Bioorganic & Medicinal Chemistry 19 (2011) 5948–5954

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis and antitumor activity of 1,2,4-triazoles having 1,4-benzodioxan fragment as a novel class of potent methionine aminopeptidase type II inhibitors

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ARTICLE INFO

Article history:
Received 24 June 2011
Revised 26 August 2011
Accepted 27 August 2011
Available online 1 September 2011

Keywords:
Triazole
Benzodioxan
Antitumor activity
Auto Dock
MetAP2

ABSTRACT

A series of 1,2,4-triazole derivatives containing 1,4-benzodioxan (**5a–5q**) have been designed, synthesized, structurally determined, and their biological activities were evaluated as potential MetAP2 inhibitors. All the synthesized compounds were first reported. Among the compounds, compound **5k** showed the most potent biological activity against HEPG2 cancer cell line (IC_{50} = 0.81 μM for HEPG2 and IC_{50} = 0.93 μM for MetAP2), which was comparable to the positive control. Docking simulation by positioning compound **5k** into the MetAP2 structure active site was performed to explore the possible binding model. The results of apoptosis and Western-blot assay demonstrated that compound **5k** possessed good antitumor activity against HEPG2 cancer cell line. Therefore, compound **5k** with potent inhibitory activity in tumor growth inhibition may be a potential antitumor agent against HEPG2 cancer cell.

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1. Introduction

Cancer is one of the degenerative diseases of old age and increases with age in both rodents and humans.[1] Cancer continues to be a worldwide killer, despite the enormous amount of research and rapid developments during the past decade.[2] According to recent statistics, cancer accounts for about 23% of the total deaths in the USA and is the second most common cause of death after heart disease.[3] Therefore, there is an increasing need for new therapies, especially those based on current knowledge of cancer biology as well as those taking advantage of the cancer cells phenotype, described by Hanahan and Weinberg.[4]

Methionine aminopeptidases (MetAPs) play an important role in removing the initiator methionine residue from nascent polypeptide chains and are believed to be one of the essential enzymes involved in protein maturation.[5] In yeasts and humans, two proteins are known to possess MetAP activity, MetAP1 and MetAP2.[6] MetAP2 appears to play a critical role in cell proliferation and tumor growth. It is expressed at higher concentrations in tumors as compared to normal cells.[7] Available reports also suggested that MetAP2 plays an important role in the growth of different types of tumors.[6]

The derivatives of 1,2,4-triazole have a high potential for biological activity. The following 1,2,4-triazole derivatives are applied in medicine: alprazolam (tranquilizer), estazolam (hypnotic, sedative, tranquilizer), rilmazafon (hypnotic, anxiolytic, used in the case of neurotic insomnia), benatradin (diuretic), trapidil (hypotensive), trazodon (antidepressant, anxiolytic), and so on.[8] The derivatives of 1,2,4-triazole possess a wide range of antimicrobial[8–11] and antitumour[8,12,13] activities. Recently, the compounds containing 1,2,4-triazole were discovered as a novel class of potent tubulin polymerization inhibitors.[14,15]

By examining the MetAP2 pocket, we hypothesized that an extra group (1,4-benzodioxan) could form an additional hydrogen bonding between oxygen atoms from 1,4-benzodioxan and the hydrogen of some amino in the active site of MetAP2, such as ARG 337, ILE 338, PHE 387, and so on. The additional binding may lead to increased activities. The 1,4-benzodioxan (14BZD) structure is an important pharmacophore and is found widely in nature.[16] 1,4-benzodioxan structure was expected to show strong biological activities.[17] Some 1,4-benzodioxan derivatives are antipsychotic agents and others are used by living species in their chemical communication systems. For example, 9-methoxystrobilurin L. having a 1,4-benzodioxan structure was suggested to have strong antifungal activity.[17] Phendioxan and mephendioxan are recognized as a selective α-adrenoceptor antagonists.[18–22]

To the best of our knowledge, few reports have been dedicated to the synthesis and MetAP2 inhibitory activity of 1,2,4-triazole

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doi:10.1016/j.bmc.2011.08.063

derivatives containing 1,4-benzodioxan fragment. Herein, in continuation to extend our research on antitumor compounds with MetAP2 structure inhibitory activity, we report in the present work the synthesis and structure–activity relationships of a series of 1,2,4-triazole derivatives containing 1,4-benzodioxan as antitumor agents. Biological evaluation indicated that some of the synthesized compounds were potent inhibitors of MetAP2.

2. Results and discussion

2.1. Chemistry

The synthesis of 5-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-4-phenyl-4*H*-1,2,4-tria zole-3-thiol **4** containing 1,4-benzodioxan was prepared as shown in Scheme 1. It was synthesized for the first time and prepared in five steps. Firstly, 2,3-dihydrobenzo[b][1,4] dioxine-6-carboxylic acids gave 2,3-dihydrobenzo[b][1,4]dioxine-6-carboxylates **1**, catalyzed by concentrated sulfuric acid in methanol. Secondly, 2,3-dihydrobenzo[b][1,4]dioxine-6-carbohydrazides were prepared by treatment of **1** with hydrazine hydrate (85%) in ethanol. Then, on treatment with phenyl isothiocyanate in presence of ethanol, **2** yielded 2-(2,3-dihydrobenzo[b][1,4]dioxine-6-carbonyl)-*N*-phenylhydrazine carbothioamides **3**. Finally, a solution of NaOH (2 N) containing **3** was stirred under refluxing for 30 min, yielding the desired compounds **4** according to the reported procedure.[11]

Seventeen 1,2,4-triazole derivatives (**5a–5q**) were prepared by refluxing in anhydrous acetonitrile of **4** with different benzyl bromide compounds as shown in Scheme 2 according to the literature.[11]

