17, 152.78, 142.70, 139.04, 136.25, 130.73, 130.13, 128.55, 126.21, 124.95, 121.02, 119.33, 117.66, 114.86, 113.79, 109.42, 66.25, 56.09, 55.03, 53.72, 45.02.

4.1.6.13

*N-(6-(3-(4-chlorophenyl)-1-(2-morpholinoethyl)ureido)benzo[d]thiazol-2-yl)-2-(3-fluoro-4-methoxyphenyl)acetamide (**18m**)*

Synthesized using the procedure for **18a**, 3-fluoro-4-methoxyphenylacetyl chloride (0.17g, 1.0 mmol) crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (100:1, v/v) to furnish **18m** as a pale yellow solid; yield: 63%; m.p.: 225.1 – 227.8 °C; HPLC purity: 96.67%, retention time = 22.612 min. MS (ESI) m/z: 598.1 $[M+H]^+$; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.08 (s, 1H), 9.07 (s, 1H), 8.13 (d, *J* = 1.0 Hz, 1H), 7.71 (d, *J* = 8.7 Hz, 1H), 7.50 (d, *J* = 8.8 Hz, 2H), 7.40 (dd, *J* = 8.7, 1.4 Hz, 1H), 7.32 (d, *J* = 8.8 Hz, 2H), 7.18 (s, 1H), 7.14 (d, *J* = 8.8 Hz, 1H), 7.07 (d, *J* = 7.9 Hz, 1H), 4.41 (s, 2H), 4.20 (s, 2H), 3.84 (s, 3H), 3.81 (s, 2H), 3.57 (s, 4H), 3.30 (m, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.24, 152.98, 143.29, 139.20, 133.71, 132.02, 130.03, 129.03, 126.45, 125.63, 121.45, 120.06, 118.40, 113.98, 110.36, 71.52, 66.66, 56.37, 54.12, 40.44.

4.1.6.14

*N-(6-(3-(4-chlorophenyl)-1-(2-morpholinoethyl)ureido)benzo[d]thiazol-2-yl)-4-methylpiperazine-1-carboxamide (**18n**)*

Synthesized using the procedure for **18a**, 4-methylpiperazine-1-carbonyl chloride (0.11g, 1.0 mmol) crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (100:1, v/v) to furnish **18n** as a pale yellow solid; yield: 70%; m.p.: 201.1 – 204.6 °C; HPLC purity: 98.92%, retention time = 19.157 min. MS (ESI) m/z: 558.4 $[M+H]^+$; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.87 (s, 1H), 8.70 (s, 1H), 7.86 (s, 1H), 7.81 (s, 1H), 7.48 (d, *J* = 6.1 Hz, 2H), 7.31 (d, *J* = 6.1 Hz, 2H), 7.17 (d, *J* = 7.4 Hz, 1H), 3.58 (s, 4H), 3.47 (s, 2H), 3.38 (s, 8H), 2.42 (s, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 165.40, 153.01, 148.34, 139.31, 133.56, 131.13, 128.96, 125.48, 119.96, 118.14, 117.67, 111.54, 66.54, 57.47, 54.89, 53.72, 45.01, 41.44, 40.43.

4.1.6.15

*N-(6-(3-(4-ethylphenyl)-1-(2-morpholinoethyl)ureido)benzo[d]thiazol-2-yl)-2-phenylacetamide (**18o**)*

Synthesized using the procedure for **18a**, phenylacetyl chloride (0.1g, 1.0 mmol) crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (100:1, v/v) to furnish **18o** as a pale yellow solid; yield:

85%; m.p.: 204.1 – 206.7 °C; HPLC purity: 97.29%, retention time = 23.403 min. MS (ESI) m/z: 544.2 $[M+H]^+$; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.95 (s, 1H), 8.77 (s, 1H), 8.15 (d, *J* = 1.7 Hz, 1H), 7.70 (d, *J* = 8.7 Hz, 1H), 7.39 (d, *J* = 1.8 Hz, 1H), 7.37 (d, *J* = 3.1 Hz, 2H), 7.36 (d, *J* = 2.4 Hz, 2H), 7.31 (d, *J* = 7.8 Hz, 3H), 7.25 (dd, *J* = 13.2, 7.0 Hz, 1H), 7.11 (d, *J* = 8.3 Hz, 2H), 4.41 (t, *J* = 5.7 Hz, 2H), 4.26 (s, 2H), 3.57 (m, 4H), 3.33 (s, 4H), 2.70 (t, *J* = 6.0 Hz, 2H), 2.54 (d, *J* = 7.6 Hz, 2H), 1.16 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.31, 153.09, 143.10, 137.74, 137.51, 136.65, 134.85, 133.74, 130.15, 129.74, 128.75, 128.60, 128.36, 127.20, 126.94, 121.44, 118.66, 118.18, 110.00, 66.65, 56.51, 54.13, 45.48, 27.89, 16.16.

4.1.6.16

*N-(6-(3-(4-ethylphenyl)-1-(2-morpholinoethyl)ureido)benzo[d]thiazol-2-yl)-2-(2-fluorophenyl)acetamide (**18p**)*

Synthesized using the procedure for **18a**, 2-fluorophenylacetyl chloride (0.13g, 1.0 mmol) crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (100:1, v/v) to furnish **18p** as a pale yellow solid; yield: 73%; m.p.: 213.1 – 215.0 °C; HPLC purity: 95.41%, retention time = 23.437 min. MS (ESI) m/z: 562.3 $[M+H]^+$; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.37 (s, 1H), 9.17 (s, 1H), 8.16 (s, 1H), 7.70 (d, *J* = 8.7 Hz, 1H), 7.41 (d, *J* = 1.3 Hz, 1H), 7.39 (s, 2H), 7.37 (s, 2H), 7.14 (d, *J* = 8.2 Hz, 2H), 7.10 (d, *J* = 8.2 Hz, 2H), 4.46 (s, 2H), 4.36 (s, 2H), 3.58 (s, 4H), 3.35 (s, 4H), 2.76 (t, *J* = 5.8 Hz, 2H), 2.54 (d, *J* = 7.8 Hz, 2H), 1.15 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 171.24, 161.88, 160.52, 153.23, 142.96, 137.95, 137.33, 132.72, 132.41, 129.57, 128.98, 128.30, 124.47, 121.44, 118.61, 118.18, 115.36, 109.81, 66.62, 56.76, 54.22, 27.89, 16.16.

