

## Synthesis and Structure-activity Relationship of Novel Tetrahydrobenzo[*d*]thiazole as Androgen Receptor Modulator

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**Abstract** Prostate cancer is one of the most commonly diagnosed cancer in men, and androgen receptor (AR) is an important target for the treatment of prostate cancer. For many reasons, existing AR antagonists fail to treat prostate cancer after long-term use. Therefore, the development of novel AR antagonists is still of great significance. A series of tetrahydrobenzo[*d*]thiazole compounds were synthesized by condensation reaction of  $\alpha, \beta$ -epoxycyclohexanones and appropriate substituted thioureas **8**, which were obtained from the corresponding anilines **7**. Their antiandrogenic activities were tested using an yeast two-hybrid (Y2H) system, and several compounds exhibited androgen receptor (AR) antagonistic behavior equal or stronger than that of flutamide ( $IC_{50} \leq 2.48$  mmol/L). Further cell viability assay demonstrated that some active compounds effectively inhibited the proliferation of androgen-sensitive LNCaP cells values with  $IC_{50}$  values of 17.1 ~ 41.4 mmol/L. Molecular docking study provide a possible model of ligand receptor interactions, which was consistent with the initial structure-activity relationship (SAR) studies. Taken together, tetrahydrobenzo[*d*]thiazoles act as effective AR modulators may represent promising leads for further development of novel and improved AR antagonists.

**Key words** thiazoles; androgen receptor; prostate cancer; structure-activity relationship

## 新型四氢苯并噻唑化合物作为雄激素受体调节剂的合成与构效关系

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**摘要** 前列腺癌是男性最常被诊断出的癌症之一, 而雄激素受体 (androgen receptor, AR) 是前列腺癌治疗的重要靶标。现有的 AR 拮抗剂在长期使用后通常由于多种原因而失效。因此, 新型 AR 拮抗剂的开发仍具有重要的意义。一系列四氢苯并噻唑类化合物, 通过  $\alpha, \beta$ -环氧环己酮与适当的

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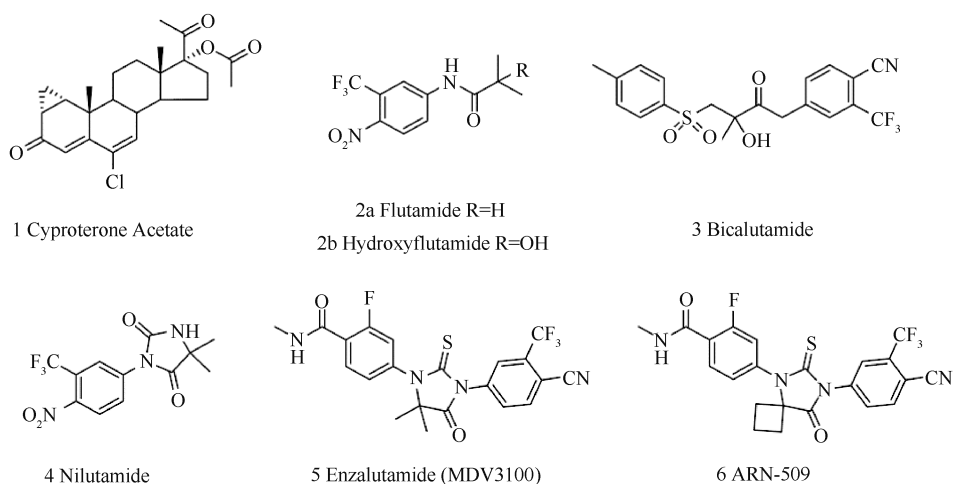
硫脲的缩合反应被合成。其中,多个化合物在酵母双杂交系统中表现出强于或相当于氟他胺的雄激素受体拮抗活性( $IC_{50} \leq 2.48$  mmol/L)。进一步的细胞活力试验表明,这些活性化合物有效地抑制了雄激素敏感的 LNCaP 细胞的增殖( $IC_{50}$  值 17.1 ~ 41.4 mmol/L)。分子对接研究提供了化合物与受体相互作用的可能模型,较好地符合了初步的构效关系研究。总之,本文的研究证明,四氢苯并噻唑可以作为有效的 AR 调节剂,可能代表了一种有前景的先导化合物,有助于进一步开发出新型的更加强效的雄激素受体拮抗剂。

**关键词** 噻唑;雄激素受体;前列腺癌;构效关系  
**中图分类号** Q71

Prostate cancer is the most common type of cancer and the second leading cause of cancer death among men in developed countries<sup>[1]</sup>. It accounts for 20% of newly-diagnosed cases and 8.4% of cancer death in the USA<sup>[2]</sup>. Androgen receptor (AR), a ligand-regulated transcription factor of the steroid hormone super family, is a key molecular target in the treatment of prostate cancer<sup>[3,4]</sup>. Upon the binding of endogenous androgens, such as testosterone (T) and 5 $\alpha$ -dihydrotestosterone (DHT), AR translocates to the nucleus and binds to the hormone response elements on DNA and regulates a variety of genes implicated in regulation of proliferation and differentiation responses<sup>[5]</sup>. Numerous studies indicated that AR and its signaling pathway contribute to the progression of metastatic prostate cancer<sup>[6-9]</sup>. Therefore, the first line of treatment of recurrent or metastatic prostate cancer is androgen withdrawal therapy that blocks either the production of androgens or their binding to the AR<sup>[10,11]</sup>.

AR antagonists, also known as antiandrogens, compete with androgens for AR occupancy<sup>[12]</sup>. Antiandrogens can be divided into two classes: steroidal and non-steroidal antiandrogens. Due to

serious side effects, steroidal antiandrogens are not suitable for clinical treatment, and cypoterone acetate (**1**) (Fig. 1) is the only one that has been widely used<sup>[13]</sup>. Non-steroidal ones, such as flutamide (**2a**) and its metabolite hydroxyflutamide (**2b**)<sup>[14-16]</sup>, bicalutamide (**3**)<sup>[17]</sup>, and nilutamide (**4**)<sup>[18,19]</sup>, have greater effectiveness, tolerability, and safety (Fig. 1). However, prostate tumors become resistant to first-line therapy after months or years, and the disease progresses in a castrate-resistant state (castrate-resistant prostate cancer, CRPC)<sup>[20,21]</sup>. The reasons for *de novo* resistance to antiandrogens are not fully understood. Studies have shown that *de novo* resistance can be contributed to the mutation of AR<sup>[22]</sup>. In fact, some antiandrogens become partial agonists because of AR mutations, such as T877 A and W741 L<sup>[23,24]</sup>. Potent antiandrogens, such as enzalutamide (**5**) and ARN-509 (**6**), have demonstrated efficacy in animal models of CRPC as well as clinical research in CRPC patients<sup>[25-28]</sup>. However, novel AR mutations have been reported to confer resistance to **5** and **6**<sup>[29]</sup>. Thus, there is still an urgent need for new classes of compounds to modulate AR for the treatment of prostate cancer.



**Fig. 1** Structure of known antiandrogens

Tetrahydrobenzo [*d*] thiazoles are heterocyclic compounds that are often embedded in some more

complex molecules with important biological activity<sup>[30,31]</sup>. As part of our ongoing research on

nuclear receptor modulators<sup>[32]</sup>, a series of fused bicyclic 2-aminothiazolyl compounds were synthesized<sup>[33]</sup>, and applied to an *in vitro* screening system. Among them, compounds bearing the tetrahydrobenzo[*d*]thiazole core exhibited remarkable antiandrogenic activities, and were selected for further SAR study. In addition, active compounds can effectively inhibit the proliferation of the androgen-sensitive LNCaP cells. We also computationally evaluated the interaction between the AR and compound **38**, and provided a possible binding model, which is qualitatively consistent with the observed SAR. Our study may provide valuable information in the design of novel potent AR modulators.

## 1 Materials and Methods

### 1.1 General

$\alpha$ ,  $\beta$ -epoxide cycloketones including 6-oxabicyclo[3.1.0]hexan-2-one, 7-oxabicyclo[4.1.0]heptan-2-one and 8-oxabicyclo[5.1.0]octan-2-one were prepared according to the reported procedures<sup>[34]</sup>. All other solvents and reagents were purchased directly from commercial suppliers and used as received without further purification. <sup>1</sup>H NMR (400 MHz), <sup>13</sup>C NMR (100 MHz) and <sup>19</sup>F NMR (377 MHz) spectra were obtained on Bruker AM-400 spectrometer with DMSO-*d*<sub>6</sub> or CDCl<sub>3</sub> as solvent and tetramethylsilane (TMS) as internal standard. Chemical shifts were reported in units (ppm) by assigning TMS resonance in the <sup>1</sup>H NMR spectra as 0.00 ppm (DMSO,  $\delta$  = 2.54 ppm or CDCl<sub>3</sub>,  $\delta$  = 7.27 ppm). Data was reported as following: chemical shift, multiplicity (*s* = single, *d* = doublet, *t* = triplet and *m* = multiplet), coupling constant (*J* = values) in Hz and integration. Chemical shifts for <sup>13</sup>C NMR spectra were recorded in ppm from tetramethylsilane using the central peak of DMSO-*d*<sub>6</sub> ( $\delta$  = 40.45 ppm) or CDCl<sub>3</sub> ( $\delta$  = 77.0 ppm) as an internal standard. High Resolution Mass Spectrometry (HRMS) were recorded under electron impact (70 eV) condition using a MicroMass GCT CA 005 instrument. Melting points were determined using a Büchi B540 Melting point apparatus. Analytical thin layer chromatography (TLC) was carried out on precoated plates (silicagel 60 F254), and spots were visualized with ultraviolet (UV) light. The yields were defined as the isolated yields. Microwave-assisted synthesis was carried out in a Biotage Initiator single-mode microwave instrument producing controlled irradiation at 2.450 GHz. Reaction times refer to hold times at the indicated temperatures, not to total irradiation times.

### 1.2 General procedure for synthesis of substituted thiourea **8**

The ammonium thiocyanate (0.76 g, 10 mmol) was added to an acetone solution (10 mL) of benzoyl

chloride (1.4 g, 10 mmol). The mixture was stirred for 15 minutes. The precipitate formed was filtered off and the filter was evaporated under reduced procedure to yield benzoyl isothiocyanate as yellow liquid without further purification. The obtained benzoyl isothiocyanate and substituted aniline (10 mmol) were added to the ethyl acetate solution (30 mL). The reaction mixture was refluxed for appropriate time. The mixture was cooled to room temperature and the solvent was removed under reduced procedure. The residue was added to a solution of ethanol (10 mL) and 2 mol/L aqueous solution of sodium hydroxide (10 mL). The mixture was refluxed for appropriate time. The solution was cooled to room temperature and then poured into ice water (30 mL). The resulting mixture was neutralized with 2 mol/L hydrochloric acid. The mixture was filtered and the solid was recrystallized from ethanol or without further purification to give thiourea **8**.

### 1.3 General procedure for synthesis of compounds **9** and **44-48**

Water (20 mL) was added to a mixture of thiourea **8** (10 mmol) and 7-oxabicyclo[4.1.0]heptan-2-one (10 mmol). The reaction was refluxed for appropriate time. The mixture was then cooled to room temperature. Precipitate formed was filtered and dried. The obtained crude product was further purified by column chromatography on silica to give desired product.

### 1.4 General procedure for synthesis of compounds **10-43**, **49-56**, **58** and **60**

Thiourea **8** (1 mmol),  $\alpha$ ,  $\beta$ -epoxy cycloketone (1.05 mmol), and corresponding alcohol (2 mL) were added to a microwave vial (2-5 mL). The sealed vial was heated in the Biotage Initiator Synthesizer for appropriate time. The mixture was then cooled to room temperature and the residue obtained after evaporating under vacuum was subjected to purification over silica gel chromatography eluting with PE/EtOAc (9:1, *V/V*) to afford target compounds.

### 1.5 Reagents for bioassays

5 $\alpha$ -dihydrotestosterone (DHT; purity > 99%) and flutamide (purity > 99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All of the test compounds were dissolved in dimethyl sulfoxide (Sigma-Aldrich, St. Louis, MO, USA) as 50 mmol/L stock solution.

Yeast Synthetic Drop-out Medium Supplements without leucine and tryptophan was purchased from Sigma-Aldrich (St. Louis, MO, USA). D (+)-glucose and p-nitrophenyl  $\alpha$ -D-galactopyranoside (PNP- $\alpha$ -Gal) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All yeast media were prepared according to the Yeast Protocols Handbook (PT3024-1, Clontech, USA). Dulbecco's minimum

essential medium (DMEM), DMEM/F12, phenol red-free RPMI-1640 medium and fetal bovine serum (FBS) were purchased from GIBCO (Rockville, USA).

