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2-Substituted-thio-*N*-(4-substituted-thiazol/1*H*-imidazol-2-yl)acetamides as BACE1 inhibitors: Synthesis, biological evaluation and docking studies

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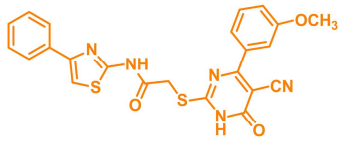
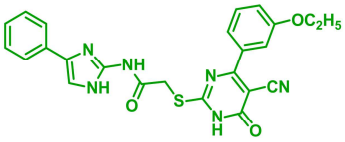


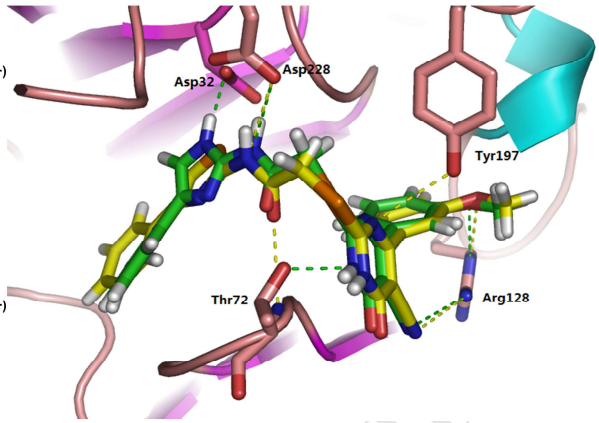
12

BACE1 IC_{50} = 37.4 μM
BACE1 K_D = 2.14 μM
BBB PAMPA P_e = 9.1 × 10^{-6} cm/s (CNS+)
CC_{50} (HEK293) = 44.47 μM
MW = 475.5



41

BACE1 IC_{50} = 4.6 μM
BACE1 K_D = 0.213 μM
BBB PAMPA P_e = 7.1 × 10^{-6} cm/s (CNS+)
CC_{50} (HEK293) > 50 μM
MW = 472.5



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Original artical

2-Substituted-thio-*N*-(4-substituted-thiazol/1*H*-imidazol-2-yl)acetamides as BACE1 inhibitors: Synthesis, biological evaluation and docking studies

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ABSTRACT

In this work, a series of 2-substituted-thio-*N*-(4-substituted-thiazol/1*H*-imidazol-2-yl)acetamide derivatives were developed as *β*-secretase (BACE-1) inhibitors. Supported by docking study, a small library of derivatives were designed, synthesized and biologically evaluated *in vitro*. In addition, the selected compounds were tested with affinity (K_D) towards BACE-1, blood brain barrier (BBB) permeability and cytotoxicity. The studies revealed that the most potent analog **41** (IC_{50} = 4.6 *μ*M) with high predicted BBB permeability and low cellular cytotoxicity, could serve as a good lead structure for further optimization.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder disease characterized by dementia and neurofunctional impairment, which will finally lead to death[1, 2]. The two pathological hallmarks which were found generally in the brains of AD patients include extracellular amyloid plaque, which is the deposition of insoluble *β*-amyloid peptides (A*β*) derived from *β*-amyloid precursor protein (APP)[3], and intracellular neurofibrillay tangle (NFT) related with hyperphosphorylated tau protein[4]. Based on the "Amyloid Hypothesis"[5, 6], the production and aggregation of A*β* are highly related with the pathology of AD. $A\beta_{42}$, which is produced from APP through successive cleavages by *β*-secretase (BACE-1) and *γ*-secretase[7], is the major component of A*β* in amyloid plaques[8]. BACE-1 catalyzes the initial step of A*β* generation, therefore, has been regarded as a potential target for the treatment of AD[9].

BACE-1 is an aspartyl protease located in the central nervous system (CNS)[10-12], which makes it hard to develop a drug towards it. In the last decade, scientists had developed many inhibitors towards BACE-1. Some of the programs were focused on peptidomimetic inhibitors such as hydroxyethylamine (HEA) and hydroxyethylene (HE)[13] in the early years. These compounds had very good potency *in vitro*, but most of them suffered from poor blood brain barrier (BBB) permeability and thus poor *in vivo* activity[14]. In recent years, more researches were dedicated to developing non-peptidomimetic inhibitors targeting BACE-1, which had good *in vitro* activity, much smaller molecular weight and better BBB permeability[15-17], and therefore became more druglike agents for the treatment of AD. Among those inhibitors, only a few of compounds had been advanced into the clinic[17-20]. However, none of them had been approved by FDA.

In this paper, a series of non-peptide BACE-1 inhibitors bearing the scaffold of 2-substituted-thio-*N*-(4-substituted-thiazol/1*H*-imidazol-2-yl) acetamide were designed and synthesized. The bioactivities of these compounds including enzyme inhibition, affinity (K_D) towards BACE1, BBB permeability and cytotoxicity towards HEK293 cell line were also evaluated.

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[1] These two authors contributed equally.

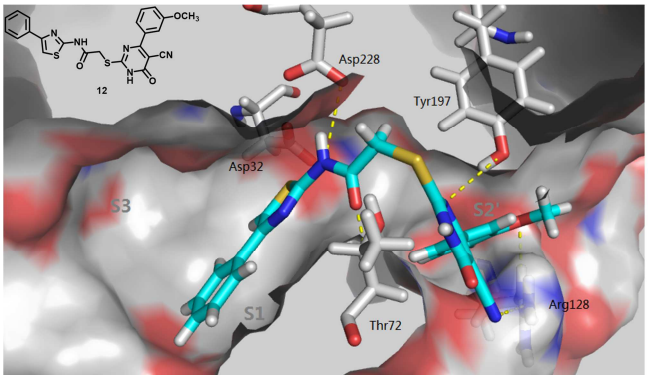


Fig. 1 Predicted binding mode of compound **12** in the active site of BACE-1 (protein PDB ID: 3VF3)

2. Design

Based on *in silico* screening and biological evaluation, a series of compounds had been found with some extent activity towards BACE-1 in our previous work[21, 22]. Among them, compound **12** (IC_{50} = 37.4 μM) was chosen as the hit compound for further optimization in this work. Molecular docking studies are powerful tools of Computer-Aided Drug Design (CADD) for designing new analogues.[23] The docking studies of compound 12 (**Fig. 1**) showed the interactions mode between BACE-1 and **12**. The nitrogen atom outside the thiazole ring formed an H-bond with catalytic Asp228, and nearby oxygen of the carbonyl group formed another H-bond with Thr72. The S1 pocket of BACE-1 was occupied by the phenyl group at 4-position of thiazole ring of **12**. And the substituted pyrimidine ring, which form three H-bonds with Tyr197 and Arg128, was extended to S2' pocket.

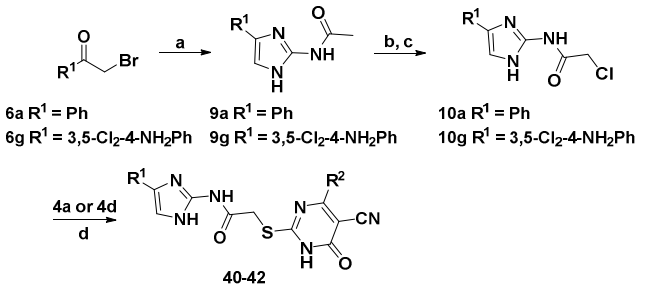
According to the above docking studies, the potency towards BACE-1 might be achieved by the introduction of proper substituents at phenyl ring in S1 pockets, which might have key interaction with nearby residues and/or extend into S3 pockets[24, 25]. A series of compounds with different substituted phenyl ring at 4-position of pyrimidine ring were also designed to find the most favorable substituent. Asp32 and Asp 228 are two key amino acids in the catalytic site.[26] However, **12** only formed one H-bond with Asp228, while with no interaction with Asp32. Docking studies showed that the distance between S atom of the thiazole ring and O atom of Asp32 was 3.07 Å. It might form another H-bond [27] by replacing –S– with a hydrogen bond donor –NH–, which might increase the activity.

3. Chemistry

As shown in **Scheme 1**, 2-substituted-thio-*N*-(4-substituted thiazol-2-yl)acetamide analogs **12-39** were prepared in 3-6 steps through convergent synthetic strategy. Starting from three commercially available materials thiourea **1**, substituted aldehydes **2a-2h** and ethyl cyanacetate **3**, compounds

Scheme 1. Synthesis of 2-thio-*N*-(thiazol-2-yl)acetamides derivatives. (Reagents and conditions: (a) K_2CO_3, EtOH, 80 °C, 6h; (b) Br_2, $CHCl_3$, reflux, 15min; (c) $HOP(OC_2H_5)_2$, TEA, THF, 0 °C to R.T., 10min; (d) thiourea, H_2O, R.T., 2h; (e) $ClCH_2COCl$, TEA or DIPEA, CH_2Cl_2, 0 °C to R.T., 1-2h; (f) (2-fluoropyridin- 3-yl)boronic acid, $Pd(PPh_3)_4$ (5 mol%), Na_2CO_3 (2 equiv.), DMF, 120 °C, 24h; (g) CH_3COCl, AcOH, 90 °C, 20min; (h) KOH, EtOH, 70 °C, 3h.)

* Only **6g** were prepared from **5g**. Other chemicals from **6a** to **6f** and **6i** are commercial available.



Scheme 2. Synthesis of 2-thio-*N*-(1*H*-imidazol-2-yl)acetamides derivatives. (Reagents and conditions: (a) *N*-acetylguanidine, CH_3CN, 100 °C, 3h; (b) HCl (40%) or H_2SO_4 (20%) in MeOH/H_2O (1:1), 100 °C, 1h; (c) $ClCH_2COCl$, TEA, CH_2Cl_2, 0 °C, 1h; (d) KOH, EtOH, 70 °C, 3h.)

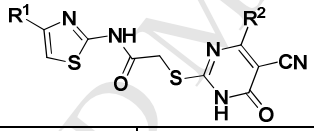
4a-4h were prepared under base condition[28, 29]. Another individual route was starting from 1-(4-amino-3,5-*d*ichlorophenyl) ethanone **5g**, it was reacted with Br_2 to form 1-(4-amino-3,5-*d*ichlorophenyl)-2-bromoethanone[30] **6g**, other substituted phenyl bromoethanones were commercially available. The substituted phenyl bromoethanone **6a - 6g** and **6i** were reacted with thiourea to form 4-substituted-2-aminothiazole[31] **7a - 7g** and **7j**, which were subsequently converted to 2-chloro-N-(thiazol-2-yl)acetamide **8a – 8g** and **8i** by reacting with chloroacetyl chloride. **8g** was reacted with acetyl chloride to obtain **8h**. **7i** was reacted with (2-fluoropyridinyl-3-yl)boronic acid to obtain **7j**, which was later converted to **8j** as mentioned above. The final compounds **12-36** were generated through a nucleophilic substitution between compounds **8a-8j** and **4a-4h**. In addition, compound **8a** could directly react with three commercially available materials **4g**, **4h** or **4i** to form three derivitives **37-39**, respectively.

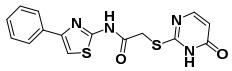
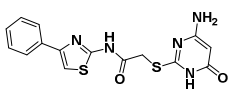
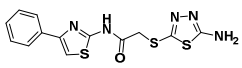
The synthesis of 2-substituted-thio-*N*-(4-substituted-1*H*-imidazol-2-yl)acetamide derivatives was shown in **Scheme 2**. Substituted bromoethanones **6a** and **6g** were transformed into *N*-(1*H*-imidazol-2-yl)acetamides **9a** and **9g** by heating with *N*-acetylguanidine[32]. The intermediates 2-chloro-*N*-(1*H*-imidazol -2-yl)acetamides **10a** and **10g** were prepared through hydrolysis reaction of compounds **9a** and **9g** under acidic condition[33], followed by reacting with chloroacetyl chloride. After that, compounds **10a** or **10g** reacted with **4a** or **4d** to form the final products **40**-**42**.

4. Results and discussion

All synthesized 2-substituted-thio-*N*-(4-substituted-thiazol-2- yl)acetamide derivatives were tested for BACE-1 inhibitory potencies with data given in **Table 1**. As for the R^1 substitutions on 2-substituted-thio-*N*-(4-substituted-thiazol-2-yl)acetamide

Table 1. BACE-1 inhibitory activities of 2-substituted-thio-*N*-(4-substituted-thiazol-2-yl)acetamide derivatives



NO.	R^1	R^2	Inhibition% (10 μM) [a]	Inhibition% (50 μM) [a]	BACE-1 IC_{50}/μM [a]	NO.	R^1	R^2	Inhibition% (10 μM) [a]	Inhibition% (50 μM) [a]	BACE-1 IC_{50}/μM [a]
12	Ph	*m*-MeOPh	10.8±5.5	50.7±3.7	37.4±1.2	**26**	*p*-MePh	*m*-EtOPh	-0.3±0.8	11.5±1.0	n.d.
13	Ph	*o*-MeOPh	11.7±1.7	25.9±0.8	n.d.[b]	**27**	*p*-MeOPh	*m*-EtOPh	-3.0±0.6	9.4±2.5	n.d.
14	Ph	*p*-MeOPh	6.3±0.1	10.7±4.1	n.d.	**28**	3,5-Cl_2-4-NH_2Ph	*m*-EtOPh	24.5±8.7	79.9±2.3	13.5±4.6
15	*p*-NO_2Ph	*o*-MeOPh	19.3±0.7	77.5±8.5	27.6±2.2	**29**	3,5-Cl_2-4-AcNHPh	*m*-MeOPh	0.3±3.6	1.4±2.9	n.d.
16	*p*-NO_2Ph	*m*-MeOPh	31.1±1.9	83.9±1.5	26.1±3.9	**30**	3,5-Cl_2-4-AcNHPh	*m*-EtOPh	2.5±0.4	4.7±3.5	n.d.
17	*p*-NO_2Ph	*p*-MeOPh	5.9±2.9	2.1±13.6	n.d.	**31**	Ph	*m*-CF_3Ph	16.8±1.1	82.5±9.4	19.1±5.9
18	*m*-NO_2Ph	*o*-MeOPh	16.8±3.9	62.4±7.1	38.6±0.8	**32**	3,5-Cl_2-4-NH_2Ph	*m*-CF_3Ph	13.0±1.8	86.1±0.6	13.4±0.6
19	*m*-NO_2Ph	*m*-MeOPh	4.4±1.0	78.5±3.7	24.3±2.1	**33**	Ph	*m*-CF_3OPh	6.8±1.6	85.8±2.3	29.6±6.6
20	*m*-NO_2Ph	*p*-MeOPh	12.3±2.6	7.5±2.0	n.d.	**34**	3,5-Cl_2-4-NH_2Ph	*m*-CF_3OPh	6.1±2.7	39.6±1.7	n.d.
21	*o*-NO_2Ph	*m*-MeOPh	-13.2±4.5	6.5±5.0	n.d.	**35**	*m*-BrPh	*m*-MeOPh	14.3±1.4	90.4±3.1	17.5±3.6
22	*p*-NO_2Ph	*m*-EtOPh	22.7±4.1	85.6±9.6	15.0±3.4	**36**	(2-F-Py- 3-yl)Ph	*m*-MeOPh	26.5±0.3	88.7±2.4	15.0±0.8
23	3,5-Cl_2-4-NH_2Ph	*m*-MeOPh	58.9±6.6	91.8±3.7	7.70±0.5	**37**			1.7±2.5	22.5±2.7	n.d.
24	*p*-MePh	*m*-MeOPh	5.6±3.4	8.1±1.5	n.d.	**38**			3.6±2.5	17.8±1.2	n.d.
25	*p*-MeOPh	*m*-MeOPh	7.7±2.2	16.5±2.7	n.d.	**39**			-0.4±7.1	21.8±3.2	n.d.

