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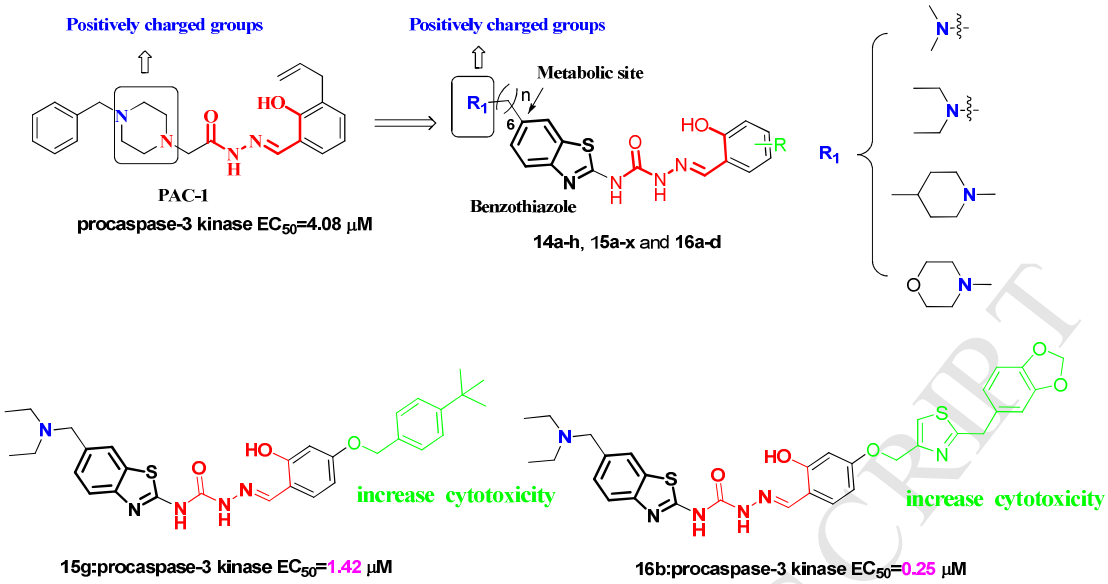

A series of novel benzothiazole derivatives bearing *ortho*-hydroxy *N*-carbamoylhydrazone moiety were synthesized and evaluated for their cytotoxic activities. Six potent compounds were further examined for their procaspase-3 kinase activity

Design, synthesis, and structure-activity relationships of novel benzothiazole derivatives bearing the *ortho*-hydroxy *N*-carbamoylhydrazone moiety as potent antitumor agents

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Abstract

A series of novel benzothiazole derivatives bearing the *ortho*-hydroxy *N*-carbamoylhydrazone moiety were designed and synthesized and their cytotoxic activities against five cancer cell lines (NCI-H226, SK-N-SH, HT29, MKN45, and MDA-MB-231) were screened *in vitro*. Most of them showed moderate to excellent activity against all the tested cell lines. Among them, compounds **15g** (procaspase-3 EC_{50} =1.42 μM) and **16b** (procaspase-3 EC_{50} = 0.25 μM) exhibited excellent antitumor activity with IC_{50} values ranging from 0.14 μM to 0.98 μM against all cancer cell lines, which were 1.8–8.7 times more active than the first procaspase activating compound (PAC-1) (procaspase-3 EC_{50} = 4.08 μM). The structure-activity relationship (SAR) analyses indicated that the introduction of a lipophilic group (a benzyloxy or heteroaryloxy group) at the 4-position of the 2-hydroxy phenyl ring was beneficial to antitumor activity, and the presence of substituents containing nitrogen that are positively charged at physiological pH could also improve antitumor activity. It was also confirmed that the steric effect of the 4-position substituent of the benzyloxy group had a significant influence on cytotoxic activity.

Keywords: antitumor; benzothiazole; *ortho*-hydroxy *N*-carbamoylhydrazone moiety; procaspase-3

1. Introduction

The primary cause of cancer development and progression is the dysregulation of apoptosis[1]. Thus, it would be an effective approach to explore novel apoptosis-inducing compounds for the treatment of cancer. Compounds such as p53 disruptors (tenovin-1)[2] and inhibitors of XIAP (GDC-0152)[3] or Bcl-2 (GDC-0199)[4] act directly on proteins in the apoptotic pathway, to induce apoptosis and lead to the death of cancer cells. However, direct activation of procaspase-3 by small molecules (**PAC-1** and **S-PAC-1**, Fig. 1) could have advantages over the above, because numerous studies have demonstrated that procaspase-3 is overexpressed in a variety of human tumors, including those of colon cancer[5], lung cancer[6], melanoma[7], hepatoma[8], breast cancer[9], lymphoma [10], and neuroblastoma[11].

Structure−activity relationship (SAR) studies revealed that the activity of PAC-1 and *S*-PAC-1 *in vitro* and in cell culture is dependent on the presence of the *ortho*-hydroxy *N*-acylhydrazone moiety, which is known to participate in metal chelation[12]. In addition, studies have also revealed that the piperazine nitrogens in **PAC-1** and **S-PAC-1** may play a very important role in their activity[5], because the autoactivation mechanism provides another channel for the activation of procaspase-3 to caspase-3[13]. It is possible that the piperazine nitrogens in **PAC-1** and **S-PAC-1** are positively charged at physiological pH, which may directly interact with the triaspartic acid "safety catch" of procaspase-3, as such inducing the autoactivation of procaspase-3 and catalyze the hydrolysis of hundreds of protein substrates, leading to cell death.

(**Fig. 1.** should be listed here)

Our group have reported a series of derivatives bearing an *ortho*-hydroxy *N*-acylhydrazone moiety (**3**, **4**, Fig. 2), the SAR of which exhibited that the introduction of either a benzyloxy or heteroaryloxy group at the 2-hydroxy phenyl ring could enhance activity *in vitro*[14-16]. Benzothiazoles have attracted much attention over many years due to their diverse biological properties, including antifungal[17], anticancer[18, 19], antiamyloid[20], and antirheumatic[21] utility, and have been widely used as important structural components in modern drug design for the treatment of cancer[22-24].

(**Fig. 2.** should be listed here)

In our continued exploration for potent and novel procaspase-3 activators as potential anticancer agents with multi-targeted molecular mechanisms, we combined benzothiazole with the *ortho*-hydroxy carbamoylhydrazone moiety in target compounds based on a hybrid pharmacophore design. Some groups (R_1) that are positively charged at physiological pH (e.g., 4-methylpiperidine, morpholine, dimethylamine, and diethylamine), were introduced at the 6-position of benzothiazole to avoid the metabolic pathway, and the effect of positively charged groups on the activity was examined by changing the number of carbon atoms (n). Substituted benzyloxy and heteroaryloxy groups (R), which were beneficial to the antitumor activity in our previous study, were also introduced into the 4-position of the 2-hydroxy phenyl ring to heighten the activity.

Herein, we describe a series of novel target compounds and their *in vitro* antitumor activities against two procaspase-3 over-expression cancer cell lines (NCI-H226, Lung cancer; and SK-N-SH, neuroblastoma) and one cancer cell line moderately sensitive to procaspase-3 (MDA-MB-231, breast cancer). Furthermore, two cancer cell lines with low-sensitivity to procaspase-3 (MKN45, human gastric cancer cell; and HT29, human colorectal cancer cell) were evaluated to rule out off-target effects[25]. Several compounds with potent activity were selected for the enzymatic assays to determine procaspase-3 kinase activation.

(**Fig. 3.** should be listed here)

2. Chemistry

The preparation of key intermediates **9a-e** is described in Scheme 1. The commercially available starting materials 4-nitrobenzyl bromide or 4-chloronitrobenzene combined with excessive secondary amines (4-methylpiperidine, morpholine, dimethylamine and diethylamine) in acetonitrile at room temperature for 3 h underwent a nucleophilic substitution reaction to provide intermediates **5a-e**. These were then reduced in the presence of 80% hydrazine, $FeCl_3.6H_2O$, and activated carbon to obtain the aryl amines **6a-e**, which were further reacted with

potassium thiocyanate and bromine to give **7a-e** in a satisfactory yield, using a known preparation method[26]. Further treatment with phenyl chloroformate was used to convert **7a-e** into the amides **8a-e**. Subsequently, the key intermediate semicarbazides **9a-e** were generated via hydrazinolysis of **8a-e** with 80% hydrazine monohydrate in 1,4-dioxane at 80°C for 6 h.

(**Scheme 1.** should be listed here)

As shown in Scheme 2, the substituted benzyloxy 2-hydroxybenzaldehydes **10a–g** were synthesized from 2,4-dihydroxy benzaldehyde through a reaction with substituted benzylchloride in acetonitrile in the presence of sodium bicarbonate and potassium iodide[27]. 3,4-(methylenedioxy)phenylacetonitrile was reacted with sodium hydrosulfide to obtain **11**[28], which was condensed with 1,3-dichloro-2-propanone at 50 °C for 4 h in acetonitrile to produce **12**. Compound **13** was prepared via a regioselective O-alkylation reaction of 2,4-dihydroxybenzaldehyde with **12**. Finally, the target compounds **14a-h**, **15a-x**, and **16a-d** were generated via the condensation of **9a-d** with appropriate 2-hydroxy aromatic aldehydes (substituted 2-hydroxy benzaldehydes, **10a-g**, and **13**) in ethanol at 78 °C with catalytic amounts of acetic acid[29].

(**Scheme 2.** should be listed here)

The chemical structures of the target compounds were confirmed by ^{1}H NMR, ^{13}C NMR, NOESY NMR, and MS spectra. All target compounds could exist in either the *E* or *Z* isomeric form due to the imino bond, so compound **15v** was selected to further confirm the stereochemistry by undergoing NOESY NMR. Results (Fig. 4 and supplementary information) showed that an evident NOE signal was observed between the H_1 (–C***H***=N–, δ=8.23 ppm) and H_2 (=N–N***H***–, δ=10.98 ppm), which existed only in the *E* isomer due to the appropriate intramolecular H–H distance; no NOE signals were observed if the target compounds existed in the *Z* isomer. Thus, there was no doubt that the configurations of target compounds were the *E* isomers.

(**Fig. 4.** should be listed here)

3. Results and discussion

3.1. *In vitro* cytotoxicity and structure-activity relationships

All target compounds were evaluated for their cytotoxicity *in vitro* against two procaspase-3 over-expression cancer cell lines (NCI-H226, Lung cancer cells and SK-N-SH, neuroblastoma cells and one cancer cell line moderately sensitive to procaspase-3 (MDA-MB-231, breast cancer cells by using an MTT assay. In addition, two cancer cell lines with low-sensitivity to procaspase-3 (MKN45, human gastric cancer cells and HT29, human colorectal cancer cells) were further evaluated to rule out off-target effects. Taking PAC-1 as the reference drug, each compound was tested in three independent experiments and the results are summarized in Table 1 and presented as IC_{50} values.

As illustrated in Table 1, all the synthesized target compounds showed moderate to excellent cytotoxic activity against all tested cancer cell lines. It was observed that compounds **15g**, **15h**, **15n**, **15p**, **15u**, and **16b** showed more potent antitumor activity against all five cancer cell lines than the other compounds, and compound **15v** displayed higher selectivity against HT29 with an IC_{50} value of 0.0018 μM, which was 760 times that of the reference drug.

By comparison of the IC_{50} values of compounds **15a-x**, **16a-d,** and compounds **14a-h**, it was clearly revealed that the introduction of a lipophilic group (e.g., a benzyloxy or heteroaryloxy group) at the 4-position of the 2-hydroxy phenyl ring could improve cytotoxicity *in vitro*, which

was consistent with the previous study in our laboratory. However, introduction of –OH at the 4-position of the 2-hydroxy phenyl ring reduced the antitumor activity against NCI-H226 and SK-N-SH. In addition, there was an obvious negative correlation between cellular concentration of procaspase-3 (NCI-H226 > SK-N-SH) and the IC_{50} values of the target compounds (NCI-H226 < SK-N-SH). This suggested that the antitumor effect of the target compounds in cultured cells was directly related to the level of procaspase-3 expression. Meanwhile, compounds **15a–x** and **16a–d** also exhibited high activity against the MKN45 cell line, indicating that compounds **15a–x** and **16a–d** with a lipophilic group might be multi-target compounds. Furthermore, compound **14g** with a morpholinyl group that is positively charged at physiological pH showed better activity against all five cancer cell lines than compound **14h** with a morpholinyl group that is not positively charged at physiological pH due to a p-π conjugation effect, thereby demonstrating that the existence of substituents containing nitrogen that are positively charged at physiological pH, could also enhance the pharmacological activity.

Further investigations were performed to study the effect on cytotoxic activity of different substituents on the benzyloxyl group (compounds **15a-x**). The results revealed that introduction of electron-withdrawing groups or electron-donating groups caused no remarkable alteration in cytotoxic activity, furthermore, introduction of a mono-chlorine or 2,4-dichlorine on the benzyloxyl group was well tolerated. It was worth noting that increasing the size of substituents at the 4-position of the benzyloxyl group could apparently enhance cytotoxic activity (**15a** *vs.* **15b**, **15f** *vs.* **15g**, **15m** *vs.* **15n**, and **15t** *vs.* **15u**). The above findings indicated that the electronic effect of the benzyloxyl group is not the main factor contributing to cytotoxicity, but rather the steric effect of the group at the 4-position of the benzyloxyl group had a significant impact.

(**Table 1.** should be listed here)

3.2. *In vitro* enzymatic assays

As shown in Table 2, the six tested compounds displayed moderate to excellent procaspase-3 enzymatic potency, suggesting that the activation of procaspase-3 may be a mechanism for the antitumor effect of these target compounds. Compound **15u** showed lower activity against procaspase-3 kinase than the other compounds; a possible reason for this is that there is no positively charged group in compound **15u**. It is worthy to mention that compounds **15g** and **16b,** with positively charged groups, showed greater enzyme activities, with EC_{50} values of 1.42μM and 0.25μM, which were 2.9 times and 16 times more active than PAC-1, respectively, indicating that the two compounds deserve further research.

(**Table 2.** should be listed here)

4. Conclusions

In summary, we designed and synthesized a series of novel benzothiazole derivatives bearing an *ortho*-hydroxy *N*-carbamoylhydrazone. The prepared compounds were evaluated for *in vitro* cytotoxic activity in five human cancer cell lines (NCI-H226, SK-N-SH, MKN45, HT29, and MDA-MB-231). Most of them had moderate to excellent activity against all tested cancer cell lines and were sensitive to the level of cellular concentration of procaspase-3. Compounds **15g** and **16b** exhibited more potent antitumor activity against all tested cancer cell lines than PAC-1, with IC_{50} values ranging from 0.14 to 0.98 μM. Furthermore, their strong procaspase-3 activation potency (**15g**, EC_{50} = 1.42 μM; **16b**, EC_{50} = 0.25 μM) suggested that they might inhibit the growth of tumor cells by activating procaspase-3 kinase. The analysis of SARs indicated that introduction

of a lipophilic group (e.g., a benzyloxy group or heteroaryloxy group) at the 4-position of the 2-hydroxy phenyl ring could enhance *in vitro* cytotoxic activity, and the introduction of substituents containing nitrogen that are positively charged at physiological pH, was beneficial to antitumor activity. In addition, the electronic effect of the benzyloxyl group showed no remarkable effect on cytotoxic activity, whereas the steric effect of a group at the 4-position of the benzyloxyl group had a major influence. Further optimization of the structure to improve the bioavailability and solubility are ongoing.

5. Experimental

5.1. Chemistry

All the mass spectra (MS) were taken in ESI mode on Agilent 1100 LC-MS (Agilent, Palo Alto, CA, USA). Proton (1H) nuclear magnetic resonance spectroscopy was performed using Bruker ARX-300, 400 MHz spectrometers (Bruker Bioscience, Billerica, MA, USA) with TMS as an internal standard. Column chromatography was run on silica gel (200-300 mesh) from Qingdao Ocean Chemicals (Qingdao, Shandong, China). The IR spectra were recorded by means of the KBr pellet technique on a Bruker FTS 135 spectrometer. Unless otherwise noted, all materials were obtained from commercially available sources and were used without further purification.

*5.2. General procedure for preparation of compounds **5a-e***

4-Nitrobenzyl bromide (10.8 g, 0.05 mol) or 4-chloronitrobenzene (7.9 g, 0.05 mol) was dissolved in acetonitrile (50 mL), excessive secondary amine (50 ml) was added, the reaction was stirred at room temperature for 3 h, water (100 mL) was added, and extracted two times with DCM. The organic extracts were combined, dried over sodium sulfate, filtered, and concentrated to obtain the compounds **5a-e**.

*5.2.1. N,N-dimethyl-1-(4-nitrophenyl)methanamine (**5a**)*

Yellow oil; Yield: 85.5%; MS (ESI) m/z: 181.4 $[M+H]^+$.

*5.2.2. N-ethyl-N-(4-nitrobenzyl)ethanamine (**5b**)*

Yellow oil; Yield: 82.7%; MS (ESI) m/z: 209.3 $[M+H]^+$.

*5.2.3. 4-methyl-1-(4-nitrobenzyl)piperidine (**5c**)*

Yellow oil; Yield: 92.5%; MS (ESI) m/z: 235.5 $[M+H]^+$.

*5.2.4. 4-(4-nitrophenyl)morpholine (**5d**)*

Yellow solid; Yield: 88.5%; MS (ESI) m/z: 209.5 $[M+H]^+$.

*5.2.5. 4-(4-nitrobenzyl)morpholine (**5e**)*

Yellow oil; Yield: 87.9%; MS (ESI) m/z: 223.1 $[M+H]^+$.

*5.3. General procedure for preparation of compounds **6a-e***

A mixture of compounds **5a-e** (0.05 mol) in ethanol was heated to 65 °C, $FeCl_3.6H_2O$ (2.8 g, 0.001 mol) and activated carbon (0.18 g, 0.015 mol) were added, and 80% hydrazine hydrate (25g, 0.5 mol) was added drop wise at such a rate to keep the temperature below 70 °C, the reaction was heated at reflux for 5 h and then cooled to room temperature and concentrated. Water (100 mL) was added, the reaction solution was extracted three times with DCM. The organic extracts were combined, dried over sodium sulfate, filtered, and concentrated to obtain the compounds **6a-e**.

*5.3.1. 4-((dimethylamino)methyl)aniline (**6a**)*

Colorless oil; Yield: 78.2%; MS (ESI) m/z: 151.2 $[M+H]^+$.

*5.3.2. 4-((diethylamino)methyl)aniline (**6b**)*

Colorless oil; Yield: 74.1%; MS (ESI) m/z: 179.3 $[M+H]^+$.

*5.3.3. 4-((4-methylpiperidin-1-yl)methyl)aniline (**6c**)*

Colorless oil; Yield: 83.5%; MS (ESI) m/z: 205.3 $[M+H]^+$.

*5.3.4. 4-morpholinoaniline (**6d**)*

White solid; Yield: 74.1%; MS (ESI) m/z: 179.2 $[M+H]^+$.

*5.3.5. 4-(morpholinomethyl)aniline (**6e**)*

White solid; Yield: 80.9%; MS (ESI) m/z: 193.2 $[M+H]^+$.