### 1.6 Plasmids and cell lines

The yeast host strain Y2H-Gold was purchased from Invitrogen (CA, USA). Yeast expression vectors pGBKT7 and pGADT7 encoding the GAL4 DNA-binding domain (DBD) and activation domain (AD), respectively, were also purchased from Invitrogen. pCMV-hAR (full length) are kind gifts from Pro. Dr. J. Trapman (Erasmus University Medical Centre, Rotterdam, Holland). pSG5-TIF2 (full length) was donated by Hinrich Gronemeyer (Institute of Genetics and Molecular and Cell Biology [IGBMC], Illkirch, France). pGBKT7-hARLBD (aa: 625-919) was constructed by cloning the hAR-LBD (aa: 625-919) in frame into the pGBKT7 vector. pGADT7-TIF2 (aa: 624-1 287) was constructed by cloning the TIF2 (aa: 624-1 287) in frame into the pGADT7 vector. The PC-3 and LNCaP cell lines were obtained from the Cell Bank at the Chinese Academy of Sciences for this study.

### 1.7 Yeast transformation and $\alpha$ -galactosidase activity assay

Recombinant plasmids of pGBKT7-hARLBD (aa: 625-919) and pGADT7-TIF2 (aa: 624-1 287) were introduced into Y2H-Gold using the lithium acetate method<sup>[35]</sup>. Selection was done on yeast selective media without leucine and tryptophan solidified with agar at 30°C for 2-3 days to get transformant AR-Y2H. The quantitative  $\alpha$ -galactosidase assay was performed by using PNP- $\alpha$ -Gal as substrate. Yeast cells were precultured in 4 mL of liquid medium overnight (16-18 hours) at 30°C with shaking (220 r/min). The overnight cultures were diluted with fresh medium, and then 0.5 mL DMSO or test compound was added into 500 mL diluted cultures independently followed by well mixing. The obtained test cultures were incubated at 30°C for 24 hours. After measuring  $A_{600}$  using EnVision Multilabel Reader (PerkinElmer, USA), cells were centrifuged and 16 mL of cell culture medium supernatants or unused culture media (as a reagent blank) was transferred into a 96-well plate (Corning, USA). Then 48 mL of assay buffer was added into each sample. After 1 hour incubation at 30°C, the reaction was terminated by the addition of 136 mL of stop buffer (1 mol/L  $\text{Na}_2\text{CO}_3$ ) and  $A_{410}$  was measured by EnVision Multilabel Reader (PerkinElmer, USA).

### 1.8 MTT assay

The effect of compounds on the viability of cells was determined by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. Human prostate cancer LNCaP and PC-3 cells were maintained in phenol red-free RPMI-1640 and DMEM medium,

respectively. Both media were supplemented with 10% fetal bovine serum (FBS). LNCaP or PC-3 cells were seeded at 5 000 cells per well in flat bottomed 96 well plates in 10% FBS-supplemented medium for 48 hours, followed by treatments with various compounds at the indicated concentrations in the presence or absence of 0.5 nmol/L DHT. Controls received DMSO at a concentration equal to that in drug-treated cells. Then the plates were incubated at 37°C under 5%  $\text{CO}_2$  for 72 hours. At the end of incubation, 20 mL MTT (5 mg/mL in PBS) was added to each well and incubated at 37°C under 5%  $\text{CO}_2$  for 4 hours. Then the solution was replaced by 150 mL DMSO and the mixture of cells and DMSO was shaken for 3 minutes. Absorbance was measured at 570 nm by EnVision Multilabel Reader (PerkinElmer, USA).

### 1.9 Molecular docking

To understand the structural basis for the inhibitory activities of compounds against AR-LBD, the binding models were studied by molecular docking simulation. We applied the crystal structure of androgen receptor ligand-binding domain (PDB code: 3V4A) to the molecular docking procedure considering its high resolution and no missing side-chain. Compound **38** was prepared with Ligprep 3.4, with OPLS3 selected as the force field, and possible states generated at target pH range from 2.0 to 9.0 using Epik 3.2<sup>[36]</sup>. Then the compound was docking in the "Extra Precision" (XP) mode of Glide 6.7<sup>[36]</sup>. The binding mode of compound was used for analysis only when the GlideScore was top ranking. The docking results were processed by PyMOL 2.0<sup>[37]</sup>.

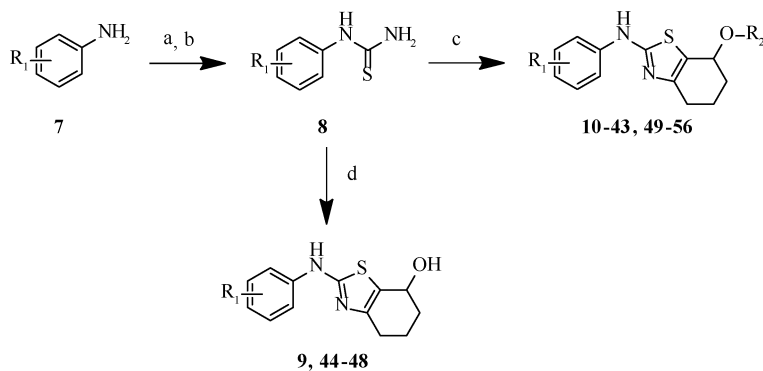
## 2 Results

### 2.1 Chemistry

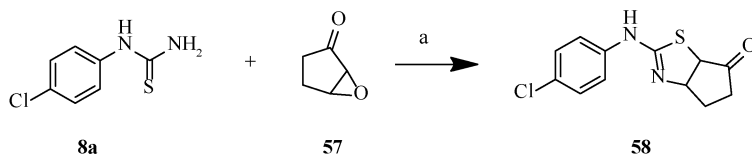
A straightforward and short synthesis of tetrahydrobenzo [*d*] thiazoles that was recently developed in our laboratories was applied to the preparation of a screening library<sup>[33]</sup>. Compounds **9-56** were prepared as racemic mixtures by condensation of  $\alpha$ ,  $\beta$ -epoxycyclohexanone and appropriate substituted thioureas **8**, obtained from the corresponding anilines **7** (Fig. 2). Unfortunately, we failed to get the corresponding five-membered ring and seven-membered ring compounds. Compounds **58** and **60** were obtained only, which were described in Fig. 3 and Fi. 4. All structures of the final products were determined by  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, and HRMS (ESI) (Supplementary data).

### 2.2 Tetrahydrobenzo [*d*] thiazoles exhibit potent AR antagonistic activity

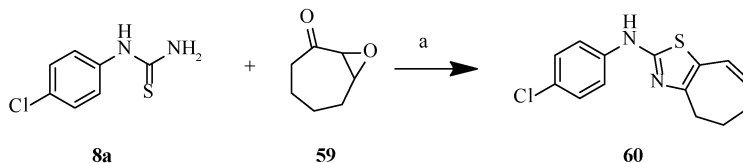
Previous studies have demonstrated that the ligand binding domain of AR (AR-LBD) interacts with the transcriptional intermediary factor 2 (TIF2) in an



**Fig. 2 General procedures for the synthesis of compounds 9-56** Reagents and conditions: (a) benzoyl isothiocyanate, EtOAc, reflux; (b) NaOH, EtOH, reflux; (c)  $\alpha$ ,  $\beta$ -epoxycyclohexanone, R<sub>2</sub>-OH, MW; (d)  $\alpha$ ,  $\beta$ -epoxycyclohexanone, H<sub>2</sub>O, reflux



**Fig. 3 Procedure for synthesis of compound 58** Reagents and conditions: (a) MeOH, 100°C, MW



**Fig.4 Procedure for synthesis of compound 60** Reagents and conditions: (a) EtOH, 100°C, MW

agonist-dependent manner and harbours a transcriptional activation function<sup>[38]</sup>. Based on the protein-protein interaction, an Y2H system was constructed for AR antagonist *in vitro* screening as previously reported<sup>[39]</sup>.

The presence of DHT effectively enhanced the protein-protein interaction, accompanied by an increased  $\alpha$ -galactosidase activity. Well known antiandrogen **2a** significantly inhibited the  $\alpha$ -galactosidase activity induced by 10 nmol/L DHT and the  $IC_{50}$  value was 2.48 mmol/L (Table 1), which is consistent with previous report<sup>[40]</sup>. Then, compounds were applied to the Y2H assay. The inhibitory rate (at 25 mmol/L) and  $IC_{50}$  values were presented in Table 1-3. Several compounds (**16**, **38** and **54**) expressed antagonistic behavior stronger than that of **2a**. No compound exhibited agonist activity at 25 mmol/L (data not shown).

The effect of the sizes of ether chains to characterize the main interactions between the receptor and the ligand had been evaluated (Table 1, 2). Branching on the ether chains (**10-17**, or **18-22**) was not conducive to enhancing antagonist potency. Therefore the methyl group at the right ether chains was the preferred substitution, and it is not worth further improving lipophilicity. But compounds having only a

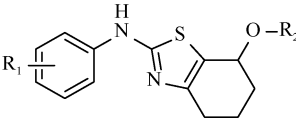
free hydroxyl group (**9**, **44-47**) were largely less active than corresponding ether derivatives, for example compound **47** and **31** ( $12.25 \pm 1.11$  *vs.*  $5.39 \pm 1.09$ ,  $P < 0.05$ ). The methoxyethoxy derivatives **42** and **43** were much less active than the corresponding alkyl analogs. Our initial SAR study indicates that the methyl group at the right ether chains was the preferred substitution.

The effect of the aromatic substituents in the left-hand side was then explored. Analogs with chlorine in the left-hand side, such as **9-17**, exhibited significant antagonism potency. Introducing a methyl or ethyl group on the benzene ring (**24-26**) slightly improved the potency ( $14.56 \pm 0.63$ ,  $10.45 \pm 0.64$ , or  $11.34 \pm 0.21$  *vs.*  $21.70 \pm 1.07$ ,  $P < 0.05$ ). Compounds bearing methoxyl, hydroxyl, nitro, cyano, *N,N*-dimethylamino or acetamido group as substituents on aromatic ring (**27**, **32-36**, **39** and **41**) only showed weak affinity for AR. Compounds having a halogen substituent (**28-30**) or trifluoromethyl group (**31**) exhibited significant inhibitory activity to AR. Interestingly, compounds which have cyano (**39**) group with trifluoromethyl, possessing the substitution pattern for **2a**, showed very weak ability to inhibit AR. The 2, 6-dichloro derivatives **37** also had no activity. Gratifyingly, the

3-chloro-4-trifluoromethyl analog **38** and 2,3-dimethyl analog **40** were more and comparable potent than **2a**. These results indicated that the introductions of strong electron-withdrawing groups, electron-donating groups or polar groups at the 4-position of aromatic rings were not beneficial for increasing inhibitory activities. Compounds **10** (*para*-Cl) and **28**, **29**

(*ortho*-Cl, *meta*-Cl), or **18** (*para*-Me) and, **24**, **25** (*ortho*-Me, *meta*-Me) showed similar potency ( $3.47 \pm 0.52$  *vs.*  $3.76 \pm 0.69$  *vs.*  $3.97 \pm 0.31$ ;  $16.70 \pm 0.42$  *vs.*  $17.45 \pm 0.72$  *vs.*  $17.54 \pm 0.86$ ), which meant that the position of substituent group in the aromatic ring seem to have no significant influence on the activities.