[a] Values are mean ± SD for BACE-1 inhibition (n ≥ 2).

[b] n.d. = not determined

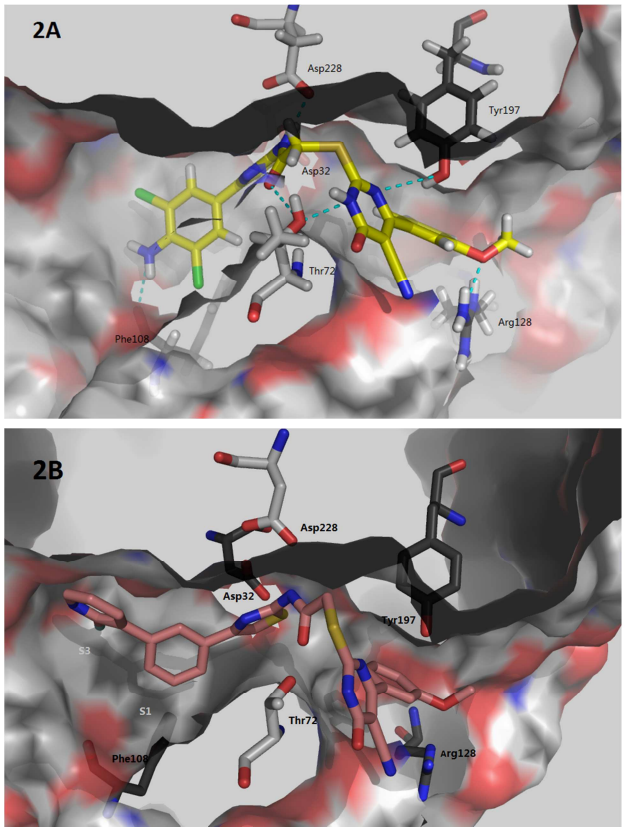


Fig. 2 Predicted binding mode of compound **23** (A) and **36** (B) in the active site of BACE-1 (protein PDB ID: 3VF3)

derivatives, compounds with *para*- (**16**) or *meta*- (**19**) substitution on the phenyl ring appeared to be more potent than *ortho*- (**21**) position. As for the various R^2, phenyl ring with *meta*- position (**12, 16, 19**) substitution showed better potency than *ortho*- position (**13, 15, 18**), which was more potent than *para*- position (**14, 17, 20**). With this conclusion, the following optimization of the hit compound **12** was focused on *p*- and *m*- position of the phenyl ring of R^1 and *m*- position of the phenyl ring of R^2. Specifically for R^1 group, The compounds with *p*-NO_2Ph (**16**, **22**), 3,5-Cl_2-4-NH_2Ph (**23**, **28**) substituents were more potent than **12.** While compounds with *p*-MeOPh (**25**, **27**) and *p*-MePh (**24**, **26**) substituents almost had no activity towards BACE-1. Docking result of **23** (**Fig. 2A**) suggested that it had similar interaction mode to **12**, while the phenyl ring was rotated so that the chlorine atom could partly extend into the S3 pocket, and the amino group had an extra H-bond with Phe108 at S1 pocket, which might contribute to the enhanced activity most. However, when the amino group was acetylated (**29**, **30**), the molecule might be too large to occupy the pocket, this might be the reason why these two compounds had no activity. Meanwhile, the docking study of compound **23** (**Fig. 2A**) suggested that the *meta*- position of phenyl ring could tolerate bulkier group, so Br atom (**35**) and another aromatic ring (**36**) at *meta*- site were designed and synthesized, and their activities were increased compared with hit compound **12**. Docking of compound **36** (**Fig. 2B**) showed that **36** had similar binding pose to **12**, while the *meta*- substituted aromatic ring was extended into S3 pocket, which might have more hydrophobic interaction with BACE-1, and this could explain the enhancement of activity. As for the phenyl ring of R^2 group, different

Table 2. BACE-1 inhibitory activities of 2-substituted-thio-*N*-(4-substituted-1*H*-imidazol-2-yl)acetamide derivatives

NO.	R^1	R^2	Inhibition% (1 μM) [a]	Inhibition% (10 μM) [a]	BACE-1 IC_{50}/μM [a]
40	Ph	*m*-MeOPh	16.8±1.1	78.6±1.8	4.2±0.5
41	Ph	*m*-EtOPh	17.9±1.8	84.5±0.7	4.6±1.1
42	3,5-Cl_2-4-NH_2Ph	*m*-MeOPh	26.4±3.2	87.7±3.5	3.2±0.6

[a] Values are mean ± SD for BACE-1 inhibition (n ≥ 2).

substituents at *meta*- position were designed and synthesized, including –OCH_3 (**12**, **16**, **23**), -OC_2H_5 (**22**, **28**), -CF_3 (**31**, **32**), -OCF_3 (**33**, **34**). But based on the enzyme activities, there was no significant difference among these substituents, except that compound **34** almost lose the activity. Relatively, when R^1 were non-substituted phenyl rings, compound with –CF_3 (**31**) was more favorable; while when R^1 were 3,5-Cl_2-4-NH_2Ph, R^2 with *m*-OCH_3Ph was better. From the docking study of compound **12** (**Fig. 1**), we could find that R^2, -CN group and pyrimidine ring all had important interactions with adjacent amino acid. From the data in scheme 1, if R^2 was replaced by -H (**37**) or -NH_2 (**38**), or the -CN group was replaced by -H, or the pyrimidine ring was replaced by 1,3,4-thiodiazole (**39**), they would lose activities.

Three synthesized 2-substituted-thio-*N*-(4-substituted-1*H*-imidazol-2-yl)aceta mide compounds were tested for BACE-1 inhibitory potencies with data given in **Table 2**. The activities of imidazole compounds **40** and **41** were 10 times more potent than the similar thiazole series compounds **12**. Compound **42** was also more favorable compared with compound **23**. This significant activity enhancement could be explained by docking study (**Fig. 3**). We could find out that compound **40** had similar interaction mode to **12** with BACE-1 when changing -S- to -NH-. While compound **40** had an additional H-bond between -NH- of the imidazole ring and Asp32. Combined with the other H-bond between amide proton and Asp228, compound **40** formed a stronger

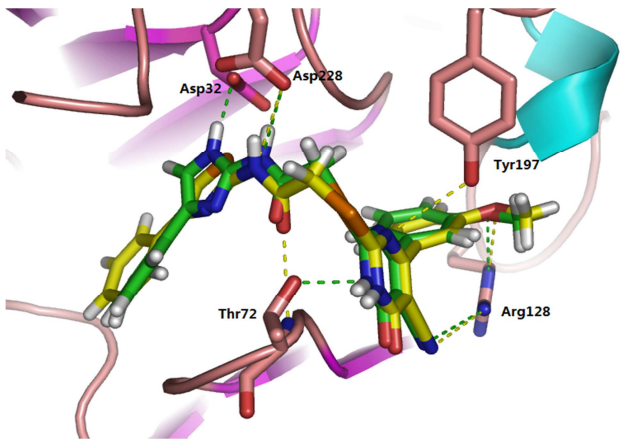


Fig. 3 Predicted binding mode of compound **12** (yellow) and **40** (green) in the active site of BACE-1 (protein PDB ID: 3VF3)

Table 3. K_D values, predicted BBB permeabilities and Cytotoxic profiles of selected compounds.

NO.	MW	K_D/μM [a]	P_e/10^{-6}cm s^{-1} [b]	Predicition	CC_{50}/μM
Verapamil	-	n.d.[c]	16.2±7.1	CNS+	n.d.
Clonidine	-	n.d.	5.9±0.37	CNS+	n.d.
Hydrocortisone	-	n.d.	2.8±2.8	CNS±	n.d.
Theophylline	-	n.d.	0.13±0.001	CNS-	n.d.
Staurosporine	-	n.d.	n.d.	n.d.	0.00354
12	475.5	2.14±1.7	9.1±1.5	CNS+	44.47
15	520.5	n.d.	1.7±0.01	CNS-	>50
16	520.5	1.84±0.62	0.42±0.11	CNS-	>50
18	520.5	1.43±1.2	3.1±1.2	CNS±	>50
19	520.5	1.75±0.74	0.46±0.03	CNS-	>50
22	534.6	1.23±0.96	0.73±0.10	CNS-	>50
23	559.5	0.861±0.37	0.11±0.01	CNS-	>50
28	573.5	n.d.	0.07±0.02	CNS-	>50
31	513.5	1.03±0.19	4.6±0.17	CNS+	>50
32	597.4	1.33±0.63	0.88±0.19	CNS-	47.73
33	529.5	1.88±0.54	3.2±0.61	CNS±	>50
35	554.4	1.74±0.43	0.94±0.10	CNS-	>50
36	570.6	1.44±0.25	0.04±0.02	CNS-	>50
40	458.5	0.554±0.24	2.8±0.47	CNS±	>50
41	472.5	0.213±0.072	7.1±2.7	CNS+	>50
42	542.4	0.169±0.068	0.34±0.05	CNS-	>50

[a] Values are mean ± SE for BACE-1 Affinity Assay (SPR assay, n ≥ 2).

[b] Values are mean ± SD for PAMPA Assay (n ≥ 2).

[c] n.d. = not determined

bidentate interaction with catalytic center of BACE1 than compound **12**. Compound **42** was the most potent compound in BACE-1 inhibitory assay.

The affinity of compounds towards BACE1 was measured by Surface Plasmon Resonance (SPR) assay (**Table 3**.). The affinity (K_D) trends of these compounds were following the same rule as that in the enzyme-inhibitory assay above. The best affinity (K_D = 0.169 μM) was also obserbed from compound **42**. The affinities of other active compounds are at μM to sub-μM level.

BBB penetration capacity plays a key role in CNS drugs[34]. The parallel artificial membrane permeability assay (PAMPA)[35] was used to study the permeability of target compounds through BBB. The *in vitro* permeability (P_e) of sixteen active compounds and control drugs (verapamil, hydrocortisone, clonidine and theophylline) were detected. According to the reference[35], the compounds can be classified:

"CNS+" (high BBB permeation): P_e (10^{-6} cm s^{-1}) > 4.0;

"CNS−" (low BBB permeation): P_e (10^{-6} cm s^{-1}) < 2.0;

"CNS±" (BBB permeation uncertain): 2.0 < P_e (10^{-6} cm s^{-1}) < 4.0.

From the results in **Table 3**, P_e values of control drugs are in accord with the reference[35, 36], which indicates that the assay used by us is reliable. From the results in **Table 3** and the ranges above, it could be concluded that compounds that had larger polarity group such as NO_2 (**15**, **16**) or NH_2 (**23**, **28**, **32**, **42**) might have low BBB permeability. In addition, we could find out that compound **12**, **31** and **41** possessed great predicted BBB permeability. However, the predicted permeabilities of other compounds were uncertain or low.

The cytotoxicities of the sixteen active compounds and positive control (Staurosporine) towards HEK293 cells were measured, and the results were listed in **Table 3**. Almost all the CC_{50} values were higher than 50 μM, suggesting these BACE-1 inhibitors had low significant cytotoxic effects.

From all the data above (**Table 3.**), **42** had the most favorable activity towards BACE-1 and low cytotoxicity, but it showed poor predicted BBB permeability and high molecular weight. Compound **41** had similar high activity (IC_{50} = 4.6 μM) and affinity (K_D = 0.213 μM), low cytotoxicity (CC_{50} < 50 μM) compared with **42**, it also showed greater BBB permeability and lower molecular weight (MW = 472.52), which made it the most potent compound.

5. Conclusions

Guided by docking study, a series of 2-substituted-thio-*N*-(4-substituted-thiazol/1*H*-imidazol-2-yl) acetamide derivatives were designed, synthesized and evaluated as BACE-1 inhibitors in enzymatic assay, SPR affinity assay, *in vitro* BBB permeability assay and cytotoxicity assay. Sixteen compounds were found to have IC_{50}s at μM level and K_Ds at μM to sub-μM level, and compound **41** exhibited optimized drug-like profiles with the relatively low IC_{50} of 4.6 μM, low molecular weight of 472, high predicted BBB permeability and low toxicity towards HEK293 cells, making it a good lead structure for further modification.

6. Experimental section

6.1. General chemical methods

Melting points were measured with an X4 apparatus and were uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker (Bruker BioSpin AG, Fällanden, Switzerland) Avance III 400 MHz system. Chemical shifts were reported in parts permillion (ppm) relative to tetramethylsilane (TMS) as internal standard. The spin multiplicities were given as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) or br (broad). MS and high-resolution mass spectra (HRMS) were obtained using Electrospray Ionization (ESI) technique on a Bruker's Fourier Transform Ion Cyclotron resonance Mass Spectrometer. Thin layer chromatography (TLC) analysis was performed on silica gel GF254 purchased from Qingdao Haiyang Chemical Co. (Qingdao, Shandong Province, China) or Merck (Darmstadt, Germany).