*5.4. General procedure for preparation of compounds **7a-e***

A mixture of compounds **6a-e** (0.05 mol) and NH_4SCN (19.03 g, 0.25 mol) in 100 mL glacial acetic acid was cooled to 10 °C in an ice bath and stirred for 10-20 min, and then bromine (2.82 ml, 0.055 mol) in glacial acetic acid was added drop wise at such a rate to keep the temperature below 10°C throughout the addition. The reaction mixture was stirred at room temperature for 4-6 h and then poured into hot water, and basified to pH 11.0 with ammonia solution (NH_4OH). The resulting precipitate was filtered, washed with water and dried to get a light yellow to brown solid. The crude product was purified by chromatography on silica gel using $MeOH/CH_2Cl_2$ to afford the compounds **7a-e**.

*5.4.1. 6-((dimethylamino)methyl)benzo[d]thiazol-2-amine (**7a**)*

Light yellow solid; Yield: 65.4%; MS (ESI) m/z: 208.4 $[M+H]^+$.

*5.4.2. 6-((diethylamino)methyl)benzo[d]thiazol-2-amine (**7b**)*

Light yellow solid; Yield: 88.6%; MS (ESI) m/z: 236.5 $[M+H]^+$.

*5.4.3. 6-((4-methylpiperidin-1-yl)methyl)benzo[d]thiazol-2-amine (**7c**)*

Yellow solid; Yield: 85.3%; MS (ESI) m/z: 262.5 $[M+H]^+$.

*5.4.4. 6-morpholinobenzo[d]thiazol-2-amine (**7d**)*

Brown solid; Yield: 57.3%; MS (ESI) m/z: 236.4 $[M+H]^+$.

*5.4.5. 6-(morpholinomethyl)benzo[d]thiazol-2-amine (**7e**)*

Yellow solid; Yield: 71.2%; MS (ESI) m/z: 250.1 $[M+H]^+$.

*5.5. General procedure for preparation of compounds **8a-e***

A mixture of compounds **7a-e** (0.01 mol) and pyridine (0.8 ml,0.02 mol) in 20 mL DCM was cooled to 0 °C in an ice bath and stirred for 0.5 h, phenyl chloroformate was added drop wise at such a rate to keep the temperature below 10 °C throughout the addition. The reaction mixture was stirred at room temperature for 4-6 h and filtered. The white to light yellow solid was collected and washed with DCM to obtain the compounds **8a-e**.

*5.5.1. Phenyl-6-((dimethylamino)methyl)benzo[d]thiazol-2-ylcarbamate (**8a**)*

White solid; Yield: 94.6%; MS (ESI) m/z: 328.5 $[M+H]^+$.

*5.5.2. Phenyl-6-((diethylamino)methyl)benzo[d]thiazol-2-ylcarbamate (**8b**)*

White solid; Yield: 92.3%; MS (ESI) m/z: 356.4$[M+H]^+$.

*5.5.3. Phenyl-6-((4-methylpiperidin-1-yl)methyl)benzo[d]thiazol-2-ylcarbamate (**8c**)*

White solid; Yield: 97.6%; MS (ESI) m/z: 382.5$[M+H]^+$.

*5.5.4. Phenyl-6-morpholinobenzo[d]thiazol-2-ylcarbamate (**8d**)*

Light yellow solid;Yield: 88.3%; MS (ESI) m/z: 356.4$[M+H]^+$.

*5.5.5. Phenyl 6-(morpholinomethyl)benzo[d]thiazol-2-ylcarbamate (**8e**)*

White solid; Yield: 88.3%; MS (ESI) m/z: 370.1$[M+H]^+$.

*5.6. General procedure for preparation of compounds **9a-e***

A mixture of the compounds **8a-e** (0.01 mol) and 80% hydrazine hydrate (1.29 ml, 0.02 mol) in 20 mL 1,4-dioxane was heated to 80 °C for 6 h, then the reaction mixture was cooled to room

temperature and concentrated, diethyl ether was added and stirred for 0.5 h and filtered, a white to gray solid was collected and washed with a small amount of water. The crude product was purified by chromatography on silica gel using MeOH/CH_2Cl_2 to get the compounds **9a-e**.

*5.6.1. N-(6-((dimethylamino)methyl)benzo[d]thiazol-2-yl)hydrazinecarboxamide (**9a**)*

White solid; Yield: 82.6%; MS (ESI) m/z: 265.7 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.37 (s, 1H), 7.77 (s, 1H), 7.56 (d, *J* = 8.2 Hz, 1H), 7.28 (d, *J* = 8.2 Hz, 1H), 3.44 (s, 2H), 2.15 (s, 6H).

*5.6.2. N-(6-((diethylamino)methyl)benzo[d]thiazol-2-yl)hydrazinecarboxamide (**9b**)*

White solid; Yield: 86.3%; MS (ESI) m/z: 607.9 $[2M+Na]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.34 (s, 1H), 8.49 (s, 1H), 8.03 (s, 1H), 7.70 (d, *J* = 8.0 Hz, 1H), 7.50 (d, *J* = 8.0 Hz, 1H), 4.31 (s, 2H), 3.04 (s, 4H), 1.22 (s, 6H).

*5.6.3. N-(6-((4-methylpiperidin-1-yl)methyl)benzo[d]thiazol-2-yl)hydrazinecarboxamide (**9c**)*

White solid; Yield: 76.8%; MS (ESI) m/z: 319.6 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.33 (s, 1H), 7.79 (s, 1H), 7.57 (d, *J* = 8.0 Hz, 1H), 7.31 (d, *J* = 7.2 Hz, 1H), 7.16 (t, *J* = 7.6 Hz, 1H), 6.76 (s, 1H), 6.74 (s, 1H), 3.54 (s, 2H), 2.81 (s, 2H), 1.94 (s, 2H), 1.58 (d, *J* = 11.6 Hz, 2H), 1.35 (s, 1H), 1.15 (m, 2H), 0.88 (d, *J* = 6.4 Hz, 3H).

*5.6.4. N-(6-morpholinobenzo[d]thiazol-2-yl)hydrazinecarboxamide (**9d**)*

Gray solid; Yield: 90.2%; MS (ESI) m/z: 608.0 $[2M+Na]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.27 (s, 1H), 7.49 (d, *J* = 8.8 Hz, 1H), 7.42 (d, *J* = 1.6 Hz, 1H), 7.05 (dd, *J* = 8.8, 1.6 Hz, 1H), 3.76 (t, *J* = 4.4 Hz, 4H), 3.10 (t, *J* = 4.4 Hz, 4H).

*5.6.5 N-(6-(morpholinomethyl)benzo[d]thiazol-2-yl)hydrazinecarboxamide (**9e**)*

White solid; Yield: 80.8%; MS (ESI) m/z: 308.1 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.39 (s, 1H), 7.80 (s, 1H), 7.57 (d, *J* = 8.0 Hz, 1H), 7.31 (d, *J* = 8.0 Hz, 1H), 3.57 (s, 4H), 3.54 (s, 2H), 2.38 (s, 4H).

*5.7. General procedure for preparation of compounds **10a-g***

A mixture of 2,4-dihydroxybenzaldehyde (1.38 g, 0.01 mol), substituted benzyl chloride (0.011 mol), $NaHCO_3$ (1.26 g, 0.015 mol) and catalytic amount of potassium iodide in 20 mL acetonitrile was heated to 60 ℃ for 36 h and hot filtered, the filtrate was cooled to 10 ℃ and filtered, the filtrated cake was collected and recrystallized with EtOH to obtain compounds **10a-g**.

*5.7.1. 4-benzyloxy-2-hydroxybenzaldehyde (**10a**)*

White solid; Yield: 77.8%; MS (ESI) m/z: 227.2 $[M-H]^-$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.48 (s, 1H), 9.72 (s, 1H), 7.44 (d, *J* = 8.8 Hz, 1H), 7.43 – 7.39 (m, 4H), 7.39 – 7.33 (m, 1H), 6.62 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.51 (d, *J* = 2.4 Hz, 1H), 5.11 (s, 2H).

*5.7.2. 4-(4-methylbenzyloxy)-2-hydroxybenzaldehyde (**10b**)*

Yellow solid; Yield: 75.4%; MS (ESI) m/z: 241.2 $[M-H]^-$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.48 (s, 1H), 9.71 (s, 1H), 7.43 (d, *J* = 8.8 Hz, 1H), 7.30 (d, *J* = 8.0 Hz, 2H), 7.21 (d, *J* = 8.0 Hz, 2H), 6.60 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.50 (d, *J* = 2.4 Hz, 1H), 5.06 (s, 2H), 2.37 (s, 3H).

*5.7.3. 4-(4-tert-butylbenzyloxy)-2-hydroxybenzaldehyde (**10c**)*

Yellow solid; Yield: 85.7%; MS (ESI) m/z: 282.6 $[M-H]^-$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.49 (s, 1H), 9.72 (s, 1H), 7.45 – 7.41 (m, 3H), 7.42 (s, 1H), 7.36 (s, 1H), 7.34 (s, 1H), 6.61 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.52 (d, *J* = 2.4 Hz, 1H), 5.07 (s, 2H), 1.33 (s, 9H).

*5.7.4. 4-(4-chlorobenzyloxy)-2-hydroxybenzaldehyde (**10d**)*

White solid; Yield: 78.2%; MS (ESI) m/z: 261.8 $[M-H]^-$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.48 (s, 1H), 9.72 (s, 1H), 7.44 (d, *J* = 8.8 Hz, 1H), 7.40-7.32 (m, 4H), 6.60 (dd, *J* = 8.8, 2.4 Hz,

1H), 6.48 (d, *J* = 2.4 Hz, 1H), 5.07 (s, 2H).

*5.7.5. 4-(3-chlorobenzyloxy)-2-hydroxybenzaldehyde (**10e**)*

White solid; Yield: 80.9%; MS (ESI) m/z: 261.2 $[M-H]^-$; ^1H NMR (400 MHz, $CDCl_3$) δ 11.47 (s, 1H), 9.73 (s, 1H), 7.50 – 7.43 (m, 2H), 7.35 (m, 1H), 7.18 (t, *J* = 7.6 Hz, 1H), 7.11 (t, *J* = 7.6 Hz, 1H), 6.62 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.53 (d, *J* = 2.0 Hz, 1H), 5.18 (s, 2H).

*5.7.6. 4-(2-chlorobenzyloxy)-2-hydroxybenzaldehyde (**10f**)*

White solid; Yield: 75.8%; MS (ESI) m/z: 261.9 $[M-H]^-$; ^1H NMR (400 MHz, $CDCl_3$) δ 11.48 (s, 1H), 9.73 (s, 1H), 7.46 (d, *J* = 8.8 Hz, 1H), 7.42 (s, 1H), 7.32 (m, 3H), 6.61 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.49 (d, *J* = 2.4 Hz, 1H), 5.09 (s, 2H).

*5.7.7. 4-(2,4-dichlorobenzyloxy)-2-hydroxybenzaldehyde (**10g**)*

Gray solid; Yield: 82.6%; MS (ESI) m/z: 295.1 $[M-H]^-$; ^1H NMR (400 MHz, $CDCl_3$) δ 11.47 (s, 1H), 9.74 (s, 1H), 7.47 (d, *J* = 8.8 Hz, 1H), 7.45 – 7.43 (m, 2H), 7.29 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.62 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.51 (d, *J* = 2.4 Hz, 1H), 5.17 (s, 2H).

*5.8. Preparation of 2-(benzo[d][1,3]dioxol-5-yl)ethanethioamide (**11**)*

A mixture of the sodium hydrosulfide (14 g, 0.25 mol), $MgCl_2{\cdot}6H_2O$ (25.4 g, 0.125 mol) and 2-(benzo[d][1,3]dioxol-5-yl)acetonitrile (16.1 g, 0.1 mol) in 140 mL DMF and 30 mL water was stirred for 15 h at room temperature, and then poured into ice-water with acutely stirring, The solution was treated with concentrated hydrochloric acid to achieve pH to 5.0. The product was separated by filtration to obtain compound **11** as a white solid (10.3 g, 53%), MS (ESI) m/z: 196.1$[M+H]^+$.

*5.9. Preparation of 2-(benzo[d][1,3]dioxol-5-ylmethyl)-4-(chloromethyl)thiazole (**12**)*

The compound **11** (10.3 g, 0.05 mol) and 1,3-dichloroacetone (7 g, 0.05 mol) were added in 100 mL acetonitrile and stirred for 4 h at 50 °C and then filtered. The filter cake was collected and washed with water to get the compound **12** as a light yellow solid (8.7 g, 61%). MS (ESI) m/z: 268.8 $[M+H]^+$.

*5.10. Preparation of 4-((2-(benzo[d][1,3]dioxol-5-ylmethyl)thiazol-4-yl)methoxy)-2-hydroxybenzaldehyde (**16**)*

A mixture of the compound **12** (8.7 g, 0.03 mol) and 2,4-dihydroxybenzaldehyde (4.5 g, 0.03mol), $NaHCO_3$ (3.2 g, 0.035 mol) and catalytic amount of potassium iodide in 50 mL acetonitrile was heated to 80 °C in an oil-bath for approximate 2 h. After cooling to ambient temperature, the contents were concentrated under reduced pressure, 30 mL methanol was added, the solution was decolorized with active carbon and hot filtered, the filtrate was cooled to room temperature and filtered, a tan solid was collected as compound **13** (7.6 g, 64%). MS (ESI) m/z: 367.2 $[M-H]^-$; ^1H NMR (400 MHz, $CDCl_3$) δ 11.47 (s, 1H), 9.73 (s, 1H), 7.45 (d, *J* = 8.8 Hz, 1H), 7.21 (s, 1H), 6.80 (s, 1H), 6.79 (s, 2H), 6.64 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.54 (d, *J* = 2.4 Hz, 1H), 5.96 (s, 2H), 5.21 (s, 2H), 4.25 (s, 2H).

*5.11 General procedure for preparation of compounds **14a-h**, **15a-x**, **16a-d***

A mixture of the compounds **9a-e** (0.001 mol), appropriate 2-hydroxy aromatic aldehydes (0.0011 mol) and a drop of glacial acetic acid in 10 mL ethanol was heated at reflux for 6 h and cooled to room temperature, separated by filtration, and the cake was washed with diethyl ether to get a white to light yellow solid. The crude product was purified by chromatography on silica gel using $MeOH/CH_2Cl_2$ to afford the compounds **14a-h**, **15a-x**, **16a-d**.

*5.11.1. (E)-N-(6-((dimethylamino)methyl)benzo[d]thiazol-2-yl)-2-(2-hydroxybenzylidene)hydrazinecarboxamide (**14a**)*

Yield: 58%; MS (ESI) m/z: 368.2 [M-H]$^-$; IR (KBr, cm^{-1}): 3424.1, 2921.9, 1725.3, 1705.8, 1630.3, 1609.1, 1565.8, 1543.6, 1501.9, 1487.9, 1465.1, 1442.7, 1384.3, 1276.5, 1246.1, 1128.1, 1036.0, 924.9, 809.1, 779.3, 619.1; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.31 (s, 2H), 8.34 (s, 1H), 8.03 (s, 1H), 7.83 (s, 1H), 7.62 (d, *J* = 7.6 Hz, 1H), 7.34 (d, *J* = 8.4 Hz, 1H), 7.25 (t, *J* = 7.6 Hz, 1H), 6.89 (m, 2H), 3.52 (s, 2H), 2.20 (s, 6H); Anal. Calcd, for $C_{18}H_{19}N_5O_2S$ (%): C, 58.52; H, 5.18; N.18.96. Found (%): 58.53; H, 5.17; N.18.94.

*5.11.2. (E)-N-(6-((dimethylamino)methyl)benzo[d]thiazol-2-yl)-2-(2,4-dihydroxybenzylidene)hydrazinecarboxamide (**14b**)*

Yield: 54%; MS (ESI) m/z: 384.1 [M-H]$^-$; IR (KBr, cm^{-1}): 3187.6, 3093.8, 2950.2, 2553.5,1695.9, 1679.2, 1629.8, 1604.0, 1564.2, 1549.3, 1501.7, 1478.0, 1460.0, 1320.2, 1288.9, 1166.5, 1123.4, 978.1, 947.6, 840.5, 795.6, 695.2, 671.3, 645.2, 608.9; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.05 (s, 2H), 9.82 (s, 2H), 8.21 (s, 1H), 7.81 (s, 2H), 7.60 (d, *J* = 8.0 Hz, 1H), 7.32 (d, *J* = 8.0 Hz, 1H), 6.32 (s, 2H), 3.48 (s, 2H), 2.17 (s, 6H); Anal. Calcd, for $C_{18}H_{19}N_5O_3S$ (%): C, 56.09; H, 4.97; N, 18.17. Found (%): C, 56.11; H, 4.96; N, 18.18.

*5.11.3. (E)-N-(6-((diethylamino)methyl)benzo[d]thiazol-2-yl)-2-(2-hydroxybenzylidene)hydrazine carboxamide (**14c**)*

Yield: 62%; MS (ESI) m/z: 396.4 [M-H]$^-$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.31 (s, 2H), 8.34 (s, 1H), 8.04 (s, 1H), 7.84 (s, 1H), 7.61 (d, *J* = 8.0 Hz, 1H), 7.36 (d, *J* = 8.0 Hz, 1H), 7.25 (t, *J* = 7.6 Hz, 1H), 6.88 (m, 2H), 3.66 (s, 2H), 2.52 (d, *J* = 6.8 Hz, 4H), 1.01 (t, *J* = 6.8 Hz, 6H); Anal. Calcd, for $C_{20}H_{23}N_5O_2S$ (%): C, 60.43; H, 5.83; N, 17.62. Found (%): C, 60.45; H, 5.82; N, 17.64.

*5.11.4. (E)-N-(6-((diethylamino)methyl)benzo[d]thiazol-2-yl)-2-(2,4-dihydroxybenzylidene)hydrazinecarboxamide (**14d**)*

Yield: 56%; MS (ESI) m/z: 412.4 [M-H]$^-$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.09 (s, 2H), 9.93 (s, 1H), 8.21 (s, 1H), 7.84 (s, 1H), 7.60 (d, *J* = 8.0 Hz, 1H), 7.36 (d, *J* = 8.0 Hz, 1H), 6.32 (m, 2H), 3.67 (s, 2H), 2.55 (d, *J* = 6.8 Hz, 4H), 1.01 (t, *J* = 6.8 Hz, 6H); Anal. Calcd, for $C_{20}H_{23}N_5O_3S$ (%): C, 58.09; H, 5.61; N, 16.94. Found (%): C, 58.10; H, 5.63; N, 16.92.