Table 1 Compound 9-47 and their antiandrogenic activities



Compound	R <sub>1</sub>	R <sub>2</sub>	Inhibitory rate <sup>a</sup> ( % )	IC <sub>50</sub> <sup>b</sup> ( mmol/L )
<b>9</b>	4-Cl	H	83.24	8.91 ± 0.38
<b>10</b>	4-Cl	Me	97.34	3.47 ± 0.52
<b>11</b>	4-Cl	Et	95.15	4.60 ± 0.62
<b>12</b>	4-Cl	<i>n</i> -Pr	82.29	7.16 ± 0.28
<b>13</b>	4-Cl	<i>i</i> -Pr	94.24	4.89 ± 0.20
<b>14</b>	4-Cl	<i>n</i> -Bu	91.59	8.71 ± 0.65
<b>15</b>	4-Cl	cyclopentyl	89.39	4.52 ± 0.21
<b>16</b>	4-Cl	cyclohexyl	95.38	1.61 ± 0.31
<b>17</b>	4-Cl	5-nonyl	92.14	8.44 ± 0.46
<b>18</b>	4-Me	Me	63.80	16.70 ± 0.42
<b>19</b>	4-Me	Et	64.23	17.45 ± 0.72
<b>20</b>	4-Me	<i>n</i> -Pr	62.77	17.54 ± 0.86
<b>21</b>	4-Me	<i>i</i> -Pr	82.96	12.43 ± 0.96
<b>22</b>	4-Me	<i>n</i> -Bu	36.92	n. d
<b>23</b>	H	Me	60.01	21.70 ± 1.07
<b>24</b>	2-Me	Me	78.18	14.56 ± 0.63
<b>25</b>	3-Me	Me	89.05	10.45 ± 0.64
<b>26</b>	4-Et	Me	87.96	11.34 ± 0.21
<b>27</b>	4-OMe	Me	1.32	n. d
<b>28</b>	2-Cl	Me	93.81	3.76 ± 0.69
<b>29</b>	3-Cl	Me	95.57	3.97 ± 0.31
<b>30</b>	4-Br	Me	94.68	4.94 ± 1.06
<b>31</b>	4-CF <sub>3</sub>	Me	93.57	5.39 ± 1.09
<b>32</b>	4-OH	Me	7.53	n. d
<b>33</b>	4-NO <sub>2</sub>	Me	30.01	n. d
<b>34</b>	4-CN	Me	30.60	n. d
<b>35</b>	4- N( CH <sub>3</sub> ) <sub>2</sub>	Me	30.56	n. d
<b>36</b>	4-NHCOCH <sub>3</sub>	Me	0.18	n. d
<b>37</b>	2,6-Cl	Me	0	n. d
<b>38</b>	2-Cl, 4-CF <sub>3</sub>	Me	95.97	1.93 ± 0.13
<b>39</b>	3-CF <sub>3</sub> , 4-CN	Me	26.08	n. d
<b>40</b>	2,3-Me	Me	98.20	2.80 ± 0.43
<b>41</b>	3,4,5-OMe	Me	0	n. d
<b>42</b>	4-Cl	( CH <sub>2</sub> ) <sub>2</sub> OCH <sub>3</sub>	86.85	11.75 ± 1.21
<b>43</b>	4-Me	( CH <sub>2</sub> ) <sub>2</sub> OCH <sub>3</sub>	22.78	n. d
<b>44</b>	4-Me	H	59.34	20.52 ± 1.98
<b>45</b>	H	H	18.87	n. d
<b>46</b>	4-F	H	75.57	13.13 ± 0.43
<b>47</b>	4-CF <sub>3</sub>	H	87.84	12.25 ± 1.11
<b>2a</b>	-	-	92.42	2.48 ± 0.33

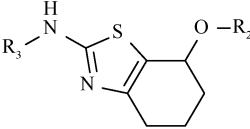
<sup>a)</sup> Inhibitory rates of test compounds (25 mmol/L) were measured with 10 nmol/L DHT. <sup>b)</sup> IC<sub>50</sub> values were measured with 10 nmol/L DHT when the inhibitory rate at 25 mmol/L was larger than 50% . n. d represented not determined. IC<sub>50</sub> values are the mean ± SD of three determinations from independent experiments.



Although the pyridinyl (**48-52**) and benzyl analogs exhibited weak antagonistic activity. Introduction of the naphthyl increased the activity remarkably (**54** and **23**,  $1.76 \pm 0.29$  *vs.*  $21.70 \pm 1.07$ ,  $P < 0.01$ ). The aromatic substituents appeared to be indispensable, since the cyclohexyl analog **55**

and allyl analog **56** was inactive. Finally, five-membered ring analog **58** and seven-membered analog **60** displayed weak potency, which indicated that the six-membered derivatives were the most potent or the unsaturated thiazole ring was indispensable (Table 3).

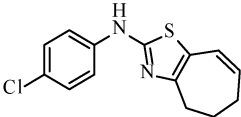
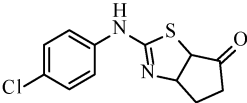
Table 2 Compound 48-56 and their antiandrogenic activities



Compound	R <sub>3</sub>	R <sub>2</sub>	Inhibitory rate <sup>a</sup> (%)	IC <sub>50</sub> <sup>b</sup> (mmol/L)
<b>48</b>	5-Cl-pyridin-2-yl	H	12.59	n. d
<b>49</b>	5-Cl-pyridin-2-yl	Me	36.31	n. d
<b>50</b>	5-Cl-pyridin-2-yl	Et	24.93	n. d
<b>51</b>	pyridin-3-yl	Me	0	n. d
<b>52</b>	2-Cl-pyridin-4-yl	Me	0	n. d
<b>53</b>	Bn	Me	0	n. d
<b>54</b>	naphthalen-1-yl	Me	98.25	$1.76 \pm 0.29$
<b>55</b>	cyclohexyl	Me	0	n. d
<b>56</b>	allyl	Me	0	n. d
<b>2a</b>	-	-	92.42	$2.48 \pm 0.33$

<sup>a)</sup> Inhibitory rates of test compounds (25 mmol/L) were measured with 10 nmol/L DHT. <sup>b)</sup> IC<sub>50</sub> values were measured with 10 nmol/L DHT when the inhibitory rate at 25 mmol/L was larger than 50%. n. d represented not determined. IC<sub>50</sub> values are the mean ± SD of three determinations from independent experiments

Table 3 Compounds 58, 60 and their antiandrogenic activities



Compound	Inhibitory rate <sup>a</sup> (%)	IC <sub>50</sub> <sup>b</sup> (mmol/L)
<b>58</b>	9.95	n. d
<b>60</b>	17.46	n. d
<b>2a</b>	92.42	$2.48 \pm 0.33$

<sup>a)</sup> Inhibitory rates of test compounds (25 mmol/L) were measured with 10 nmol/L DHT. <sup>b)</sup> IC<sub>50</sub> values were measured with 10 nmol/L DHT when the inhibitory rate at 25 mmol/L was larger than 50%. n. d represented not determined. IC<sub>50</sub> values are the mean ± SD of three determinations from independent experiments

2.3 Active compounds inhibit the proliferation of androgen-sensitive prostate cancer cells

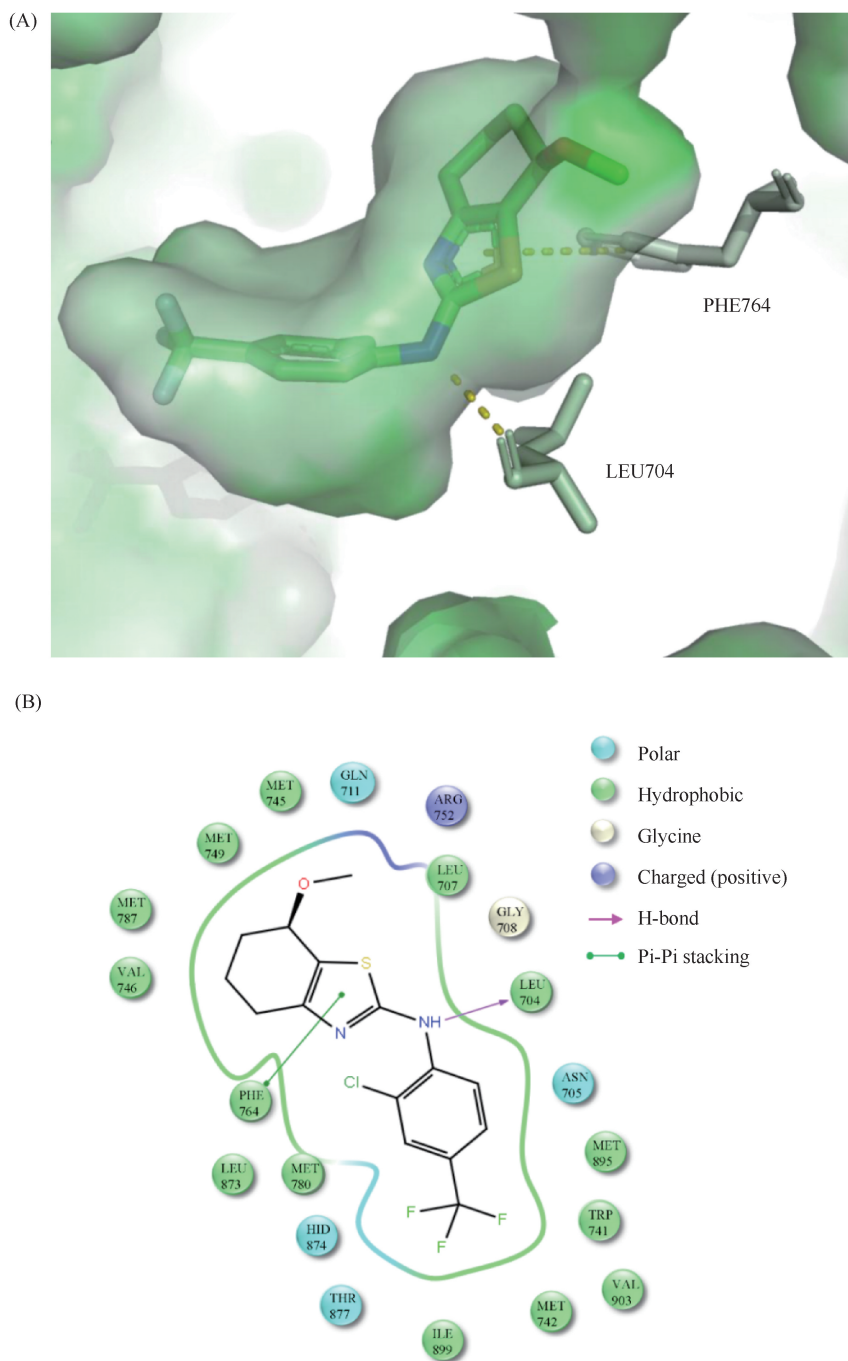
Growth of prostate cancer cells is driven by the transcriptional activity of AR<sup>[41]</sup>. Thus, we wanted to know if our compounds inhibit the proliferation of prostate cancer cells. Active compounds in the Y2H experiment (IC<sub>50</sub> < 10 mmol/L) were further applied to the cell viability assay using PC3 (without DHT) and LNCaP cell lines (with DHT). The PC-3 is an

androgen-independent human prostate cancer cell line that does not express functional AR, and the LNCaP is an androgen-sensitive cell line<sup>[42]</sup>.

All tested compounds inhibited DHT-induced LNCaP cell proliferation effectively, with mean IC<sub>50</sub> values ranging from 17.6 μmol/L to 41.4 mmol/L (Table 4). Compound **38** showed the most potent inhibitory effect with an IC<sub>50</sub> value of 17.6 mmol/L, which was stronger than that of **2a**. As expected, the androgen-dependent LNCaP cell line was more sensitive to the compounds than the androgen-independent PC3 cell line, which indicated that the inhibition of LNCaP cell proliferation arose from their AR antagonistic activity. Interestingly, some compounds (**9** and **30**) were found to significantly inhibit the proliferation of PC3 cell (Table 4), which might be due to the off-target effect.

2.4 Molecular docking

To better elucidate the mechanism of antagonism at the molecular level, molecular docking was performed using the Glide program. Compound **38**, which exhibited considerable inhibitory activity in previous experiments, was modeled in AR-LBD binding site. Three-dimensional crystal structure of AR-LBD was retrieved from RCBS PDB database (PDB ID: 3V4A). The lowest energy conformation was showed in Fig. 5. In our binding model, the NH



**Fig. 5 Structural analysis of ligand-protein interactions between compound 38 and AR-LBD** (A) Docking poses of compounds 38 in the ligand binding pocket of AR-LBD. Molecular docking was performed using the Glide program. The depth of the color represents the hydrophobicity of the surface. The dotted line represents the force between compound and residues. (B) The 2D-binding model of AR-LBD and 38

moiety between the thiazole ring and the benzene ring interacts with Leu704 by forming a hydrogen bond, which plays a critical role in enhancing binding affinity. In addition, the thiazole ring forms a  $\pi$ - $\pi$  interaction with Phe764. It is consistent with the fact that compound 58, which lack of the critical aromatic ring, exhibited weak antagonistic activity. Our initial

SAR investigation has demonstrated that compounds with a free hydroxyl group are largely less active, which may be explained by the hydrophobic surface around the oxygen atom. Taken together, our study provided a possible binding model of AR and tetrahydrobenzo[*d*]thiazoles, and it corresponded with the biological evaluation well.