4.1.6.17

*N-(6-(3-(3-bromo-4-fluorophenyl)-1-(2-morpholinoethyl)ureido)benzo[d]thiazol-2-yl)-2-phenylacetamide (**18q**)*

Synthesized using the procedure for **18a**, phenylacetyl chloride (0.1g, 1.0 mmol) crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (100:1, v/v) to furnish **18q** as a pale yellow solid; yield: 53%; m.p.: 225.6 – 227.9 °C; HPLC purity: 98.27%, retention time = 23.441 min. MS

(ESI) m/z: 612.4 $[M+H]^+$; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.22 (s, 1H), 9.18 (s, 1H), 8.15 (d, *J* = 1.8 Hz, 1H), 7.95 (dd, *J* = 6.3, 2.5 Hz, 1H), 7.71 (d, *J* = 8.7 Hz, 1H), 7.40 (m, 1H), 7.37 (s, 1H), 7.36 (s, 1H), 7.31 (s, 2H), 7.30 (s, 2H), 7.26 (s, 1H), 7.25 (s, 1H), 4.41 (t, *J* = 5.8 Hz, 2H), 4.26 (s, 2H), 3.57 (d, *J* = 4.1 Hz, 4H), 3.56 (s, 2H), 3.34 (s, 4H), 2.70 (t, *J* = 6.3 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.10, 172.34, 153.04, 143.34, 137.74, 136.25, 134.83, 130.15, 129.73, 128.75, 128.60, 127.21, 126.93, 122.53, 121.44, 119.46, 118.44, 117.10, 110.44, 108.06, 66.65, 56.51, 54.20, 45.49.

4.1.6.18

*N-(6-(3-(3-bromo-4-fluorophenyl)-1-(2-morpholinoethyl)ureido)benzo[d]thiazol-2-yl) -2-(2-fluorophenyl)acetamide (**18r**)*

Synthesized using the procedure for **18a**, 2-fluorophenylacetyl chloride (0.13g, 1.0 mmol) crude product was purified by column chromatography on silica using mobile phase dichloromethane−methanol (100:1, v/v) to furnish **18r** as a pale yellow solid; yield: 64%; m.p.: 220.5 – 223.2 °C; HPLC purity: 96.10%, retention time = 24.009 min. MS (ESI) m/z: 630.2 $[M+H]^+$; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.32 (s, 1H), 9.27 (s, 1H), 8.15 (d, *J* = 1.5 Hz, 1H), 7.95 (dd, *J* = 6.3, 2.4 Hz, 1H), 7.72 (d, *J* = 8.7 Hz, 1H), 7.41 (d, *J* = 1.6 Hz, 1H), 7.38 (s, 2H), 7.30 (d, *J* = 8.7 Hz, 1H), 7.24 (d, *J* = 9.6 Hz, 1H), 7.21 (s, 1H), 7.15 (d, *J* = 7.9 Hz, 1H), 4.46 (s, 2H), 4.36 (s, 2H), 3.58 (m, 4H), 3.33 (s, 4H), 2.76 (t, *J* = 6.0 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 171.30, 162.14, 160.52, 153.06, 152.92, 143.30, 137.80, 136.33, 133.66, 132.75, 129.61, 124.71, 122.51, 122.32, 121.47, 119.39, 118.44, 117.09, 115.39, 110.39, 108.05, 107.90, 66.62, 56.75, 54.22, 45.57.

4.2 Biochemical characterization

4.2.1 sEH activity assay

The sEH inhibitory values of the compounds were determined by a fluorescence-based assay system of 96-well format. As substrate, nonfluorescent PHOME (3-phenylcyano-(6-methoxy-2-naphthalenyl)methyl ester 2-oxiraneacetic acid, Cayman Chemicals) was used, which can be hydrolyzed by the sEH to the fluorescent 6-methoxynaphthaldehyde. The fluorescence of the product was measured

(λem = 330 nm, λex = 465 nm). Therefore, human recombinant sEH in bis-Tris buffer, pH 7, with 0.1 mg/mL BSA and different concentrations of compounds in DMSO were were mixed in the wells (sEH and DMSO with the final concentrations of 0.25 μg/mL and 5%, respectively). PHOME was added to the wells (with the final concentration of 0.25 μM)to initiate the reaction and fluorescence was read at 15 min, and IC_{50} values were calculated. A background (noprotein and no compound) as well as a positive control (no compound) was executed. All measurements were performed in triplicate.

4.2.2 Enzyme inhibitory kinetics

Enzyme kinetics studies were employed to explore the mode of action and Ki value. The human sEH in 25 mM bis-Tris buffer and various concentrations of compound dissolved in DMSO were mixed in the wells. PHOME at indicated concentrations was added to the wells to initiate the reaction and fluorescence was measured as above. The initial reaction velocity was obtained from the slope of the line for each substrate concentration, and analyzed using Lineweaver-Burk plot to determine Ki values.

4.2.3 Solubility Assay

The water solubility of each tested compound was measured using a turbidimetric assay via Cyprotex (Watertown, MA). Each test compound was prepared as a 100-X concentrated stock solution in DMSO, from which serial dilutions were performed to yield eight solutions with final test compound concentrations of 1.6, 3.21, 6.25, 12.5, 25, 50, 100, and 200 mM. Each test compound solution was introduced into a 96-well plate, diluted 100-fold with PBS buffer (pH 7.4), and mixed. The solutions were incubated for two h, and absorbance was measured at 540 nm. An absorbance value > 3X standard deviation of the average blank absorption value was considered to exhibit turbidity. The highest test compound concentration with no sign of turbidity was indicative of a compound's kinetic water solubility.

4.2.4 Cytotoxicity

The target compounds were screened in HepG2 cells by a standard MTT assay in vitro. HepG2 cells were cultured in RPMI1640 medium supplemented with 10% fetal bovine serum (FBS). Approximately 4 ×104 cells, suspended in MEM medium, were plated onto each well of a 96-well plate and incubated in 5% CO_2 at 37 C for 24 h. The tested compounds were added to the culture medium at the indicated final concentrations, and the cell cultures were continued for 48 h. Fresh MTT was added to each well at a final concentration of 0.5 mg/mL and incubated with cells at 37 ^{o}C for 4 h. The formazan crystals were dissolved in 150 mL DMSO per each well, and the absorbency at 492 nm (for the absorbance of MTT formazan) and 630 nm (for the reference wavelength) was measured with the ELISA reader. All of the compounds were tested three times in each of the cell lines. The results expressed as IC_{50} (inhibitory concentration of 50%) were the mean ± SD and were calculated by using the Ghaphapad prism 6.02.