*5.11.5. (E)-N-(6-((4-methylpiperidin-1-yl)methyl)benzo[d]thiazol-2-yl)-2-(2-hydroxybenzylidene) hydrazinecarboxamide (**14e**)*

Yield: 64%; MS (ESI) m/z: 422.2 [M-H]$^-$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.24 (s, 2H), 8.34 (s, 1H), 8.03 (s, 1H), 7.80 (s, 1H), 7.60 (d, *J* = 8.0 Hz, 1H), 7.33 (d, *J* = 8.0 Hz, 1H), 7.29-7.20 (m, 1H), 6.94-6.84 (m, 2H), 3.52 (s, 2H), 2.79 (d, *J* = 11.2 Hz, 2H), 1.93 (t, *J* = 10.8 Hz, 2H), 1.56 (d, *J* = 11.2 Hz, 2H), 1.32 (m, 1H), 1.23-1.06 (m, 2H), 0.88 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.24, 156.87, 153.43, 147.76, 141.87, 134.01, 131.75, 131.44, 128.46, 127.49, 121.92, 120.32, 119.69, 119.35, 116.57, 62.80, 53.68(2C), 34.34(2C), 30.73, 22.27; Anal. Calcd, for $C_{22}H_{25}N_5O_2S$ (%): C, 62.39; H, 5.95; N, 16.54. Found (%): C, 62.37; H, 5.96; N, 16.52.

*5.11.6. (E)-N-(6-((4-methylpiperidin-1-yl)methyl)benzo[d]thiazol-2-yl)-2-(2,4-dihydroxybenzylidene)hydrazinecarboxamide (**14f**)*

Yield: 71%; MS (ESI) m/z: 438.3 [M-H]$^-$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.24 (s, 2H), 10.57 (s, 2H), 8.23 (s, 1H), 8.12 (s, 1H), 7.94 (s, 1H), 7.70 (d, *J* = 8.0 Hz,, 1H), 7.64 (d, *J* = 8.0 Hz, 1H), 6.35 (m, 2H), 4.31 (s, 2H), 3.29 (d, *J* = 11.2 Hz, 2H), 2.88 (d, *J* = 11.2 Hz, 2H), 1.73 (d, *J* = 12.4 Hz, 2H), 1.59-1.41 (m, 3H), 0.88 (d, *J* = 4.8 Hz, 3H); Anal. Calcd, for $C_{22}H_{25}N_5O_3S$ (%): C, 60.12; H, 5.73; N, 15.93. Found (%): C, 60.14; H, 5.74; N, 15.91.

5.11.7. (E)-N-(6-(morpholinomethyl)benzo[d]thiazol-2-yl)-2-(2-hydroxybenzylidene) hydrazinec-

*arboxamide (**14g**)*

Yield: 76%; MS (ESI) m/z: 410.1 [M-H]$^-$; IR (KBr, cm^{-1}): 3421.7, 3200.2, 3074.0, 2925.1, 2855.6, 2806.3, 1695.0, 1679.3, 1608.8, 1571.7, 1541.7, 1488.4, 1461.3, 1332.8, 1311.4, 1274.3, 1115.0, 1007.2, 864.5, 808.4, 760.8, 684.7; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.24 (s, 2H), 10.02 (s, 1H), 8.34 (s, 1H), 8.09 (s, 1H), 7.84 (s, 1H), 7.62 (d, *J* = 5.6 Hz, 1H), 7.36 (d, *J* = 5.6 Hz, 1H), 7.25 (t, *J* = 7.6 Hz,1H), 6.88 (m, 2H), 3.58 (s, 4H), 3.54 (s, 2H), 2.37 (s, 4H); Anal. Calcd, for $C_{20}H_{21}N_5O_3S$ (%): C, 58.38; H, 5.14; N, 17.02. Found (%): C, 58.38; H, 5.15; N, 17.01.

*5.11.8. (E)-N-(6-morpholinobenzo[d]thiazol-2-yl)-2-(2-hydroxybenzylidene)hydrazinecarboxamide (**14h**)*

Yield: 82%; MS (ESI) m/z: 396.1 [M-H]$^-$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.31 (s, 2H), 8.34 (s, 1H), 8.03 (s, 1H), 7.83 (s, 1H), 7.62 (d, *J* = 7.6 Hz, 1H), 7.34 (d, *J* = 8.8 Hz, 1H), 7.25 (t, *J* = 7.6 Hz, 1H), 6.89 (m, 2H), 3.52 (s, 2H), 2.20 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 156.80, 153.18, 148.33, 133.16, 131.45, 128.33, 120.35, 119.71, 116.54, 116.14, 107.57, 66.62(2C), 49.99(2C); Anal. Calcd, for $C_{19}H_{19}N_5O_3S$ (%): C, 57.42; H, 4.82; N, 17.62. Found (%): C, 57.41; H, 4.83; N, 17.60.

*5.11.9. (E)-N-(6-((dimethylamino)methyl)benzo[d]thiazol-2-yl)-2-(2-hydroxy-4-(4-methylbenzyloxy)benzylidene)hydrazinecarboxamide (**15a**)*

Yield: 71%; MS (ESI) m/z: 488.2 [M-H]$^-$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.15 (s, 2H), 8.23 (s, 1H), 7.94 (s, 1H), 7.81 (s, 1H), 7.60 (d, *J* = 8.4 Hz, 1H), 7.34 (d, *J* = 7.6 Hz, 2H), 7.31 (s, 1H), 7.21 (d, *J* = 7.6 Hz, 2H), 6.55 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.51 (d, *J* = 2.0 Hz, 1H), 5.06 (s, 2H), 3.47 (s, 2H), 2.31 (s, 3H), 2.17 (s, 6H).; Anal. Calcd, for $C_{26}H_{27}N_5O_3S$ (%): C, 63.78; H, 5.56; N, 14.30. Found (%): C, 63.80; H, 5.55; N, 14.31.

*5.11.10. (E)-N-(6-((dimethylamino)methyl)-benzo[d]thiazol-2-yl)-2-(4-(4-tert-butylbenzyloxy)-2-hydroxybenzylidene)hydrazinecarboxamide (**15b**)*

Yield: 69%; MS (ESI) m/z: 530.6 [M-H]$^-$, 566.6 [M+Cl]$^-$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.38 (s, 2H), 8.26 (s, 1H), 8.08 (s, 1H), 8.00 (s, 1H), 7.73 (s, 1H), 7.58 (d, *J* = 8.4 Hz, 1H), 7.42 (d, *J* = 8.4 Hz, 2H), 7.38 (d, *J* = 8.4 Hz, 2H), 6.56 (s, 1H), 6.54 (s, 1H), 5.07 (s, 2H), 4.36 (s, 2H), 2.72 (s, 6H), 1.29 (s, 9H); Anal. Calcd, for $C_{29}H_{33}N_5O_3S$ (%): C, 65.51; H, 6.26; N, 13.17. Found (%): C, 65.54; H, 6.25; N, 13.16.

*5.11.11. (E)-N-(6-((dimethylamino)methyl)ben-zo[d]thiazol-2-yl)-2-(4-(4-chlorobenzyloxy)-2-hydroxybenzylidene)hydrazinecarboxamide (**15c**)*

Yield: 65%; MS (ESI) m/z: 508.1 [M-H]$^-$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.13 (s, 2H), 8.24 (s, 1H), 8.00 (s, 1H), 7.81 (s, 1H), 7.60 (d, *J* = 8.0 Hz, 1H), 7.48 (s, 4H), 7.32 (d, *J* = 8.0 Hz, 1H), 6.56 (dd, *J* = 8.4, 2.4 Hz, 1H), 6.51 (d, *J* = 2.4 Hz, 1H), 5.12 (s, 2H), 3.47 (s, 2H), 2.17 (s, 6H); Anal. Calcd, for $C_{25}H_{24}ClN_5O_3S$ (%): C, 58.88; H, 4.74; N, 13.73. Found (%): C, 58.89; H, 4.75; N, 13.70.

*5.11.12. (E)-N-(6-((dimethylamino)methyl)benzo[d]thiazol-2-yl)-2-(4-(3-chlorobenzyloxy)-2-hydroxybenzylidene)hydrazinecarboxamide (**15d**)*

Yield: 69%; MS (ESI) m/z: 508.2 [M-H]$^-$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.16 (s, 2H), 8.24 (s, 1H), 7.98 (s, 1H), 7.81 (s, 1H), 7.60 (d, *J* = 8.0 Hz, 1H), 7.53 (s, 1H), 7.46 – 7.39 (m, 3H), 7.32 (d, *J* = 8.0 Hz, 1H), 6.57 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.52 (d, *J* = 2.4 Hz, 1H), 5.14 (s, 2H), 3.47 (s, 2H), 2.17 (s, 6H); Anal. Calcd, for $C_{25}H_{24}ClN_5O_3S$ (%): C, 58.88; H, 4.74; N, 13.73. Found (%): C, 58.85; H, 4.77; N, 13.72.

5.11.13. (E)-N-(6-((dimethylamino)methyl)benzo[d]thiazol-2-yl)-2-(4-(2-chlorobenzyloxy)-2hydr-

*oxybenzylidene)hydrazinecarboxamide (**15e**)*

Yield: 75%; MS (ESI) m/z: 508.2 $[M-H]^-$; IR (KBr, cm^{-1}): 3178.0, 3077.6, 2976.5, 2940.0, 2856.4, 2814.6, 2766.8, 1690.7, 1679.0, 1628.3, 1609.5, 1571.9, 1556.5, 1538.5, 1492.3, 1457.2, 1384.2, 1360.5, 1318.4, 1280.3, 1115.8, 1037.9, 1021.5, 845.7, 835.6, 814.3, 804.1, 752.5; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.10 (s, 2H), 8.25 (s, 1H), 7.85 (s, 1H), 7.81 (s, 1H), 7.64 – 7.53 (m, 2H), 7.48 – 7.40 (m, 1H), 7.33 (d, *J* = 8.4 Hz, 1H), 7.30 – 7.21 (m, 2H), 6.58 (dd, *J* = 8.4, 2.4 Hz, 1H), 6.55 (d, *J* = 2.4 Hz, 1H), 5.15 (s, 2H), 3.51 (s, 2H), 2.19 (s, 6H); Anal. Calcd, for $C_{25}H_{24}ClN_5O_3S$ (%): C, 58.88; H, 4.74; N, 13.73. Found (%): C, 58.86; H, 4.78; N, 13.71.

*5.11.14. (E)-N-(6-((diethylamino)methyl)benzo[d]thiazol-2-yl)-2-(2-hydroxy-4-(4-methylbenzyloxy)benzylidene)hydrazinecarboxamide (**15f**)*

Yield: 55%; MS (ESI) m/z: 516.4 $[M-H]^-$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.15 (s, 2H), 8.24 (s, 1H), 7.94 (s, 1H), 7.85 (s, 1H), 7.60 (d, *J* = 8.0 Hz, 1H), 7.37 (d, *J* = 8.0 Hz, 1H), 7.34 (d, *J* = 7.6 Hz, 2H), 7.21 (d, *J* = 7.6 Hz, 2H), 6.55 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.51 (d, *J* = 2.0 Hz, 1H), 5.05 (s, 2H), 3.70 (s, 2H), 2.55 (q, *J* = 7.2 Hz, 4H), 2.31 (s, 3H), 1.02 (t, *J* = 7.2 Hz, 6H).; Anal. Calcd, for $C_{28}H_{31}N_5O_3S$ (%): C, 64.97; H, 6.04; N, 13.53; Found (%): C, 64.99; H, 6.03; N, 13.52.

*5.11.15. (E)-N-(6-((diethylamino)methyl)benzo[d]thiazol-2-yl)-2-(4-(4-tert-butylbenzyloxy)-2-hydroxybenzylidene)hydrazinecarboxamide (**15g**)*

Yield: 75%; MS (ESI) m/z: 558.6 $[M-H]^-$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.37 (s, 1H), 11.19 (s, 1H), 8.26 (s, 1H), 8.13 (s, 1H), 8.00 (s, 1H), 7.75 (d, *J* = 7.8 Hz, 1H), 7.61 (d, *J* = 7.8 Hz, 1H), 7.42 (d, *J* = 8.4 Hz, 2H), 7.37 (d, *J* = 8.4 Hz, 2H), 6.56 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.53 (d, *J* = 2.0 Hz, 1H), 5.07 (s, 2H), 4.38 (s, 2H), 3.08 (q, *J* = 7.2 Hz, 4H), 1.29 (s, 9H), 1.26 (t, *J* = 7.2 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.41, 161.05, 158.50, 152.99, 150.86, 150.09, 141.72, 134.25, 132.23, 129.89, 129.47, 128.11(2C), 125.65(2C), 125.20, 124.78, 120.26, 113.35, 107.36, 102.34, 69.55, 55.16, 46.03(2C), 34.76, 31.59(3C), 8.79(2C); Anal. Calcd, for $C_{31}H_{37}N_5O_3S$ (%): C, 66.52; H, 6.66; N, 12.51; Found (%): C, 66.55; H, 6.63; N, 12.50.

*5.11.16. (E)-N-(6-((diethylamino)methyl)benzo[d]thiazol-2-yl)-2-(4-(4-chlorobenzyloxy)-2-hydroxybenzylidene)hydrazinecarboxamide (**15h**)*

Yield: 72%; MS (ESI) m/z: 536.3 $[M-H]^-$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.15 (s, 2H), 8.25 (s, 1H), 7.96 (s, 2H), 7.66 (d, *J* = 7.6 Hz, 1H), 7.48 (s, 5H), 6.56 (dd, *J* = 8.8, 2.0 Hz, 1H), 6.53 (d, *J* = 2.0 Hz, 1H), 5.12 (s, 2H), 3.96 (s, 2H), 2.75 (s, 4H), 1.11 (s, 6H); Anal. Calcd, for $C_{27}H_{28}ClN_5O_3S$ (%): C, 60.27; H, 5.25; N, 13.02; Found (%): C, 60.27; H, 5.27; N, 13.03.

*5.11.17. (E)-N-(6-((diethylamino)methyl)benzo[d]thiazol-2-yl)-2-(4-(3-chlorobenzyloxy)-2-hydroxybenzylidene) hydrazinecarboxamide (**15i**)*

Yield: 81%; MS (ESI) m/z: 536.4 $[M-H]^-$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.16 (s, 2H), 8.25 (s, 1H), 7.96 (s, 2H), 7.69 (d, *J* = 8.4 Hz, 1H), 7.53 (s, 1H), 7.50-7.37 (m, 4H), 6.57 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.54 (d, *J* = 2.0 Hz, 1H), 5.14 (s, 2H), 3.96 (s, 2H), 2.76 (s, 4H), 1.12 (s, 6H); Anal. Calcd, for $C_{27}H_{28}ClN_5O_3S$ (%): C, 60.27; H, 5.25; N, 13.02; Found (%): C, 60.24; H, 5.26; N, 13.04.

*5.11.18. (E)-N-(6-((diethylamino)methyl)benzo[d]thiazol-2-yl)-2-(4-(2-chlorobenzyloxy)-2-hydroxybenzylidene) hydrazinecarboxamide (**15j**)*

Yield: 78%; MS (ESI) m/z: 536.3 $[M-H]^-$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.37 (s, 2H), 8.88 (s, 1H), 8.27 (s, 1H), 8.11 (s, 1H), 7.96 (s, 1H), 7.72 (d, *J* = 8.0 Hz, 1H), 7.62 (s, 1H), 7.56 (d, *J* = 8.0 Hz, 1H), 7.48 – 7.41 (m, 1H), 7.29 – 7.22 (m, 2H), 6.61 – 6.55 (m, 2H), 5.15 (s, 2H), 4.31 (s, 2H), 3.02 (s, 4H), 1.24 (s, 6H); Anal. Calcd, for $C_{27}H_{28}ClN_5O_3S$ (%): C, 60.27; H, 5.25; N,

13.02; Found (%): C, 60.28; H, 5.26; N, 13.01.

*5.11.19. (E)-N-(6-((diethylamino)methyl)benzo[d]thiazol-2-yl)-2-(4-(2,4-dichlorobenzyloxy)-2-hydroxybenzylidene)hydrazinecarboxamide (**15k**)*

Yield: 85%; MS (ESI) m/z: 570.2 $[M-H]^-$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.16 (s, 2H), 8.25 (s, 1H), 8.04 (s, 1H), 7.85 (s, 1H), 7.72 (d, *J* = 1.2 Hz, 1H), 7.61 (m, 2H), 7.50 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.37 (d, *J* = 8.0 Hz, 1H), 6.58 (d, *J* = 8.8 Hz, 1H), 6.53 (s, 1H), 5.16 (s, 2H), 3.68 (s, 2H), 2.54 (d, *J* = 7.2 Hz, 4H), 1.02 (t, *J* = 7.2 Hz, 6H); Anal. Calcd, for $C_{27}H_{27}Cl_2N_5O_3S$ (%): C, 56.64; H, 4.75; N, 12.23; Found (%): C, 56.66; H, 4.74; N, 12.22.

*5.11.20. (E)-N-(6-((diethylamino)methyl)benzo[d]thiazol-2-yl)-2-(2-hydroxy-4-benzyloxybenzylidene)hydrazinecarboxamide (**15l**)*

Yield: 84%; MS (ESI) m/z: 504.1 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.40 (s, 1H), 11.16 (s, 1H), 8.26 (s, 1H), 8.09 (s, 2H), 7.76 (d, *J* = 7.2 Hz, 1H), 7.55 (m, 1H), 7.46 (d, *J* = 7.2 Hz, 2H), 7.41 (t, *J* = 7.2 Hz, 2H), 7.36 (d, *J* = 7.2 Hz, 1H), 6.57 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.53 (d, *J* = 2.4 Hz, 1H), 5.12 (s, 2H), 4.40 (s, 2H), 3.11 (q, *J* = 7.2 Hz, 4H), 1.25 (t, *J* = 7.2 Hz, 6H); Anal. Calcd, for $C_{27}H_{29}N_5O_3S$ (%): C, 64.39; H, 5.80; N, 13.91; Found (%): C, 64.41; H, 5.81; N, 13.90.

*5.11.21. (E)-N-(6-((4-methylpiperidin-1-yl)methyl)benzo[d]thiazol-2-yl)-2-(2-hydroxy-4-(4-methylbenzyloxy)benzylidene) hydrazinecarboxamide (**15m**)*

Yield: 67%; MS (ESI) m/z: 542.3 $[M-H]^-$; 578.3$[M+Cl]^-$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.11 (s, 2H), 8.24 (s, 1H), 7.98 (s, 1H), 7.82 (s, 1H), 7.61 (d, *J* = 8.4 Hz, 1H), 7.34 (d, *J* = 8.0 Hz, 3H), 7.21 (d, *J* = 8.0 Hz, 2H), 6.55 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.51 (d, *J* = 2.0 Hz, 1H), 5.06 (s, 2H), 3.61 (s, 2H), 2.85 (d, *J* = 9.2 Hz, 2H), 2.31 (s, 3H), 2.04 (s, 2H), 1.58 (d, *J* = 11.2 Hz, 2H), 1.35 (s, 1H), 1.16 (m, 2H), 0.89 (d, *J* = 6.4 Hz, 3H); Anal. Calcd, for $C_{30}H_{33}N_5O_3S$ (%): C, 66.27; H, 6.12; N, 12.88; Found (%): C, 66.24; H, 6.14; N, 12.89.