*6.1.1. 6-(3-methoxyphenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (**4a**)*

To a vial was added thiourea (0.83 g, 11 mmol), ethyl cyanoacetate (1.13 g, 11 mmol), 3-methoxybenzaldehyde (1.36 g, 10 mmol), K_2CO_3 (1.52 g, 11 mmol) and EtOH (10 mL). The mixture was stirring at 80 °C for 6 h. After that, the

reaction mixtures were poured into ice-water, then was adjusted to pH = 3~5 with 1 mol/L HCl. The precipitate was filtered off, dried and then purified by column chromatography on silica gel to give **4a** as a yellowish solid (0.91 g, 35% yield). m.p. 232-233 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.29 (s, 1H), 13.18 (s, 1H), 7.49 (t, *J* = 7.5 Hz, 1H), 7.19 – 7.26 (m, 3H), 3.83 (s, 3H); HRMS (ESI) *m/z* calcd for $C_{12}H_8N_3O_2S^-$ [M - H$^+$] 258.0343, found 258.0390.

*6.1.2. 6-(2-methoxyphenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (**4b**)*

The procedure described for **4a** was used, starting from thiourea (0.83 g, 11 mmol), ethyl cyanoacetate (1.13 g, 11 mmol), 2-methoxybenzaldehyde (1.36 g, 10 mmol) and K_2CO_3 (1.52 g, 11 mmol), and **4b** was obtained as a yellowish solid (0.54g, 21% yield). m.p. 241-242 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.26 (s, 1H), 13.17 (s, 1H), 7.58 (ddd, *J* = 8.5, 7.4, 1.7 Hz, 1H), 7.47 (dd, *J* = 7.6, 1.7 Hz, 1H), 7.21 (d, *J* = 8.3 Hz, 1H), 7.10 (td, *J* = 7.5, 0.9 Hz, 1H), 3.85 (s, 3H). MS (ESI) *m/z* calcd for $C_{12}H_8N_3O_2S^-$ [M - H$^+$] 258.03, found 258.18.

*6.1.3. 6-(4-methoxyphenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (**4c**)*

The procedure described for **4a** was used, starting from thiourea (0.83 g, 11 mmol), ethyl cyanoacetate (1.13 g, 11 mmol), 4-methoxybenzaldehyde (1.36 g, 10 mmol) and K_2CO_3 (1.52 g, 11 mmol), and **4c** was obtained as a yellowish solid (0.90 g, 35% yield). m.p. 254-255 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.19 (s, 1H), 13.11 (s, 1H), 7.66 (d, *J* = 8.8 Hz, 2H), 7.12 (d, *J* = 8.8 Hz, 2H), 3.85 (s, 3H). ^{13}C NMR (100MHz, DMSO-d_6): δ 176.64, 162.82, 160.92, 159.11, 131.34, 121.51, 115.53, 114.34, 90.26, 56.05. MS (ESI) *m/z* calcd for $C_{12}H_8N_3O_2S^-$ [M - H$^+$] 258.03, found 258.10.

*6.1.4. 6-(3-ethoxyphenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (**4d**)*

The procedure described for **4a** was used, starting from thiourea (0.83 g, 11 mmol), ethyl cyanoacetate (1.13 g, 11 mmol), 3-ethoxybenzaldehyde (1.50 g, 10 mmol) and K_2CO_3 (1.52 g, 11 mmol), and **4d** was obtained as a yellowish solid (0.93 g, 34% yield). m.p. 210-211 °C; ^{1}H NMR (400 MHz, DMSO-*d*6): δ 13.27 (s, 1H), 13.18 (s, 1H), 7.47 (t, *J* = 7.5 Hz, 1H), 7.17 – 7.24 (m, 3H), 4.11 (q, *J* = 7.5 Hz, 2H), 1.37 (t, *J* = 7.5 Hz, 3H); MS (ESI) *m/z* calcd for $C_{13}H_{10}N_3O_2S^-$ [M - H$^+$] 272.05, found 272.13.

*6.1.5. 4-oxo-2-thioxo-6-(3-(trifluoromethyl)phenyl)-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (**4e**)*

The procedure described for **4a** was used, starting from thiourea (0.83 g, 11 mmol), ethyl cyanoacetate (1.13 g, 11 mmol), 3-(trifluoromethyl)benzaldehyde (1.74 g, 10 mmol) and K_2CO_3 (1.52 g, 11 mmol), and **4e** was obtained as a yellow solid (1.66 g, 56% yield). m.p. 224-225 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.47 (s, 1H), 13.26 (s, 1H), 8.08 (s, 1H), 8.04 – 7.91 (m, 2H), 7.82 (t, *J* = 7.9 Hz, 1H); MS (ESI) *m/z* calcd for $C_{12}H_6F_3N_3OS^-$ [M - H$^+$] 296.01, found 296.06.

*6.1.6. 4-oxo-2-thioxo-6-(3-(trifluoromethoxy)phenyl)-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (**4f**)*

The procedure described for **4a** was used, starting from thiourea (0.83 g, 11 mmol), ethyl cyanoacetate (1.13 g, 11 mmol), 3-(trifluoromethoxy)benzaldehyde (1.90 g, 10 mmol) and K_2CO_3 (1.52 g, 11 mmol), and **4f** was obtained as a yellow solid (1.28 g, 41% yield). m.p. 189-190 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.44 (s, 1H), 13.23 (s, 1H), 7.77 – 7.69 (m, 3H), 7.69 – 7.61 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 176.59, 159.73, 158.73, 148.28, 131.77, 131.23, 128.46, 124.92, 124.32, 122.04, 120.49 (q, *J* = 256 Hz), 114.77, 91.97; MS (ESI) *m/z* calcd for $C_{12}H_6F_3N_3O_2S^-$ [M - H$^+$] 312.01, found 312.03.

*6.1.7. 1-(4-amino-3,5-dichlorophenyl)-2-bromoethanone (**6g**)*

To a vial was added chloroform (120 mL) and 1-(4-amino-3,5-*d*ichlorophenyl)ethanone (6.12 g, 30 mmol). The mixtures were heated to reflux and then bromine (2.04 ml, 39 mmol) was slowly added. The mixture was stirred for 15 min and then cooled to room temperature. The solvent was evaporated to afford the crude product. Then crude product was dissolved in THF (120 ml), and cooled to 0 °C. To the solution was slowly added a solution of diethylphosphite (0.51 ml, 4 mmol) and triethylamine (TEA, 3.3 ml, 24 mmol) in THF (30 ml). The mixture was warmed to room temperature and stirred for 10 min. After completion of the reaction, the mixture was concentrated in vacuo and poured into crushed ice. The precipitate was filtered out, washed with water and air-dried to give the pure product **6g** as light brown solid (6.78 g, 80% yield). m.p. 140-141 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.88 (s, 2H), 6.60 (s, 2H), 4.79 (s, 2H); MS (ESI) *m/z* calcd for $C_8H_7BrCl_2NO^+$ [M + H$^+$] 281.91, found 281.88.

*6.1.8. 4-phenylthiazol-2-amine (**7a**)*

To a solution of thiourea (1.67 g, 22 mmol) in water (100 ml) was added 2-bromo-1-phenylethanone (3.98 g, 20 mmol). The mixture was stirred at room temperature for 2h. After the completion of the reaction, the mixture was filtered to obtain the crude solid product. The crude product was further purified by recrystalization using EtOH/H_2O to afford the pure product **7a** as white acicular crystal (3.20 g, 91% yield). m.p. 150-151 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.61 (s, 2H), 7.82 – 7.57 (m, 2H), 7.57 – 7.28 (m, 3H), 7.22 (s, 1H); HRMS (ESI) *m/z* calcd for $C_9H_9N_2S^+$ [M + H$^+$] 177.0481, found 177.1929.

*6.1.9. 4-(4-nitrophenyl)thiazol-2-amine (**7b**)*

The procedure described for **7a** was used, starting from 2-bromo-1-(4-nitrophenyl)ethanone (4.88 g, 20 mmol) and thiourea (1.67 g, 22 mmol), the recrystalization condition was DMF/H_2O, and **7b** was obtained as an orange solid (4.11 g, 93% yield). m.p. 285-286 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.23 (d, *J* = 9.0 Hz, 2H), 8.05 (d, *J* = 8.9 Hz, 2H), 7.41 (s, 1H), 7.22 (s, 2H); MS (ESI) *m/z* calcd for $C_9H_8N_3O_2S^+$ [M + H$^+$] 222.03, found 222.10.

*6.1.10. 4-(3-nitrophenyl)thiazol-2-amine (**7c**)*

The procedure described for **7b** was used, starting from 2-bromo-1-(3-nitrophenyl)ethanone (4.88 g, 20 mmol) and thiourea (1.67 g, 22 mmol), and **7c** was obtained as yellow acicular crystal (3.71 g, 84% yield). m.p. 189-190 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.61 (t, *J* = 2.0 Hz, 1H), 8.24 (ddd, *J* = 7.8, 1.7, 1.0 Hz, 1H), 8.11 (ddd, *J* = 8.2, 2.4, 1.0 Hz, 1H), 7.66 (t, *J* = 8.0 Hz, 1H), 7.34 (s, 1H), 7.23 (s, 2H); ^{13}CNMR (100 MHz, DMSO-*d*6): δ 168.58, 148.21, 147.37, 136.38, 131.49, 130.03, 121.64, 119.92, 104.23; MS (ESI) *m/z* calcd for $C_9H_8N_3O_2S^+$ [M + H$^+$] 222.03, found 222.10.

*6.1.11. 4-(2-nitrophenyl)thiazol-2-amine (**7d**)*

The procedure described for **7a** was used, starting from 2-bromo-1-(2-nitrophenyl)ethanone (4.88 g, 20 mmol) and thiourea (1.67 g, 22 mmol), and **7d** was obtained as orange acicular crystal (4.07 g, 92% yield). m.p. 105-106 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.78 (dd, *J* = 7.5, 1.6 Hz, 1H), 7.73 (dd, *J* = 7.5, 1.6 Hz, 1H), 7.66 (dt, *J* = 7.5, 1.6 Hz, 1H), 7.53 (dt, *J* = 7.5, 1.6 Hz, 1H), 7.07 (s, 2H), 6.91 (s, 1H); MS (ESI) *m/z* calcd for $C_9H_8N_3O_2S^+$ [M + H$^+$] 222.03, found 222.10.

*6.1.12. 4-(p-tolyl)thiazol-2-amine (**7e**)*

The procedure described for **7a** was used, starting from 2-bromo-1-(p-tolyl)ethanone (4.26 g, 20 mmol) and thiourea (1.67 g, 22 mmol), and **7e** was obtained as white acicular crystal (3.43 g, 90% yield). m.p. 124-125 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.70 (s, 2H), 7.63 (d, *J* = 8.2 Hz, 2H), 7.30 (d, *J* = 8.1 Hz, 2H), 7.15 (s, 1H), 2.35 (s, 3H); MS (ESI) *m/z* calcd for $C_{10}H_{11}N_2S^+$ [M + H$^+$] 191.06, found 191.11.

*6.1.13. 4-(4-methoxyphenyl)thiazol-2-amine (**7f**)*

The procedure described for **7a** was used, starting from 2-bromo-1-(4-methoxyphenyl)ethanone (4.58 g, 20 mmol) and thiourea (1.67 g, 22 mmol), and **7f** was obtained as white acicular crystal (2.64 g, 64% yield). m.p. 206-207 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.67 (s, 2H), 7.67 (d, *J* = 8.8 Hz, 2H), 7.11 – 7.00 (m, 3H), 3.81 (s, 3H); MS (ESI) *m/z* calcd for $C_{10}H_{11}N_2OS^+$ [M + H$^+$] 207.06, found 207.10.

*6.1.14. 4-(4-amino-3,5-dichlorophenyl)thiazol-2-amine (**7g**)*

The procedure described for **7a** was used, starting from 1-(4-amino-3,5-dichlorophenyl)-2-bromoethanone (**6g**, 5.66 g, 20 mmol) and thiourea (1.67 g, 22 mmol), and **7g** was obtained as a light brown solid (3.95 g, 76% yield). m.p. 192-193 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.80 (s, 2H), 7.68 (s, 2H), 7.14 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.34, 142.39, 125.94, 118.56, 101.09; MS (ESI) *m/z* calcd for $C_9H_8Cl_2N_3S^+$ [M + H$^+$] 259.98, found 259.98.

*6.1.15. 4-(3-bromophenyl)thiazol-2-amine (**7i**)*

The procedure described for **7a** was used, starting from 2-bromo-1-(3-bromophenyl)ethanone (5.66 g, 20 mmol) and thiourea (1.67 g, 22 mmol), and **7i** was obtained as white acicular crystal (4.59 g, 90% yield). m.p. 116-118 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.46 (s, 2H), 7.97 (s, 1H), 7.76 (d, *J* = 7.8 Hz, 1H), 7.61 (d, *J* = 7.9 Hz, 1H), 7.44 (t, *J* = 7.9 Hz, 1H), 7.38 (s, 1H); MS (ESI) *m/z* calcd for $C_9H_8BrN_2S^+$ [M + H$^+$] 254.96, found 254.95.