**Table 4** MTT assay of active compounds in LNCaP and PC-3 cells

Compound	LNCaP	PC3
	IC <sub>50</sub> <sup>a</sup> (mmol/L)	IC <sub>50</sub> <sup>b</sup> (mmol/L)
9	19.1 ± 2.3	32.4 ± 3.1
10	33.6 ± 1.5	> 50
11	27.9 ± 3.7	> 50
12	30.1 ± 2.1	> 50
13	37.5 ± 0.9	> 50
14	27.3 ± 1.9	> 50
15	41.4 ± 2.4	> 50
16	21.4 ± 1.7	> 50
17	35.9 ± 3.1	> 50
25	33.5 ± 2.6	> 50
28	34.5 ± 1.4	> 50
29	30.2 ± 3.2	> 50
30	26.3 ± 2.0	34.2 ± 4.3
31	37.9 ± 2.7	> 50
38	17.6 ± 1.1	> 50
40	28.0 ± 3.9	> 50
54	25.6 ± 1.8	> 50
2a	32.3 ± 2.2	> 50

<sup>a)</sup> LNCaP cells were incubated with 0.5 nmol/L DHT. <sup>b)</sup> PC-3 cells were incubated without DHT. Cells were incubated with different concentrations of compounds for 72 hours. The viability of cells was determined by MTT assay. All IC<sub>50</sub> values are the mean ± SD of three determinations from independent experiments

3 Discussion and Conclusions

A series of tetrahydrobenzo [d] thiazoles were prepared by varying substituents on the aromatic rings and changing the sizes of the alcohol chains. All the compounds were tested for their anti-androgenic activities using yeast two-hybrid system, and several compounds exhibited potent activities. Further cell viability assay demonstrated their ability to inhibit the proliferation of LNCaP cells. An initial structure-activity relationship (SAR) study was also conducted. Generally, the methyl group at the right ether chains was the preferred substitution. Compounds with a free hydroxyl group were largely less active than corresponding ether derivatives. On the left-hand aromatic ring, the halogens, alkyl groups (methyl and ethyl) and trifluoromethyl were preferred. Strong electron-withdrawing and electron-donating groups, or polar groups at the 4-position were not tolerated. The nitrogen preferred a directly aromatic ring substitution pattern. Our docking study provides a possible binding model of AR and tetrahydrobenzo [d] thiazoles. A qualitative consistency of the model with the observed SAR validates our model and merits its use for further design of novel androgen receptor modulators. In

conclusion, tetrahydrobenzo [d] thiazoles are expected to be versatile lead compounds for treatment of human prostate cancer, and our SAR and docking study may contribute to the discovery of novel potent AR modulators.

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## Supplementary Data for

Synthesis and Structure-activity Relationship Studies of Novel Tetrahydrobenzo[*d*]thiazoles as Androgen Receptor Modulators

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## 1 Chemistry: <sup>1</sup>H NMR, <sup>13</sup>C NMR, and HRMS (ESI) data of compounds.

### 1.1 2-((4-chlorophenyl) amino)-4, 5, 6, 7-tetrahydrobenzo[*d*]thiazol-7-ol (9)

Yield 90%; mp 159.7 – 163.1°C; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ = 10.18 (s, 1H), 7.65 (d, *J* = 8.6 Hz, 2H), 7.33 (d, *J* = 8.6 Hz, 2H), 5.24 (d, *J* = 6.0 Hz, 1H), 4.64 (t, *J* = 4.2 Hz, 1H), 2.58 – 2.45 (m, 2H), 1.96 – 1.88 (m, 2H), 1.70 – 1.64 (m, 2H); <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ = 162.0, 147.5, 140.7, 129.1, 124.5, 122.9, 118.6, 63.4, 33.6, 27.0, 19.7; HRMS(ESI): *m/z* [M + H]<sup>+</sup> calcd for C<sub>13</sub>H<sub>14</sub><sup>35</sup>ClN<sub>2</sub>OS<sup>+</sup>: 281.0515, found: 281.0519; calcd for C<sub>13</sub>H<sub>14</sub><sup>37</sup>ClN<sub>2</sub>OS<sup>+</sup>: 283.0486, found: 283.0488.

### 1.2 2-(*p*-tolylamino)-4, 5, 6, 7-tetrahydrobenzo[*d*]thiazol-7-ol (44)

White solid; Yield 89%; mp 129.5 – 130.3°C; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ = 9.91 (s, 1H), 7.48 (d, *J* = 8.4 Hz, 2H), 7.08 (d, *J* = 8.4 Hz, 2H), 5.20 (d, *J* = 6.4 Hz, 1H), 4.66 – 4.60 (m, 1H), 2.57 – 2.38 (m, 2H), 2.33 (s, 3H), 1.94 – 1.86 (m, 2H), 1.70 – 1.64 (m, 2H); <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ = 162.6, 147.4, 139.4, 130.1, 129.7, 121.9, 117.3, 63.4, 33.6, 27.0, 20.8, 19.7; HRMS(ESI): *m/z* [M + H]<sup>+</sup> calcd for C<sub>14</sub>H<sub>17</sub>N<sub>2</sub>OS<sup>+</sup>: 261.1056, found: 261.1056.

### 1.3 2-(phenylamino)-4, 5, 6, 7-tetrahydrobenzo[*d*]thiazol-7-ol (45)

White solid; Yield 88%; mp 134.5 – 135.4°C; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ = 10.08 (s, 1H), 7.60 (d, *J* = 7.8 Hz, 2H), 7.29 (t, *J* = 8.0 Hz, 2H), 6.92 (t, *J* = 7.4 Hz, 1H), 5.24 (d, *J* = 6.1 Hz, 1H), 4.64 (t, *J* = 4.2 Hz, 1H), 2.58 – 2.45 (m, 2H), 1.96 – 1.88 (m, 2H), 1.70 – 1.64 (m, 2H); <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ = 162.7, 148.8, 141.7, 129.4, 121.5, 117.9, 117.3, 63.4, 33.6, 27.0, 19.7; HRMS(ESI): *m/z* [M + H]<sup>+</sup> calcd for C<sub>13</sub>H<sub>15</sub>N<sub>2</sub>OS<sup>+</sup>: 247.0900, found: 247.0901.

### 1.4 2-((4-fluorophenyl) amino)-4, 5, 6, 7-tetrahydrobenzo[*d*]thiazol-7-ol (46)

White solid; Yield 85%; mp 140.5 – 141.6°C; <sup>1</sup>H

NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ = 10.05 (s, 1H), 7.76 – 7.48 (m, 2H), 7.16 – 7.09 (m, 2H), 5.22 (d, *J* = 6.4 Hz, 1H), 4.63 (t, *J* = 2.4 Hz, 1H), 2.58 – 2.39 (m, 2H), 1.95 – 1.86 (m, 2H), 1.81 – 1.60 (m, 2H); <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ = 162.4, 157.1 (d, <sup>1</sup>*J*<sub>CF</sub> = 235.6 Hz), 147.3, 138.3 (d, <sup>4</sup>*J*<sub>CF</sub> = 1.7 Hz), 122.4, 118.6 (q, d, <sup>3</sup>*J*<sub>CF</sub> = 7.5 Hz), 115.8 (q, <sup>2</sup>*J*<sub>CF</sub> = 22.0 Hz), 63.4, 33.6, 27.0, 19.7; <sup>19</sup>F NMR (376 MHz, DMSO-*d*<sub>6</sub>) δ = -122.6 (m, 1F); HRMS(ESI): *m/z* [M + H]<sup>+</sup> calcd for C<sub>13</sub>H<sub>14</sub>FN<sub>2</sub>OS<sup>+</sup>: 265.0805, found: 265.0805.

### 1.5 2-((4-(trifluoromethyl) phenyl) amino)-4, 5, 6, 7-tetrahydrobenzo[*d*]thiazol-7-ol (47)

White solid; Yield 88%; mp 155.4 – 155.9°C; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ = 10.47 (s, 1H), 7.80 (d, *J* = 8.6 Hz, 2H), 7.63 (d, *J* = 8.6 Hz, 2H), 5.28 (s, 1H), 4.66 (s, 1H), 2.53 (m, 2H), 1.93 (dt, *J* = 10.5, 3.4 Hz, 2H), 1.69 (m, 2H); <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ = 161.5, 147.6, 145.0, 126.7 (q, *J* = 3.8 Hz), 125.2 (q, *J* = 269 Hz), 123.9, 121.0 (q, *J* = 31.7 Hz), 116.7, 63.4, 33.5, 27.0, 19.7; <sup>19</sup>F NMR (376 MHz, DMSO-*d*<sub>6</sub>) δ = -59.79 (s, 3F); HRMS(ESI): *m/z* [M + H]<sup>+</sup> calcd for C<sub>14</sub>H<sub>14</sub>F<sub>3</sub>N<sub>2</sub>OS<sup>+</sup>: 315.0773, found: 315.0771.

### 1.6 2-((5-chloropyridin-2-yl) amino)-4, 5, 6, 7-tetrahydrobenzo[*d*]thiazol-7-ol (48)

White solid; Yield 95%; mp 180.3 – 181.0°C; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ = 11.23 (s, 1H), 8.29 (d, *J* = 2.6 Hz, 1H), 7.77 (dd, *J* = 9.0, 2.6 Hz, 1H), 7.08 (d, *J* = 8.8 Hz, 1H), 5.21 (s, 1H), 4.70 (s, 1H), 2.60 – 2.40 (m, 2H), 1.91 (m, 2H), 1.74 – 1.60 (m, 2H); <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ = 158.4, 151.1, 145.1, 138.1, 125.0, 122.2, 119.8, 112.6, 63.4, 33.6, 26.6, 19.8; HRMS(ESI): *m/z* [M + H]<sup>+</sup> calcd for C<sub>13</sub>H<sub>14</sub><sup>35</sup>ClN<sub>2</sub>OS<sup>+</sup>: 283.0515, found: 283.0519; calcd for C<sub>13</sub>H<sub>14</sub><sup>37</sup>ClN<sub>2</sub>OS<sup>+</sup>: 285.0486, found: 285.0488.

### 1.7 N-(4-chlorophenyl)-7-methoxy-4, 5, 6, 7-tetrahydrobenzo[*d*]thiazol-2-amine (10)

White solid; Yield 93%; mp 113.5 – 114.2°C; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ = 10.25 (s, 1H), δ

7.65 (d,  $J = 8.6$  Hz, 2H), 7.34 (d,  $J = 8.6$  Hz, 2H), 4.37 (t,  $J = 4.2$  Hz, 1H), 3.33 (s, 3H), 2.62 – 2.48 (m, 2H), 1.89 – 1.78 (m, 4H);  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta = 162.4$ , 148.8, 140.5, 129.2, 124.8, 118.7, 118.5, 72.6, 55.9, 28.7, 27.1, 19.3; HRMS (ESI):  $m/z$  [ $M + H$ ] $^+$  calcd for  $\text{C}_{14}\text{H}_{16}^{35}\text{ClN}_2\text{OS}^+$ : 295.0672, found: 295.0674; calcd for  $\text{C}_{14}\text{H}_{16}^{37}\text{ClN}_2\text{OS}^+$ : 297.0642, found: 297.0642.

### 1.8 N-(4-chlorophenyl)-7-ethoxy-4, 5, 6, 7-tetrahydrobenzo[*d*]thiazol-2-amine (11)

White solid; Yield 95%; mp 133.0 – 134.2°C;  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta = 10.24$  (s, 1H), 7.65 (d,  $J = 8.6$  Hz, 2H), 7.34 (d,  $J = 8.6$  Hz, 2H), 4.45 (t,  $J = 4.8$  Hz, 1H), 3.54 (q, 7.2 Hz, 2H), 2.62 – 2.47 (m, 2H), 1.87 – 1.71 (m, 4H), 1.13 (t,  $J = 7.2$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta = 162.3$ , 148.6, 140.6, 129.2, 124.8, 118.9, 118.7, 71.0, 63.5, 29.5, 27.1, 19.4, 16.1; HRMS (ESI):  $m/z$  [ $M + H$ ] $^+$  calcd for  $\text{C}_{15}\text{H}_{18}^{35}\text{ClN}_2\text{OS}^+$ : 309.0828, found: 309.0830; calcd for  $\text{C}_{15}\text{H}_{18}^{37}\text{ClN}_2\text{OS}^+$ : 311.0799, found: 311.0802.

### 1.9 N-(4-chlorophenyl)-7-propoxy-4, 5, 6, 7-tetrahydrobenzo[*d*]thiazol-2-amine (12)

White solid; Yield 92%; mp 136.7 – 137.5°C;  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta = 10.23$  (s, 1H), 7.65 (d,  $J = 8.6$  Hz, 2H), 7.34 (d,  $J = 8.6$  Hz, 2H), 4.45 (t,  $J = 4.8$  Hz, 1H), 3.45 (t,  $J = 7.2$  Hz, 2H), 2.62 – 2.47 (m, 2H), 1.88 – 1.71 (m, 4H), 1.56 – 1.47 (m, 2H), 0.89 (t,  $J = 7.6$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta = 162.3$ , 148.6, 140.6, 129.2, 124.7, 119.1, 118.7, 71.2, 69.8, 29.4, 27.1, 23.3, 19.5, 11.1; HRMS (ESI):  $m/z$  [ $M + H$ ] $^+$  calcd for  $\text{C}_{16}\text{H}_{20}^{35}\text{ClN}_2\text{OS}^+$ : 323.0985, found: 323.0985; calcd for  $\text{C}_{16}\text{H}_{20}^{37}\text{ClN}_2\text{OS}^+$ : 325.0955, found: 325.0956.