*5.11.21. (E)-N-(6-((4-methylpiperidin-1-yl)-methyl)benzo[d]thiazol-2-yl)-2-(4-(4-tert-butylbenzyloxy)-2-hydroxybenzylidene) hydrazinecarboxamide (**15n**)*

Yield: 71%; MS (ESI) m/z: 584.6 $[M-H]^-$; 620.6$[M+Cl]^-$; IR (KBr, cm^{-1}): 3198.0, 3088.5, 2947.0, 2928.1, 2866.7, 2791.1, 2753.5, 1693.2, 1629.7, 1608.5, 1569.6, 1549.7, 1503.6, 1465.3, 1294.1, 1165.2, 1131.9, 1119.4, 1045.2, 1017.6, 865.0, 817.5, 789.8, 617.5, 545.7; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.10 (s, 2H), 8.24 (s, 1H), 8.00 (s, 1H), 7.80 (s, 1H), 7.59 (d, *J* = 7.2 Hz, 1H), 7.42 (d, *J* = 8.4 Hz, 2H), 7.37 (d, *J* = 8.4 Hz, 2H), 7.32 (d, *J* = 8.4 Hz, 1H), 6.55 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.51 (d, *J* = 2.0 Hz, 1H), 5.07 (s, 2H), 3.52 (s, 2H), 2.79 (d, *J* = 10.4 Hz, 2H), 1.93 (s, 2H), 1.56 (d, *J* = 11.2 Hz, 2H), 1.29 (s, 10H), 1.13 (m, 2H), 0.88 (d, *J* = 6.4 Hz, 3H); Anal. Calcd, for $C_{33}H_{39}N_5O_3S$ (%): C, 67.66; H, 6.71; N, 11.96; Found (%): C, 67.63; H, 6.72; N, 11.94.

*5.11.22. (E)-N-(6-((4-methylpiperidin-1-yl)methyl)benzo[d]thiazol-2-yl)-2-(4-(4-chlorobenzyloxy)-2-hydroxybenzylidene) hydrazinecarboxamide (**15o**)*

Yield: 78%; MS (ESI) m/z: 562.2 $[M-H]^-$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.15 (s, 2H), 8.25 (s, 1H), 7.96 (s, 2H), 7.69 (d, *J* = 8.0 Hz, 1H), 7.49-7.46 (m, 5H), 6.56 (dd, *J* = 8.0, 2.4 Hz, 1H), 6.52 (d, *J* = 2.4 Hz, 1H), 5.12 (s, 2H), 4.10 (s, 2H), 3.17 (s, 2H), 2.67 (s, 2H), 1.71 (d, *J* = 12.4 Hz, 2H), 1.52 (s, 1H), 1.28 (s, 2H), 0.90 (d, *J* = 6.4 Hz, 3H); Anal. Calcd, for $C_{29}H_{30}ClN_5O_3S$ (%): C, 61.75; H, 5.36; N, 12.42; Found (%): C, 61.78; H, 5.35; N, 12.40.

*5.11.23. (E)-N-(6-((4-methylpiperidin-1-yl)methyl)benzo[d]thiazol-2-yl)-2-(4-(3-chlorobenzyloxy)-2-hydroxybenzylidene) hydrazinecarboxamide (**15p**)*

Yield: 75%; MS (ESI) m/z: 562.2 $[M-H]^-$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.12 (s, 2H), 8.24 (s, 1H), 8.04 (s, 1H), 7.82 (s, 1H), 7.61 (d, *J* = 7.6 Hz, 1H), 7.53 (s, 1H), 7.43 (m, 3H), 7.34

(d, *J* = 8.4 Hz, 1H), 6.57 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.52 (*J* = 2.0 Hz, 1H), 5.14 (s, 2H), 3.62 (s, 2H), 2.85 (d, *J* = 10.0 Hz, 2H), 2.05 (s, 2H), 1.59 (d, *J* = 12.4 Hz, 2H), 1.35 (s, 1H), 1.16 (m, 2H), 0.88 (d, *J* = 6.4 Hz, 3H); Anal. Calcd, for $C_{29}H_{30}ClN_5O_3S$ (%): C, 61.75; H, 5.36; N, 12.42; Found (%): C, 61.79; H, 5.34; N, 12.43.

*5.11.24. (E)-N-(6-((4-methylpiperidin-1-yl)methyl)benzo[d]thiazol-2-yl)-2-(4-(2-chlorobenzyloxy)-2-hydroxybenzylidene) hydrazinecarboxamide (**15q**)*

Yield: 71%; MS (ESI) m/z: 562.2 $[M-H]^-$; IR (KBr, cm^{-1}): 3420.5, 2922.8, 1698.9, 1631.2, 1572.6, 1557.4, 1510.2, 1461.7, 1384.8, 1250.1, 1212.4, 1182.2, 1129.0, 1113.9, 1011.2, 975.0, 824.6, 797.4, 651.3; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.10 (s, 2H), 8.25 (s, 1H), 8.00 (s, 1H), 7.80 (s, 1H), 7.62-7.54 (m, 2H), 7.49 – 7.40 (m, 1H), 7.35-7.29 (m, 1H), 7.29-7.23 (m, 2H), 6.58 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.54 (d, *J* = 2.0 Hz, 1H), 5.15 (s, 2H), 3.52 (s, 2H), 2.79 (d, *J* = 10.0 Hz, 2H), 1.93 (s, 2H), 1.56 (d, *J* = 12.0 Hz, 2H), 1.33 (s, 1H), 1.14 (m, 2H), 0.88 (d, *J* = 6.4 Hz, 3H); Anal. Calcd, for $C_{29}H_{30}ClN_5O_3S$(%): C, 61.75; H, 5.36; N, 12.42; Found (%): C, 61.72; H, 5.37; N, 12.45.

*5.11.25. (E)-N-(6-((4-methylpiperidin-1-yl)methyl)benzo[d]thiazol-2-yl)-2-(4-(2,4-dichlorobenzyloxy)-2-hydroxybenzylidene) hydrazinecarboxamide (**15r**)*

Yield: 88%; MS (ESI) m/z: 596.2 $[M-H]^-$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.14 (s, 2H), 8.25 (s, 1H), 7.99 (s, 1H), 7.79 (s, 1H), 7.71 (s, 1H), 7.62 (d, *J* = 8.2 Hz, 1H), 7.59 (s, 1H), 7.50 (d, *J* = 8.4 Hz, 1H), 7.32 (d, *J* = 8.2 Hz, 1H), 6.58 (d, *J* = 8.4 Hz, 1H), 6.53 (s, 1H), 5.16 (s, 2H), 3.51 (s, 2H), 2.79 (d, *J* = 10.8 Hz, 2H), 1.92 (t, *J* = 10.8 Hz, 2H), 1.56 (d, *J* = 12.0 Hz, 2H), 1.32 (s, 1H), 1.15 (m, 2H), 0.88 (d, *J* = 6.4 Hz, 3H); Anal. Calcd, for $C_{29}H_{29}Cl_2N_5O_3S$(%): C, 58.19; H, 4.88; N, 11.70; Found (%): C, 58.21; H, 4.86; N, 11.69.

*5.11.26. (E)-N-(6-((4-methylpiperidin-1-yl)methyl)benzo[d]thiazol-2-yl)-2-(4-benzyloxy-2-hydroxybenzylidene) hydrazinecarboxamide (**15s**)*

Yield: 58%; MS (ESI) m/z: 528.2 $[M-H]^-$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.12 (s, 2H), 8.24 (s, 1H), 7.97 (s, 1H), 7.83 (s, 1H), 7.61 (d, *J* = 8.4 Hz, 1H), 7.46 (d, *J* = 7.2 Hz, 2H), 7.41 (t, *J* = 7.2 Hz, 2H), 7.37 – 7.32 (m, 2H), 6.57 (dd, *J* = 8.4, 2.4 Hz, 1H), 6.53 (d, *J* = 2.4 Hz, 1H), 5.11 (s, 2H), 3.61 (s, 2H), 2.85 (*J* = 6.8 Hz, 2H), 2.05 (s, 2H), 1.58 (d, *J* = 10.8 Hz, 2H), 1.35 (s, 1H), 1.16 (m, 2H), 0.88 (d, *J* = 6.4 Hz, 3H); Anal. Calcd, for $C_{29}H_{31}N_5O_3S$ (%): C, 65.76; H, 5.90; N, 13.22; Found (%): C, 65.78; H, 5.91; N, 13.20.

*5.11.27. (E)-N-(6-morpholinobenzo[d]thiazol-2-yl)-2-(2-hydroxy-4-(4-methylbenzyloxy)benzylidene)hydrazinecarboxamide (**15t**)*

Yield: 65%; MS (ESI) m/z: 516.3 $[M-H]^-$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.97 (s, 2H), 8.23 (s, 1H), 7.86 (s, 1H), 7.52 (d, *J* = 8.4 Hz, 1H), 7.44 (d, *J* = 2.4 Hz, 1H), 7.33 (d, *J* = 8.0 Hz, 2H), 7.21 (d, *J* = 8.0 Hz, 2H), 7.09 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.55 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.51 (d, *J* = 2.4 Hz, 1H), 5.06 (s, 2H), 3.76 (t, *J* = 4.4 Hz, 4H), 3.12 (t, *J* = 4.4 Hz, 4H), 2.31 (s, 3H); Anal. Calcd, for $C_{27}H_{27}N_5O_4S$ (%): C, 62.65; H, 5.26; N, 13.53; Found (%): C, 62.68; H, 5.24; N, 13.52.

*5.11.28. (E)-N-(6-morpholinobenzo[d]thiazol-2-yl)-2-(4-(4-tert-butylbenzyloxy)-2-hydroxybenzylidene) hydrazinecarboxamide (**15u**)*

Yield: 73%; MS (ESI) m/z: 558.6 $[M-H]^-$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.97 (s, 2H), 8.23 (s, 1H), 7.87 (s, 1H), 7.52 (d, *J* = 8.8 Hz, 1H), 7.44 (d, *J* = 2.4 Hz, 1H), 7.42 (d, *J* = 8.4 Hz, 2H), 7.37 (d, *J* = 8.4 Hz, 2H), 7.09 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.55 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.51 (d, *J* = 2.4 Hz, 1H), 5.07 (s, 2H), 3.76 (t, *J* = 4.4 Hz, 4H), 3.12 (t, *J* = 4.4 Hz, 4H), 1.29 (s, 9H) ;

Anal. Calcd, for $C_{30}H_{33}N_5O_4S$ (%): C, 64.38; H, 5.94; N, 12.51; Found (%): C, 64.39; H, 5.95; N, 12.49.

*5.11.29. (E)-N-(6-morpholinobenzo[d]thiazol-2-yl)-2-(4-(4-chlorobenzyloxy)-2-hydroxybenzylidene) hydrazinecarboxamide (**15v**)*

Yield: 70%; MS (ESI) m/z: 536.3 $[M-H]^-$; IR (KBr, cm^{-1}): 3421.7, 3183.4, 3072.2, 2960.5, 2924.8, 2857.1, 1897.5, 1693.1, 1679.7, 1628.0, 1607.9, 1555.2, 1492.5, 1467.2, 1449.3, 1313.1, 1279.3, 1221.6, 1184.4, 1118.0, 1039.9, 1014.9, 946.6, 825.9, 805.1, 747.6; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.98 (s, 2H), 8.23 (s, 1H), 7.92 (s, 1H), 7.60-7.40 (m, 6H), 7.09 (d, *J* = 8.8 Hz, 1H), 6.56 (d, *J* = 8.8 Hz, 1H), 6.51 (s, 1H), 5.11 (s, 2H), 3.76 (t, *J* = 4.4 Hz, 4H), 3.12 (t, *J* = 4.4 Hz, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.06, 158.32, 157.52, 153.06, 148.33, 142.78, 136.36, 133.31, 132.94, 129.98(2C), 129.84, 128.92(2C), 120.38, 116.10, 113.72, 107.56, 107.38, 102.38, 68.87, 66.63(2C), 50.00(2C); Anal. Calcd, for $C_{26}H_{23}ClN_5O_4S$ (%): C, 58.04; H, 4.50; N, 13.02; Found (%): C, 58.06; H, 4.52; N, 13.01.

*5.11.30. (E)-N-(6-morpholinobenzo[d]thiazol-2-yl)-2-(4-(3-chlorobenzyloxy)-2-hydroxybenzylidene) hydrazinecarboxamide (**15w**)*

Yield: 74%; MS (ESI) m/z: 536.3 $[M-H]^-$; IR (KBr, cm^{-1}): 3414.3, 3183.9, 3067.5, 2958.4, 2919.9, 2852.5, 1690.8, 1679.4, 1628.6, 1607.9, 1554.2, 1499.6, 1473.4, 1448.4, 1383.9, 1298.6 1223.0, 1171.5, 1120.7, 1041.5, 947.5, 828.0, 781.6, 746.2, 680.8, 617.1; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.98 (s, 2H), 8.23 (s, 1H), 7.81 (s, 1H), 7.52 (s, 2H), 7.43 (m, 4H), 7.09 (d, *J* = 8.8 Hz, 1H), 6.57 (d, *J* = 8.8 Hz, 1H), 6.52 (s, 1H), 5.13 (s, 2H), 3.76 (s, 4H), 3.12 (s, 4H); Anal. Calcd, for $C_{26}H_{23}ClN_5O_4S$ (%): C, 58.04; H, 4.50; N, 13.02; Found (%): C, 58.02; H, 4.51; N, 13.03.

*5.11.31. (E)-N-(6-morpholinobenzo[d]thiazol-2-yl)-2-(4-(2,4-dichlorobenzyloxy)-2-hydroxybenzylidene) hydrazinecarboxamide (**15x**)*

Yield: 75%; MS (ESI) m/z: 570.2 $[M-H]^-$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.99 (s, 2H), 8.24 (s, 1H), 7.92 (s, 1H), 7.71 (d, *J* = 2.0 Hz, 1H), 7.62 (d, *J* = 8.4 Hz, 1H), 7.55-7.47 (m, 2H), 7.44 (d, *J* = 2.0 Hz, 1H), 7.09 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.58 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.53 (d, *J* = 2.4 Hz, 1H), 5.16 (s, 2H), 3.76 (t, *J* = 4.4 Hz, 4H), 3.12 (t, *J* = 4.4 Hz, 4H); Anal. Calcd, for $C_{26}H_{23}Cl_2N_5O_4S$ (%): C, 54.55; H, 4.05; N, 12.23; Found (%): C, 54.58; H, 4.05; N, 12.21.

*5.11.32. (E)-N-(6-((dimethylamino)methyl)benzo[d]thiazol-2-yl)-2-(4-((2-(benzo[d][1,3]dioxol-5-ylmethyl)thiazol-4-yl)methoxy)-2-hydroxybenzylidene) hydrazinecarboxamide (**16a**)*

Yield: 81%; MS (ESI) m/z: 615.1 $[M-H]^-$, 651.1$[M+Cl]^-$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.11 (s, 2H), 8.25 (s, 1H), 8.04 (s, 1H), 7.85 (s, 1H), 7.62 (d, *J* = 8.8 Hz, 1H), 7.58 (s, 1H), 7.37 (d, *J* = 8.4 Hz, 1H), 6.93 (d, *J* = 1.2 Hz, 1H), 6.89 (d, *J* = 8.0 Hz, 1H), 6.83 (dd, *J* = 8.0, 1.2 Hz, 1H), 6.58 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.55 (d, *J* =2.4 Hz, 1H), 6.01 (s, 2H), 5.12 (s, 2H), 4.25 (s, 2H), 3.63 (s, 2H), 2.27 (s, 6H); Anal. Calcd, for $C_{30}H_{28}N_6O_5S_2$ (%): C, 58.43; H, 4.58; N, 13.63; Found (%): C, 58.46; H, 4.57; N, 13.61.

*5.11.33. (E)-N-(6-((diethylamino)methyl)benzo[d]thiazol-2-yl)-2-(4-((2-(benzo[d][1,3]dioxol-5-ylmethyl)thiazol-4-yl)methoxy)-2-hydroxybenzylidene) hydrazinecarboxamide (**16b**)*

Yield: 79%; MS (ESI) m/z: 643.2 $[M-H]^-$, 679.2$[M+Cl]^-$; IR (KBr, cm^{-1}): 3339.7, 2961.7, 2919.9, 1703.6, 1613.2, 1578.0, 1557.7, 1484.1, 1462.6, 1416.3, 1404.2, 1384.8, 1277.5, 1236.8, 1151.0, 1047.1, 942.9, 879.6, 847.7, 808.8, 756.1, 734.1,658.6, 644.5; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.38 (s, 1H), 11.21 (s, 1H), 8.26 (s, 1H), 8.14 (s, 1H), 8.01 (s, 1H), 7.74 (d, *J* = 8.8 Hz, 1H), 7.62 (d, *J* = 8.8 Hz, 1H), 7.59 (s, 1H), 6.93 (s, 1H), 6.89 (d, *J* = 8.0 Hz, 1H), 6.83 (d, *J* =

8.0 Hz, 1H), 6.58 (m, 1H), 6.56 (s, 1H), 6.01 (s, 2H), 5.12 (s, 2H), 4.38 (s, 2H), 4.25 (s, 2H), 3.08 (q, *J* = 7.2 Hz, 4H), 1.26 (t, *J* = 7.2 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.31, 165.17, 163.48, 161.29, 161.12, 152.98, 151.57, 150.99, 147.87, 146.67, 132.13, 129.49, 125.22, 124.79, 122.68, 119.98, 119.35, 118.87, 116.94, 109.91, 108.84, 108.24, 107.23, 102.30, 101.42, 65.70, 55.13, 46.02(2C), 38.58, 8.79(2C); Anal. Calcd, for $C_{32}H_{32}N_6O_5S_2$ (%): C, 59.61; H, 5.00; N, 13.03; Found (%): C, 59.63; H, 5.01; N, 13.01.

*5.11.34. (E)-N-(6-((4-methylpiperidin-1-yl)methyl)benzo[d]thiazol-2-yl)-2-(4-((2-(benzo[d][1,3]-dioxol-5-ylmethyl)thiazol-4-yl)methoxy)-2-hydroxybenzylidene)hydrazinecarboxamide (**16c**)*

Yield: 82%; MS (ESI) m/z: 669.3 $[M-H]^-$,705.2$[M+Cl]^-$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.11 (s, 2H), 8.88 (s, 1H), 8.25 (s, 1H), 7.95 (s, 1H), 7.83 (s, 1H), 7.61 (d, *J* = 8.8 Hz, 1H), 7.58 (s, 1H), 7.35 (d, *J* = 8.0 Hz, 1H), 6.93 (s, 1H), 6.89 (d, *J* = 8.0 Hz, 1H), 6.83 (d, *J* = 8.0 Hz, 1H), 6.58 (d, *J* = 8.8 Hz, 1H), 6.55 (s, 1H), 6.00 (s, 2H), 5.12 (s, 2H), 4.25 (s, 2H), 3.64 (s, 2H), 2.86 (s, 2H), 2.07 (s, 2H), 1.59 (d, *J* = 12.0 Hz, 2H), 1.36 (s, 1H), 1.17 (m, 2H), 0.89 (d, *J* = 6.4 Hz, 3H); Anal. Calcd, for $C_{34}H_{34}N_6O_5S_2$ (%): C, 60.88; H, 5.11; N, 12.53; Found (%): C, 60.86; H, 5.10; N, 12.52.