*6.1.16. 4-(3-(2-fluoropyridin-3-yl)phenyl)thiazol-2-amine (**7j**)*

To a schlenk flask was added **7i** (2.55 g, 10 mmol), (2-fluoropyridin-3-yl)boronic acid (1.97 g, 14 mmol), $Pd(PPh_3)_4$ (1.16 g, 1 mmol) and Na_2CO_3 (2.12 g, 20 mmol). The flask was evacuated and backfilled with argon (×3). Then DMF (50 ml) and H_2O (2 ml) was added afterwaards. The mixture was stirred at 120 °C for 24 h. After completion of the reaction, the mixture was diluted with ethyl acetate and then washed with saturated Na_2CO_3 and brine. The organic layer was collected and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and the residual mass was purified by column chromatography to obtain **7j** as white solid (1.36 g, 50% yield). m.p. 153-154 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.27 (dt, *J* = 4.7, 1.5 Hz, 1H), 8.21 – 8.10 (m, 1H), 8.05 (s, 1H), 7.93 – 7.83 (m, 1H), 7.54 – 7.43 (m, 3H), 7.14 (s, 1H), 7.12 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.76, 160.12 (d, *J* = 235 Hz), 149.73, 147.00 (d, *J* = 15 Hz), 141.93 (d, *J* = 4 Hz), 135.86, 134.15 (d, *J* = 5 Hz), 129.44, 127.97, 126.44, 125.99, 123.47 (d, *J* = 28 Hz), 123.16 (d, *J* = 4 Hz), 102.77; MS (ESI) *m/z* calcd for $C_{14}H_{11}FN_3S^+$ [M + H$^+$] 272.07, found 272.21.

*6.1.17. 2-chloro-N-(4-phenylthiazol-2-yl)acetamide (**8a**)*

To a vial was added **7a** (1.76 g, 10 mmol) and DIPEA (2.3 ml, 13 mmol). The mixture was stirred at 0 °C in CH_2Cl_2 (50 ml), then a solution of chloroacetic chloride (0.83 ml, 11 mmol) in CH_2Cl_2 (10 mL) was added to the mixture slowly. The mixture was then warm to room temperature and stirred for 1 h. After completion of the reaction, the mixture was diluted with CH_2Cl_2 and washed thrice with saturated NaCl. The organic layer was dried over anhydrous sodium sulfate, the solvent was removed under reduced pressure and the residual mass was purified by column chromatography to obtain **8a** as white solid (2.25 g, 89% yield). m.p. 143-144 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.66 (s, 1H), 7.94 – 7.87 (m, 2H), 7.70 (s, 1H), 7.48 – 7.39 (m, 2H), 7.37 – 7.29 (m, 1H), 4.42 (s, 2H); MS (ESI) *m/z* calcd for $C_{11}H_{10}ClN_2OS^+$ [M + H$^+$] 253.02, found 253.00.

*6.1.18. 2-chloro-N-(4-(4-nitrophenyl)thiazol-2-yl)acetamide (**8b**)*

The procedure described for **8a** was used, starting from **7b** (2.21 g, 10 mmol) and TEA (2.8 ml, 15 mmol), and **8b** was obtained as yellow solid (1.67 g, 56% yield). m.p. 211-212 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.77 (s, 1H), 8.31 (d, *J* = 8.8 Hz, 2H), 8.16 (d, *J* = 8.8 Hz, 2H), 8.06 (s, 1H), 4.44 (s, 2H); MS (ESI) *m/z* calcd for $C_{11}H_7ClN_3O_3S^-$ [M - H$^+$] 295.99, found 296.03.

*6.1.19. 2-chloro-N-(4-(3-nitrophenyl)thiazol-2-yl)acetamide (**8c**)*

The procedure described for **8a** was used, starting from **7c** (2.21 g, 10 mmol) and TEA (2.8 ml, 15 mmol), and **8c** was obtained as yellow solid (1.73 g, 58% yield). m.p. 215-217 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.77 (s, 1H), 8.74 (s, 1H), 8.36 (d, *J* = 7.5 Hz, 1H), 8.19 (d, *J* = 7.5 Hz, 1H), 8.02 (s, 1H), 7.75 (t, *J* = 7.5 Hz, 1H), 4.44 (s, 2H); MS (ESI) *m/z* calcd for $C_{11}H_7ClN_3O_3S^-$ [M - H$^+$] 295.99, found 296.03.

*6.1.20. 2-chloro-N-(4-(2-nitrophenyl)thiazol-2-yl)acetamide (**8d**)*

The procedure described for **8a** was used, starting from **7d** (2.21 g, 10 mmol) and TEA (2.8 ml, 15 mmol), and **8d** was obtained as yellowish solid (1.52 g, 51% yield). m.p. 140-141 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.57 (s, 1H), 7.89 (dd, *J* = 8.0, 1.3 Hz, 1H), 7.80 (dd, *J* = 7.7, 1.5 Hz, 1H), 7.74 (td, *J* = 7.5, 1.3 Hz, 1H), 7.63 (s, 1H), 7.63 – 7.58 (m, 1H), 4.42 (s, 2H); MS (ESI) *m/z* calcd for $C_{11}H_9ClN_3O_3S^+$ [M + H$^+$] 298.00, found 298.05.

*6.1.21. 2-chloro-N-(4-(p-tolyl)thiazol-2-yl)acetamide (**8e**)*

The procedure described for **8a** was used, starting from **7e** (1.90 g, 10 mmol) and DIPEA(2.3 ml, 13 mmol), and **8e** was obtained as white solid (1.68 g, 63% yield). m.p. 157-158 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.62 (s, 1H),

7.79 (d, *J* = 8.1 Hz, 2H), 7.61 (s, 1H), 7.25 (d, *J* = 7.9 Hz, 2H), 4.41 (s, 2H), 2.33 (s, 3H); MS (ESI) *m/z* calcd for $C_{12}H_{12}ClN_2OS^+$ [M + H^+] 267.04, found 267.03.

*6.1.22. 2-chloro-N-(4-(4-methoxyphenyl)thiazol-2-yl)acetamide (**8f**)*

The procedure described for **8a** was used, starting from **7f** (2.06 g, 10 mmol) and DIPEA (2.3 ml, 13 mmol), and **8f** was obtained as white solid (1.70 g, 60% yield). m.p. 161-162 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.60 (s, 1H), 7.83 (d, *J* = 8.8 Hz, 2H), 7.53 (s, 1H), 7.00 (d, *J* = 8.8 Hz, 2H), 4.41 (s, 2H), 3.79 (s, 3H); MS (ESI) *m/z* calcd for $C_{12}H_{10}ClN_2O_2S^-$ [M - H^+] 281.02, found 281.16.

*6.1.23. N-(4-(4-amino-3,5-dichlorophenyl)thiazol-2-yl)-2-chloroacetamide (**8g**)*

The procedure described for **8a** was used, starting from **7g** (2.60 g, 10 mmol) and DIPEA (2.3 ml, 13 mmol), and **8g** was obtained as light brown solid (2.05 g, 61% yield). m.p. 208-209 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.57 (s, 1H), 7.78 (s, 2H), 7.60 (s, 1H), 5.68 (s, 2H), 4.40 (s, 2H); MS (ESI) *m/z* calcd for $C_{11}H_7Cl_3N_3OS^-$ [M - H^+] 333.94, found 333.90.

*6.1.24. N-(4-(4-acetamido-3,5-dichlorophenyl)thiazol-2-yl)-2-chloroacetamide (**8h**)*

To a solution of **8g** (1.68 g, 5 mmol) in AcOH (5 ml) was added acetyl chloride (430 mg, 5.5 mmol). The mixture was stirred at 90 °C for 20min. After completion of the reaction, the mixture was pured into ice water, and the crude product was precipitated. Then the solid was filtered out and air-dried. The crude product was purified by column chromatography to obtain **8h** as light brown solid (1.65 g, 87% yield). m.p. 258-259 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.69 (s, 1H), 9.87 (s, 1H), 8.03 (s, 2H), 7.97 (s, 1H), 4.43 (s, 2H), 2.09 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.72, 165.79, 158.31, 146.28, 135.14, 134.55, 132.81, 125.64, 111.73, 42.74, 22.89; MS (ESI) *m/z* calcd for $C_{13}H_9Cl_3N_3O_2S^-$ [M - H^+] 375.95, found 375.96.

*6.1.25. N-(4-(3-bromophenyl)thiazol-2-yl)-2-chloroacetamide (**8i**)*

The procedure described for **8a** was used, starting from **7i** (2.55 g, 10 mmol) and DIPEA (2.3 ml, 13 mmol), and **8i** was obtained as white solid (2.69 g, 81% yield). m.p. 147-148 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.66 (s, 1H), 8.10 (t, *J* = 1.9 Hz, 1H), 7.90 (dt, *J* = 7.8, 1.3 Hz, 1H), 7.83 (s, 1H), 7.52 (ddd, *J* = 8.1, 2.1, 1.1 Hz, 1H), 7.40 (t, *J* = 7.9 Hz, 1H), 4.42 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.68, 158.10, 147.79, 136.81, 131.41, 130.95, 128.78, 125.02, 122.66, 110.52, 42.75; MS (ESI) *m/z* calcd for $C_{11}H_9BrClN_2OS^+$ [M + H^+] 330.93, found 331.00.

*6.1.26. 2-chloro-N-(4-(3-(2-fluoropyridin-3-yl)phenyl)thiazol-2-yl)acetamide (**8j**)*

The procedure described for **8a** was used, starting from **7j** (2.71 g, 10 mmol) and DIPEA (2.3 ml, 13 mmol), and **8j** was obtained as white solid (2.47 g, 71% yield). m.p. 187-188 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.66 (s, 1H), 8.28 (d, *J* = 4.7 Hz, 1H), 8.24 – 8.10 (m, 2H), 7.99 (t, *J* = 3.5 Hz, 1H), 7.83 (s, 1H), 7.64 – 7.55 (m, 2H), 7.55 – 7.46 (m, 1H), 4.43 (s, 2H); MS (ESI) *m/z* calcd for $C_{16}H_{12}ClFN_3OS^+$ [M + H^+] 348.04, found 348.01.

*6.1.27. N-(4-phenyl-1H-imidazol-2-yl)acetamide (**9a**)*

To a vial was added 2-bromo-1-phenylethanone (3.98 g, 20 mmol), *N*-acetylguanidine (6.06 g, 60 mmol) and CH_3CN (500 ml). The mixture was stirred at 100 °C for 3h. After completion of the reaction, the solution was cooled to room temperature and the solvent was removed to obtain the crude product. The pure product **9a** was obtained through column chromatography as white solid (2.57 g, 64% yield). m.p. 218-219 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.63 (s, 1H), 11.23 (s, 1H), 7.71 (d, *J* = 7.6 Hz, 2H), 7.32 (t, *J* = 7.6 Hz, 2H), 7.25 (s, 1H), 7.16 (t, *J* = 7.3 Hz, 1H), 2.07 (s, 3H); MS (ESI) *m/z* calcd for $C_{11}H_{10}N_3O^-$ [M - H^+] 200.08, found 200.19.

*6.1.28. N-(4-(4-amino-3,5-dichlorophenyl)-1H-imidazol-2-yl)acetamide (**9g**)*

The procedure described for **9a** was used, starting from **6g** (5.66 g, 20 mmol) and *N*-acetylguanidine (6.06 g, 60 mmol), **9g** was obtained as yellow solid (4.62 g, 81% yield). m.p. 188-189 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.62 (s, 1H), 11.18 (s, 1H), 7.59 (s, 2H), 7.21 (s, 1H), 5.40 (s, 2H), 2.06 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ =168.94, 141.54, 139.52, 134.26, 124.76, 124.04, 118.95, 109.45, 23.25; MS (ESI) *m/z* calcd for $C_{11}H_{11}Cl_2N_4O^+$ [M + H^+] 285.03, found 285.11.

*6.1.29. 2-chloro-N-(4-phenyl-1H-imidazol-2-yl)acetamide (**10a**)*

To a 25 mL mixture of a 1:1 MeOH:H_2O containing 20% H_2SO_4 was added **9a** (1.01 g, 5 mmol). The mixture was heated at 100 °C for 1 h. The mixture was then concentrated under reduced pressure, and the white sulfate salt was crystallized from solution, then the solid was filtered out, washed with MeOH and air-dried. After that, the solid was added into a mixture of CH_2Cl_2 (20 ml) and TEA (2.8 ml, 15 mmol). After that, a solution of chloroacetic chloride (0.83 ml, 5 mmol) in CH_2Cl_2 (5 mL) was added into the above mixture slowly under ice bath. The mixture was stirred for 1 h under ice bath. After the reaction, the mixture was diluted with CH_2Cl_2 and then washed thrice with saturated NaCl. The organic layer was dried over anhydrous sodium sulfate, the solvent was removed under reduced pressure and the residual mass was purified by column chromatography to obtain **10a** as white solid (200 mg, 17% yield in total). m.p. 171-172 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.81 (s, 1H), 11.62 (s, 1H,), 7.72 (d, *J* = 7.5 Hz, 2H), 7.34 (d, *J* = 7.5 Hz, 2H), 7.32 (s, 1H), 7.18 (t, *J* = 7.5 Hz, 1H), 4.30 (s, 2H); MS (ESI) *m/z* calcd for $C_{11}H_{11}ClN_3O^+$ [M + H^+] 236.06, found 235.97.

*6.1.30. N-(4-(4-amino-3,5-dichlorophenyl)-1H-imidazol-2-yl)-2-chloroacetamide (**10g**)*

The procedure described for **10a** was used, starting from **9g** (1.43 g, 5 mmol), except that 40% HCl in 1:1 MeOH/H_2O was used as the solvent instead of 20% H_2SO_4, the **10g** was obtained as yellow solid (160 mg, 10% yield). m.p. 232-233 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.76 (s, 1H), 11.56 (s, 1H), 7.60 (s, 2H), 7.28 (s, 1H), 5.43 (s, 2H), 4.30 (s, 2H); MS (ESI) *m/z* calcd for $C_{11}H_{10}Cl_3N_4O^+$ [M + H^+] 318.99, found 318.98.