4.2.5 In vitro drug metabolism in rat liver microsomes.

A solution of the test compound (1 mM) was prepared in 100% DMSO. 432 μL of phosphate buffer (0.1 M, pH 7.4) and 50 μL of NADPH regenerating system (30 mM glucose 6-phosphate, 4 U/mL glucose 6-phosphate dehydrogenase, 10 mM NADP, 30 mM $MgCl_2$) and 5 μL of the corresponding test compound were preincubated at 37 °C. The final concentration of the investigated compound is 10 μM. After 5 min the reaction was started by the addition of 13 μL of microsome mix from the liver of Sprague-Dawley rats (Gibco, Darmstadt, Germany; 20 mg of protein/mL in 0.1 M phosphate buffer). The incubation was performed in a shaking water bath at 37 °C. The reaction was stopped by the addition of 500 μL of ice-cold methanol at 0, 15, 30, and 60 min. The samples were centrifuged at 10 000g for 5 min at 4 °C. The supernatants were analyzed and quantified by HPLC. Control samples were always performed to check the stability of the compounds in the reaction mixture. The first control was without NADPH, which is needed for the enzymatic activity of the microsomes. The second control was with inactivated microsomes (microsomes that were incubated for 20 min at 90 °C). Third control was without test compounds (to determine the baseline). As a positive control, a solution of 7-ethoxycoumarin (1 mM)

was used. The final concentration of the control compound, under assay conditions, was again 10 μM. The amounts of the test compounds were quantified by an external calibration curve.

4.2.6 In vivo anti-inflammatory assay

Male BALB/c mice weighing 18-25 g were purchased from the Experimental Animal Center of Hebei Medical University and kept in the animal house for at least one week before the experiments under standard conditions of light and temperature. All animals were accessed to standard laboratory diet. All test compounds were dissolved in dimethyl sulfoxide. Compounds **15a**, **17p**, **18d** and **t-AUCB** were evaluated for their in vivo anti-inflammatory activity applying the carrageenan-induced mice paw edema screening protocol as an acute inflammation model. The mice were marked and divided into 4 experimental groups of six mice each. The first group received 0.1 mL dimethyl sulfoxide and served as the vehicle-treated group. The second and third group received 100 mg/kg of tested compounds **15a**, **17p** and **18d**.The fourth group received 100 mg/kg **t-AUCB** and served as a positive control group. After 1 h of intraperitoneal injection of test compounds, a sub-plantar injection of 50 μL of 1% carrageenan solution to the right hind paw of each animal was performed. Mice paw thickness were measured before and 1,2,3,4 h after the injection of carrageenan. The right hind paw edema was measured by calibre, and the % edema were calculated. In all tests, adequate considerations were adopted to reduce pain or discomfort of animals.

4.2.7 Molecular docking

The molecules were built using Maestro, version 8.0.308, or converted to 3D structures from the 2D structure using LigPrep, version 2.1.207. The sEH (PDB entry 5ALW) X-ray structure was downloaded from the Protein Data Bank (PDB, http://www.rcsb.org/). The protein structures were prepared using the protein preparation wizard in Maestro with standard settings. Grids were generated using Glide, version 4.5.208, following the standard procedure recommended by Schrödinger. The conformational ensembles were docked flexibly using Glide with

standard settings in both standard and extra precision mode. Only poses with low energy conformations and good hydrogen bond geometries were considered. Figures were drawn using PyMOL (version 1.7).

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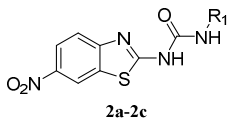
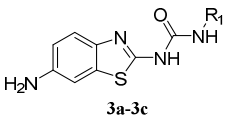
Tables

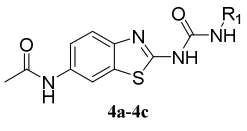
Table 1. In *vitro* sEH activities assay of series A compounds.



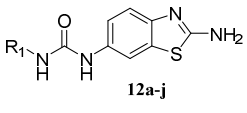
2a-2c



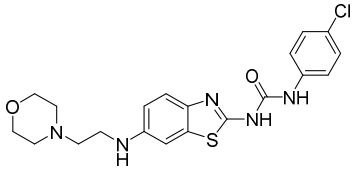
3a-3c



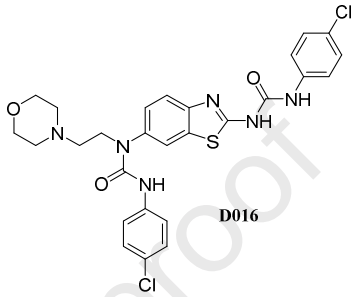
4a-4c



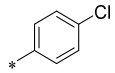
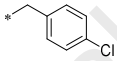
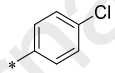
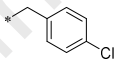
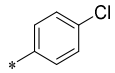
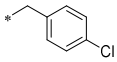
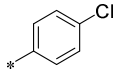
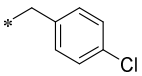
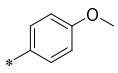
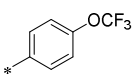
12a-j

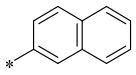


9



D016

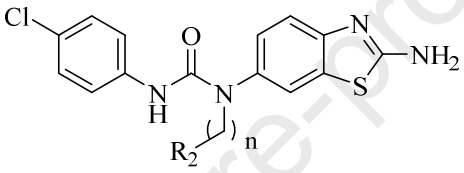
Compd.	R_1	sEH inhibitory activity [a]	
		Inhibition @100nM	IC_{50} (nM)
2a		**7.2%**	**ND** [b]
2b		**10.9%**	**ND**
3a		**6.7%**	**ND**
3b		**14.2%**	**ND**
4a		**12.5%**	**ND**
4b		**14.5%**	**ND**
9		**16.2%**	**ND**
12a		**76.1%**	**10.1**
12b		**47%**	**ND**
12c		**77.3%**	**822**
12d		**40.1%**	**ND**
12e		**39.3%**	**ND**
12f		**60.2%**	**ND**

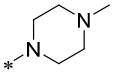
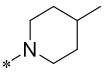
12g		**59.9%**	**4.1**
12h		**20.5%**	**ND**
12i		**34.1%**	**ND**
12j	H	**25.3%**	**ND**
D016		**90.2%**	**0.1**
t-AUCB	-	**87.8%**	**6.7**

[a] All compounds were assayed at least twice, and the inhibitory values were averaged.