### 1.10 N-(4-chlorophenyl)-7-isopropoxy-4, 5, 6, 7-tetrahydrobenzo[*d*]thiazol-2-amine (13)

White solid; Yield 88%; mp 123.9 – 124.7°C;  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta = 10.24$  (s, 1H), 7.65 (d,  $J = 8.6$  Hz, 2H), 7.34 (d,  $J = 8.6$  Hz, 2H), 4.48 (t,  $J = 4.8$  Hz, 1H), 3.80 – 3.70 (m, 1H), 2.60 – 2.45 (m, 2H), 1.88 – 1.72 (m, 4H), 1.12 (d,  $J = 5.8$  Hz, 6H);  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta = 162.2$ , 148.3, 140.6, 129.2, 124.7, 119.9, 118.7, 69.5, 68.8, 30.6, 27.0, 23.5, 23.1, 19.5; HRMS (ESI):  $m/z$  [ $M + H$ ] $^+$  calcd for  $\text{C}_{16}\text{H}_{20}^{35}\text{ClN}_2\text{OS}^+$ : 323.0985, found: 323.0988; calcd for  $\text{C}_{16}\text{H}_{20}^{37}\text{ClN}_2\text{OS}^+$ : 325.0955, found: 325.0958.

### 1.11 7-butoxy-N-(4-chlorophenyl)-4, 5, 6, 7-tetrahydrobenzo[*d*]thiazol-2-amine (14)

White solid; Yield 82%; mp 119.6 – 120.6°C;  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta = 10.24$  (s, 1H),

7.65 (d,  $J = 8.6$  Hz, 2H), 7.34 (d,  $J = 8.6$  Hz, 2H), 4.45 (t,  $J = 4.8$  Hz, 1H), 3.45 (t,  $J = 7.2$  Hz, 2H), 2.61 – 2.46 (m, 2H), 1.87 – 1.71 (m, 4H), 1.52 – 1.45 (m, 2H), 1.39 – 1.30 (m, 2H), 0.89 (t,  $J = 7.4$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta = 162.3$ , 148.6, 140.6, 129.2, 124.7, 119.0, 118.7, 71.3, 67.8, 32.1, 29.4, 27.1, 19.4, 14.2; HRMS (ESI):  $m/z$  [ $M + H$ ] $^+$  calcd for  $\text{C}_{17}\text{H}_{22}^{35}\text{ClN}_2\text{OS}^+$ : 337.1141, found: 349.1141; calcd for  $\text{C}_{17}\text{H}_{22}^{37}\text{ClN}_2\text{OS}^+$ : 339.1112, found: 339.1110.

### 1.12 N-(4-chlorophenyl)-7-(cyclopentyloxy)-4, 5, 6, 7-tetrahydrobenzo[*d*]thiazol-2-amine (15)

White solid; Yield 76%; mp 108.9 – 109.5°C;  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta = 10.24$  (s, 1H), 7.65 (d,  $J = 8.6$  Hz, 2H), 7.34 (d,  $J = 8.6$  Hz, 2H), 4.45 (t,  $J = 4.8$  Hz, 1H), 4.10 (t,  $J = 4.8$  Hz, 1H), 2.60 – 2.45 (m, 2H), 2.03 – 1.40 (m, 12H);  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta = 162.2$ , 148.4, 140.6, 129.2, 124.7, 119.8, 118.7, 79.0, 69.3, 33.1, 32.9, 30.1, 27.0, 23.6, 23.4, 19.5; HRMS (ESI):  $m/z$  [ $M + H$ ] $^+$  calcd for  $\text{C}_{18}\text{H}_{22}^{35}\text{ClN}_2\text{OS}^+$ : 349.1141, found: 349.1143; calcd for  $\text{C}_{18}\text{H}_{22}^{37}\text{ClN}_2\text{OS}^+$ : 351.1112, found: 351.1114.

### 1.13 N-(4-chlorophenyl)-7-(cyclohexyloxy)-4, 5, 6, 7-tetrahydrobenzo[*d*]thiazol-2-amine (16)

White solid; Yield 72%; mp 104.5 – 105.2°C;  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta = 10.24$  (s, 1H), 7.65 (d,  $J = 8.6$  Hz, 2H), 7.34 (d,  $J = 8.6$  Hz, 2H), 4.45 (t,  $J = 4.8$  Hz, 1H), 3.46 (t,  $J = 4.8$  Hz, 1H), 2.62 – 2.45 (m, 2H), 1.99 – 1.62 (m, 8H), 1.48 – 1.26 (m, 6H);  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta = 162.2$ , 148.3, 140.6, 129.2, 124.7, 120.0, 118.7, 75.0, 68.6, 33.4, 32.7, 30.6, 27.0, 25.8, 24.0, 23.9, 19.5; HRMS (ESI):  $m/z$  [ $M + H$ ] $^+$  calcd for  $\text{C}_{19}\text{H}_{24}^{35}\text{ClN}_2\text{OS}^+$ : 363.1298, found: 363.1298; calcd for  $\text{C}_{19}\text{H}_{24}^{37}\text{ClN}_2\text{OS}^+$ : 365.1268, found: 365.1269.

### 1.14 N-(4-chlorophenyl)-7-(nonan-5-yloxy)-4, 5, 6, 7-tetrahydrobenzo[*d*]thiazol-2-amine (17)

White solid; Yield 75%; mp 98.2 – 99.1°C;  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta = 10.24$  (s, 1H), 7.65 (d,  $J = 8.6$  Hz, 2H), 7.34 (d,  $J = 8.6$  Hz, 2H), 4.45 (t,  $J = 4.8$  Hz, 1H), 3.43 (t,  $J = 5.6$  Hz, 1H), 2.61 – 2.45 (m, 2H), 1.91 – 1.64 (m, 4H), 1.48 – 1.19 (m, 12H), 0.93 – 0.85 (m, 6H);  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta = 162.2$ , 148.4, 140.6, 129.1, 124.7, 119.8, 118.7, 77.2, 69.5, 34.3, 34.1, 30.3, 27.8, 27.2, 27.1, 23.0, 22.8, 19.4, 14.5, 14.5; HRMS (ESI):  $m/z$  [ $M + H$ ] $^+$  calcd for  $\text{C}_{22}\text{H}_{32}^{35}\text{ClN}_2\text{OS}^+$ : 407.1924, found: 407.1923; calcd for  $\text{C}_{22}\text{H}_{32}^{37}\text{ClN}_2\text{OS}^+$ : 409.1894, found: 409.1891.

### 1.15 7-methoxy-N-(p-tolyl)-4, 5, 6, 7-tetrahydrobenzo[*d*]thiazol-2-amine (18)

White solid; Yield 95%; mp 112.1 – 112.7°C;  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  = 10.00 (s, 1H), 7.48 (d,  $J$  = 7.8 Hz, 2H), 7.10 (d,  $J$  = 7.8 Hz, 2H), 4.34 (t,  $J$  = 4.8 Hz, 1H), 3.31 (s, 3H), 2.51 (m, 2H), 2.24 (s, 3H), 1.86 – 1.70 (m, 4H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO}-d_6$ )  $\delta$  = 163.0, 148.8, 139.3, 130.4, 129.8, 117.5, 117.4, 72.7, 55.9, 28.8, 27.1, 20.8, 19.4; HRMS(ESI):  $m/z$   $[\text{M} + \text{H}]^+$  calcd for  $\text{C}_{15}\text{H}_{19}\text{N}_2\text{OS}^+$ : 275.1218, found: 275.1219.

**1.16 7-ethoxy-N-(p-tolyl)-4, 5, 6, 7-tetrahydrobenzo[d]thiazol-2-amine (19)**

White solid; Yield 91%; mp 126.0 – 126.8°C;  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  = 9.97 (s, 1H), 7.48 (d,  $J$  = 7.8 Hz, 2H), 7.10 (d,  $J$  = 7.8 Hz, 2H), 4.44 (t,  $J$  = 4.8 Hz, 1H), 3.53 (q,  $J$  = 8.2 Hz, 2H), 2.52 (m, 2H), 2.24 (s, 3H), 1.86 – 1.70 (m, 4H), 1.13 (t,  $J$  = 7.0 Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO}-d_6$ )  $\delta$  = 162.9, 148.6, 139.3, 130.4, 129.8, 118.0, 117.5, 71.0, 63.4, 29.6, 27.1, 20.8, 19.4, 16.1; HRMS(ESI):  $m/z$   $[\text{M} + \text{H}]^+$  calcd for  $\text{C}_{16}\text{H}_{21}\text{N}_2\text{OS}^+$ : 288.1375, found: 288.1364.

**1.17 7-propoxy-N-(p-tolyl)-4, 5, 6, 7-tetrahydrobenzo[d]thiazol-2-amine (20)**

White solid; Yield 92%; mp 118.9 – 119.8°C;  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  = 9.97 (s, 1H), 7.48 (d,  $J$  = 7.8 Hz, 2H), 7.10 (d,  $J$  = 7.8 Hz, 2H), 4.42 (t,  $J$  = 4.8 Hz, 1H), 3.45 (t,  $J$  = 7.2 Hz, 2H), 2.51 (m, 2H), 2.24 (s, 3H), 1.88 – 1.71 (m, 4H), 1.52 (m, 2H), 0.89 (t,  $J$  = 7.6 Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO}-d_6$ )  $\delta$  = 162.9, 148.6, 139.3, 130.3, 129.8, 118.1, 117.5, 71.3, 69.8, 29.5, 27.1, 23.3, 20.8, 19.5, 11.1; HRMS(ESI):  $m/z$   $[\text{M}-\text{H}]^-$  calcd for  $\text{C}_{17}\text{H}_{21}\text{N}_2\text{OS}^-$ : 301.1375, found: 301.1376.

**1.18 7-isopropoxy-N-(p-tolyl)-4, 5, 6, 7-tetrahydrobenzo[d]thiazol-2-amine (21)**

White solid; Yield 88%; mp 121.5 – 122.2°C;  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  = 9.97 (s, 1H), 7.48 (d,  $J$  = 7.8 Hz, 2H), 7.10 (d,  $J$  = 7.8 Hz, 2H), 4.42 (t,  $J$  = 4.8 Hz, 1H), 3.75 (m, 1H), 2.54 (m, 2H), 2.24 (s, 3H), 1.88 – 1.72 (m, 4H), 1.12 (d,  $J$  = 5.8 Hz, 6H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO}-d_6$ )  $\delta$  = 162.8, 148.3, 139.3, 130.3, 129.8, 118.9, 117.5, 69.4, 68.8, 30.6, 27.0, 23.5, 23.1, 20.8, 19.5; HRMS(ESI):  $m/z$   $[\text{M}-\text{H}]^-$ : calcd for  $\text{C}_{17}\text{H}_{21}\text{N}_2\text{OS}^-$  301.1375, found: 301.1374.

**1.19 7-butoxy-N-(p-tolyl)-4, 5, 6, 7-tetrahydrobenzo[d]thiazol-2-amine (22)**

White solid; Yield 87%; mp 117.3 – 117.7°C;  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  = 9.97 (s, 1H), 7.48 (d,  $J$  = 7.8 Hz, 2H), 7.10 (d,  $J$  = 7.8 Hz, 2H), 4.42 (t,  $J$  = 4.8 Hz, 1H), 3.45 (t,  $J$  = 7.2

Hz, 2H), 2.51 (m, 2H), 2.24 (s, 3H), 1.87 – 1.71 (m, 4H), 1.47 (m, 2H), 1.35 (m, 2H), 0.89 (t,  $J$  = 7.4 Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO}-d_6$ )  $\delta$  = 162.8, 148.3, 139.3, 130.3, 129.8, 118.9, 117.5, 69.4, 68.8, 30.6, 27.0, 23.5, 23.1, 20.8, 19.5; HRMS(ESI):  $m/z$   $[\text{M}-\text{H}]^-$  calcd for  $\text{C}_{18}\text{H}_{23}\text{N}_2\text{OS}^-$ : 315.1531, found: 315.1530.

**1.20 7-methoxy-N-phenyl-4,5,6,7-tetrahydrobenzo[d]thiazol-2-amine (23)**

White solid; Yield 95%; mp 101.7 – 102.4°C;  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  = 10.08 (s, 1H), 7.60 (d,  $J$  = 7.8 Hz, 2H), 7.29 (t,  $J$  = 8.0 Hz, 2H), 6.92 (t,  $J$  = 7.4 Hz, 1H), 4.36 (t,  $J$  = 3.8 Hz, 1H), 3.32 (s, 3H), 2.64 – 2.42 (m, 2H), 1.93 – 1.66 (m, 4H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO}-d_6$ )  $\delta$  = 162.7, 148.8, 141.7, 129.4, 121.5, 117.9, 117.3, 72.7, 55.9, 28.8, 27.1, 19.4; HRMS(ESI):  $m/z$   $[\text{M} + \text{H}]^+$  calcd for  $\text{C}_{14}\text{H}_{17}\text{N}_2\text{OS}^+$ : 261.1062, found: 261.1062.