*6.1.31. 2-((5-cyano-4-(3-methoxyphenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(4-phenylthiazol-2-yl)acetamide (**12**)*

To a vial was added **4a** (52 mg, 0.2 mmol), **8a** (50 mg, 0.2 mmol) and KOH (13 mg, 0.22 mmol). The mixture was stirred in EtOH (5 mL) at 70 °C for 3 h. After the reaction, the mixture was purified by column chromatography and **12** was obtained as white solid (87 mg, 91% yield). m.p. 205-206 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.99 (s, 1H), 12.61 (s, 1H), 7.91 (d, *J* = 7.6 Hz, 2H), 7.64 (s, 1H), 7.45 (t, *J* = 7.6 Hz, 2H), 7.34 (d, *J* = 4.2 Hz, 3H), 7.20 (t, *J* = 7.9 Hz, 1H), 7.02 (d, *J* = 8.2 Hz, 1H), 4.32 (s, 2H), 3.64 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.43, 166.34, 166.01, 161.73, 159.46, 158.18, 149.44, 136.81, 134.74, 129.89, 129.22, 128.28, 126.13, 121.42, 117.62, 116.26, 114.40, 108.72, 93.82, 55.60, 34.82; HRMS (ESI) *m/z* calcd for $C_{23}H_{17}KN_5O_3S_2^+$ [M + K$^+$] 514.04044, found 514.04064; $C_{23}H_{17}N_5NaO_3S_2^+$ [M + Na$^+$] 498.06650, found 498.06647; $C_{23}H_{18}N_5O_3S_2^+$ [M + H$^+$] 476.08456, found 476.08431; HPLC 97.5% (R_t = 6.02 min, 50-80% CH_3OH in water for 7 min and 80% CH_3OH in water for 3 min, 10 min in total).

*6.1.32. 2-((5-cyano-4-(2-methoxyphenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(4-phenylthiazol-2-yl)acetamide (**13**)*

The procedure described for **12** was used, starting from **4b** (52 mg, 0.2 mmol), **8a** (50 mg, 0.2 mmol) and KOH (13 mg, 0.22 mmol), **13** was obtained as white solid (84 mg, 88% yield). m.p. 209-210 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.46 (s, 1H), 7.89 (d, *J* = 7.6 Hz, 2H), 7.64 (s, 1H), 7.44 (t, *J* = 7.6 Hz, 2H), 7.33 (t, *J* = 7.7 Hz, 2H), 7.19 (d, *J* = 7.5 Hz, 1H), 7.07 (d, *J* = 8.4 Hz, 1H), 6.79 (t, *J* = 7.4 Hz, 1H), 4.03 (s, 2H), 3.76 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.78, 169.09, 168.22, 166.98, 158.22, 156.73, 149.35, 134.75, 131.08, 130.12, 129.18, 128.24, 127.38, 126.15, 120.44, 119.07, 112.03, 108.49, 93.86, 55.77, 34.42; HRMS (ESI) *m/z* calcd for $C_{23}H_{17}KN_5O_3S_2^+$ [M + K$^+$] 514.04044, found 514.04096; $C_{23}H_{17}N_5NaO_3S_2^+$ [M + Na$^+$] 498.06650, found 498.06669; $C_{23}H_{18}N_5O_3S_2^+$ [M + H$^+$] 476.08456, found 476.08444; HPLC 98.2% (R_t = 4.98 min, 50-80% CH_3OH in water for 7 min and 80% CH_3OH in water for 3 min, 10 min in total).

*6.1.33. 2-((5-cyano-4-(4-methoxyphenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(4-phenylthiazol-2-yl)acetamide (**14**)*

The procedure described for **12** was used, starting from **4c** (52 mg, 0.2 mmol), **8a** (50 mg, 0.2 mmol) and KOH (13 mg, 0.22 mmol), **14** was obtained as white solid (88 mg, 93% yield). m.p. 216-217 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.80 (s, 1H), 12.63 (s, 1H), 7.99 – 7.86 (m, 2H), 7.82 (d, *J* = 8.9 Hz, 2H), 7.67 (s, 1H), 7.45 (t, *J* = 7.6 Hz, 2H), 7.35 (t, *J* = 7.4 Hz, 1H), 6.77 (d, *J* = 9.0 Hz, 2H), 4.26 (s, 2H), 3.55 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.97, 166.71, 163.89, 162.10, 158.33, 149.42, 134.69, 130.99, 129.21, 128.32, 128.13, 126.13, 117.54, 114.03, 108.71, 91.42, 55.49, 34.75; HRMS (ESI) *m/z* calcd for $C_{23}H_{17}KN_5O_3S_2^+$ [M + K$^+$] 514.04044, found 514.04092; $C_{23}H_{17}N_5NaO_3S_2^+$ [M + Na$^+$] 498.06650, found 498.06668; $C_{23}H_{18}N_5O_3S_2^+$ [M + H$^+$] 476.08456, found 476.08440; HPLC 98.2% (R_t = 5.47 min, 50-80% CH_3OH in water for 7 min and 80% CH_3OH in water for 3 min, 10 min in total).

*6.1.34. 2-((5-cyano-4-(2-methoxyphenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(4-(4-nitrophenyl)thiazol-2-yl)acetamide (**15**)*

The procedure described for **12** was used, starting from **4b** (52 mg, 0.2 mmol), **8b** (60 mg, 0.2 mmol) and KOH (13 mg, 0.22 mmol), **15** was obtained as yellowish solid (90 mg, 86% yield). m.p. 206-207 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.59 (s, 1H), 8.32 (d, *J* = 8.5 Hz, 2H), 8.15 (d, *J* = 8.6 Hz, 2H), 8.02 (s, 1H), 7.32 (t, *J* = 7.2 Hz, 1H), 7.17 (d, *J* = 6.6 Hz, 1H), 7.07 (d, *J* = 8.4 Hz, 1H), 6.78 (t, *J* = 7.5 Hz, 1H), 4.04 (s, 2H), 3.76 (s, 3H); ^{13}C NMR (100MHz, DMSO-d_6): δ 166.76, 166.72, 158.79, 156.77, 147.29, 146.98, 140.69, 132.75, 130.40, 127.00, 124.71, 120.39, 115.44, 113.14, 112.30, 97.04, 55.81, 34.78; HRMS (ESI) *m/z* calcd for $C_{23}H_{16}KN_6O_5S_2^+$ [M + K$^+$] 559.02552, found 559.01728; $C_{23}H_{16}N_6NaO_5S_2^+$ [M + Na$^+$] 543.05158, found 543.05260; $C_{23}H_{17}N_6O_5S_2^+$ [M + H$^+$] 521.06964, found 521.07068. HPLC 99.2% (R_t = 5.36 min, 50-80% CH_3OH in water for 7 min and 80% CH_3OH in water for 3 min, 10 min in total).

*6.1.35. 2-((5-cyano-4-(3-methoxyphenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(4-(4-nitrophenyl)thiazol-2-yl)acetamide (**16**)*

The procedure described for **12** was used, starting from **4a** (52 mg, 0.2 mmol), **8b** (60 mg, 0.2 mmol) and KOH (13 mg, 0.22 mmol), **16** was obtained as yellowish solid (94 mg, 90% yield). m.p. 187-188 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 14.00 (s, 1H) , 12.73 (s, 1H), 8.33 (d, *J* = 7.5 Hz, 2H) , 8.18 (d, *J* = 7.5 Hz, 2H), 8.02 (s, 1H) , 7.30-7.35 (m, 2H), 7.19 (t, *J* = 7.5 Hz, 1H), 7.01 (dd, *J* = 7.5, 1.5 Hz, 1H), 4.33 (s, 2H), 3.62 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.44, 167.02, 159.42, 158.79, 147.25, 146.96, 140.70, 137.24, 129.84, 126.99, 124.73, 121.28, 117.20, 116.97, 114.37, 113.17, 93.20, 55.59, 34.76; HRMS (ESI) *m/z* calcd for $C_{23}H_{16}KN_6O_5S_2^+$ [M + K$^+$] 559.02552, found 559.01886; $C_{23}H_{16}N_6NaO_5S_2^+$ [M + Na$^+$] 543.05158, found 543.05286; $C_{23}H_{17}N_6O_5S_2^+$ [M + H$^+$] 521.06964, found 521.07087. HPLC 95.2% (R_t = 5.88 min, 50-80% CH_3OH in water for 7 min and 80% CH_3OH in water for 3 min, 10 min in total).

*6.1.36. 2-((5-cyano-4-(4-methoxyphenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(4-(4-nitrophenyl)thiazol-2-yl)acetamide (**17**)*

The procedure described for **12** was used, starting from **4c** (52 mg, 0.2 mmol), **8b** (60 mg, 0.2 mmol) and KOH (13 mg, 0.22 mmol), **17** was obtained as yellowish solid (95 mg, 91% yield). m.p. 245-246 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.90 (s, 1H), 12.82 (s, 1H) , 8.34 (d, *J* = 7.5 Hz, 2H), 8.19 (d, *J* = 7.5 Hz, 2H), 8.05 (s, 1H) 7.83 (t, *J* = 7.5 Hz, 2H), 6.76 (d, *J* = 7.5 Hz, 2H), 4.32 (s, 2H), 3.55 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.80, 165.18, 162.43, 158.90, 147.24, 146.99, 140.63, 131.19, 127.48, 126.99, 124.72, 116.57, 114.09, 113.28, 92.18, 55.57, 34.83; HRMS (ESI) *m/z* calcd for $C_{23}H_{16}KN_6O_5S_2^+$ [M + K$^+$] 559.02552, found 559.02596. HPLC 95.5% (R_t = 5.96 min, 50-80% CH_3OH in water for 7 min and 80% CH_3OH in water for 3 min, 10 min in total).

*6.1.37. 2-((5-cyano-4-(2-methoxyphenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(4-(3-nitrophenyl)thiazol-2-yl)acetamide (**18**)*

The procedure described for **12** was used, starting from **4b** (52 mg, 0.2 mmol), **8c** (60 mg, 0.2 mmol) and KOH (13 mg, 0.22 mmol), **18** was obtained as yellowish solid (91 mg, 87% yield). m.p. 192-193 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.56 (s, 1H), 8.72 (t, *J* = 2.0 Hz, 1H), 8.35 (dd, *J* = 7.9, 1.3 Hz, 1H), 8.19 (ddd, *J* = 8.2, 2.4, 1.0 Hz, 1H), 7.97 (s, 1H), 7.76 (t, *J* = 8.0 Hz, 1H), 7.31 (td, *J* = 8.1,

7.4, 1.8 Hz, 1H), 7.15 (dd, *J* = 7.5, 1.8 Hz, 1H), 7.06 (d, *J* = 8.3 Hz, 1H), 6.78 (t, *J* = 7.4 Hz, 1H), 4.02 (s, 2H), 3.75 (s, 3H); ^{13}C NMR (100MHz, DMSO-d_6): δ 166.68, 166.07, 158.70, 156.77, 148.83, 147.02, 136.26, 132.76, 132.23, 130.89, 130.41, 122.83, 120.58, 120.41, 115.41, 112.29, 111.26, 97.09, 55.81, 34.78; HRMS (ESI) *m/z* calcd for $C_{23}H_{16}KN_6O_5S_2^+$ [M + K^+] 559.02552, found 559.02312; $C_{23}H_{16}N_6NaO_5S_2^+$ [M + Na^+] 543.05158, found 543.05288; $C_{23}H_{17}N_6O_5S_2^+$ [M + H^+] 521.06964, found 521.07094. HPLC 96.2% (R_t = 4.94 min, 50-80% CH_3OH in water for 7 min and 80% CH_3OH in water for 3 min, 10 min in total).

*6.1.38. 2-((5-cyano-4-(3-methoxyphenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(4-(3-nitrophenyl)thiazol-2-yl)acetamide (**19**)*

The procedure described for **12** was used, starting from **4a** (52 mg, 0.2 mmol), **8c** (60 mg, 0.2 mmol) and KOH (13 mg, 0.22 mmol), **19** was obtained as yellowish solid (96 mg, 92% yield). m.p. 226-227 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.97 (s, 1H), 12.72 (s, 1H), 8.75 (t, *J* = 2.0 Hz, 1H), 8.37 (dt, *J* = 8.0, 1.3 Hz, 1H), 8.25 – 8.15 (m, 1H), 7.97 (s, 1H), 7.76 (t, *J* = 8.0 Hz, 1H), 7.39 – 7.28 (m, 2H), 7.19 (t, *J* = 7.9 Hz, 1H), 7.05 – 6.95 (m, 1H), 4.33 (s, 2H), 3.63 (s, 3H); ^{13}C NMR (100MHz, DMSO-d_6): δ 167.53, 166.54, 159.44, 158.68, 148.85, 146.99, 136.75, 136.23, 132.20, 130.91, 129.91, 122.83, 121.38, 120.56, 117.56, 116.14, 114.46, 111.30, 93.99, 55.60, 34.83; HRMS (ESI) *m/z* calcd for $C_{23}H_{16}KN_6O_5S_2^+$ [M + K^+] 559.02552, found 559.02623; $C_{23}H_{17}N_6O_5S_2^+$ [M + H^+] 521.06964, found 521.07068.

*6.1.39. 2-((5-cyano-4-(4-methoxyphenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(4-(3-nitrophenyl)thiazol-2-yl)acetamide (**20**)*

The procedure described for **12** was used, starting from **4c** (52 mg, 0.2 mmol), **8c** (60 mg, 0.2 mmol) and KOH (13 mg, 0.22 mmol), **20** was obtained as yellowish solid (98 mg, 94% yield). m.p. 224-225 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.86 (s, 1H), 12.77 (s, 1H), 8.76 (t, *J* = 2.0 Hz, 1H), 8.38 (d, *J* = 7.9 Hz, 1H), 8.27 – 8.13 (m, 1H), 7.98 (s, 1H), 7.82 (d, *J* = 8.9 Hz, 2H), 7.76 (t, *J* = 8.0 Hz, 1H), 6.76 (d, *J* = 8.7 Hz, 2H), 4.30 (s, 2H), 3.53 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.71, 165.17, 162.41, 161.51, 158.81, 148.83, 146.96, 136.17, 132.20, 131.18, 130.92, 127.50, 122.86, 120.53, 116.55, 114.09, 111.39, 92.23, 55.50, 34.83; HRMS (ESI) *m/z* calcd for $C_{23}H_{16}KN_6O_5S_2^+$ [M + K^+] 559.02552, found 559.02678; $C_{23}H_{16}N_6NaO_5S_2^+$ [M + Na^+] 543.05158, found 543.05237; $C_{23}H_{17}N_6O_5S_2^+$ [M + H^+] 521.06964, found 521.07053. HPLC 96.6% (R_t = 5.72 min, 50-80% CH_3OH in water for 7 min and 80% CH_3OH in water for 3 min, 10 min in total).