[b] Not determined.

Table 2. In *vitro* sEH enzymatic assay of target compounds(15a-15h).

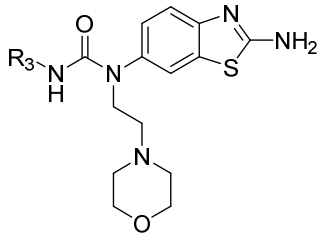


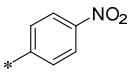
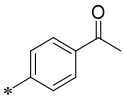
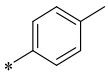
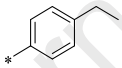
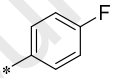
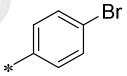
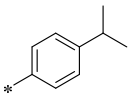
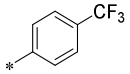
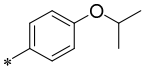
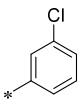
Compd.	n	R_2	sEH inhibitory activity [a]	
			Inhibition @100nM	IC_{50} (nM)
15a	**2**		**89.1%**	**2.8**
15b	**2**		**86.3%**	**24.5**
15c	**2**		**48.3%**	**ND** [b]
15d	**2**		**84.6%**	**6.5**
15e	**2**		**45.9%**	**ND**
15f	**2**		**39.1%**	**ND**
15g	**2**		**77.5%**	**20.2**
15h	**3**		**9.44%**	**ND**
t-AUCB	-	-	**87.8%**	**6.7**

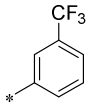
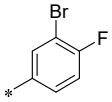
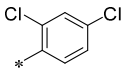
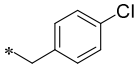
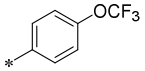
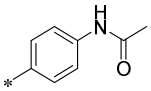
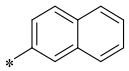
[a] All compounds were assayed at least twice, and the inhibitory values were averaged.

[b] Not determined.

Table 3. In *vitro* sEH enzymatic assay of target compounds (17a-17v).



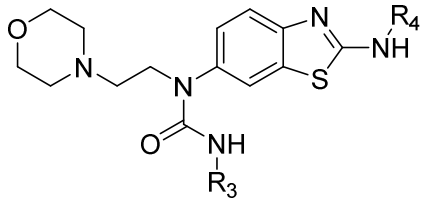
Cpd.	R_3	sEH inhibitory activity [a]	
		Inhibition @100nM	IC_{50} (nM)
17a		**40.4%**	**ND** [b]
17b		**19.2%**	**ND**
17c		**28.2%**	**ND**
17d		**73.1%**	**8.3**
17e		**84.7%**	**1.9**
17f		**74.3%**	**ND**
17g		**83.8%**	**16.2**
17h		**75.2%**	**11.2**
17i		**62%**	**ND**
17j		**56.6%**	**122**
17k		**59.7%**	**ND**
17l		**36.5%**	**ND**
17m		**26.3%**	**ND**

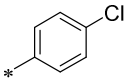
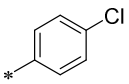
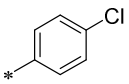
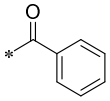
17n		**61.7%**	**101.3**
17o		**6.18%**	**ND**
17p		**90.5%**	**0.25**
17q		**58.4%**	**ND**
17r		**14.5%**	**ND**
17s		**61.3%**	**ND**
17t		**59.4%**	**ND**
17u		**16.2%**	**ND**
17v		**39.0%**	**150.8**
D016	-	**90.2%**	**0.1**
t-AUCB	-	**87.8%**	**6.7**

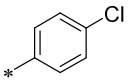
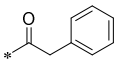
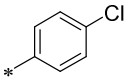
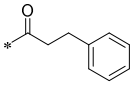
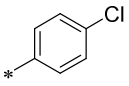
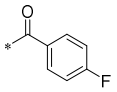
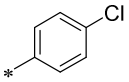
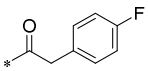
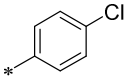
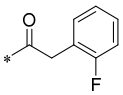
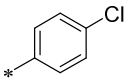
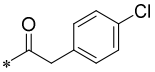
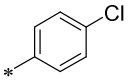
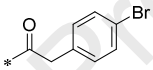
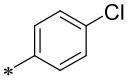
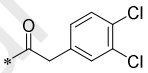
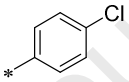
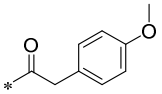
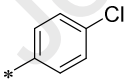
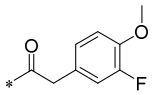
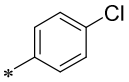
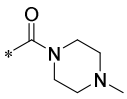
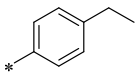
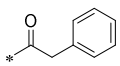
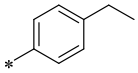
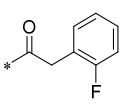
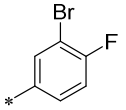
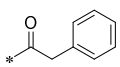
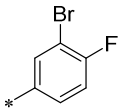
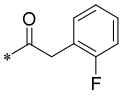
[a] All compounds were assayed at least twice, and the inhibitory values were averaged.

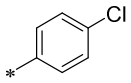
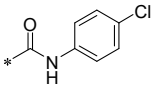
[b] Not determined.

Table 4. In *vitro* sEH enzymatic assay of target compounds (18a-18r).



Cpd.	R_3	R_4	sEH inhibitory activity [a]	
			Inhibition @100nM	IC_{50} (nM)
18a			**68.1%**	**ND** [b]
18b			**32.5%**	**ND**
18c			**48.8%**	**ND**

18d			**84.7%**	**0.082**
18e			**77.7%**	**ND**
18f			**64%**	**ND**
18g			**74.9%**	**ND**
18h			**82.5%**	**4.08**
18i			**71.9%**	**ND**
18j			**70.6%**	**ND**
18k			**71.8%**	**ND**
18l			**65.8%**	**ND**
18m			**58.1%**	**ND**
18n			**64%**	**ND**
18o			**76.8%**	**ND**
18p			**85.2%**	**5.4**
18q			**74.2%**	**ND**
18r			**73.8%**	**ND**

D016			**90.2%**	**0.1 nM**
t-AUCB	-	-	**87.8%**	**6.7 nM**

[a] All compounds were assayed at least twice, and the inhibitory values were averaged.