**1.21 7-methoxy-N-(o-tolyl)-4, 5, 6, 7-tetrahydrobenzo[d]thiazol-2-amine (24)**

White solid; Yield 91%; mp 85.8 – 86.4°C;  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  = 9.16 (s, 1H), 7.75 (d,  $J$  = 8.0 Hz, 1H), 7.18 (m, 2H), 7.00 (m, 1H), 4.30 (t,  $J$  = 4.2 Hz, 1H), 3.29 (s, 3H), 2.55 – 2.38 (m, 2H), 2.24 (s, 3H), 1.90 – 1.60 (m, 4H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO}-d_6$ )  $\delta$  = 165.2, 148.6, 139.9, 131.0, 129.9, 126.9, 124.0, 122.2, 117.6, 72.7, 55.9, 28.9, 27.0, 19.3, 18.4; HRMS(ESI):  $m/z$   $[\text{M} + \text{H}]^+$  calcd for  $\text{C}_{15}\text{H}_{19}\text{N}_2\text{OS}^+$ : 275.1218, found: 275.1208.

**1.22 7-methoxy-N-(m-tolyl)-4, 5, 6, 7-tetrahydrobenzo[d]thiazol-2-amine (25)**

White solid; Yield 92%; mp 95.4 – 96.1°C;  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  = 10.00 (s, 1H), 7.45 (dd,  $J$  = 8.2, 2.0 Hz, 1H), 7.35 (s, 1H), 7.17 (t,  $J$  = 8.2, 1H), 6.75 (d,  $J$  = 7.6 Hz, 1H), 4.34 (t,  $J$  = 4.0 Hz, 1H), 3.31 (s, 3H), 2.62 – 2.43 (m, 2H), 2.28 (s, 3H), 1.98 – 1.62 (m, 4H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO}-d_6$ )  $\delta$  = 162.8, 148.8, 141.6, 138.5, 129.2, 122.4, 117.9, 117.8, 114.6, 72.7, 55.9, 28.8, 27.1, 21.8, 19.4; HRMS(ESI):  $m/z$   $[\text{M} + \text{H}]^+$  calcd for  $\text{C}_{15}\text{H}_{19}\text{N}_2\text{OS}^+$ : 275.1218, found: 275.1219.

**1.23 N-(4-ethylphenyl)-7-methoxy-4, 5, 6, 7-tetrahydrobenzo[d]thiazol-2-amine (26)**

White solid; Yield 90%; mp 83.1 – 84.1°C;  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  = 9.98 (s, 1H), 7.50 (d,  $J$  = 8.4 Hz, 2H), 7.12 (d,  $J$  = 8.4 Hz, 2H), 4.34 (t,  $J$  = 4.0 Hz, 1H), 3.31 (s, 3H), 2.61 – 2.40 (m, 2H), 1.93 – 1.66 (m, 4H), 1.15 (t,  $J$  = 7.6 Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO}-d_6$ )  $\delta$  = 163.0, 148.8, 139.5, 137.0, 128.5, 117.6, 117.5, 72.7, 55.9, 28.8, 28.0, 27.1, 19.4, 16.3; HRMS(ESI):  $m/z$   $[\text{M} + \text{H}]^+$  calcd for  $\text{C}_{16}\text{H}_{21}$



$\text{N}_2\text{OS}^+$ ; 289.1375, found; 289.1364.

**1.24 7-methoxy-N-(4-methoxyphenyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2-amine (27)**

White solid; Yield 88%; mp 131.4 – 132.1°C;  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  = 9.98 (s, 1H), 7.63 (d,  $J$  = 8.0 Hz, 2H), 7.25 (t,  $J$  = 8.2 Hz, 1H), 4.20 (t,  $J$  = 4.2 Hz, 1H), 3.25 (s, 3H), 2.62 – 2.45 (m, 2H), 1.82 – 1.66 (m, 4H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO}-d_6$ )  $\delta$  = 162.6, 154.5, 146.7, 135.1, 121.9, 119.2, 114.6, 72.7, 55.7, 49.1, 28.8, 27.1, 19.4; HRMS (ESI):  $m/z$   $[\text{M} + \text{H}]^+$  calcd for  $\text{C}_{15}\text{H}_{19}\text{N}_2\text{O}_2\text{S}^+$ : 291.1167, found; 291.1169.

**1.25 N-(2-chlorophenyl)-7-methoxy-4,5,6,7-tetrahydrobenzo[d]thiazol-2-amine (28)**

White solid; Yield 90%; mp 81.8 – 82.9°C;  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  = 9.51 (s, 1H), 8.19 (d,  $J$  = 8.1 Hz, 1H), 7.44 (d,  $J$  = 8.0 Hz, 1H), 7.31 (t,  $J$  = 7.8 Hz, 1H), 7.03 (t,  $J$  = 7.8 Hz, 1H), 4.34 (t,  $J$  = 4.6 Hz, 1H), 3.30 (s, 3H), 2.57 – 2.43 (m, 2H), 1.85 – 1.68 (m, 4H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO}-d_6$ )  $\delta$  = 163.4, 148.1, 138.3, 130.0, 128.1, 124.0, 123.4, 122.1, 119.2, 72.7, 55.9, 28.8, 26.9, 19.3; HRMS (ESI):  $m/z$   $[\text{M} + \text{H}]^+$  calcd for  $\text{C}_{14}\text{H}_{16}^{35}\text{ClN}_2\text{OS}^+$ : 295.0672, found; 295.0661; calcd for  $\text{C}_{14}\text{H}_{16}^{37}\text{ClN}_2\text{OS}^+$ : 297.0642, found; 297.0644.

**1.26 N-(3-chlorophenyl)-7-methoxy-4,5,6,7-tetrahydrobenzo[d]thiazol-2-amine (29)**

White solid; Yield 92%; mp 105.3 – 106.5°C;  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  = 10.32 (s, 1H), 7.85 (t,  $J$  = 2.1 Hz, 1H), 7.45 (m, 1H), 7.30 (t,  $J$  = 8.2 Hz, 1H), 6.96 (m, 1H), 4.37 (t,  $J$  = 4.4 Hz, 1H), 3.31 (s, 3H), 2.54 (m, 2H), 1.87 – 1.71 (m, 4H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO}-d_6$ )  $\delta$  = 162.2, 148.9, 143.0, 133.8, 130.9, 121.0, 118.8, 116.5, 115.7, 72.6, 55.9, 28.7, 27.1, 19.4; HRMS (ESI):  $m/z$   $[\text{M} + \text{H}]^+$  calcd for  $\text{C}_{14}\text{H}_{16}^{35}\text{ClN}_2\text{OS}^+$ : 295.0672, found; 295.0675; calcd for  $\text{C}_{14}\text{H}_{16}^{37}\text{ClN}_2\text{OS}^+$ : 297.0642, found; 297.0642.

**1.27 N-(4-bromophenyl)-7-methoxy-4,5,6,7-tetrahydrobenzo[d]thiazol-2-amine (30)**

White solid; Yield 92%; mp 132.8 – 133.0°C;  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  = 10.26 (s, 1H), 7.60 (d,  $J$  = 8.8 Hz, 1H), 7.45 (d,  $J$  = 8.8 Hz, 1H), 4.36 (t,  $J$  = 4.2 Hz, 1H), 3.31 (s, 2H), 2.63 – 2.45 (m, 2H), 1.87 – 1.71 (m, 4H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO}-d_6$ )  $\delta$  = 162.3, 148.8, 140.9, 132.0, 119.2, 118.5, 112.6, 72.6, 55.9, 28.7, 27.1, 19.3; HRMS (ESI):  $m/z$   $[\text{M} + \text{H}]^+$  calcd for  $\text{C}_{15}\text{H}_{18}^{79}\text{BrN}_2\text{OS}^+$ : 339.0167, found; 339.0167; calcd for  $\text{C}_{15}\text{H}_{18}^{81}\text{BrN}_2\text{OS}^+$ : 341.0146, found; 341.0148.

**1.28 7-methoxy-N-(4-(trifluoromethyl)phenyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2-amine (31)**

White solid; Yield 92%; mp 108.9 – 109.7°C;  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  = 10.56 (s, 1H), 7.81 (d,  $J$  = 8.4 Hz, 2H), 7.65 (d,  $J$  = 8.4 Hz, 2H), 4.39 (t,  $J$  = 4.8 Hz, 1H), 3.33 (s, 3H), 2.65 – 2.48 (m, 2H), 1.89 – 1.73 (m, 4H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO}-d_6$ )  $\delta$  = 161.9, 148.9, 144.9, 126.7 (q,  $^4J_{\text{CF}}$  = 3.3 Hz), 125.1 (q,  $^1J_{\text{CF}}$  = 269.4 Hz), 121.2 (q,  $^2J_{\text{CF}}$  = 31.9 Hz), 119.4, 116.9, 72.6, 56.0, 28.7, 27.1, 19.3;  $^{19}\text{F}$  NMR (376 MHz,  $\text{DMSO}-d_6$ )  $\delta$  = -58.8 (s, 3F); HRMS (ESI):  $m/z$   $[\text{M} + \text{H}]^+$  calcd for  $\text{C}_{15}\text{H}_{16}\text{F}_3\text{N}_2\text{OS}^+$ : 329.0935, found; 329.0937.

**1.29 4-((7-methoxy-4,5,6,7-tetrahydrobenzo[d]thiazol-2-yl)amino)phenol (32)**

Bright yellow solid; Yield 85%; mp 82 – 83.5°C;  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  = 9.82 (s, 1H), 9.82 (s, 1H), 7.45 (d,  $J$  = 8.8 Hz, 1H), 6.89 (d,  $J$  = 8.8 Hz, 1H), 4.36 (t,  $J$  = 4.2 Hz, 1H), 3.31 (s, 3H), 2.60 – 2.43 (m, 2H), 1.87 – 1.71 (m, 4H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO}-d_6$ )  $\delta$  = 164.0, 152.7, 148.8, 133.7, 126.6, 119.8, 115.8, 72.7, 55.8, 28.8, 27.1, 19.4; HRMS (ESI):  $m/z$   $[\text{M} + \text{H}]^+$  calcd for  $\text{C}_{14}\text{H}_{17}\text{N}_2\text{O}_2\text{S}^+$ : 277.1011, found; 277.1009.

**1.30 7-methoxy-N-(4-nitrophenyl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2-amine (33)**

Yellow solid; Yield 93%; mp 164.6 – 165.7°C;  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  = 10.94 (s, 1H), 8.21 (d,  $J$  = 9.2 Hz, 1H), 7.81 (d,  $J$  = 9.2 Hz, 1H), 4.41 (t,  $J$  = 4.2 Hz, 1H), 3.33 (s, 3H), 2.68 – 2.52 (m, 2H), 1.90 – 1.73 (m, 4H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO}-d_6$ )  $\delta$  = 161.3, 149.1, 147.4, 140.6, 126.0, 120.7, 116.6, 72.6, 56.0, 28.6, 27.1, 19.3; HRMS (ESI):  $m/z$   $[\text{M} + \text{H}]^+$  calcd for  $\text{C}_{14}\text{H}_{16}\text{N}_3\text{O}_3\text{S}^+$ : 306.0912, found; 306.0912.

**1.31 4-((7-methoxy-4,5,6,7-tetrahydrobenzo[d]thiazol-2-yl)amino)benzonitrile (34)**

White solid; Yield 91%; mp 143.7 – 144.3°C;  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  = 10.68 (s, 1H), 7.76 (m, 4H), 4.39 (t,  $J$  = 4.4 Hz, 1H), 3.32 (s, 3H), 2.55 (m, 3H), 1.88 – 1.72 (m, 4H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO}-d_6$ )  $\delta$  = 161.5, 149.0, 145.3, 133.9, 120.0, 117.1, 102.5, 72.6, 56.0, 28.6, 27.1, 19.3; HRMS (ESI):  $m/z$   $[\text{M} + \text{H}]^+$  calcd for  $\text{C}_{15}\text{H}_{16}\text{N}_3\text{OS}^+$ : 286.1014, found; 286.1014.

**1.32 N1-(7-methoxy-4,5,6,7-tetrahydrobenzo[d]thiazol-2-yl)-N4,N4-dimethylbenzene-1,4-diamine (35)**

White solid; Yield 88%; mp 149.0 – 149.8°C;  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  = 9.66 (s, 1H), 7.38 (d,  $J$  = 8.6 Hz, 1H), 6.73 (d,  $J$  = 7.2 Hz, 1H), 4.31 (t,  $J$  = 4.2 Hz, 1H), 3.29 (s, 3H), 2.83 (s, 6H), 2.49 (m, 2H), 1.85 – 1.68 (m, 4H);  $^{13}\text{C}$



NMR (100 MHz, DMSO- $d_6$ )  $\delta$  = 163.7, 148.3, 146.2, 131.6, 119.4, 115.9, 113.3, 72.3, 55.3, 40.7, 28.4, 26.6, 18.9; HRMS (ESI):  $m/z$  [M + H]<sup>+</sup> calcd for C<sub>16</sub>H<sub>22</sub>N<sub>3</sub>OS<sup>+</sup>: 304.1484, found: 304.1484.