*6.1.40. 2-((5-cyano-4-(3-methoxyphenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(4-(2-nitrophenyl)thiazol-2-yl)acetamide (**21**)*

The procedure described for **12** was used, starting from **4a** (52 mg, 0.2 mmol), **8d** (60 mg, 0.2 mmol) and KOH (13 mg, 0.22 mmol), **21** was obtained as yellowish solid (84 mg, 81% yield). m.p. 231-232 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.96 (s, 1H), 12.54 (s, 1H), 7.90 (d, *J* = 8.0 Hz, 1H), 7.81 (d, *J* = 7.7 Hz, 1H), 7.76 (t, *J* = 7.5 Hz, 1H), 7.62 (t, *J* = 7.9 Hz, 1H), 7.58 (s, 1H), 7.41 – 7.28 (m, 2H), 7.23 (t, *J* = 8.0 Hz, 1H), 7.09 – 7.00 (m, 1H), 4.32 (s, 2H), 3.67 (s, 3H); ^{13}C NMR (100MHz, DMSO-d_6): δ 167.41, 166.45, 165.76, 161.45, 159.44, 158.24, 149.07, 145.50, 136.71, 132.98, 131.27, 130.00, 129.77, 128.63, 124.45, 121.42, 117.89, 116.13, 114.22, 112.70, 93.86, 55.61, 34.81; HRMS (ESI) *m/z* calcd for $C_{23}H_{16}KN_6O_5S_2^+$ [M + K^+] 559.02552, found 559.02704; $C_{23}H_{16}N_6NaO_5S_2^+$ [M + Na^+] 543.05158, found 543.05289; $C_{23}H_{17}N_6O_5S_2^+$ [M + H^+] 521.06964, found 521.07087; HPLC 92.0% (R_t = 4.11 min, 50-80% CH_3OH in water for 7 min and 80% CH_3OH in water for 3 min, 10 min in total).

*6.1.41. 2-((5-cyano-4-(3-ethoxyphenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(4-(4-nitrophenyl)thiazol-2-yl)acetamide (**22**)*

The procedure described for **12** was used, starting from **4d** (55 mg, 0.2 mmol), **8b** (60 mg, 0.2 mmol) and KOH (13 mg, 0.22 mmol), **22** was obtained as yellowish solid (92 mg, 86% yield). m.p. 220-221 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 14.02 (s, 1H), 12.74 (s, 1H), 8.33 (d, *J* = 8.7 Hz, 2H), 8.17 (d, *J* = 8.7 Hz, 2H), 8.04 (s, 1H), 7.32 (d, *J* = 7.9 Hz, 1H), 7.28 (t, *J* = 2.1 Hz, 1H), 7.17 (t, *J* = 8.0 Hz, 1H), 7.00 (dd, *J* = 8.3, 2.5 Hz, 1H), 4.33 (s, 2H), 3.89 (q, *J* = 6.9 Hz, 2H), 1.17 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (100MHz, DMSO-d_6): δ 167.60, 166.60, 165.91, 161.64, 158.82, 158.71, 147.30, 147.00, 140.69, 136.82, 129.89, 127.00, 124.75, 121.34, 118.44, 116.20, 114.40, 113.25, 93.92, 63.60, 34.82, 14.91; HRMS (ESI) *m/z* calcd for $C_{24}H_{19}N_6O_5S_2^+$ [M + H^+] 535.08529, found 535.08372; HPLC 96.2% (R_t = 7.30 min, 50-80% CH_3OH in water for 7 min and 80% CH_3OH in water for 3 min, 10 min in total).

*6.1.42. N-(4-(4-amino-3,5-dichlorophenyl)thiazol-2-yl)-2-((5-cyano-4-(3-methoxyphenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)thio)acetamide (**23**)*

The procedure described for **12** was used, starting from **4a** (52 mg, 0.2 mmol), **8g** (67 mg, 0.2 mmol) and KOH (13 mg, 0.22 mmol), **23** was obtained as light orange solid (96 mg, 86% yield). m.p. 190-191 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.48 (s, 1H), 7.79 (s, 2H), 7.54 (s, 1H), 7.36 – 7.28 (m, 2H), 7.22 (t, *J* = 7.9 Hz, 1H), 7.05 – 6.99 (m, 1H), 5.68 (s, 2H), 4.19 (s, 2H), 3.68 (s, 3H); ^{13}C NMR (100MHz, DMSO-d_6): δ 167.39, 167.08, 159.42, 158.11, 147.35, 141.14, 137.78, 129.75, 125.73, 124.28, 121.22, 118.82, 117.79, 116.91, 114.26, 106.77, 92.34, 55.60, 34.66; HRMS (ESI) *m/z* calcd for $C_{23}H_{16}Cl_2KN_6O_3S_2^+$ [M + K^+] 596.97339, found 596.97199; $C_{23}H_{16}Cl_2N_6NaO_3S_2^+$ [M + Na^+] 580.99946, found 580.99964; $C_{23}H_{17}Cl_2N_6O_3S_2^+$ [M + H^+] 559.01751, found 559.01645; HPLC 95.8% (R_t = 6.14 min, 50-80% CH_3OH in water for 7 min and 80% CH_3OH in water for 3 min, 10 min in total).

*6.1.43. 2-((5-cyano-4-(3-methoxyphenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(4-(p-tolyl)thiazol-2-yl)acetamide (**24**)*

The procedure described for **12** was used, starting from **4a** (52 mg, 0.2 mmol), **8e** (58 mg, 0.2 mmol) and KOH (13 mg, 0.22 mmol), **24** was obtained as white solid (90 mg, 92% yield). m.p. 234-235 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.99 (s, 1H), 12.59 (s, 1H), 7.80 (d, *J* = 7.7 Hz, 2H), 7.56 (s, 1H), 7.44 – 7.29 (m, 2H), 7.25 (d, *J* = 7.8 Hz, 2H), 7.19 (t, *J* = 8.1 Hz, 1H), 7.02 (d, *J* = 8.4 Hz, 1H), 4.32 (s, 2H), 3.63 (s, 3H), 2.34 (s, 3H); ^{13}C NMR (100MHz, DMSO-d_6): δ 167.48, 166.20, 165.81, 161.39, 159.46, 158.06, 149.53, 137.60, 136.73, 132.10, 129.90, 129.79, 126.07, 121.43, 117.68, 116.11, 114.42, 107.84, 93.95, 55.60, 34.82, 21.27; HRMS (ESI) *m/z* calcd for $C_{24}H_{19}KN_5O_3S_2^+$ [M + K^+] 528.05609, found 528.05718;

$C_{24}H_{19}N_5NaO_3S_2^+$ [M + Na^+] 512.08215, found 512.08155; $C_{24}H_{20}N_5O_3S_2^+$ [M + H^+] 490.10021, found 490.09982; HPLC 98.1% (R_t = 6.98 min, 50-80% CH_3OH in water for 7 min and 80% CH_3OH in water for 3 min, 10 min in total).

*6.1.44. 2-((5-cyano-4-(3-methoxyphenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(4-(4-methoxyphenyl)thiazol-2-yl)acetamide (**25**)*

The procedure described for **12** was used, starting from **4a** (52 mg, 0.2 mmol), **8f** (62 mg, 0.2 mmol) and KOH (13 mg, 0.22 mmol), **26** was obtained as white solid (78 mg, 77% yield). m.p. 214-215 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.99 (s, 1H), 12.57 (s, 1H), 7.84 (d, *J* = 8.2 Hz, 2H), 7.48 (s, 1H), 7.41 – 7.27 (m, 2H), 7.19 (t, *J* = 8.1 Hz, 1H), 7.11 – 6.89 (m, 3H), 4.32 (s, 2H), 3.80 (s, 3H), 3.64 (s, 3H); ^{13}C NMR (100MHz, DMSO-d_6): δ 166.16, 165.83, 159.48, 158.02, 149.35, 136.75, 129.92, 127.61, 127.47, 121.44, 117.73, 116.10, 114.61, 114.41, 106.69, 93.92, 55.63, 34.82; HRMS (ESI) *m/z* calcd for $C_{24}H_{19}KN_5O_4S_2^+$ [M + K^+] 544.05100, found 544.05057; $C_{24}H_{19}N_5NaO_4S_2^+$ [M + Na^+] 528.07707, found 528.07617; $C_{24}H_{20}N_5O_4S_2^+$ [M + H^+] 506.09512, found 506.09427; HPLC 99.0% (R_t = 5.73 min, 50-80% CH_3OH in water for 7 min and 80% CH_3OH in water for 3 min, 10 min in total).

*6.1.45. 2-((5-cyano-4-(3-ethoxyphenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(4-(p-tolyl)thiazol-2-yl)acetamide (**26**)*

The procedure described for **12** was used, starting from **4d** (55 mg, 0.2 mmol), **8e** (58 mg, 0.2 mmol) and KOH (13 mg, 0.22 mmol), **26** was obtained as white solid (88 mg, 87% yield). m.p. 238-239 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 14.05 (s, 1H), 12.60 (s, 1H), 7.79 (d, *J* = 7.9 Hz, 2H), 7.57 (s, 1H), 7.36 – 7.32 (m, 1H), 7.31 (t, *J* = 2.1 Hz, 1H), 7.25 (d, *J* = 7.9 Hz, 2H), 7.17 (t, *J* = 8.0 Hz, 1H), 7.01 (dd, *J* = 8.4, 2.5 Hz, 1H), 4.32 (s, 2H), 3.90 (q, *J* = 6.9 Hz, 2H), 2.34 (s, 3H), 1.19 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (100MHz, DMSO-d_6): δ 167.53, 166.22, 165.86, 161.42, 158.72, 158.09, 149.54, 137.61, 136.75, 132.07, 129.89, 129.78, 126.07, 121.40, 118.59, 116.18, 114.28, 107.86, 93.89, 63.60, 34.81, 21.28, 14.91; HRMS (ESI) *m/z* calcd for $C_{25}H_{21}KN_5O_3S_2^+$ [M + K^+] 542.07174, found 542.07020; $C_{25}H_{22}N_5O_3S_2^+$ [M + H^+] 504.11586, found 504.11451; HPLC 96.7% (R_t = 7.58 min, 50-80% CH_3OH in water for 7 min and 80% CH_3OH in water for 3 min, 10 min in total).

*6.1.46. 2-((5-cyano-4-(3-ethoxyphenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(4-(4-methoxyphenyl)thiazol-2-yl)acetamide (**27**)*

The procedure described for **12** was used, starting from **4d** (55 mg, 0.2 mmol), **8f** (62 mg, 0.2 mmol) and KOH (13 mg, 0.22 mmol), **27** was obtained as white solid (80 mg, 77% yield). m.p. 231-232 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.94 (s, 1H), 12.57 (s, 1H) , 7.84 (d, *J* = 7.5 Hz, 2H), 7.48 (s, 1H), 7.33 (t, *J* = 7.5 Hz, 2H), 7.18 (t, *J* = 7.5 Hz, 1H), 7.01 (d, *J* = 7.5 Hz, 3H), 4.32 (s, 2H), 3.90 (s, 3H), 3.80 (q, *J* = 6.9 Hz, 2H), 1.19 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (100MHz, DMSO-d_6): δ 166.12, 159.49, 158.72, 158.05, 149.36, 129.90, 127.57, 127.45, 121.40, 118.60, 116.12, 114.58, 114.30, 106.70, 93.92, 63.61, 55.62, 34.79, 14.90; HRMS (ESI) *m/z* calcd for $C_{25}H_{21}KN_5O_4S_2^+$ [M + K^+] 558.06665, found 558.06594; $C_{25}H_{21}N_5NaO_4S_2^+$ [M + Na^+] 542.09272, found 542.09167; $C_{25}H_{22}N_5O_4S_2^+$ [M + H^+] 520.11077, found 520.10980; HPLC 95.8% (R_t = 6.70 min, 50-80% CH_3OH in water for 7 min and 80% CH_3OH in water for 3 min, 10 min in total).

*6.1.47. N-(4-(4-amino-3,5-dichlorophenyl)thiazol-2-yl)-2-((5-cyano-4-(3-ethoxyphenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)thio)acetamide (**28**)*

The procedure described for **12** was used, starting from **4d** (55 mg, 0.2 mmol), **8g** (67 mg, 0.2 mmol) and KOH (13 mg, 0.22 mmol), **28** was obtained as yellowish solid (94 mg, 82% yield). m.p. 221-222 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 14.00 (s, 1H), 12.53 (s, 1H), 7.79 (s, 2H), 7.56 (s, 1H), 7.39 – 7.25 (m, 2H), 7.17 (t, *J* = 7.9 Hz, 1H), 7.01 (dd, *J* = 8.1, 2.5 Hz, 1H), 5.70 (s, 2H), 4.31 (s, 2H), 3.90 (q, *J* = 6.9 Hz, 2H), 1.20 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (100MHz, DMSO-d_6): δ 167.55, 167.55, 166.20, 158.73, 158.09, 147.40, 141.18, 136.76, 129.87, 125.73, 124.20, 121.36, 118.79, 118.51, 116.15, 114.36, 106.86, 93.90, 63.62, 34.79, 14.92; HRMS (ESI) *m/z* calcd for $C_{24}H_{18}Cl_2KN_6O_3S_2^+$ [M + K^+] 610.98904, found 610.98743; $C_{24}H_{18}Cl_2N_6NaO_3S_2^+$ [M + Na^+] 595.01511, found 595.01430; $C_{24}H_{19}Cl_2N_6O_3S_2^+$ [M + H^+] 573.03316, found 573.03197; HPLC 99.0% (R_t = 7.23 min, 50-80% CH_3OH in water for 7 min and 80% CH_3OH in water for 3 min, 10 min in total).