*5.11.35. (E)-N-(6-morpholinobenzo[d]thiazol-2-yl)-2-(4-((2-(benzo[d][1,3]dioxol-5-ylmethyl)thiazol-4-yl)methoxy)-2-hydroxybenzylidene)hydrazinecarboxamide (**16d**)*

Yield: 85%; MS (ESI) m/z: 643.3 $[M-H]^-$; IR (KBr, cm^{-1}): 3186.4, 3075.9, 2957.1, 1691.4, 1626.7, 1607.3, 1570.0, 1543.1, 1501.5, 1489.0, 1444.6, 1277.6, 1246.3, 1223.6, 1185.4, 1116.6, 1038.3, 947.2, 925.8, 809.6, 743.2, 705.7, 650.5, 534.7; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.00 (s, 2H), 8.24 (s, 1H), 7.95 (s, 1H), 7.58 (s, 1H), 7.53 (m, 1H), 7.45 (s, 1H), 7.10 (d, *J* = 8.8 Hz, 1H), 6.93 (s, 1H), 6.89 (d, *J* = 8.0 Hz, 1H), 6.83 (d, *J* = 8.0 Hz, 1H), 6.58 (dd, *J* = 8.8, 2.0 Hz, 1H), 6.54 (d, *J* = 2.0 Hz, 1H), 6.01 (s, 2H), 5.12 (s, 2H), 4.25 (s, 2H), 3.76 (t, *J* = 4.4 Hz, 4H), 3.12 (t, *J* = 4.4 Hz, 4H); Anal. Calcd, for $C_{31}H_{28}N_6O_6S_2$ (%): C, 57.75; H, 4.38; N, 13.04; Found (%): C, 57.73; H, 4.39; N, 13.05.

5.12. Pharmacology

5.12.1. MTT assay in vitro

The anti-proliferative activities of compounds **14a-h**, **15a-x** and **16a-d** were evaluated against NCI-H226, SK-N-SH, HT29, MKN-45 and MDA-MB-231 cell lines using the standard MTT assay *in vitro*, with PAC-1 as the positive control. The cancer cell lines were cultured in minimum essential medium (MEM) supplemented with 10% foetal bovine serum (FBS). Approximate 4 $\times 10^3$ cells, suspended in MEM medium, were plated onto each well of a 96-well plate and incubated in 5% CO_2 at 37 °C for 24 h. The compounds tested at the indicated final concentrations were added to the culture medium and the cell cultures were continued for 72 h. Fresh MTT was added to each well at aterminal concentration of 5 mg/mL, and incubated with cells at 37 °C for 4 h. The formazan crystals were dissolved in 100 mL of DMSO each well, and the absorbance at 492 nm (for absorbance of MTT formazan) and 490 nm (for the reference wavelength) was measured with an ELISA reader. All compounds were tested three times in each cell line. The results expressed as IC_{50} (inhibitory concentration 50%) were the averages of three determinations and calculated by using the Bacus Laboratories Incorporated Slide Scanner (Bliss) software.

5.12.2. Procaspase-3 kinase assay

The procaspase-3 activity was evaluated using this method as previously reported[5]. (a) 100μM target compounds and PAC-1 were dissolved in DMSO and stored at -20 °C as mother liquor, which were diluted to corresponding concentration by using corresponding buffer when

used. (b) 40μL procaspase-3, which was dissolved in buffer (50 mM HEPES, 0.1% CHAPS, 10% glycerol, 100 mM NaCl, 0.1 mM EDTA, 10 mM DTT, pH 7.4), was added in 384-well plates. 10μL five times the final concentration of target compounds were added, the final concentration of procaspase-3 was 1μM, and procaspase-3 was incubated in buffer at 37 °C for 2 h. (c) 0.4 mM Ac-DEVD-pNa was added in the buffer (50 mM HEPES, 0.1% CHAPS, 10% glycerol, 100 mM NaCl, 0.1 mM EDTA, 10 mM DTT, pH 7.4) of every plates, absorbance values were read in every two minute for 1 h, and absorbance values of per minute were calculated as enzyme activity of detection index. (d) all data were detected and analyzed by using SPSS 16.0, each data was presented as mean±S.E. The activation rate (%) was calculated using the following equation: $(A_{sample}-A_{dmso})/(A_{pac-1}-A_{dmso})\times 100$. EC_{50} was calculated from the activation curves with probability regression method.

5.12.3. Explanation about anticancer activity and enzymatic activity of PAC-1

There were many factors that affected the anticancer activity and enzymatic activity of PAC-1, which were different of reported data. Of them, the main reason about biological assay difference was the difference in test methods. In our work, the anti-proliferative activity of PAC-1 was evaluated by using the standard MTT assay, however, PAC-1 was evaluated in the literature (Nature chemical biology 2006, 2 (10): 543-50) by using the MTS-PMS-assay. In addition, although the test method of enzymatic activity was mainly refer to this method reported in the literature (Nature chemical biology 2006, 2 (10): 543-50), there were some differences in the details, for example, concentration of procaspase-3 and Ac-DEVD-pNa were different, absorbance values were read at different time frequency, detected and analyzed software and calculation method were different. All above could be a possible reason that made our data be different of reported data.

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Legends

Fig. 1. Structures of procaspase-3 PAC-1 and SPAC-1

Fig. 2. Structures of derivatives bearing an *ortho*-hydroxy *N*-acylhydrazone moiety

Fig. 3. The design of target compounds

Scheme 1. Reagents and conditions: (i) acetonitrile, amine, rt, 3 h; (ii) 80% hydrazine monohydrate, $FeCl_3 \cdot 6H_2O$, activated carbon, ethanol, 65 °C to 78 °C, 5 h;(iii) Br_2, NH_4SCN, HOAC, 10°C to rt, 4-6 h; (iv) phenyl chloroformate, pyridine, CH_2Cl_2, 0°C to rt, 4-6 h; (v) 80%hydrazine monohydrate, 1,4-dioxane, 80°C, 6 h.

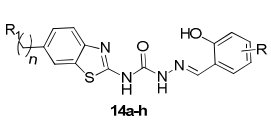
Scheme 2. Reagents and conditions: (i) Na_2CO_3, KI, 65 °C,30 h; (ii) NaHS, $MgCl_2 \cdot 6H_2O$, DMF, H_2O, rt, 15 h; (iii) 1,3-dichloro-2-propanon, acetonitrile, 50 °C, 4 h; (iv) 2,4-dihydroxy benzaldehyde, $NaCO_3$, KI, acetonitrile, 80 °C, 2 h; (v) acetic acid, ethanol, reflux.

Fig. 4. NOE of the representative compound **15v**

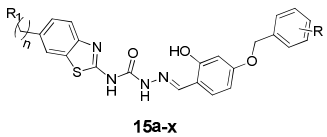
Table 1. cytotoxicity of target compounds **14a-h**, **15a-x**, **16a-d** against H226, SK-N-SH, HT-29, MKN45 and MDA-MB-231 cancer cell lines.

Table 2. procaspase-3 activity of selected compounds **15g**, **15h**, **15n**, **15p**, **15u**, **16b** and PAC-1 *in vitro*.

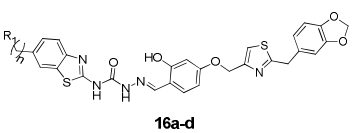
Table 1. cytotoxicity of target compounds **14a-h**, **15a-x**, **16a-d** against H226, SK-N-SH, HT-29, MKN45 and MDA-MB-231 cancer cell lines.



14a-h



15a-x



16a-d

Compd.	R_1	n	R	IC_{50} (μmol/L) ±SD[a]				
				H226	SK-N-SH	HT29	MKN45	MDA-MB-231
14a		1	H	2.03 ±0.38	5.14±1.02	5.96±0.52	1.54±1.01	5.95±0.21
14b		1	4-OH	2.98±0.34	14.31±1.35	77.92±5.40	2.85±0.13	>100
14c		1	H	1.81±0.11	5.79±1.01	4.03±0.54	1.71±0.08	11.33±0.23
14d		1	4-OH	5.16±0.19	6.05±0.17	23.73±1.67	1.74±0.26	4.11±0.16
14e		1	H	2.96±0.28	4.36±1.52	10.40±2.62	0.68±0.06	12.29±0.03
14f		1	4-OH	4.61±0.15	7.74±1.26	2.73±0.05	4.09±0.16	1.66±0.31
14g		1	H	2.71±0.18	7.05±0.76	10.32±1.06	2.34±0.32	12.04±1.21
14h		0	H	4.26±0.11	11.68±0.78	67.93±2.11	9.31±0.51	>100
15a		1	4-CH_3	1.08±0.05	2.58±0.11	2.13±0.04	1.27±0.02	2.65±0.02
15b		1	4-$C(CH_3)_3$	0.83±0.17	1.66±0.15	1.51±0.11	1.04±0.01	1.14±0.01
15c		1	4-Cl	1.17±0.10	2.74±0.12	2.15±0.18	0.70±0.09	0.60±0.05
15d		1	3-Cl	0.36±0.07	1.19±0.18	2.94±0.11	0.19±0.01	0.54±0.04
15e		1	2-Cl	0.58±0.01	2.94±0.15	1.61±0.25	0.33±0.01	0.68±0.12
15f		1	4-CH_3	1.01±0.02	1.43±0.09	1.33±0.13	0.58±0.01	0.71±0.03
15g		1	4-$C(CH_3)_3$	**0.16±0.01**	**0.48±0.02**	**0.77±0.01**	**0.50±0.02**	**0.98±0.03**
15h		1	4-Cl	**0.43±0.01**	**0.74±0.05**	**1.02±0.02**	**0.54±0.08**	**0.61±0.01**
15i		1	3-Cl	1.30±0.14	1.83±0.13	1.88±0.01	0.42±0.02	0.57±0.02
15j		1	2-Cl	0.56±0.10	2.01±0.01	2.23±0.16	0.42±0.01	0.74±0.03

15k		1	2,4-$(Cl)_2$	0.70±0.02	1.04±0.05	2.43±0.03	0.52±0.03	1.52±0.03
15l		1	H	0.88±0.01	1.67±0.07	1.44±0.23	ND	ND
15m		1	4-CH_3	1.06±0.05	1.56±0.02	1.04±0.03	0.31±0.02	1.51±0.02
15n		1	4-$C(CH_3)_3$	**0.18±0.02**	**0.27±0.03**	**0.87±0.03**	**0.17±0.02**	**0.56±0.01**
15o		1	4-Cl	0.41±0.06	1.06±0.07	0.95±0.10	0.21±0.01	0.88±0.03
15p		1	3-Cl	**0.36±0.03**	**0.34±0.01**	1.72±0.04	0.67±0.09	1.12±0.02
15q		1	2-Cl	0.79±0.06	0.85±0.07	1.03±0.14	0.76±0.06	2.3±0.05
15r		1	2,4-$(Cl)_2$	0.39±0.06	1.03±0.19	1.00±0.09	0.28±0.03	0.30±0.03
15s		1	H	0.61±0.11	1.32±0.05	1.57±0.02	ND	ND
15t		0	4-CH_3	0.53±0.05	2.51±0.15	1.58±0.17	0.75±0.03	19.34±0.31
15u		0	4-$C(CH_3)_3$	**0.24±0.03**	**0.92±0.33**	0.92±0.15	0.46±0.04	1.04±0.05
15v		0	4-Cl	0.26±0.01	1.37±0.03	**0.0018±0.0002**	0.29±0.01	4.46±0.03
15w		0	3-Cl	0.79±0.11	1.48±0.13	0.59±0.04	0.28±0.02	1.18±0.01
15x		0	2,4-$(Cl)_2$	1.22±0.23	3.14±0.08	4.72±0.13	1.75±0.10	15.06±1.01
16a		1	-	0.72±0.03	0.79±0.01	1.88±0.03	0.62±0.01	1.04±0.11
16b		1	-	**0.14±0.01**	**0.48±0.03**	**0.72±0.02**	**0.51±0.01**	**0.55±0.01**
16c		1	-	0.81±0.02	1.25±0.01	1.12±0.15	0.55±0.03	0.88±0.02
16d		0	-	2.14±0.11	6.21±0.23	2.31±0.16	1.01±0.01	4.03±0.04
PAC-1[b]				1.02±0.01	3.06±0.04	1.36±0.02	2.61±0.05	4.8±0.02

Bold values show the IC_{50} values of the target compounds lower than the values of the positive control. ND: Not determined.

[a] IC_{50}: concentration of the compound (μM) producing 50% cell growth inhibition after 72 h of drug exposure, as determined by the MTT assay. Each experiment was carried out in triplicate.

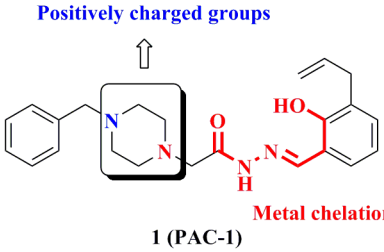
[b] Used as the positive control.

Table 2. procaspase-3 activity of selected compounds **15g**, **15h**, **15n**, **15p**, **15u**, **16b** and PAC-1 *in vitro*.

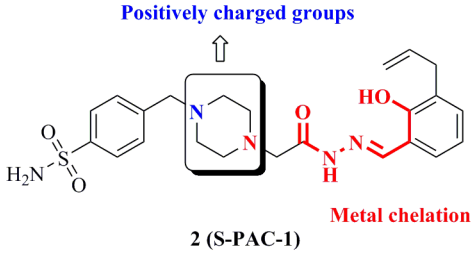
Compd.	EC_{50} on procaspase-3 (μM)
15g	1.42
15h	4.96
15n	2.42
15p	4.26
15u	18.23
16b	0.25
PAC-1[a]	4.08[b]

[a] Used as the positive control.

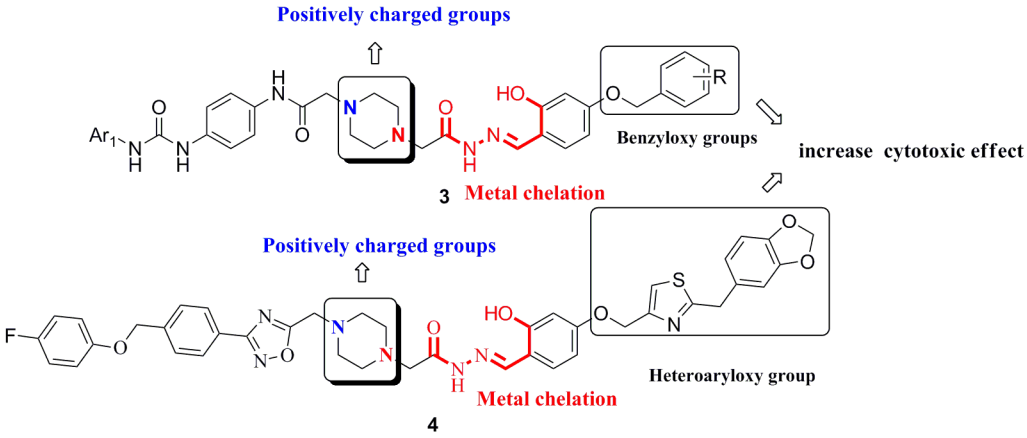
[b] Reported value is 0.22 μM [5].

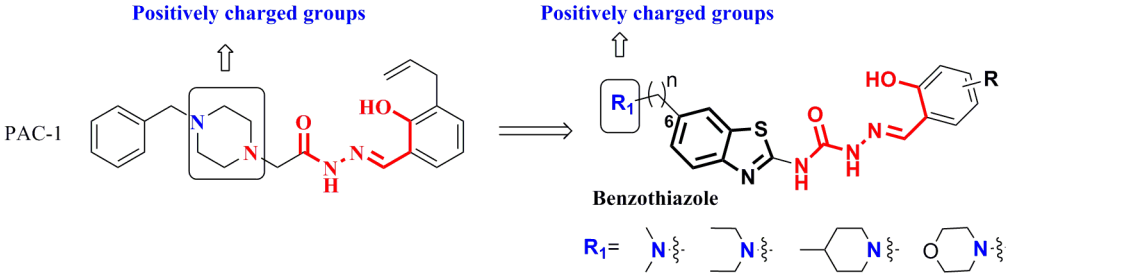


1 (PAC-1)

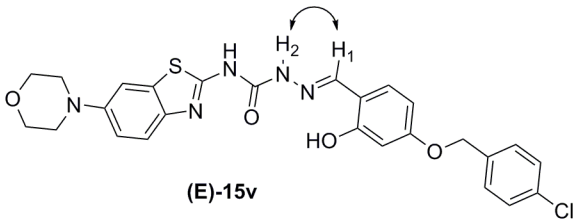


2 (S-PAC-1)

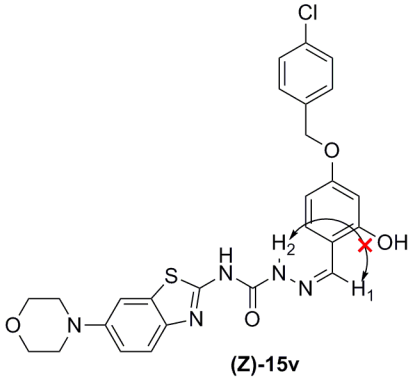




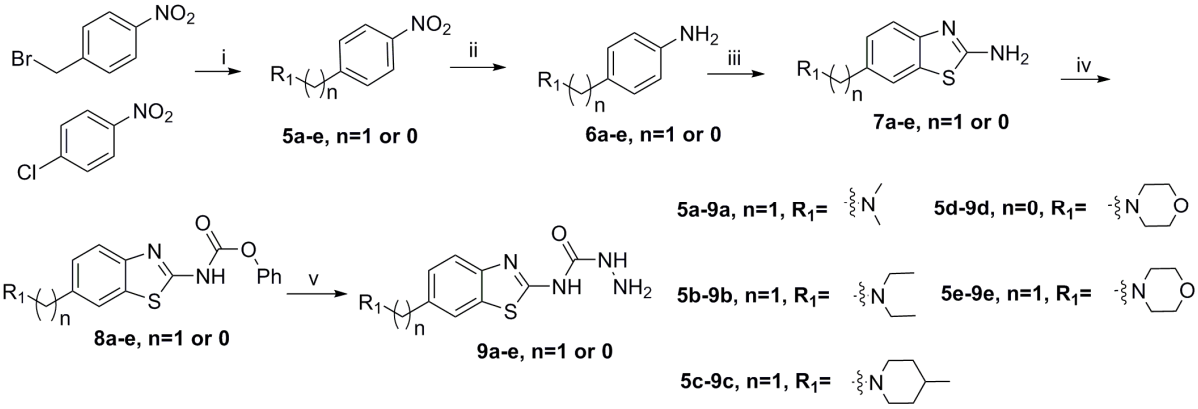
R= substituted benzyloxy and heteroaryloxy groups

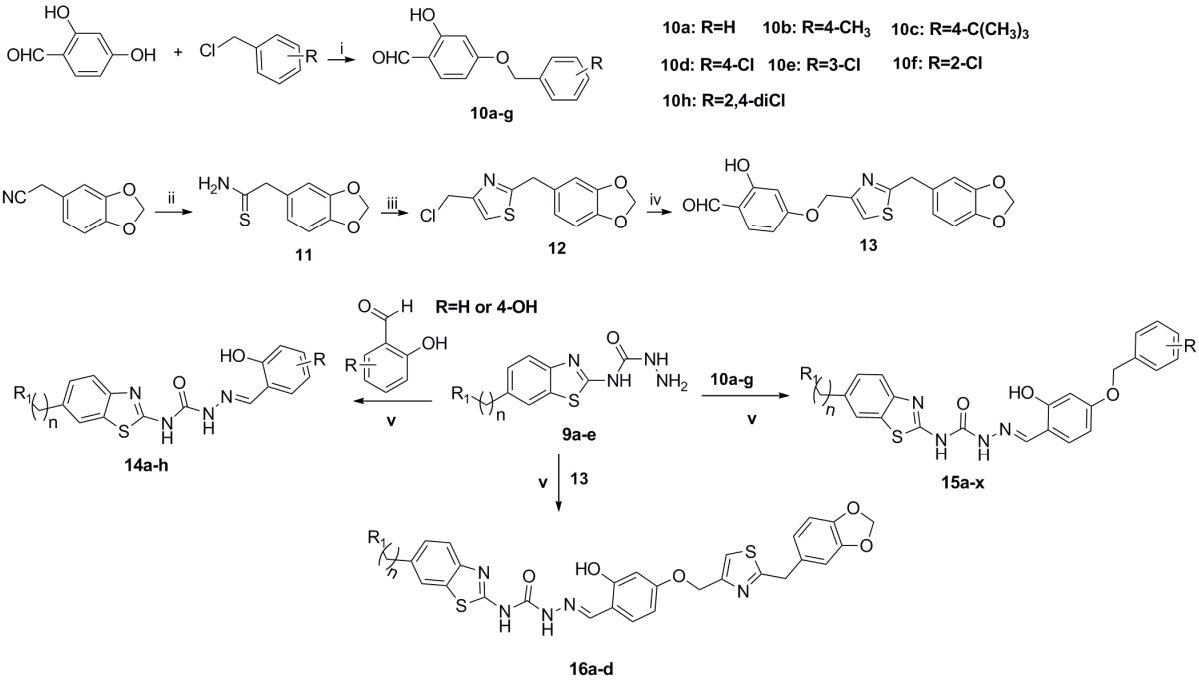


(E)-15v



(Z)-15v





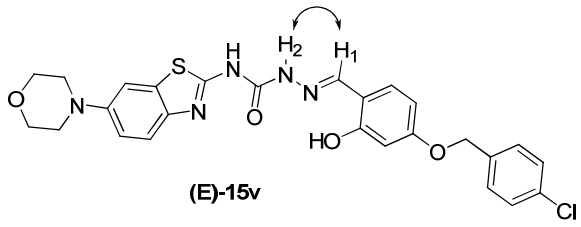
1. 36 novel benzothiazole derivatives were designed and synthesized.