**1.33 N-(4-((7-methoxy-4, 5, 6, 7-tetrahydrobenzo[*d*]thiazol-2-yl)amino)phenyl)acetamide (36)**

White solid; Yield 87%; mp 169.3 – 170.5°C; <sup>1</sup>H NMR (400 MHz, DMSO- $d_6$ )  $\delta$  = 9.98 (s, 1H), 9.81 (s, 1H), 7.66 – 7.38 (m, 4H), 4.34 (t,  $J$  = 4.2 Hz, 1H), 3.33 (s, 3H), 2.60 – 2.44 (m, 2H), 2.01 (s, 3H), 1.86 – 1.72 (m, 4H); <sup>13</sup>C NMR (100 MHz, DMSO- $d_6$ )  $\delta$  = 168.2, 163.0, 148.8, 137.2, 133.6, 120.3, 117.8, 117.5, 72.7, 55.9, 28.8, 27.1, 24.3, 19.4; HRMS (ESI):  $m/z$  [M + H]<sup>+</sup> calcd for C<sub>16</sub>H<sub>20</sub>N<sub>3</sub>O<sub>2</sub>S<sup>+</sup>: 318.1271, found: 318.1275.

**1.34 N-(2,6-dichlorophenyl)-7-methoxy-4,5,6,7-tetrahydrobenzo[*d*]thiazol-2-amine (37)**

White solid; Yield 89%; mp 141.4 – 142.4°C; <sup>1</sup>H NMR (400 MHz, DMSO- $d_6$ )  $\delta$  = 9.98 (s, 1H), 7.53 (d,  $J$  = 8.0 Hz, 2H), 6.95 (t,  $J$  = 8.2 Hz, 1H), 4.20 (t,  $J$  = 4.2 Hz, 1H), 3.81 (s, 3H), 3.25 (s, 3H), 2.46 – 2.28 (m, 2H), 1.81 – 1.66 (m, 4H); <sup>13</sup>C NMR (100 MHz, DMSO- $d_6$ )  $\delta$  = 164.6, 144.8, 138.8, 132.7, 129.4, 128.0, 115.5, 72.7, 56.0, 28.6, 25.9, 19.0; HRMS (ESI):  $m/z$  [M + H]<sup>+</sup> calcd for C<sub>14</sub>H<sub>15</sub><sup>35</sup>Cl<sub>2</sub>N<sub>2</sub>OS<sup>+</sup>: 329.0282, found: 329.0281; calcd for C<sub>14</sub>H<sub>15</sub><sup>35</sup>Cl<sup>37</sup>ClN<sub>2</sub>OS<sup>+</sup>: 331.0253, found: 331.0253; calcd for C<sub>14</sub>H<sub>15</sub><sup>37</sup>Cl<sub>2</sub>N<sub>2</sub>OS<sup>+</sup>: 333.0223, found: 333.0224.

**1.35 N-(2-chloro-4-(trifluoromethyl)phenyl)-7-methoxy-4,5,6,7-tetrahydrobenzo[*d*]thiazol-2-amine (38)**

White solid; Yield 90%; mp 106.0 – 106.6°C; <sup>1</sup>H NMR (400 MHz, DMSO- $d_6$ )  $\delta$  = 9.90 (s, 1H), 8.62 (m, 1H), 7.82 (d,  $J$  = 2.0 Hz, 1H), 7.68 (dd,  $J$  = 8.8, 2.2 Hz, 1H), 4.40 (t,  $J$  = 4.2 Hz, 1H), 3.33 (s, 1H), 2.53 (m, 2H), 1.88 – 1.72 (m, 4H); <sup>13</sup>C NMR (100 MHz, DMSO- $d_6$ )  $\delta$  = 161.9, 128.2, 127.0, 125.5, 125.4, 122.8, 122.5, 122.2, 112.0, 72.6, 56.0, 28.6, 26.9, 19.3; <sup>19</sup>F NMR (376 MHz, DMSO- $d_6$ )  $\delta$  = -60.20; HRMS (EI):  $m/z$  calcd for C<sub>15</sub>H<sub>14</sub><sup>35</sup>ClF<sub>3</sub>N<sub>2</sub>OS[M]<sup>+</sup>: 362.0467, found 362.0466. calcd for C<sub>15</sub>H<sub>14</sub><sup>37</sup>ClF<sub>3</sub>N<sub>2</sub>OS[M]<sup>+</sup>: 364.0438, found 364.0450.

**1.36 4-((7-methoxy-4, 5, 6, 7-tetrahydrobenzo[*d*]thiazol-2-yl)amino)-2-(trifluoromethyl)benzonitrile (39)**

White solid; Yield 95%; mp 133.5 – 134.2°C; <sup>1</sup>H NMR (400 MHz, DMSO- $d_6$ )  $\delta$  = 11.10 (s, 1H), 8.21 (s, 1H), 8.05 (m, 2H), 4.42 (t,  $J$  = 4.6 Hz, 1H), 3.33 (s, 3H), 2.60 (m, 2H), 1.89 – 1.73 (m, 4H); <sup>13</sup>C NMR (100 MHz, DMSO- $d_6$ )  $\delta$  =

159.7, 146.8, 145.4, 137.0, 132.4 (d, <sup>2</sup> $J_{CF}$  = 31.2 Hz), 124.4, 123.0 (d, <sup>1</sup> $J_{CF}$  = 271.9 Hz), 119.5, 119.1, 119.0, 118.5, 116.8, 114.5 (d, <sup>3</sup> $J_{CF}$  = 5.1 Hz), 98.5 (d, <sup>4</sup> $J_{CF}$  = 1.9 Hz), 49.0, 24.6, 23.8; <sup>19</sup>F NMR (376 MHz, DMSO- $d_6$ )  $\delta$  = -61.37; HRMS (EI):  $m/z$  calcd for C<sub>16</sub>H<sub>14</sub>F<sub>3</sub>N<sub>3</sub>OS[M]<sup>+</sup>: 353.0810, found 353.0814.

**1.37 N-(2,3-dimethylphenyl)-7-methoxy-4,5,6,7-tetrahydrobenzo[*d*]thiazol-2-amine (40)**

White solid; Yield 94%; mp 123.0 – 123.5°C; <sup>1</sup>H NMR (400 MHz, DMSO- $d_6$ )  $\delta$  = 9.18 (s, 1H), 7.39 (dd,  $J$  = 7.9, 1.2 Hz, 1H), 7.07 (t,  $J$  = 7.7 Hz, 1H), 6.97 (d,  $J$  = 7.4 Hz, 1H), 4.27 (t,  $J$  = 3.6 Hz, 1H), 3.27 (s, 3H), 2.47 – 2.41 (m, 2H), 2.26 (s, 3H), 2.12 (s, 3H), 1.83 – 1.67 (m, 4H); <sup>13</sup>C NMR (100 MHz, DMSO- $d_6$ )  $\delta$  = 166.4, 148.7, 139.8, 137.8, 130.2, 126.6, 126.2, 121.7, 117.1, 72.8, 55.9, 28.9, 27.0, 20.7, 19.3, 14.32; HRMS (ESI):  $m/z$  [M + H]<sup>+</sup> calcd for C<sub>16</sub>H<sub>21</sub>N<sub>2</sub>OS<sup>+</sup>: 289.1375; found: 289.1376.

**1.38 7-methoxy-N-(3,4,5-trimethoxyphenyl)-4,5,6,7-tetrahydrobenzo[*d*]thiazol-2-amine (41)**

Gray solid; Yield 83%; mp 130.8 – 131.2°C; <sup>1</sup>H NMR (400 MHz, DMSO- $d_6$ )  $\delta$  = 9.99 (s, 1H), 6.97 (s, 2H), 3.75 (s, 6H), 3.61 (s, 3H), 3.18 (s, 3H), 2.53 (m, 2H), 1.86 – 1.68 (m, 4H); <sup>13</sup>C NMR (100 MHz, DMSO- $d_6$ )  $\delta$  = 162.9, 153.5, 148.8, 137.9, 132.5, 117.7, 95.6, 72.7, 60.6, 56.2, 55.9, 28.8, 27.1, 19.3; HRMS (ESI):  $m/z$  [M + H]<sup>+</sup> calcd for C<sub>17</sub>H<sub>23</sub>N<sub>2</sub>O<sub>4</sub>S<sup>+</sup>: 351.1379, found: 351.1379.

**1.39 N-(4-chlorophenyl)-7-(2-methoxyethoxy)-4,5,6,7-tetrahydrobenzo[*d*]thiazol-2-amine (42)**

Light yellow solid; Yield 83%; mp 96.3 – 97.1°C; <sup>1</sup>H NMR (400 MHz, DMSO- $d_6$ )  $\delta$  = 10.23 (s, 1H), 7.64 (d,  $J$  = 8.6 Hz, 2H), 7.34 (d,  $J$  = 8.6 Hz, 2H), 4.49 (t,  $J$  = 4.0 Hz, 1H), 3.64 – 3.56 (m, 2H), 3.51 – 3.41 (m, 2H), 3.26 (s, 3H), 2.63 – 2.40 (m, 2H), 1.91 – 1.66 (m, 4H); <sup>13</sup>C NMR (100 MHz, DMSO- $d_6$ )  $\delta$  = 161.9, 148.3, 140.1, 128.7, 124.3, 118.2, 71.6, 71.0, 67.1, 58.1, 28.9, 26.6, 18.8; HRMS (ESI):  $m/z$  [M + H]<sup>+</sup> calcd for C<sub>16</sub>H<sub>20</sub><sup>35</sup>ClN<sub>2</sub>O<sub>2</sub>S<sup>+</sup>: 339.0929, found: 339.0931; calcd for C<sub>16</sub>H<sub>20</sub><sup>37</sup>ClN<sub>2</sub>O<sub>2</sub>S<sup>+</sup>: 341.0899, found: 339.0901.

**1.40 7-(2-methoxyethoxy)-N-(p-tolyl)-4,5,6,7-tetrahydrobenzo[*d*]thiazol-2-amine (43)**

Light yellow solid; Yield 82%; mp 85.2 – 85.9°C; <sup>1</sup>H NMR (400 MHz, DMSO- $d_6$ )  $\delta$  = 9.96 (s, 1H), 7.47 (d,  $J$  = 8.2 Hz, 2H), 7.08 (d,  $J$  = 8.2 Hz, 2H), 4.47 (t,  $J$  = 4.0 Hz, 1H), 3.64 – 3.57 (m, 2H), 3.48 – 3.41 (m, 2H), 3.26 (s, 3H), 2.61 – 2.39 (m, 2H), 2.23 (s, 3H), 1.88 – 1.66 (m, 4H); <sup>13</sup>C NMR (100 MHz, DMSO- $d_6$ )  $\delta$  = 162.5,

148.3, 138.8, 129.9, 129.2, 117.2, 117.0, 71.6, 71.0, 67.1, 58.1, 28.9, 26.6, 20.3, 18.8; HRMS (ESI):  $m/z$  [M + H]<sup>+</sup> calcd for C<sub>17</sub>H<sub>23</sub>N<sub>2</sub>O<sub>2</sub>S<sup>+</sup>: 319.1475, found: 315.1479.

**1.41 N-(5-chloropyridin-2-yl)-7-methoxy-4,5,6,7-tetrahydrobenzo[d]thiazol-2-amine (49)**

White solid; Yield 96%; mp 153.5 – 154.3°C; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ = 11.34 (s, 1H), 8.32 (d, *J* = 2.4 Hz, 1H), 7.78 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.08 (d, *J* = 8.8 Hz, 1H), 4.42 (t, *J* = 4.8 Hz, 1H), 3.34 (s, 3H), 2.54 (m, 2H), 1.87 – 1.71 (m, 4H); <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ = 158.7, 151.0, 147.2, 145.1, 138.2, 122.3, 120.7, 112.7, 72.7, 56.0, 28.8, 26.7, 19.4; HRMS (ESI):  $m/z$  [M-H]<sup>-</sup> calcd for C<sub>13</sub>H<sub>13</sub><sup>35</sup>ClN<sub>2</sub>O<sub>2</sub>S<sup>-</sup>: 294.0468, found: 294.0469; calcd for C<sub>13</sub>H<sub>13</sub><sup>37</sup>ClN<sub>2</sub>O<sub>2</sub>S<sup>-</sup>: 296.0438, found: 296.0441.