[b] Not determined.

Table 5. Water solubility and cLogP of selected potent compounds

Compounds	sEH IC_{50} (nM)	Solubility [a] (μg/mL)	cLogP [b]
15a	2.8	39	3.38
17p	0.25	68	3.69
18d	0.082	61	5.28
D016	0.1	30	5.96

[a] Tested using UV Spectrophotometer (UV-2600).

[b] Calculated using instant JChem.

Table 6. In *vitro* cytotoxicity and parameters of Rat Liver Microsomes Stability

Cpd	HepG-2 cytotoxicity $IC_{50}(\mu M)$	$T_{1/2}$ (min)	$CL_{int(mic)}$ (μL/min/mg)[a]	$CL_{int(liver)}$ (μL/min/mg)[b]	Remaining (T=60min)	Remaining (NCF=60min)[c]
15a	>25	34.7	40.0	71.9	30.1%	99.5%
17p	>25	44.7	31.0	55.8	36.2%	85.0%
18d	>25	1.7	820.4	1476.7	0%	115.5%
Propafenone	ND [d]	1.3	1047.3	1885.1	0.4%	98%
Diclofenac	ND [d]	23.4	59.3	106.7	16.9%	96.3%

[a] $CL_{int(mic)}$: intrinsic clearance; $CL_{int(mic)}=0.693/T_{1/2}$/mg microsomal protein per mL

[b] $CL_{int(liver)}$: $CL_{int(mic)}$ × mg microsomal protein/g liver weight × g liver weight/kg body weight

[c] NCF (no co-factor): No NADPH regenerating system is added to NCF samples during the 60 min incubation.

[d] Not determined.

Table 7. In *vivo* anti-inflammatory activities of compounds 15a, 17p, 18d and *t*-AUCB (100mg/kg) in BALB/c mice (n=6).

	0h	1h		2h		3h		4h	
	paw *thickness* [a,b] (mm)	paw *thickness* (mm)	Edema%	paw *thickness* (mm)	Edema%	paw *thickness* (mm)	Edema%	paw *thickness* (mm)	Edema%
Control	2.64±0.09	3.51±0.19	32.82	3.56±0.29	34.58	3.61±0.36	36.49	3.60±0.12	36.24
15a	2.57±0.08	3.11±0.17	20.99*[c]	3.06±0.09	19.06*	3.13±0.21	21.64*	3.17±0.12	23.39*
17p	2.40±0.06	3.15±0.04	30.86	3.16±0.16	31.49	3.06±0.07	27.39*	3.06±0.10	27.12*
18d	2.74±0.09	3.33±0.16	21.56*	3.22±0.21	17.58*	3.19±0.19	16.48*	3.30±0.11	20.32*
***t*-AUCB**	2.64±0.13	3.30±0.15	25.01*	3.23±0.15	22.61*	3.29±0.22	24.98*	3.28±0.15	24.71*

[a] Paw thickness were measured before (0 h) or 1, 2, 3, 4 h after injection of 50 μL of carrageenan in mice right hind paw and were significantly different compared to vehicle-treated group.

[b] Data are expressed as mean ± SEM. The data were compared using two-way analysis of variance (ANOVA) followed by Bonferroni's test for multiple comparisons.

[c] $*P < 0.05$ *vs*. vehicle.

Figures

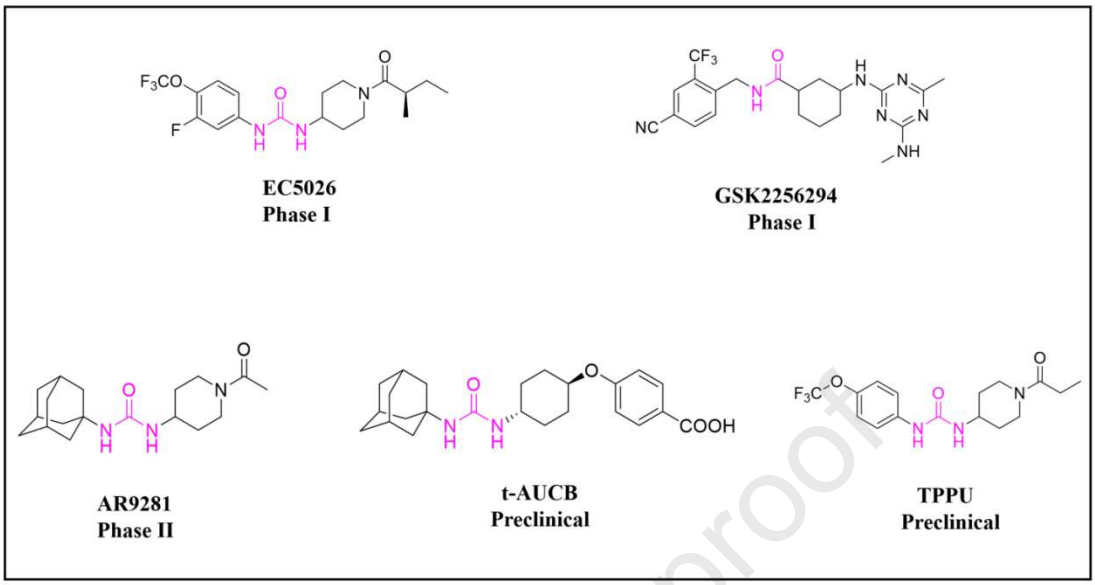
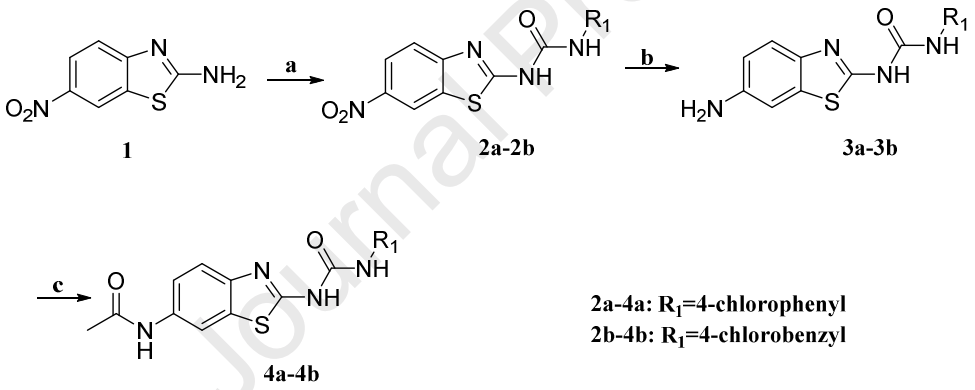
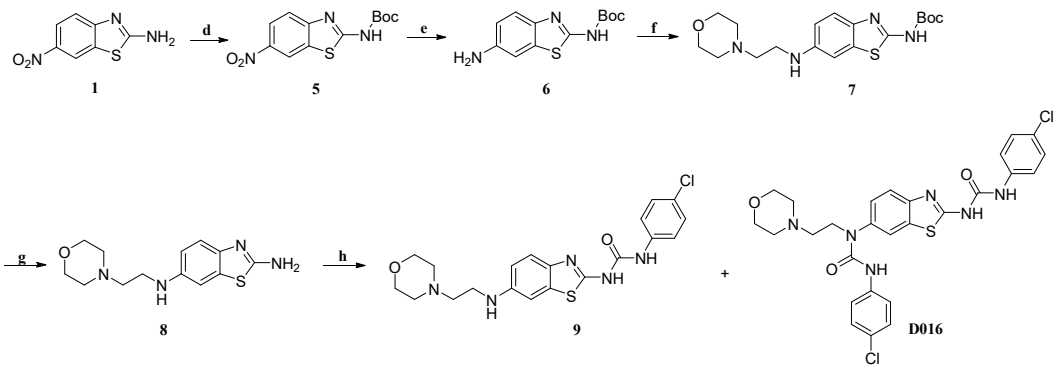