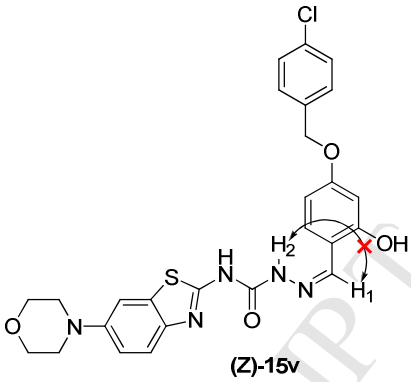
2. Target compounds showed excellent antitumor potency *in vitro* against 5 cancer cell lines.

3. The cytotoxic activities of **15g** and **16b** were 1.8–8.7 times more active than PAC-1.

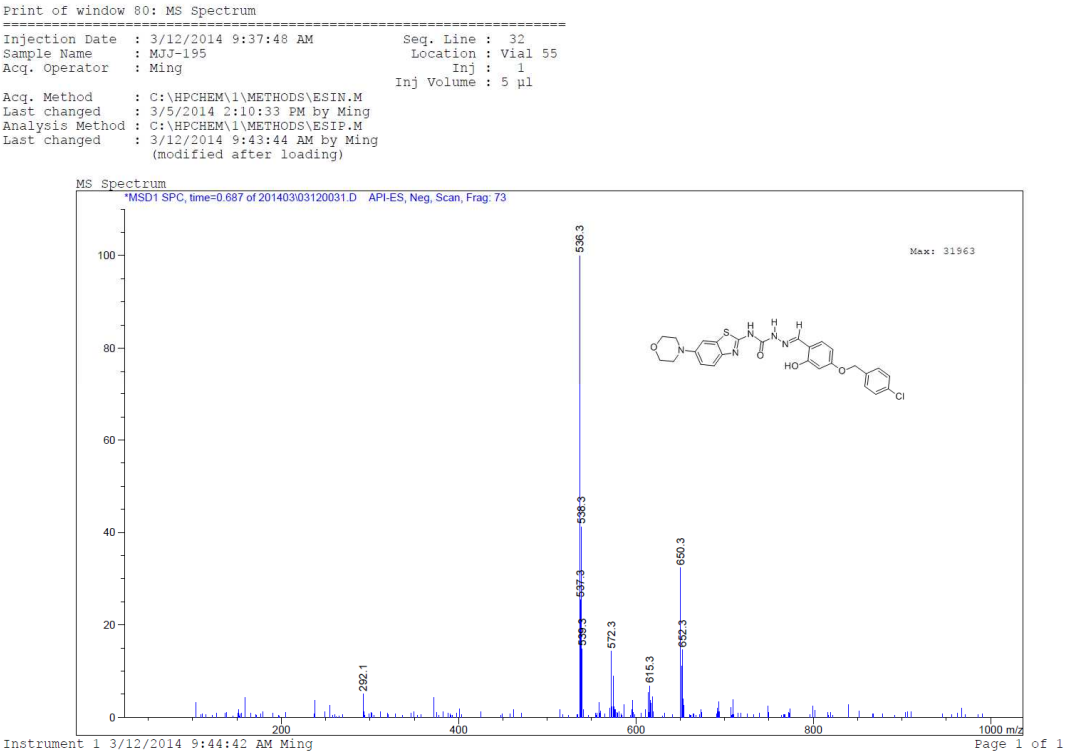
4. Enzymatic activities of **15g** and **16b** were 2.9 times and 16 times better than PAC-1.

1. The structure of compound **(*E*)-15v**

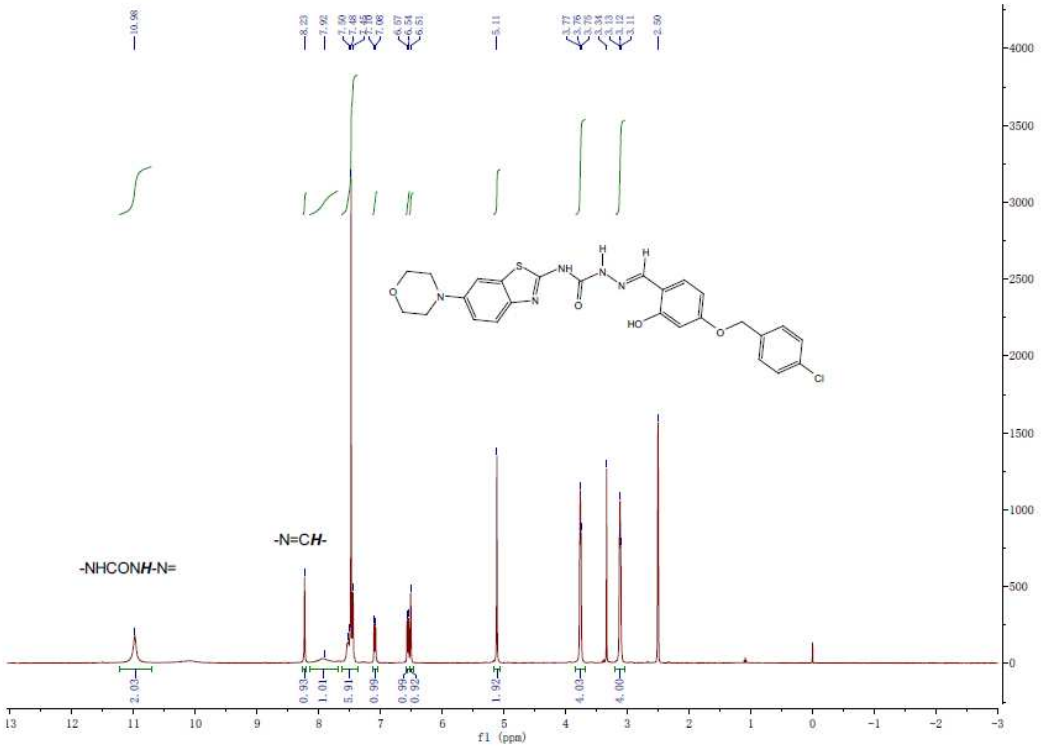




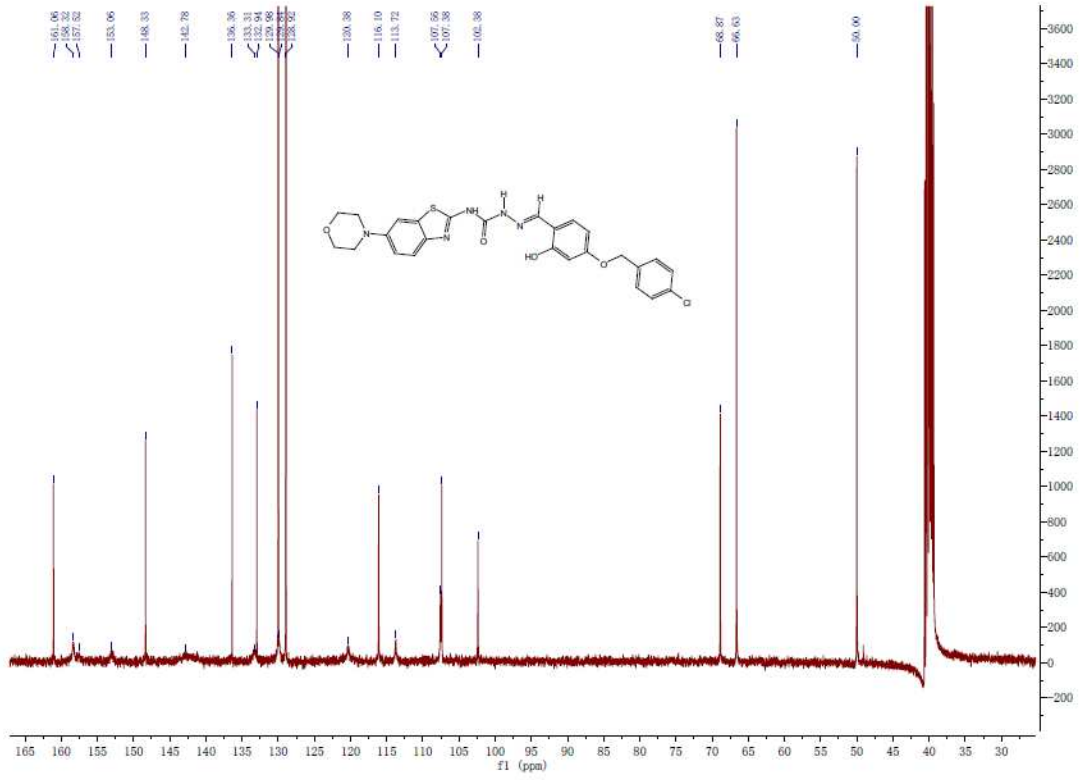
2. The MS of compound **(*E*)-15v**



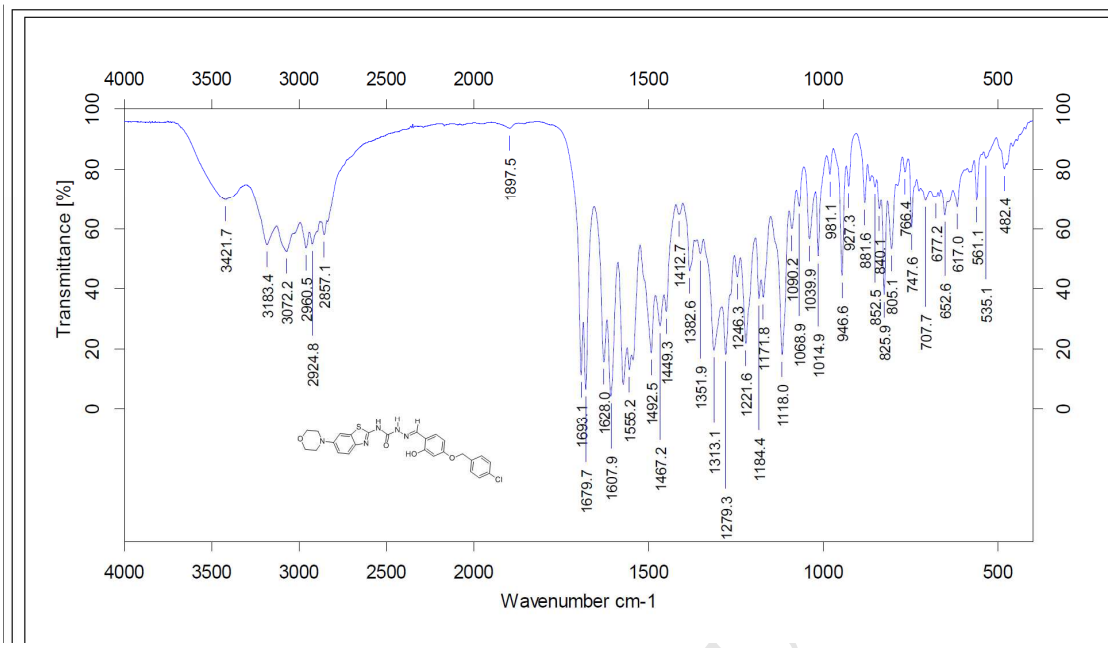
3. The ^{1}H-NMR (600 MHz, DMSO-d_6) of compound **(*E*)-15v**



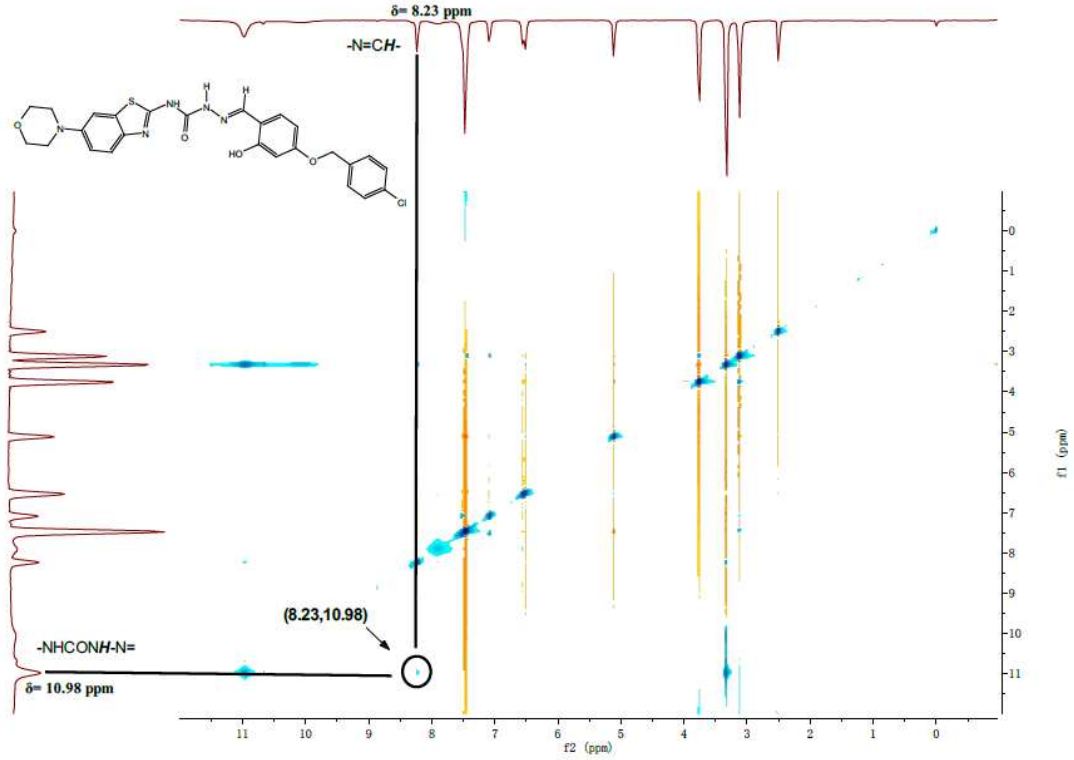
4. The ^{13}C-NMR (DMSO-d_6) of Compound **(*E*)-15v**