**1.42 N-(5-chloropyridin-2-yl)-7-ethoxy-4,5,6,7-tetrahydrobenzo[d]thiazol-2-amine (50)**

White solid; Yield 91%; mp 161.6 – 162.5°C; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ = 11.34 (s, 1H), 8.32 (d, *J* = 2.4 Hz, 1H), 7.78 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.08 (d, *J* = 8.8 Hz, 1H), 4.51 (t, *J* = 4.8 Hz, 1H), 3.57 (q, *J* = 8.2 Hz, 2H), 2.51 (m, 2H), 1.90 – 1.71 (m, 4H), 1.14 (t, *J* = 7.0 Hz, 3H); <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ = 158.6, 151.0, 147.0, 145.2, 138.2, 122.3, 121.1, 112.7, 71.0, 63.5, 29.6, 26.7, 19.5, 16.1; HRMS (ESI):  $m/z$  [M + H]<sup>+</sup> calcd for C<sub>14</sub>H<sub>17</sub><sup>35</sup>ClN<sub>3</sub>O<sub>2</sub>S<sup>+</sup>: 310.0781, found: 310.0783; calcd for C<sub>14</sub>H<sub>17</sub><sup>37</sup>ClN<sub>3</sub>O<sub>2</sub>S<sup>+</sup>: 312.0751, found: 312.0754.

**1.43 7-methoxy-N-(pyridin-3-yl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2-amine (51)**

White solid; Yield 94%; mp 146.3 – 147.4°C; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ = 10.68 (s, 1H), 8.74 (d, *J* = 2.7 Hz, 1H), 8.18 – 8.16 (m, 1H), 8.16 – 8.12 (m, 1H), 7.34–7.30 (m, 1H), 4.37 (t, *J* = 4.2 Hz, 1H), 3.32 (s, 3H), 2.642.47 (m, 2H), 1.88 – 1.71 (m, 4H); <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ = 162.3, 148.9, 142.3, 139.3, 138.3, 124.1, 123.6, 118.9, 72.6, 55.9, 28.7, 27.1, 19.3; HRMS (ESI):  $m/z$  [M-H]<sup>-</sup> calcd for C<sub>13</sub>H<sub>14</sub>N<sub>3</sub>O<sub>2</sub>S: 260.0858, found: 260.0855.

**1.44 N-(2-chloropyridin-4-yl)-7-methoxy-4,5,6,7-tetrahydrobenzo[d]thiazol-2-amine (52)**

White solid; Yield 93%; mp 142.4 – 143.3°C; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ = 10.86 (s, 1H), 8.17 (d, *J* = 5.8 Hz, 1H), 7.79 (d, *J* = 2.0 Hz, 1H), 7.41 (dd, *J* = 5.8, 2.0 Hz, 1H), 4.41 (t, *J* = 4.4 Hz, 1H), 3.33 (s, 3H), 2.69 – 2.53 (m, 2H), 1.89 – 1.73 (m, 4H); <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ = 161.0, 151.5, 150.4, 149.7, 149.1, 120.9, 111.2, 110.3, 72.5, 56.0, 28.6, 27.0, 19.3; HRMS (ESI):  $m/z$  [M + H]<sup>+</sup> calcd for C<sub>13</sub>H<sub>15</sub><sup>35</sup>ClN<sub>3</sub>O<sub>2</sub>S<sup>+</sup>: 296.0624,

found: 296.0628. calcd for C<sub>13</sub>H<sub>15</sub><sup>37</sup>ClN<sub>3</sub>O<sub>2</sub>S<sup>+</sup>: 298.0595, found: 298.0599.

**1.45 N-benzyl-7-methoxy-4,5,6,7-tetrahydrobenzo[d]thiazol-2-amine (53)**

White solid; Yield 96%; mp 72.3 – 73.5°C; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ = 8.00 (t, *J* = 4.8 Hz, 1H), 7.33 (d, *J* = 4.2 Hz, 4H), 7.27 – 7.22 (m, 1H), 4.41 (d, *J* = 5.2 Hz, 2H), 4.23 (t, *J* = 4.2 Hz, 1H), 3.26 (s, 3H), 2.48 – 2.30 (m, 2H), 1.80 – 1.64 (m, 4H); <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ = 168.0, 148.7, 139.8, 128.7, 127.7, 127.3, 116.0, 72.9, 55.8, 47.9, 28.9, 27.1, 19.3; HRMS (ESI):  $m/z$  [M + H]<sup>+</sup> calcd for C<sub>15</sub>H<sub>19</sub>N<sub>2</sub>O<sub>2</sub>S<sup>+</sup>: 275.1218, found: 275.1219.

**1.46 7-methoxy-N-(naphthalen-1-yl)-4,5,6,7-tetrahydrobenzo[d]thiazol-2-amine (54)**

White solid; Yield 94%; mp 115.8 – 116.5°C; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ = 9.98 (s, 1H), 8.30 – 8.20 (m, 1H), 8.08 (d, *J* = 7.4 Hz, 1H), 7.97 – 7.89 (m, 1H), 7.65 (d, *J* = 8.2 Hz, 1H), 7.59 – 7.52 (m, 2H), 7.51 – 7.46 (m, 1H), 4.33 (t, *J* = 4.2 Hz, 1H), 3.30 (d, 3H), 2.60 – 2.42 (m, 2H), 1.86 – 1.70 (m, 4H); <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ = 165.2, 148.5, 137.4, 134.5, 128.7, 126.8, 126.5, 126.5, 126.1, 123.8, 122.7, 118.1, 117.4, 72.8, 55.9, 28.9, 27.0, 19.4; HRMS (ESI):  $m/z$  [M + H]<sup>+</sup> calcd for C<sub>18</sub>H<sub>19</sub>N<sub>2</sub>O<sub>2</sub>S<sup>+</sup>: 311.1218, found: 311.1216.

**1.47 N-cyclohexyl-7-methoxy-4,5,6,7-tetrahydrobenzo[d]thiazol-2-amine (55)**

Light yellow sticky solid; Yield 82%; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ = 7.37 (d, *J* = 7.6 Hz, 1H), 4.22 (t, *J* = 3.6 Hz, 1H), 3.26 (s, 3H), 2.47 – 2.27 (m, 2H), 1.94 – 1.50 (m, 7H), 1.33 – 1.09 (m, 8H); <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ = 166.6, 148.2, 114.5, 72.4, 55.2, 52.8, 32.3, 28.4, 26.6, 25.3, 24.4, 28.8; HRMS (ESI):  $m/z$  [M + H]<sup>+</sup> calcd for C<sub>14</sub>H<sub>23</sub>N<sub>2</sub>O<sub>2</sub>S<sup>+</sup>: 267.1531, found: 267.1536.

**1.48 N-allyl-7-methoxy-4,5,6,7-tetrahydrobenzo[d]thiazol-2-amine (56)**

Light yellow sticky solid; Yield 85%; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ = 7.62 (t, *J* = 4.8 Hz, 1H), 5.92 – 5.82 (m, 1H), 5.25 – 5.17 (m, 1H), 5.11 – 5.06 (m, 1H), 4.24 (t, *J* = 3.8 Hz, 1H), 3.82 (s, 2H), 3.27 (s, 3H), 2.48 – 2.28 (m, 2H), 1.79 – 1.60 (m, 4H), 1.70 – 1.58 (m); <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ = 167.4, 148.2, 135.1, 115.5, 115.4, 72.4, 55.3, 46.3, 28.4, 26.6, 18.8; HRMS (ESI):  $m/z$  [M + H]<sup>+</sup> calcd for C<sub>11</sub>H<sub>17</sub>N<sub>2</sub>O<sub>2</sub>S<sup>+</sup>: 225.1062, found: 225.1065.

**1.49 2-((4-chlorophenyl)amino)-3a,4,5,6a-tetrahydro-6H-cyclopenta[d]thiazol-6-one (58)**

White solid; Yield 85%; mp 147.0 – 148.2°C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ = 7.26 (d, *J* = 8.8 Hz,

2H), 7.12 (d,  $J$  = 8.8 Hz, 2H), 6.68 (s, 1H), 4.89–4.84 (m, 1H), 3.96 (d,  $J$  = 7.2 Hz, 1H), 2.64–2.52 (m, 1H), 2.43–2.32 (m, 1H), 2.32–2.17 (m, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  = 214.0, 159.4, 143.7, 129.2, 129.0, 122.2, 66.6, 54.1, 34.4, 27.9; HRMS (ESI):  $m/z$  [ $\text{M} + \text{H}$ ] + calcd for  $\text{C}_{12}\text{H}_{12}^{35}\text{ClN}_2\text{OS}^+$ : 267.0359, found: 267.0366. calcd for  $\text{C}_{12}\text{H}_{12}^{37}\text{ClN}_2\text{OS}^+$ : 269.0329, found: 269.0335.

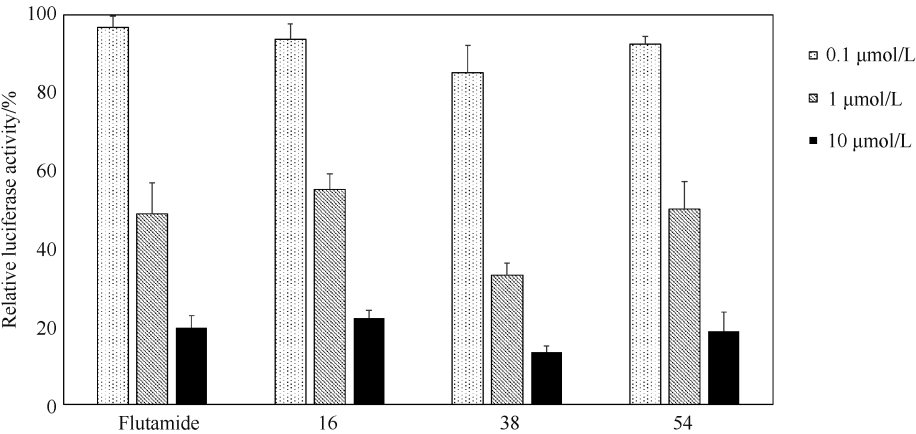
1.50 N-( 4-chlorophenyl )-5, 6-dihydro-4H-cyclohepta[ *d* ]thiazol-2-amine (60)

Light yellow solid; Yield 69%; mp 126.5 – 128.0°C;  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  = 10.21 (s, 1H), 7.62 (d,  $J$  = 8.8 Hz, 2H), 7.33 (d,  $J$  = 8.8 Hz, 2H), 6.17 (d,  $J$  = 11.6 Hz, 1H), 5.54 (dt,  $J$  = 11.6, 5.2 Hz, 1H, 1H), 2.92 (t,  $J$  = 5.6 Hz, 2H), 2.39 (m, 2H), 1.85 (dt,  $J$  = 11.2, 5.6

Hz, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO}-d_6$ )  $\delta$  = 160.2, 150.6, 140.5, 129.4, 129.2, 124.8, 118.7, 118.3, 118.0, 33.5, 30.6, 22.9; HRMS (ESI):  $m/z$  [ $\text{M} + \text{H}$ ] + calcd for  $\text{C}_{14}\text{H}_{14}^{35}\text{ClN}_2\text{S}^+$ : 277.0566, found: 277.0568. calcd for  $\text{C}_{14}\text{H}_{14}^{37}\text{ClN}_2\text{S}^+$ : 279.0537, found: 279.0542.

2 Active compounds inhibit the transcriptional activity of AR

To verify the AR inhibitory effect of our compounds, an AR-dependent luciferase reporter gene assay was conducted, in which the transcriptional activity of AR was reflected by the luciferase activity. Consistent with data from flutamide, all compounds effectively reduced the luciferase activity when the final concentration was greater than 1 mmol/L (Fig. 1-SI). The above results further demonstrated that these tetrahydrobenzo[ *d* ]thiazoles were AR antagonists.



**Fig. 1 -SI Active compounds inhibited the transcriptional activity of AR induced by DHT** CHO cells were transfected with hAR expression vector, ARE/Luc (firefly luciferase) and pRL/CMV (Renilla luciferase) and incubated with the test compounds ( $10^{-7}$ - $10^{-5}$  mol/L) and DHT (0.5 nmol/L). Relative luciferase activities were standardized to Renilla luciferase control and normalized to the relative luciferase units with 0.5 nmol/L DHT. The results are reported as mean ± SD.

3 Verification of docking method

Molecular docking study was performed with the original ligand (PK1) using the same procedure. PK1 was prepared with Ligprep 3.4, with OPLS3 selected as the force field, and possible states generated at target pH range from 2.0 to 9.0 using Epik 3.2. Then the compound was docking in the “Extra Precision” (XP) mode of Glide 6.7. The RMSD value (Table 1-

SI) was less than 2 angstroms, which indicated that our docking method is relatively reliable.

**Table 1 -SI. Result of docking study using the original ligand**

Compound	Docking Score	Glide Score	RMSD (Å)
PK1	-6.795	-6.795	1.0599