Fig.1. Representative examples of sEH inhibitors.

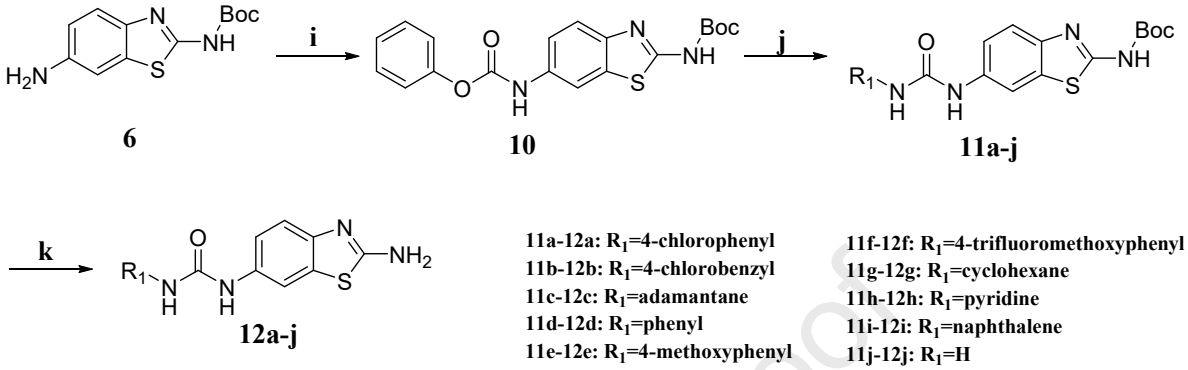


Scheme 1 Reagents and conditions: (a) substituted phenyl benzoate, DBU, 1,4-dioxane, 80 ^{o}C, 12 h; (b) 80% $N_2H_4{\cdot}H_2O$, $FeCl_3{\cdot}6H_2O$, active carbon, 1,4-dioxane, 80 ^{o}C, 12 h; (c) acetyl chloride, Et_3N, DCM, 0 ^{o}C, 2 h.

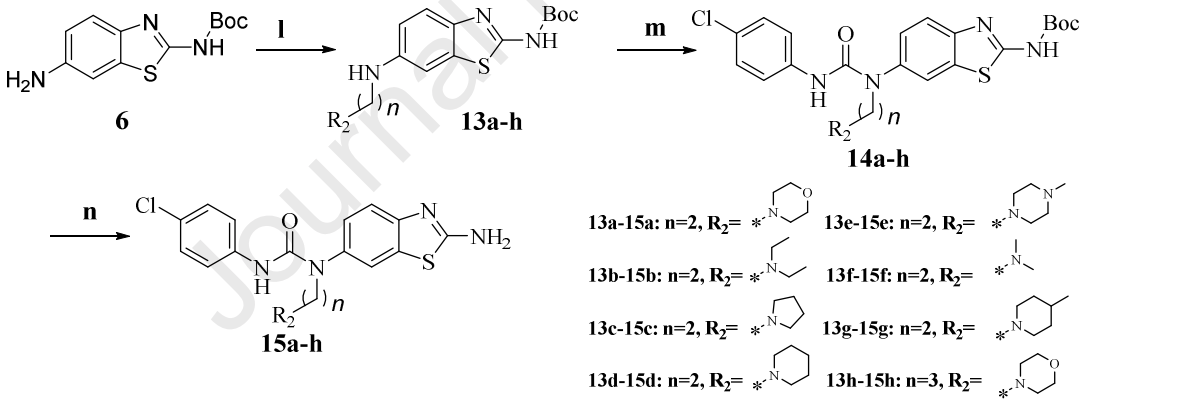


Scheme 2 Reagents and conditions: (d) $(Boc)_2O$, DMAP, DMF, 120 ^{o}C, 12 h; (e) 80% $N_2H_4{\cdot}H_2O$,

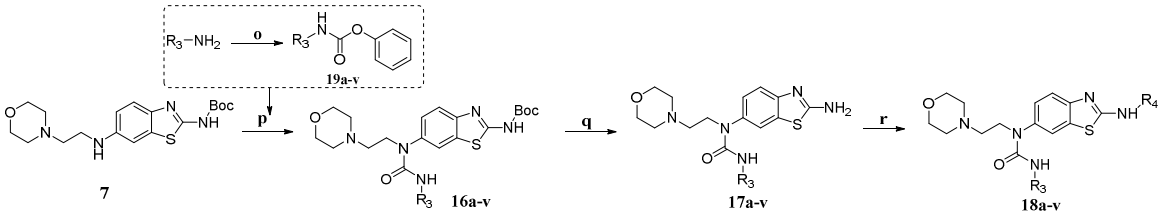
$FeCl_3{\cdot}6H_2O$, active carbon, 1,4-dioxane, 80 °C, 12 h; (f) 4-(2-chloroethyl)morpholine, K_2CO_3, 1,4-dioxane, 90 °C, 12 h; (g) CF_3COOH, DCM, rt, 3 h; (h) phenyl (4-chlorophenyl) carbamate, DBU, 1,4-dioxane, 80 °C, 12 h.