5. The IR spectra of compound **(*E*)-15v**



6. 2D NOESY Spectrum (600 MHz, DMSO-d_6) of compound **(*E*)-15v**



7. The MS of compound **15g**

```
Print of window 80: MS Spectrum
=====================================================================
Injection Date  : 3/11/2014 8:56:07 AM          Seq. Line :  15
Sample Name     : MJJ-230                       Location : Vial 19
Acq. Operator   : Ming                             Inj :   1
                                                Inj Volume : 5 µl

Acq. Method     : C:\HPCHEM\1\METHODS\ESIN.M
Last changed    : 3/11/2014 8:49:43 AM by Ming
                  (modified after loading)
Analysis Method : C:\HPCHEM\1\METHODS\ESIP.M
Last changed    : 3/10/2014 3:17:07 PM by Ming
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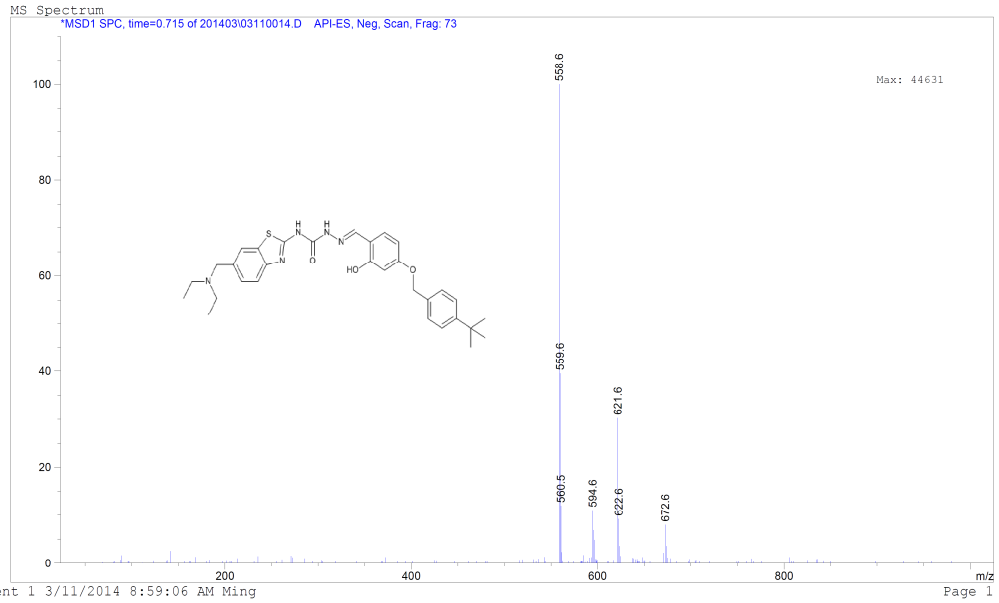
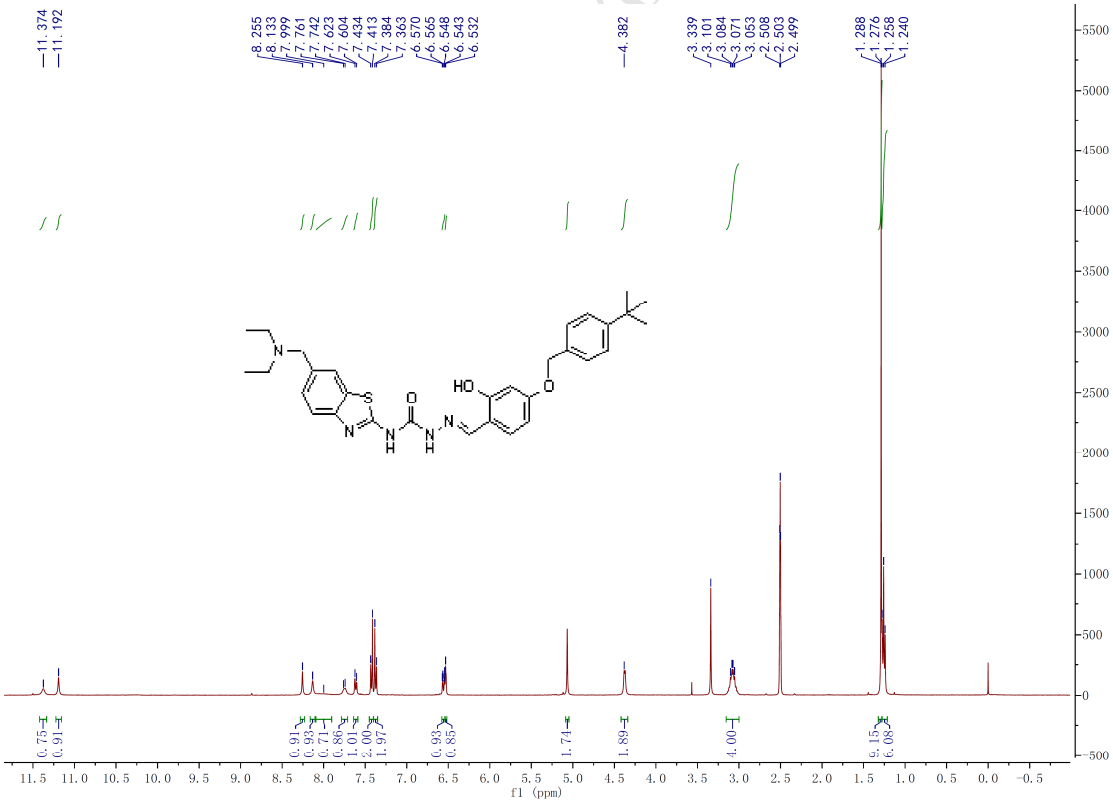


Instrument 1 3/11/2014 8:59:06 AM Ming

8. The ^{1}H-NMR of compound **15g**



9. The MS of compound **16b**

```
Print of window 80: MS Spectrum
=====================================================================
Injection Date  : 3/11/2014 11:40:35 AM          Seq. Line :  86
Sample Name     : MJJ-305                        Location : Vial 78
Acq. Operator   : Ming                              Inj :   1
                                                 Inj Volume : 5 µl

Acq. Method     : C:\HPCHEM\1\METHODS\ESIN.M
Last changed    : 3/5/2014 2:10:33 PM by Ming
Analysis Method : C:\HPCHEM\1\METHODS\ESIP.M
Last changed    : 3/11/2014 1:37:56 PM by Ming
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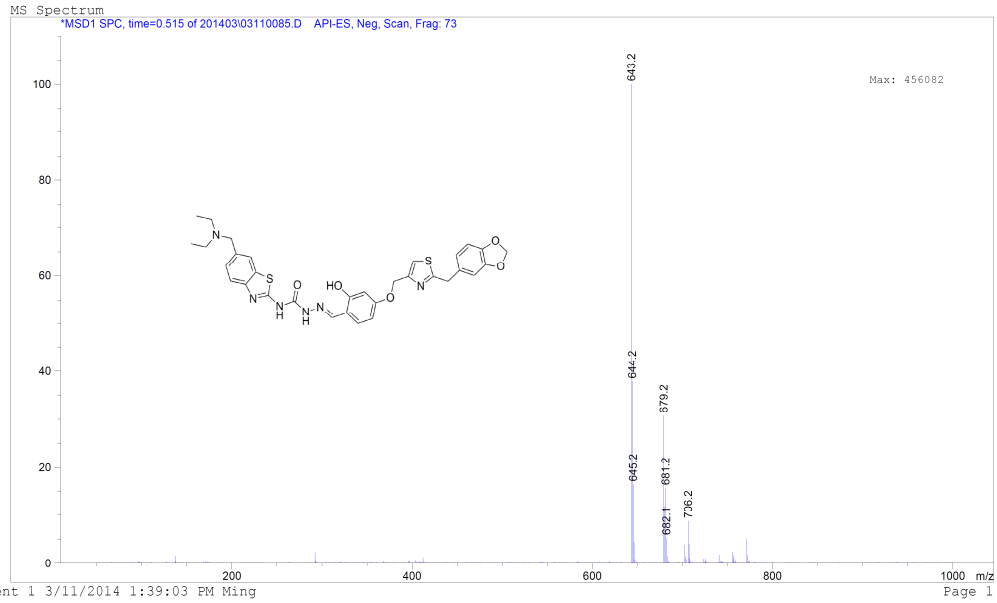
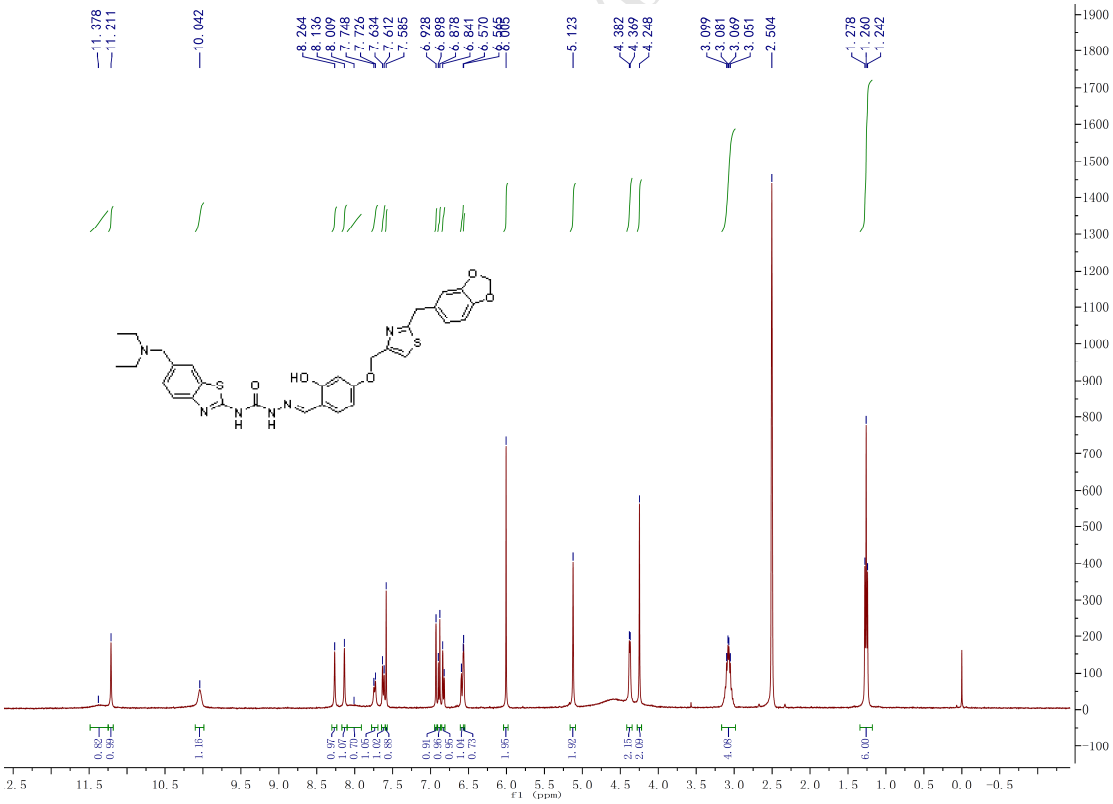


Instrument 1 3/11/2014 1:39:03 PM Ming Page 1 of 1

10. The ^{1}H-NMR of compound **16b**



11. The MS of compound **9a**

```
Print of window 80: MS Spectrum
=====================================================================
Injection Date  : 4/18/2014 10:31:05 AM          Seq. Line :  29
Sample Name     : MJJ-265                        Location : Vial 38
Acq. Operator   : Ming                              Inj :   1
                                                 Inj Volume : 6 µl

Acq. Method     : C:\HPCHEM\1\METHODS\ESIP.M
Last changed    : 4/17/2014 8:15:01 AM by Ming
Analysis Method : C:\HPCHEM\1\METHODS\ESIP.M
Last changed    : 4/18/2014 10:30:49 AM by Ming
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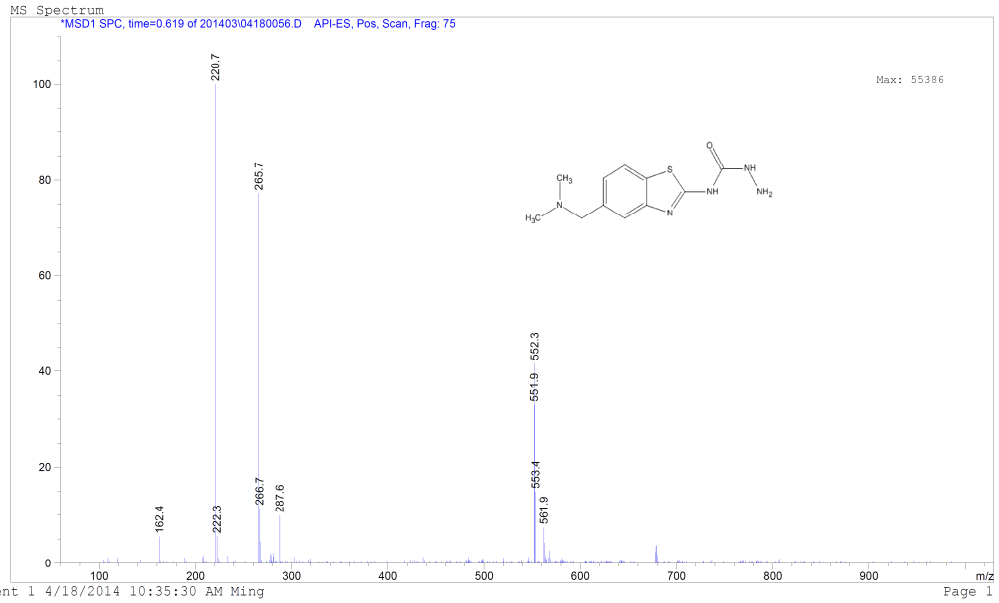
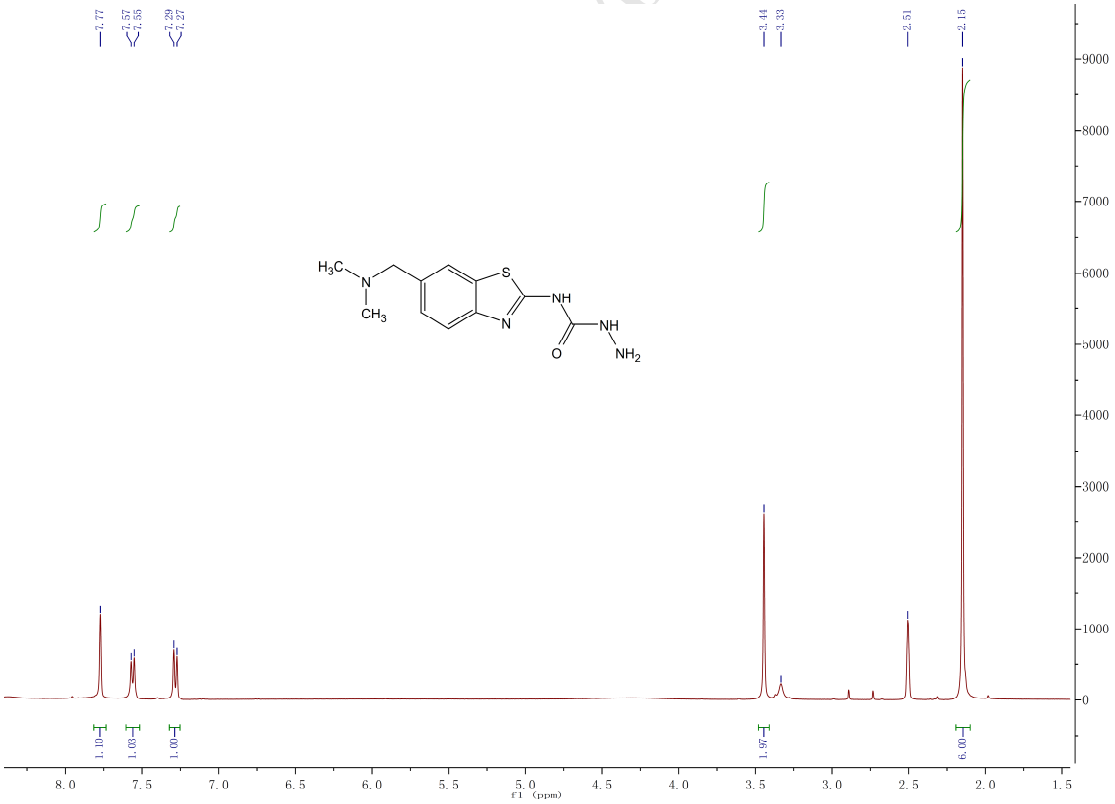



```
Instrument 1 4/18/2014 10:35:30 AM Ming                    Page 1 of 1
```

12. The ^{1}H-NMR of compound **9a**



13.The MS of compound **9b**

```
Print of window 80: MS Spectrum
================================================================
Injection Date  : 4/18/2014 10:54:17 AM          Seq. Line :  39
Sample Name     : MJJ-293                        Location : Vial 40
Acq. Operator   : Ming                              Inj :   1
                                                 Inj Volume : 6 µl

Acq. Method     : C:\HPCHEM\1\METHODS\ESIP.M
Last changed    : 4/17/2014 8:15:01 AM by Ming
Analysis Method : C:\HPCHEM\1\METHODS\ESIP.M
Last changed    : 4/19/2014 10:06:56 AM by Ming
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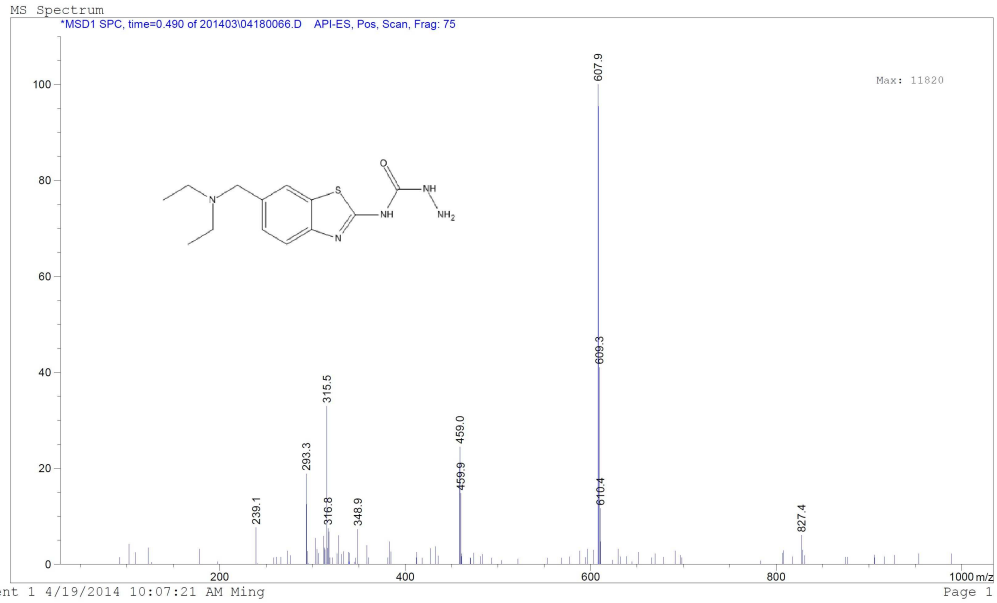
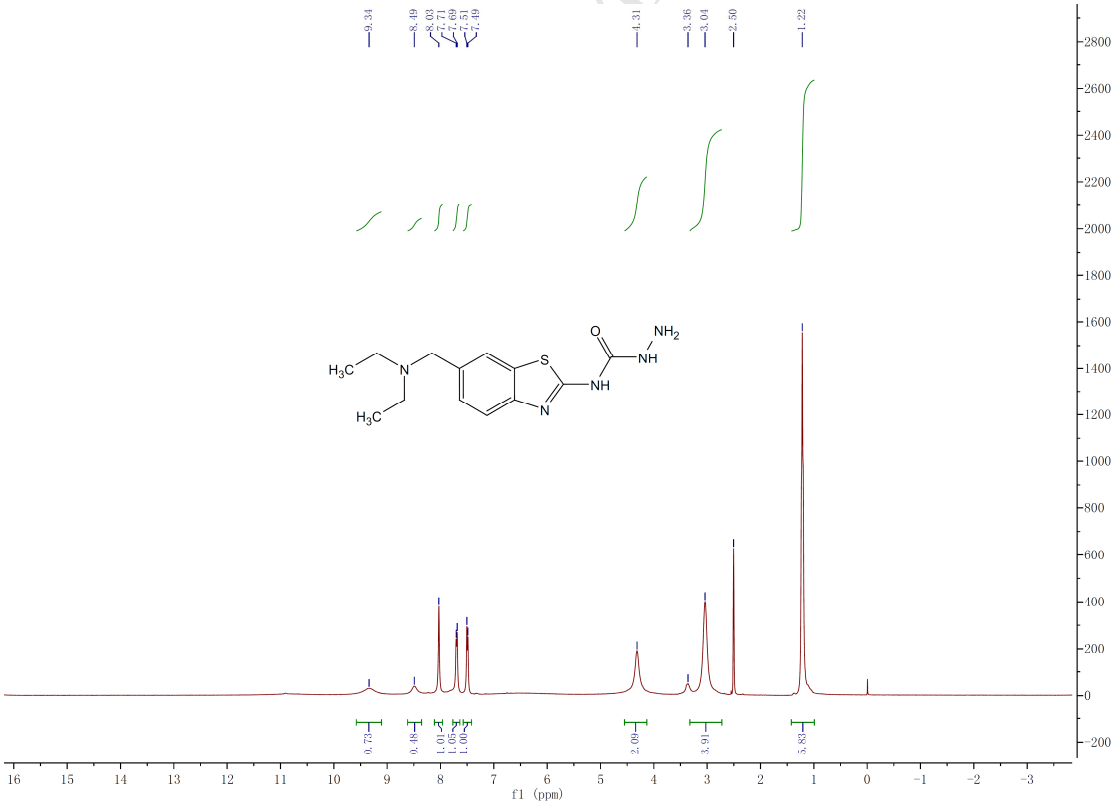