*6.1.48. N-(4-(4-acetamido-3,5-dichlorophenyl)thiazol-2-yl)-2-((5-cyano-4-(3-methoxyphenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)thio)acetamide (**29**)*

The procedure described for **12** was used, starting from **4a** (52 mg, 0.2 mmol), **8h** (76 mg, 0.2 mmol) and KOH (13 mg, 0.22 mmol), **29** was obtained as white solid (103 mg, 86% yield). m.p. 191-193 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 14.00 (s, 1H), 12.65 (s, 1H), 9.89 (s, 1H), 8.04 (s, 2H), 7.94 (s, 1H), 7.38 – 7.28 (m, 2H), 7.17 (t, *J* = 8.2 Hz, 1H), 7.09 – 6.99 (m, 1H), 4.32 (s, 2H), 3.65 (s, 3H), 2.09 (s, 3H); ^{13}C NMR (100MHz, DMSO-d_6): δ 168.75, 167.49, 166.50, 165.79, 161.38, 159.46, 158.57, 146.16, 136.73, 135.28, 134.57, 132.76, 129.86, 125.60, 121.39, 117.63, 116.08, 114.46, 111.43, 94.00, 55.63, 34.83, 22.90; HRMS (ESI) *m/z* calcd for $C_{25}H_{19}Cl_2N_6O_4S_2^+$ [M + H^+] 601.02808, found 601.02756; HPLC 95.0% (R_t = 3.37 min, 50-80% CH_3OH in water for 7 min and 80% CH_3OH in water for 3 min, 10 min in total).

*6.1.49. N-(4-(4-acetamido-3,5-dichlorophenyl)thiazol-2-yl)-2-((5-cyano-4-(3-ethoxyphenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)thio)acetamide (**30**)*

The procedure described for **12** was used, starting from **4d** (55 mg, 0.2 mmol), **8h** (76 mg, 0.2 mmol) and KOH (13 mg, 0.22 mmol), **30** was obtained as white solid (104 mg, 86% yield). m.p. 203-205 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 14.04 (s, 1H), 12.65 (s, 1H), 9.88 (s, 1H), 8.04 (s, 2H), 7.94 (s, 1H), 7.39 – 7.24 (m, 2H), 7.15 (t, *J* = 7.9 Hz, 1H), 7.07 – 6.94 (m, 1H), 4.32 (s, 2H), 3.91 (q, *J* = 6.9 Hz, 2H), 2.09 (s, 3H), 1.21 (t, *J* = 7.0 Hz, 3H).; ^{13}C NMR (100MHz, DMSO-d_6): δ 168.74, 167.56, 166.46, 165.76, 161.40, 158.73, 158.60, 146.17, 136.74, 135.26, 134.55, 132.75, 129.84, 125.59, 121.34, 118.49, 116.10, 114.41, 111.44, 93.95, 63.63, 34.81, 22.90, 14.92; HRMS (ESI) *m/z* calcd for $C_{26}H_{21}Cl_2N_6O_4S_2^+$ [M + H^+] 615.04373, found 615.04309; HPLC 97.0% (R_t = 4.64 min, 50-80% CH_3OH in water for 7 min and 80% CH_3OH in water for 3 min, 10 min in total).

*6.1.50. 2-((5-cyano-6-oxo-4-(3-(trifluoromethyl) phenyl)-1,6-dihydropyrimidin-2-yl)thio)-N-(4-phe nylthiazol-2-yl)acetamide (**31**)*

The procedure described for **12** was used, starting from **4e** (59 mg, 0.2 mmol), **8a** (50 mg, 0.2 mmol) and KOH (13 mg, 0.22 mmol), **31** was obtained as yellowish solid (91 mg, 89% yield). m.p. 149-150 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.51 (s, 1H), 8.07 (s, 1H), 8.04 (d, *J* = 7.9 Hz, 1H), 7.89 (d, *J* = 7.3 Hz, 2H), 7.81 (d, *J* = 7.8 Hz, 1H), 7.63 (s, 1H), 7.58 (t, *J* = 7.8 Hz, 1H), 7.44 (t, *J* = 7.6 Hz, 2H), 7.33 (t, *J* = 7.3 Hz, 1H), 4.13 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.51, 167.75, 166.01, 158.21, 149.37, 138.20, 134.74, 132.78, 129.84, 129.66, 129.35, 129.16, 128.22, 127.22, 126.13, 125.14 (q, *J* = 3.8 Hz), 124.36 (q, *J* = 271 Hz), 118.89, 108.53, 91.30, 34.58; HRMS (ESI) *m/z* calcd for $C_{23}H_{14}F_3KN_5O_2S_2^+$ [M + K$^+$] 552.01726, found 552.01705; $C_{23}H_{14}F_3N_5NaO_2S_2^+$ [M + Na$^+$] 536.04332, found 536.04245; $C_{23}H_{15}F_3N_5O_2S_2^+$ [M + H$^+$] 514.06138, found 514.06083; HPLC 97.3% (R_t = 6.90 min, 50-80% CH_3OH in water for 7 min and 80% CH_3OH in water for 3 min, 10 min in total).

*6.1.51. N-(4-(4-amino-3,5-dichlorophenyl)thiazo l-2-yl)-2-((5-cyano-6-oxo-4-(3-(trifluoromethyl)p henyl)-1,6-dihydropyrimidin-2-yl)thio)acetamide (**32**)*

The procedure described for **12** was used, starting from **4e** (59 mg, 0.2 mmol), **8g** (67 mg, 0.2 mmol) and KOH (13 mg, 0.22 mmol), **32** was obtained as yellowish solid (103 mg, 86% yield). m.p. 202-203 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.55 (s, 1H), 8.12 (s, 1H), 8.01 (d, *J* = 8.0 Hz, 1H), 7.83 (d, *J* = 8.0 Hz, 1H), 7.78 (s, 2H), 7.54 (s, 1H), 7.48 (t, *J* = 7.9 Hz, 1H), 6.07 (s, 2H), 4.32 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.45, 166.25, 161.14, 157.97, 147.41, 141.16, 136.43, 133.12, 129.98, 129.87, 129.55, 128.44, 125.74, 125.50 (q, *J* = 4 Hz), 124.20, 124.08 (q, *J* = 271 Hz), 118.79, 115.83, 106.82, 94.85, 34.87; HRMS (ESI) *m/z* calcd for $C_{23}H_{13}Cl_2F_3KN_6O_2S_2^+$ [M + K$^+$] 634.95021, found 634.95043; $C_{23}H_{14}Cl_2F_3N_6O_2S_2^+$ [M + H$^+$] 596.99433, found 596.99382; HPLC 94.8% (R_t = 7.70 min, 50-80% CH_3OH in water for 7 min and 80% CH_3OH in water for 3 min, 10 min in total).

*6.1.52. 2-((5-cyano-6-oxo-4-(3-(trifluoromethox y)phenyl)-1,6-dihydropyrimidin-2-yl)thio)-N-(4-p henylthiazol-2-yl)acetamide (**33**)*

The procedure described for **12** was used, starting from **4f** (63 mg, 0.2 mmol), **8a** (50 mg, 0.2 mmol) and KOH (13 mg, 0.22 mmol), **33** was obtained as yellowish solid (96 mg, 91% yield). m.p. 149-150 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.52 (s, 1H), 7.90 (d, *J* = 7.7 Hz, 2H), 7.80 (d, *J* = 7.6 Hz, 1H), 7.71 (s, 1H), 7.63 (s, 1H), 7.46 (dt, *J* = 14.6, 7.8 Hz, 4H), 7.34 (q, *J* = 7.4, 6.1 Hz, 1H), 4.14 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.34, 167.69, 165.74, 158.18, 149.40, 148.51, 139.33, 134.77, 130.68, 129.14, 128.89, 128.21, 127.88, 126.13, 123.22, 121.20, 120.48 (q, *J* = 255 Hz), 118.68, 108.49, 91.39, 34.60; HRMS (ESI) *m/z* calcd for $C_{23}H_{15}F_3N_5O_3S_2^+$ [M + H$^+$] 530.05629, found 530.05563; HPLC 96.4% (R_t = 7.45 min, 50-80% CH_3OH in water for 7 min and 80% CH_3OH in water for 3 min, 10 min in total).

*6.1.53. N-(4-(4-amino-3,5-dichlorophenyl)thiazo l-2-yl)-2-((5-cyano-6-oxo-4-(3-(trifluoromethoxy) phenyl)-1,6-dihydropyrimidin-2-yl)thio)acetamid e (**34**)*

The procedure described for **12** was used, starting from **4f** (63 mg, 0.2 mmol), **8g** (67 mg, 0.2 mmol) and KOH (13 mg, 0.22 mmol), **34** was obtained as yellowish solid (104 mg, 85% yield). m.p. 139-140 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.51 (s, 1H), 7.79 (s, 2H), 7.79 – 7.76 (m, 1H), 7.73 (s, 1H), 7.54 (s, 1H), 7.50 – 7.45 (m, 1H), 7.41 (t, *J* = 8.0 Hz, 1H), 5.69 (s, 2H).; ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.36, 166.24, 165.95, 161.22, 157.94, 148.53, 147.44, 141.16, 137.57, 130.85, 128.21, 125.73, 124.49, 124.22, 120.38 (q, *J* = 255 Hz), 118.76, 115.79, 106.77, 94.71, 34.85; HRMS (ESI) *m/z* calcd for $C_{23}H_{14}Cl_2F_3N_6O_3S_2^+$ [M + H$^+$] 612.98925, found 612.98761; HPLC 97.2% (R_t = 7.94 min, 50-80% CH_3OH in water for 7 min and 80% CH_3OH in water for 3 min, 10 min in total).

*6.1.54. N-(4-(3-bromophenyl)thiazol-2-yl)-2-((5-cyano-4-(3-methoxyphenyl)-6-oxo-1,6-dihydropy rimidin-2-yl)thio)acetamide (**35**)*

The procedure described for **12** was used, starting from **4a** (52 mg, 0.2 mmol), **8i** (66 mg, 0.2 mmol) and KOH (13 mg, 0.22 mmol), **35** was obtained as white solid (103 mg, 93% yield). m.p. 212-213 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.99 (s, 1H), 12.63 (s, 1H), 8.12 (t, *J* = 1.8 Hz, 1H), 7.92 (dt, *J* = 7.8, 1.3 Hz, 1H), 7.80 (s, 1H), 7.54 (d, *J* = 8.1 Hz, 1H), 7.43 (t, *J* = 7.9 Hz, 1H), 7.37 – 7.30 (m, 2H), 7.19 (t, *J* = 7.9 Hz, 1H), 7.03 (dd, *J* = 8.5, 2.0 Hz, 1H), 4.33 (s, 2H), 3.64 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.49, 166.41, 165.79, 161.40, 159.45, 158.36, 147.68, 136.96, 136.74, 131.45, 130.90, 129.90, 128.77, 125.00, 122.69, 121.41, 117.59, 116.12, 114.46, 110.23, 94.01, 55.60, 34.83; HRMS (ESI) *m/z* calcd for $C_{23}H_{16}{}^{81}BrKN_5O_3S_2^+$ [M + K$^+$] 593.94891, found 593.94716; $C_{23}H_{16}BrN_5NaO_3S_2^+$ [M + Na$^+$] 575.97701, found 575.97594; $C_{23}H_{17}BrN_5O_3S_2^+$ [M + H$^+$] 553.99507, found 553.99384; HPLC 99.4% (R_t = 7.31 min, 50-80% CH_3OH in water for 7 min and 80% CH_3OH in water for 3 min, 10 min in total).

*6.1.55. 2-((5-cyano-4-(3-methoxyphenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(4-(3-(2-fluoro pyridin-3-yl)phenyl)thiazol-2-yl)acetamide (**36**)*

The procedure described for **12** was used, starting from **4a** (52 mg, 0.2 mmol), **8j** (70 mg, 0.2 mmol) and KOH (13 mg, 0.22 mmol), **36** was obtained as white solid (81 mg, 71% yield). m.p. 146-147 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.64 (s, 1H), 8.29 (dt, *J* = 4.8, 1.5 Hz, 1H), 8.23 – 8.18 (m, 1H), 8.18 – 8.12 (m, 1H), 8.04 – 7.94 (m, 1H), 7.78 (s, 1H), 7.66 – 7.55 (m, 2H), 7.52 (ddd, *J* = 7.1, 4.8, 1.8 Hz, 1H), 7.41 – 7.30 (m, 2H), 7.20 (t, *J* = 7.9 Hz, 1H), 7.06 – 6.95 (m, 1H), 4.34 (s, 2H), 3.64 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.47, 166.37, 165.83, 161.41, 160.11 (d, *J* = 236 Hz), 159.46, 158.30, 148.86, 147.13 (d, *J* = 14 Hz), 141.94 (d, *J* = 4 Hz), 136.74, 135.20, 134.43 (d, *J* = 5 Hz), 129.90, 129.77, 128.62, 126.55, 126.15, 123.44, 123.16 (d, *J* = 4 Hz), 121.44, 117.67, 116.12, 114.41, 109.51, 93.94, 55.60, 34.84; HRMS (ESI) *m/z* calcd for $C_{28}H_{19}FKN_6O_3S_2^+$ [M + K$^+$] 609.05757, found 609.04987; $C_{28}H_{19}FN_6NaO_3S_2^+$ [M + Na$^+$] 593.08363, found 593.08289; $C_{28}H_{20}FN_6O_3S_2^+$ [M + H$^+$] 571.10168, found 571.10112; HPLC 98.4% (R_t = 6.33 min, 50-80% CH_3OH in water for 7 min and 80% CH_3OH in water for 3 min, 10 min in total).

*6.1.56. 2-((6-oxo-1,6-dihydropyrimidin-2-yl)thio) -N-(4-phenylthiazol-2-yl)acetamide (**37**)*

The procedure described for **12** was used, starting from 2-thioxo-2,3-*d*ihydropyrimidin-4(*1H*)-one (26 mg, 0.2 mmol), **8a** (50 mg, 0.2 mmol) and KOH (13 mg, 0.22 mmol), **37** was obtained as white solid (61 mg, 89% yield). m.p. 194-195 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.58 (s, 1H), 7.91 (d, *J* = 7.2 Hz, 2H), 7.88 (d, *J* = 6.6 Hz, 1H), 7.64 (s, 1H), 7.44 (t, *J* = 7.6 Hz, 2H), 7.33 (t, *J* = 7.3 Hz, 1H), 6.16 (d, *J* = 6.5 Hz, 1H), 4.24 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.86, 163.97, 163.11, 158.26, 154.36, 149.37, 134.67, 129.22, 128.31, 126.14, 109.90, 108.69, 34.22; HRMS (ESI) *m/z* calcd for $C_{15}H_{12}KN_4O_2S_2^+$ [M + K^+] 383.00333, found 382.99370; $C_{15}H_{12}N_4NaO_2S_2^+$ [M + Na^+] 367.02939, found 367.02913; $C_{15}H_{13}N_4O_2S_2^+$ [M + H^+] 345.04744, found 345.04717; HPLC 97.2% (R_t = 6.07 min, 50-80% CH_3OH in water for 7 min and 80% CH_3OH in water for 3 min, 10 min in total).