Scheme 3 Reagents and conditions: (i) phenyl chloroformate, acetone, rt, 1 h; (j) R_1-NH_2, Et_3N, 1,4-dioxane, 60 °C, 2 h; (k) CF_3COOH, DCM, rt, 3 h.



Scheme 4 Reagents and conditions: (l) $R_2(CH_2)_nCl$, alkyl halide, K_2CO_3, 1,4-dioxane, 90 °C, 12 h; (m) phenyl (4-chlorophenyl) carbamate, Et_3N, 1,4-dioxane, 60 °C, 12 h; (n) CF_3COOH, DCM, rt, 3 h.



16a-17a: R_3=phenyl
16b-17b: R_3=4-nitrophenyl
16c-17c: R_3=4-acetylphenyl
16d-17d: R_3=4-methylphenyl
16e-17e: R_3=4-ethylphenyl
16f-17f: R_3=4-fluorophenyl
16g-17g: R_3=4-bromophenyl
16h-17h: R_3=4-isopropylphenyl
16i-17i: R_3=4-trifluoromethylphenyl
16j-17j: R_3=4-isopropanolphenyl
16k-17k: R_3=2-chlorophenyl

16l-17l: R_3=3-chlorophenyl
16m-17m: R_3=2-methylphenyl
16n-17n: R_3=3-methylphenyl
16o-17o: R_3=3-trifluoromethylphenyl
16p-17p: R_3=3-bromo-4-fluorophenyl
16q-17q: R_3=2,4-dichlorophenyl
16r-17r: R_3=4-chlorobenzyl
16s-17s: R_3=4-trifluoromethoxyphenyl
16t-17t: R_3=4-aminoacetylphenyl
16u-17u: R_3=pyridine
16v-17v: R_3=naphthalene

18a: R_3=4-chlorophenyl, R_4=propan-2-one
18b: R_3=4-chlorophenyl, R_4=1-cyclopropylethan-1-one
18c: R_3=4-chlorophenyl, R_4=acetophenone
18d: R_3=4-chlorophenyl, R_4=1-phenylpropan-2-one
18e: R_3=4-chlorophenyl, R_4=4-phenylbutan-2-one
18f: R_3=4-chlorophenyl, R_4=1-(4-fluorophenyl)ethan-1-one
18g: R_3=4-chlorophenyl, R_4=1-(4-fluorophenyl)propan-2-one
18h: R_3=4-chlorophenyl, R_4=1-(2-fluorophenyl)propan-2-one
18i: R_3=4-chlorophenyl, R_4=1-(4-chlorophenyl)propan-2-one
18j: R_3=4-chlorophenyl, R_4=1-(4-bromophenyl)propan-2-one
18k: R_3=4-chlorophenyl, R_4=1-(2,4-dichlorophenyl)propan-2-one
18l: R_3=4-chlorophenyl, R_4=1-(4-methoxyphenyl)propan-2-one
18m: R_3=4-chlorophenyl, R_4=1-(3-fluoro-4-methoxyphenyl)propan-2-one
18n: R_3=4-chlorophenyl, R_4=1-(4-methylpiperazin-1-yl)ethan-1-one
18o: R_3=4-ethylphenyl, R_4=1-phenylpropan-2-one
18p: R_3=4-ethylphenyl, R_4=1-(2-fluorophenyl)propan-2-one
18q: R_3=3-bromo-4-fluorophenyl, R_4=1-phenylpropan-2-one
18r: R_3=3-bromo-4-fluorophenyl, R_4=1-(2-fluorophenyl)propan-2-one

Scheme 5 Reagents and conditions: (o) **19a-v**, sodium carbonate, THF/EA/H_2O, rt, 12 h; (p) Et_3N, 1,4-dioxane, 60 ^oC, 12h; (q) CF_3COOH, DCM, rt, 3 h. (r) R_4-COCl, K_2CO_3, EA, rt, 5 h.

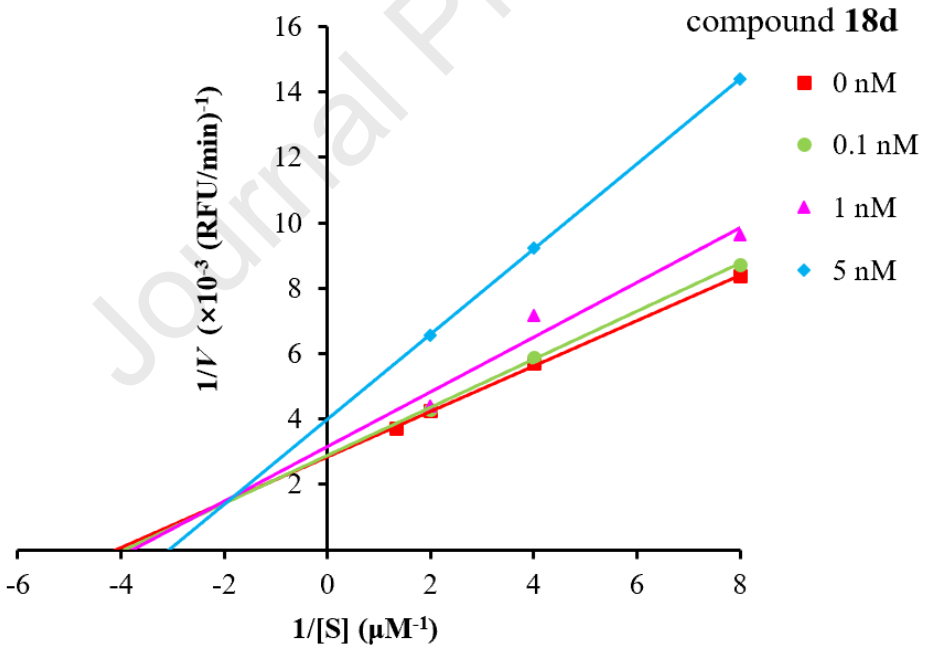


Fig 2. Lineweaver-Burk plot of fluorogenic substrate (PHOME) in the presence of compound **18d**. **[S]**: the concentrations of PHOME (μM); ***V*:** initial reaction velocity (RFU/min).