Instrument 1 4/19/2014 10:07:21 AM Ming Page 1 of 1

14. The ^{1}H-NMR of compound **9b**



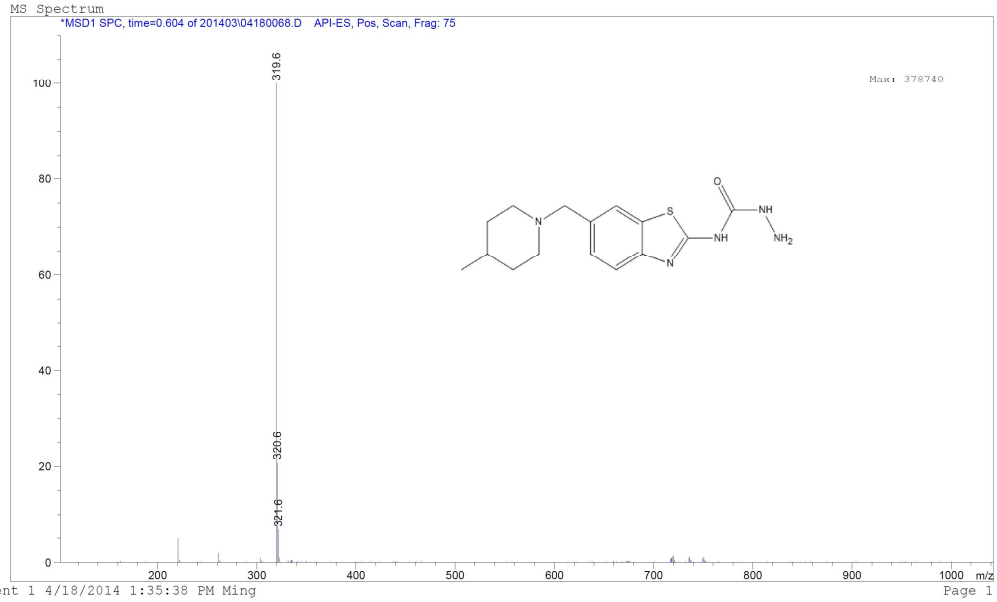
14. The MS of compound **9c**

Print of window 80: MS Spectrum

Injection Date : 4/18/2014 10:58:52 AM Seq. Line : 41
Sample Name : MJJ-319 Location : Vial 42
Acq. Operator : Ming Inj : 1
Inj Volume : 6 µl

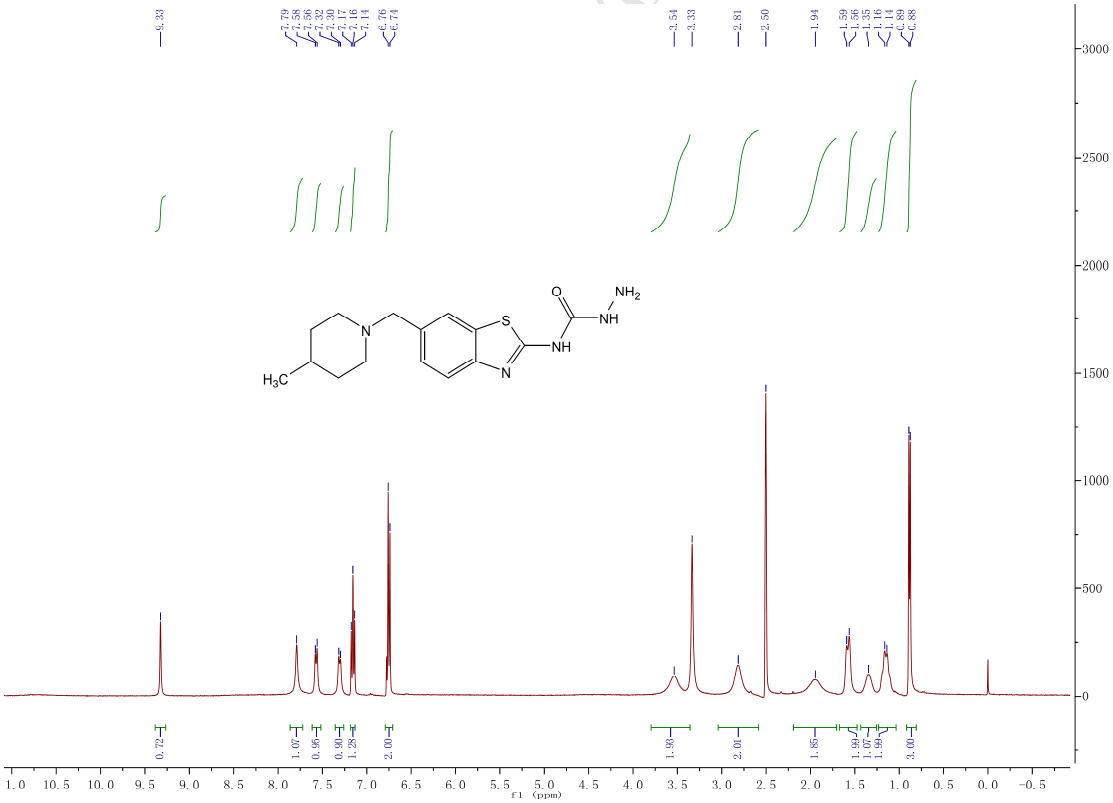
Acq. Method : C:\HPCHEM\1\METHODS\ESIP.M
Last changed : 4/17/2014 8:15:01 AM by Ming
Analysis Method : C:\HPCHEM\1\METHODS\ESIP.M
Last changed : 4/18/2014 1:34:58 PM by Ming
(modified after loading)

MS Spectrum



Instrument 1 4/18/2014 1:35:38 PM Ming

15. The ^{1}H-NMR of compound **9c**



16. The MS of compound **9d**

```
Print of window 80: MS Spectrum
=====================================================================
Injection Date  : 4/18/2014 10:28:45 AM          Seq. Line :  28
Sample Name     : MJJ-293-1                      Location : Vial 37
Acq. Operator   : Ming                              Inj :   1
                                                 Inj Volume : 6 µl

Acq. Method     : C:\HPCHEM\1\METHODS\ESIP.M
Last changed    : 4/17/2014 8:15:01 AM by Ming
Analysis Method : C:\HPCHEM\1\METHODS\ESIP.M
Last changed    : 4/19/2014 10:07:50 AM by Ming
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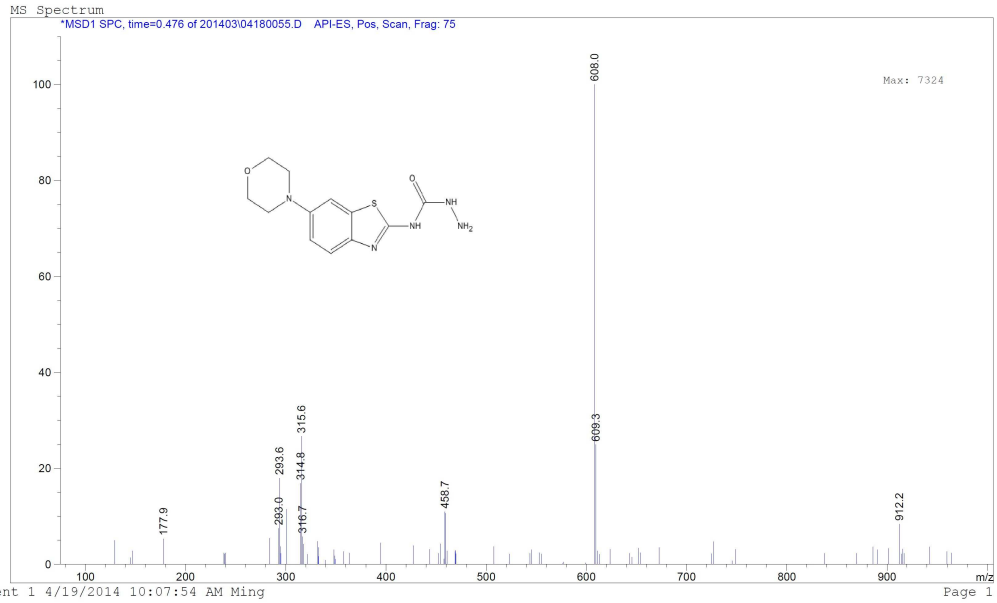

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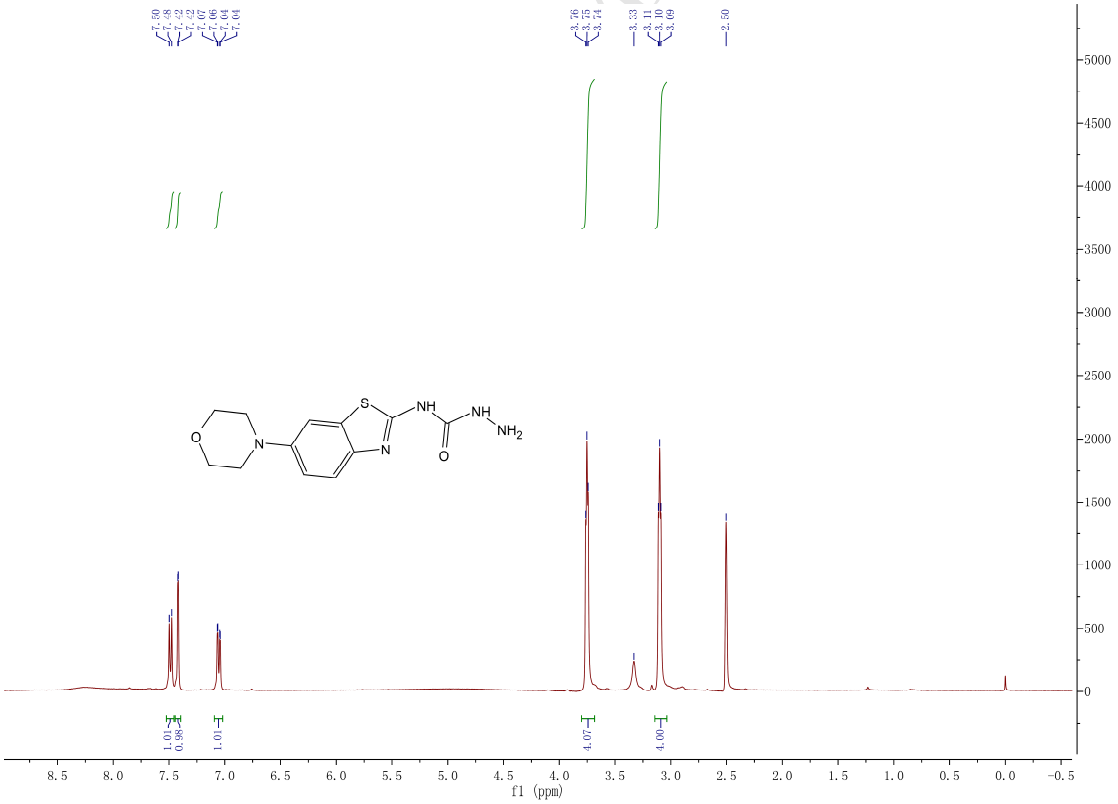
17. The ^{1}H-NMR of compound **9d**



18. The MS of compound **10c**

```
Print of window 80: MS Spectrum
=====================================================================
Injection Date  : 4/18/2014 10:47:18 AM          Seq. Line :  36
Sample Name     : MJJ-284                        Location : Vial 36
Acq. Operator   : Ming                              Inj :   1
                                                 Inj Volume : 5 µl

Acq. Method     : C:\HPCHEM\1\METHODS\ESIN.M
Last changed    : 4/18/2014 10:38:19 AM by Ming
                  (modified after loading)
Analysis Method : C:\HPCHEM\1\METHODS\ESIP.M
Last changed    : 4/18/2014 10:44:01 AM by Ming
                  (modified after loading)
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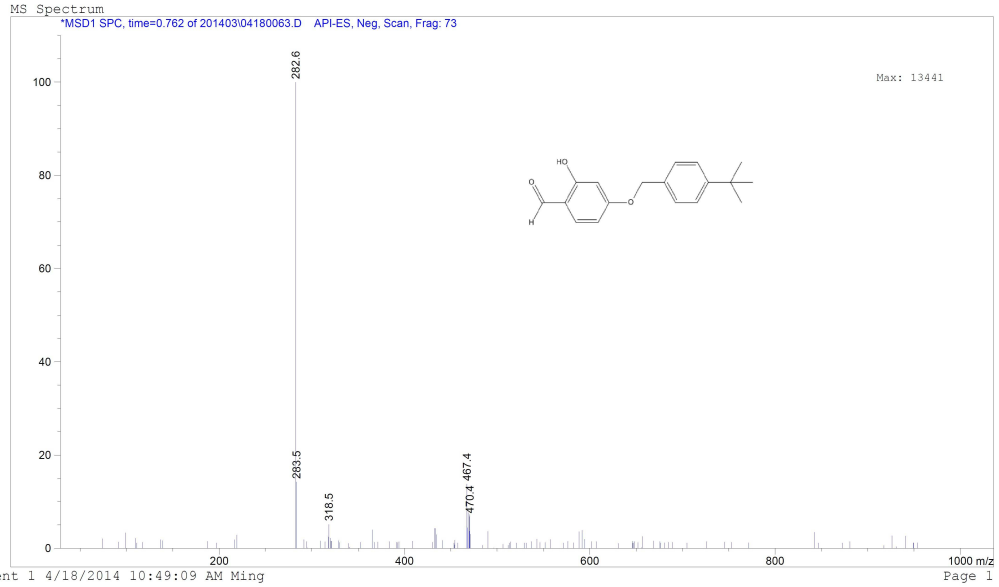
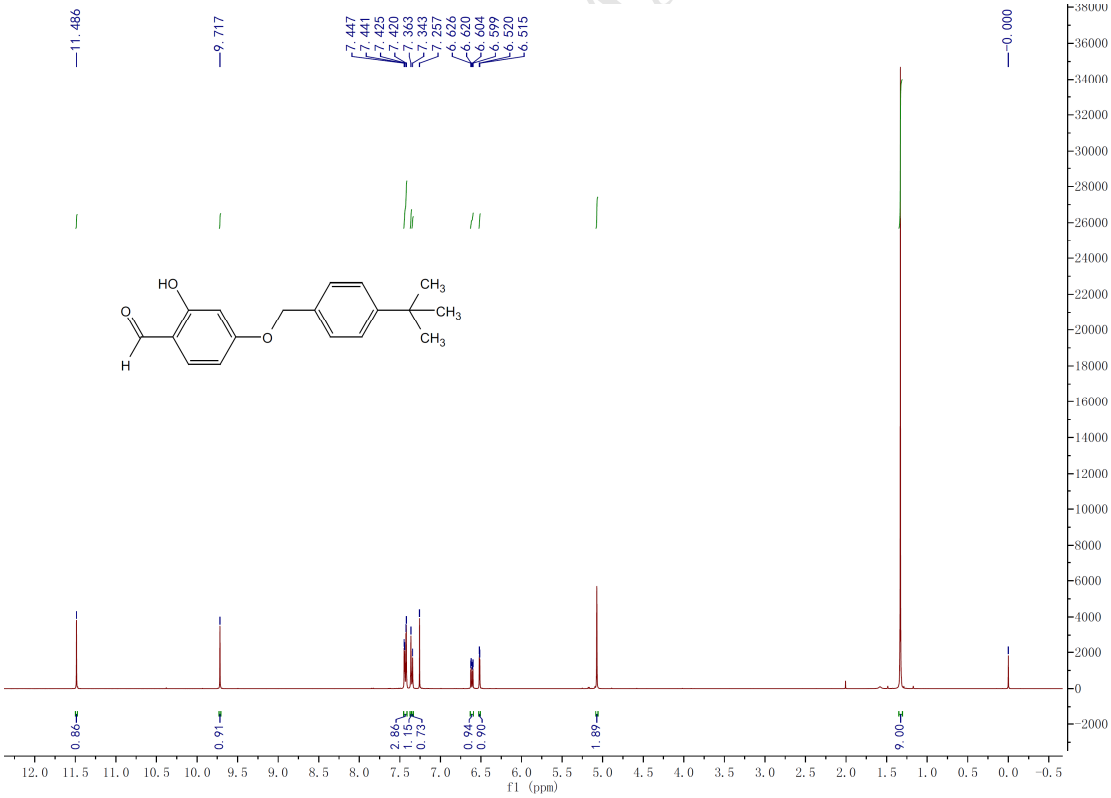


Instrument 1 4/18/2014 10:49:09 AM Ming Page 1 of 1

19. The ^{1}H-NMR of compound **10c**



20. The MS of compound **13**

```
Print of window 80: MS Spectrum
=====================================================================
Injection Date  : 4/18/2014 10:45:01 AM          Seq. Line :  35
Sample Name     : MJJ-369                        Location : Vial 35
Acq. Operator   : Ming                              Inj :   1
                                                 Inj Volume : 5 µl

Acq. Method     : C:\HPCHEM\1\METHODS\ESIN.M
Last changed    : 4/18/2014 10:38:19 AM by Ming
                  (modified after loading)
Analysis Method : C:\HPCHEM\1\METHODS\ESIP.M
Last changed    : 4/18/2014 10:44:01 AM by Ming
                  (modified after loading)
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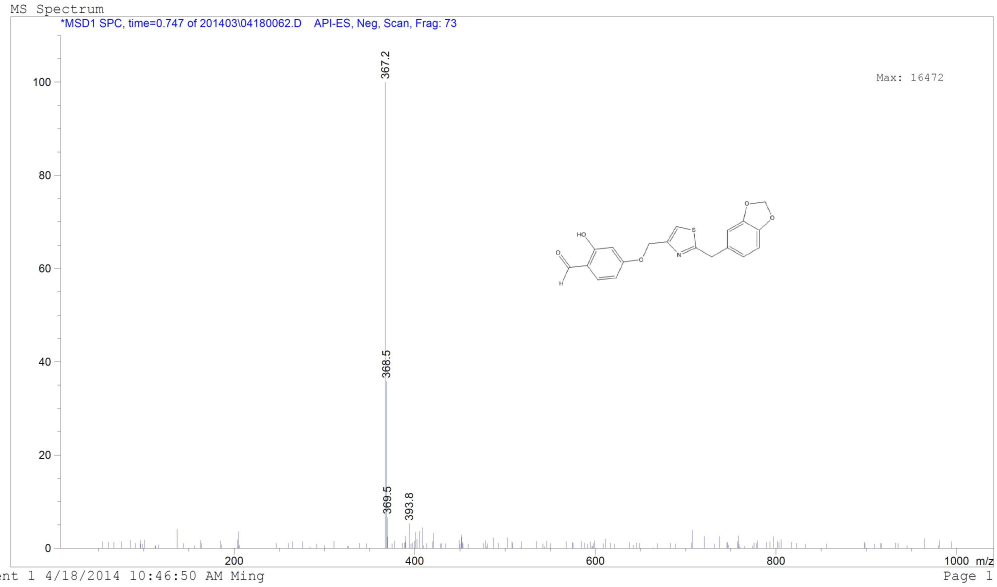

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21. The ^{1}H-NMR of compound **13**