*6.1.57. 2-((4-amino-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(4-phenylthiazol-2-yl)acetamide (**38**)*

The procedure described for **12** was used, starting from 6-amino-4-hydroxypyrimidine-2(*1H*)-thione (29 mg, 0.2 mmol), **8a** (50 mg, 0.2 mmol) and KOH (13 mg, 0.22 mmol), **38** was obtained as light brown solid (62 mg, 86% yield). m.p. 239-240 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.43 (s, 1H), 11.54 (s, 1H), 7.90 (d, *J* = 7.6 Hz, 2H), 7.63 (s, 1H), 7.43 (t, *J* = 7.6 Hz, 2H), 7.33 (t, *J* = 7.4 Hz, 1H), 6.48 (s, 2H), 5.06 (s, 1H), 4.14 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.48, 165.91, 164.30, 158.26, 149.38, 134.70, 129.20, 128.28, 126.15, 109.05, 108.69, 81.94, 33.82; HRMS (ESI) *m/z* calcd for $C_{15}H_{13}KN_5O_2S_2^+$ [M + K^+] 398.01422, found 398.00396; $C_{15}H_{13}N_5NaO_2S_2^+$ [M + Na^+] 382.04029, found 382.03961; $C_{15}H_{14}N_5O_2S_2^+$ [M + H^+] 360.05834, found 360.05781; HPLC 95.4% (R_t = 5.48 min, 50-80% CH_3OH in water for 7 min and 80% CH_3OH in water for 3 min, 10 min in total).

*6.1.58. 2-((5-amino-1,3,4-thiadiazol-2-yl)thio)-N -(4-phenylthiazol-2-yl)acetamide (**39**)*

The procedure described for **12** was used, starting from 5-amino-1,3,4-thiadiazole-2-thiol (27 mg, 0.2 mmol), **8a** (50 mg, 0.2 mmol) and KOH (13 mg, 0.22 mmol), **39** was obtained as yellowish solid (57 mg, 82% yield). m.p. 224-225 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.56 (s, 1H), 7.90 (d, *J* = 7.7 Hz, 2H), 7.66 (s, 1H), 7.44 (t, *J* = 7.6 Hz, 2H), 7.41 – 7.23 (m, 3H), 4.12 (s, 2H).; ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.61, 166.99, 158.09, 149.44, 149.30, 134.65, 129.21, 128.31, 126.15, 108.82, 37.81; HRMS (ESI) *m/z* calcd for $C_{13}H_{11}KN_5OS_3^+$ [M + K^+] 387.97573; found 387.97517; $C_{13}H_{12}N_5OS_3^+$ [M + H^+] 350.01985; found 350.01943; HPLC 99.0% (R_t = 6.29 min, 50-80% CH_3OH in water for 7 min and 80% CH_3OH in water for 3 min, 10 min in total).

*6.1.59. 2-((5-cyano-4-(3-methoxyphenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(4-phenyl-1H-imidazol-2-yl)acetamide (**40**)*

The procedure described for **12** was used, starting from **4a** (52 mg, 0.2 mmol), **10a** (47 mg, 0.2 mmol) and KOH (13 mg, 0.22 mmol), **40** was obtained as white solid (88 mg, 96% yield). m.p. 269-270 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ11.68 (s, 1H), 11.45 (s, 1H) , 7.71 (d, *J* = 7.5 Hz, 2H), 7.32 (m, PhH, 5H) , 7.26 (s, 1H), 7.16 (t, *J* = 7.5 Hz, 1H) , 7.00 (d, *J* = 7.5 Hz, 1H), 3.93 (s, 2H), 3.75 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.59, 170.63, 168.11, 167.32, 159.32, 141.40, 139.38, 136.80, 135.14, 129.64, 128.88, 126.37, 124.48, 121.00, 120.41, 115.95, 113.88, 109.92, 89.83, 55.57, 34.64; HRMS (ESI) *m/z* calcd for $C_{23}H_{18}KN_6O_3S^+$ [M + K^+] 497.07927, found 497.07838; $C_{23}H_{19}N_6O_3S^+$ [M + H^+] 459.12339, found 459.12291; HPLC 98.1% (R_t = 8.11 min, 40-60% CH_3OH in water for 10 min).

*6.1.60. 2-((5-cyano-4-(3-ethoxyphenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(4-phenyl-1H-imidazol-2-yl)acetamide (**41**)*

The procedure described for **12** was used, starting from **4d** (55 mg, 0.2 mmol), **10a** (47 mg, 0.2 mmol) and KOH (13 mg, 0.22 mmol), **41** was obtained as white solid (81 mg, 86% yield). m.p. 213-214 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.78 (s, 1H), 11.45 (s, 1H), 7.72 (d, *J* = 7.5 Hz, 2H), 7.41 – 7.23 (m, 6H), 7.17 (t, *J* = 7.2 Hz, 1H), 7.00 (d, *J* = 8.0 Hz, 1H), 4.00 (t, *J* = 7.1 Hz, 4H), 1.28 (t, *J* = 6.0 Hz, 4H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.91, 167.70, 158.62, 141.46, 138.85, 136.27, 134.80, 129.71, 128.90, 126.42, 124.48, 121.04, 119.62, 117.01, 114.18, 110.85, 90.66, 63.56, 34.76, 15.05; HRMS (ESI) *m/z* calcd for $C_{24}H_{20}KN_6O_3S^+$ [M + K^+] 511.09492, found 511.09466; $C_{24}H_{20}N_6NaO_3S^+$ [M + Na^+] 495.12098, found 495.12022; $C_{24}H_{21}N_6O_3S^+$ [M + H^+] 473.13904, found 473.13818; HPLC 97.9% (R_t = 10.01 min, 40-60% CH_3OH in water for 10 min and 60% CH_3OH in water for 5 min, 15min in total).

*6.1.61. N-(4-(4-amino-3,5-dichlorophenyl)-1H-imidazol-2-yl)-2-((5-cyano-4-(3-methoxyphenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)thio)acetamide (**42**)*

The procedure described for **12** was used, starting from **4a** (52 mg, 0.2 mmol), **10g** (64 mg, 0.2 mmol) and KOH (13 mg, 0.22 mmol), **42** was obtained as yellow solid (80 mg, 74% yield). m.p. 210-211 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.93 (s, 3H), 7.63 (s, 2H), 7.43 (d, *J* = 7.5 Hz, 2H), 7.41 (s, 1H), 7.28 (t, *J* = 7.5 Hz, 1H), 7.09 (d, *J* = 7.5 Hz, 1H), 5.45 (s, 2H), 4.26 (s, 2H), 3.69 (s, 3H); ^{13}C NMR (100MHz, DMSO-d_6): δ 167.49, 166.11, 165.87, 161.68, 159.48, 140.88, 139.75, 136.78, 134.17, 130.05, 124.17, 121.56, 118.95, 117.96, 116.28, 114.25, 110.11, 93.69, 55.62, 35.00; HRMS (ESI) *m/z* calcd for $C_{23}H_{17}Cl_2KN_7O_3S^+$ [M + K^+] 580.01222, found 580.01111; $C_{23}H_{18}Cl_2N_7O_3S^+$ [M + H^+] 542.05634, found 542.05512; HPLC 95.7% (R_t = 9.59 min, 40-60% CH_3OH in water for 10 min and 60% CH_3OH in water for 2 min, 12min in total).

6.2. In Vitro BACE-1 inhibition assays.

BACE-1 inhibition assays were carried out using a Fluorescense Resonance Energy Transfer (FRET) assay kit purchased from invitrogen (P2985) in the 384-well black round-bottomed microplate (Corning 3677) with a final volume of 18 *μ*L/well. A statin peptide (H-Lys-Thr-Glu-Glu-Ile-Ser-Glu-Val-Asn-Sta-Val-Ala-Glu-Phe-OH, AnaSpec 23959) derivative was used in the assay as a positive control.The assays were run under the following step: 1. Add 6 µL 3X BACE1 Substrate (750 nM) to 10 µL 3X Test Compound (or BACE1 Assay Buffer if preparing a control well) and mix gently; 2. Add 6 µL of 3X BACE1 Enzyme (1 Units/mL); 3. Incubate 60 minutes at room temperature and read the fluorescence. Compounds **12**-**39** were tested at a concentration of 10 *μ*M and 50*μ*M, and compounds **40**-**42**

were tested at a concentration of 1 μM and 10 μM. The IC_{50} values were then determined on all the compounds that displayed 50% or higher inhibition rate at 50 μM or 10 μM. IC_{50} values were calculated using Graph Pad Prism Software. All the IC_{50} measurements were done in duplicate.

6.3. Molecular docking

The molecular docking procedure of compound **12**, **23**, **36** and **40** was performed with GOLD 3.0 (Cambridge Crystallographic Data Centre, Chembridge, UK) using the default parameter settings and GOLDScore was used as the fitness function. The three-dimensional (3D) structure of BACE1 (PDB ID: 3VF3) was downloaded from Protein Data Bank (PDB). The ligand was built by ChemDraw (ChembridgeSoft Inc, Chembridge, MA) and initial 3D conformation was constructed by Chem3D. Next, the ligand was prepared for docking using Discovery Studio 2.5 (Accelrys Software Inc, San Diego, CA). The binding modes were visually inspected by PyMol 0.99 (DeLano Scientific LLC Inc, San Carlos, CA).

6.4. SPR based assays and binding Affinity of BACE-1

BACE-1 was immobilized on a CM5 sensor chip using standard amine coupling procedure at a concentration of 20 μg mL^{-1} (in 10 mM acetic acid, pH 4.5) to a level of 7500-8000 response units (RU). Typically, 2 flow cells of the sensor were used: flow cell 3 served as a reference cell was activated and deactivated, flow cell 4 contained BACE-1.

Affinity experiments were performed at 25 °C using Biacore T200 instrument by initial injection of 20 buffer blanks to equilibrate the surfaces followed by the injection of analyte solutions at different concentrations in 1.5-fold dilutions over all flow cells. The running buffer for the binding experiments was PBS, pH 7.4 with 5% DMSO with a flow rate of 30 μL min^{-1}. Running buffer samples containing 4.5-5.8% DMSO were also injected to create a DMSO calibration plot. Affinity constants (K_D) were determined using the Biacore T200 evaluation software V.2.0 by curve fitting using steady state model.

6.5. In Vitro Blood−Brain Barrier Permeability Assay.

The ability to cross the BBB was predicted and evaluated using PAMPA. The 96-well filter plate (catalog no. MAIPN4550) and the donor plate (catalog no. MATRNPS50) were both purchased from Millipore. The porcine polar brain lipid (PBL) was purchased from Avanti Polar Lipids. Dodecane was obtained from J&K®. Verapamil, clonidine, hydrocortisone and theophylline were purchased from Energy Chemical and used as positive control. Tested compounds were dissolved in DMSO at 5 mg ml^{-1} as stock solutions, which were diluted in PBS to make a final solution (final concentration 25 μg ml^{-1}). The PBL was dissolved in dodecane at 20 mg ml^{-1}. A 300 μL final solution was added to the donor well, the filter membrane was coated with 4 μL of PBL solution, and the acceptor well was filled with 150 μL of PBS. The acceptor filter plate was carefully put on the donor plate to form a “sandwich” which was composed of the donor with tested compounds on the bottom, artificial lipid membrane in the middle, and the acceptor on the top. The sandwich was incubated undisturbed at room temperature for 18 h. The donor plate was removed after incubation. The concentrations of tested compounds in the acceptor and reference solutions were determined by HPLC. Reference solutions were prepared by diluting the sample secondary stock solution to the same concentration as that with no membrane barrier. Every sample was analyzed at three wavelengths, in three wells, and in two independent runs. The P_e was obtained by the following equation:

$$P_e = -\frac{V_{dn}V_{ac}}{st(V_{dn}+V_{ac})}\ln\left(1-\frac{[drug]_{ac}}{[drug]_{ref}}\right)$$

where V_{dn} (mL) = volume of the donor compartment, V_{ac} (mL) = volume of the acceptor compartment, $[drug]_{ac}$ = compound concentration of the solution of the acceptor compartment, $[drug]_{ref}$ = compound concentration of the reference solution, s (cm^2) = membrane area (0.3 cm^2), and t (s) = incubation time.

6.6. In Vitro Cytotoxicity assay.

The assay was performed with the Human Embryonic Kidney 293 cells (HEK293) which were purchased from the cell bank of the Shanghai Institute of Cell Biology. The HEK293 cell line was cultured in the DMEM medium. The media was supplemented with 10% fetal bovine serum (FBS), 100 units/ ml penicillin, and 100 units/ ml streptomycin (Invitrogen). The cells were maintained at 37 °C in a humidified environment with 5% CO_2. The cell viability was determined by using the CellTiter Glo™ luminescent cell viability assay (Promega). Briefly, the cells were seeded into 384-well plates at an initial density of 1000 cells/well in 45 mlof medium. Then the cells were treated with compounds at varying concentrations. The first compound work concentration is 100 mM, and then three-fold dilution ten more times. Staurosporine (SigmaeAldrich, catalog No. S4400) was used as a positive control. After incubation for 72 h, 10% of CellTiter Glo™ reagent was added and luminescent signals were read on a VeriScan reader (Thermo Fisher Scientific). The IC_{50} value was calculated from the curves generated by plotting the percentage of the viable cells versus test concentrations on a logarithmic scale using Sigma Plot 10.0 software.

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Appendix And. Supplementary data

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- Guided by docking studies, a series of compounds were designed and synthesized
- Enzyme activity, affinity, permeability and cytotoxicity have been evaluated
- Sixteen compounds were found to have μM to sub-μM level activities towards BACE1
- Compound **41** exhibited high activities and the most optimized drug-like profiles