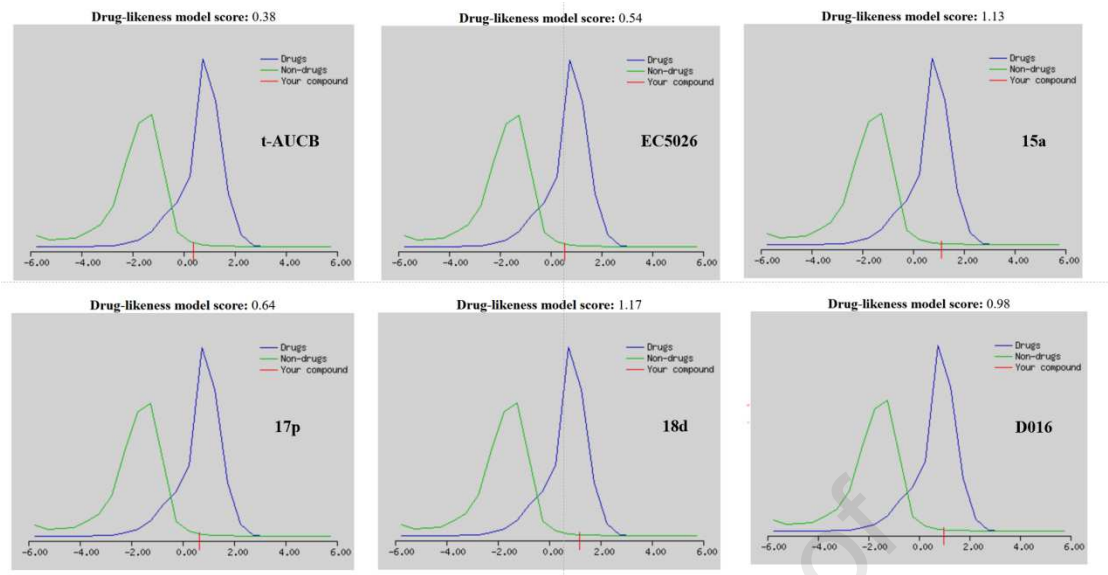


Fig 3. Representative drug-likeness model score results of **t-AUCB**, **EC5026**, **15a**, **17p**, **18d** and **D016**. (The green curve indicates that a compound is possibly not a drug, while the blue curve suggests the compound may possess drug-likeness properties.). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

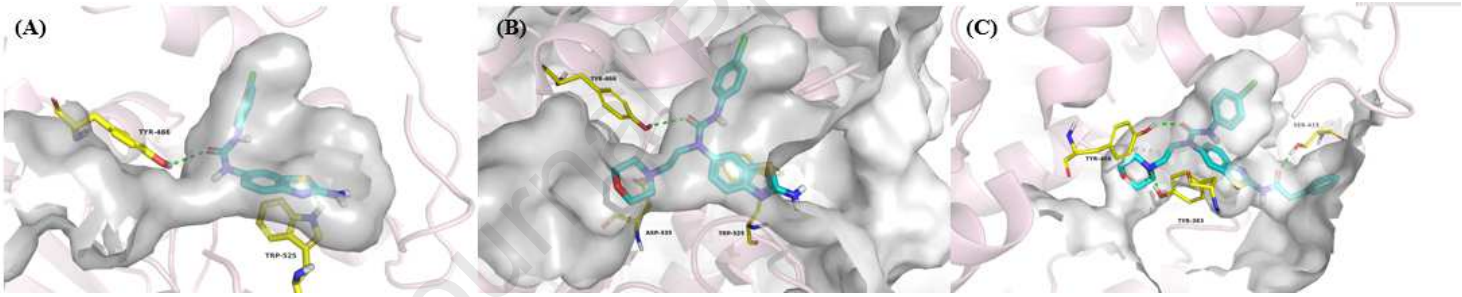


Fig. 4. The proposed binding mode of compound **12a**, **15a** and **18d** to sEH hydrolase catalytic domain (PDB code: 5ALW), hydrogen bonds were shown as green dashes.

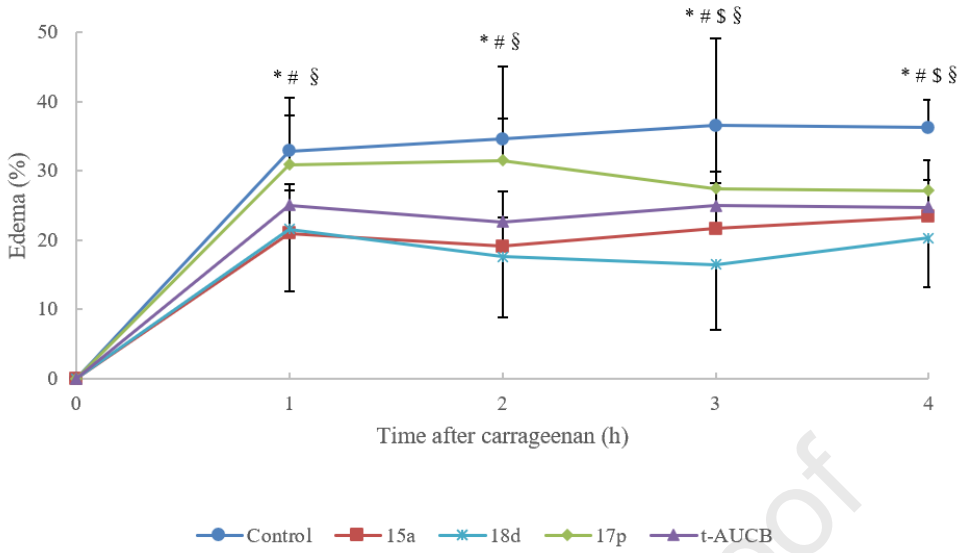


Fig.5. Graphical representation of in *vivo* anti-inflammatory activities of the selected compounds in carrageenan-induced mice paws edema.

Highlights

1. Three series of 2-aminobenzo[*d*]thiazole derivatives were designed and synthesized.
2. Compound **18d** showed the activity against sEH with an IC_{50} value of 0.082 nM.
3. Although **18d** was unstable in rat liver microsomes, it might serve as “prodrug”.
4. **18d** exhibited more effective in *vivo* anti-inflammatory effect than ***t*-AUCB**.

Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.