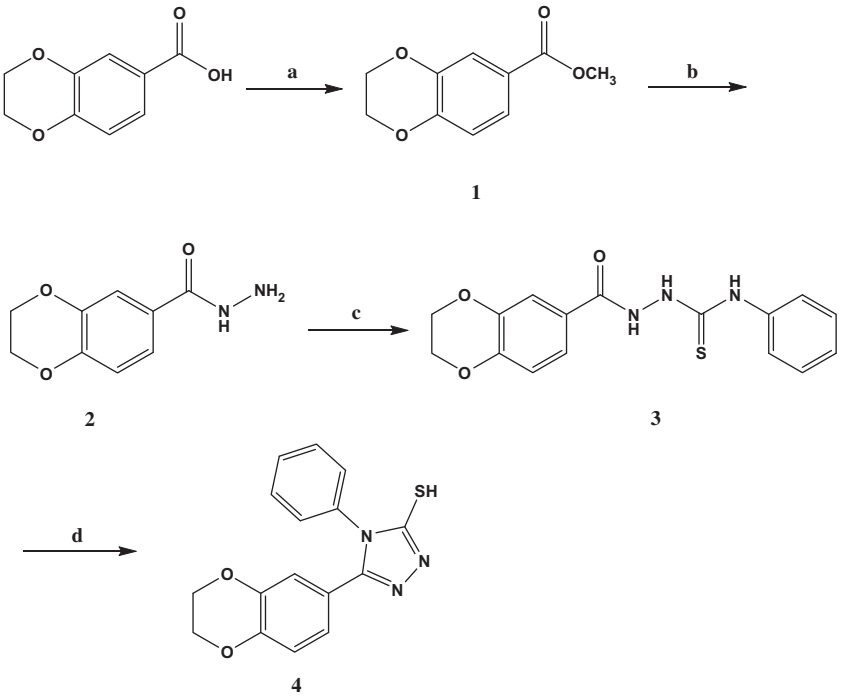
2.2. Biological activity

2.2.1. Antiproliferation assay

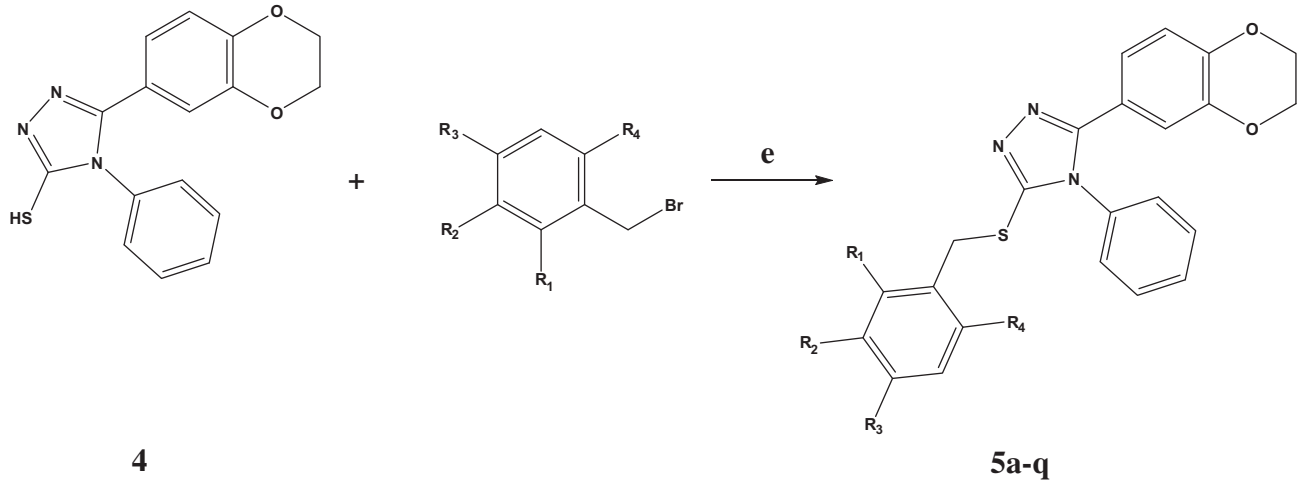
All the synthesized derivatives (**5a–5q**) were evaluated for their ability to antiproliferative activity against HEPG2, HELA, SW1116 and BGC823. The results were summarized in Table 1.

As illustrated in Table 1, the active analogs showed a distinctive potential pattern of selectivity as well as broad-spectrum antitumor activity. With regard to selectivity against individual cell lines, most of the compounds showed effectiveness against cell line Human hepatocellular liver carcinoma HEPG2 with IC_{50} values range of 0.81–60.01 μM comparative to TNP-470 (0.86 μM). Among these compounds, compound **5k** showed the most potent biological activity (IC_{50} = 0.81 μM). HELA human cervical cancer cell line proved to be sensitive toward compounds **5f** and **5m** with IC_{50} concentration range of 15.03–18.60 μg/mL comparative to TNP-470 (2.02 μM). Regarding SW1116 human colorectal carcinoma cell line, higher sensitivity was observed with compounds **5o** and **5q** with IC_{50} values range of 5.81–7.35 μM, comparable to TNP-470 (5.54 μM). BGC823 human gastric cancer cell line was proved to be a little sensitive toward compounds **5b**, **5e** and **5k** with IC_{50} concentration range of 17.43–26.45 μM comparative to TNP-470 (1.48 μM). Compounds **5i** and **5j** were proved to be ineffective against the four cell lines.

The activity of the tested compounds could be correlated to structure variations. Through investigating on the selectivity of the tested compounds over the four cell lines, it was clear that the tested compounds showed stronger activities against HEPG2 than other three cancer cell lines. Among the tested compounds, compound **5k** showed the most potent biological activity against HEPG2 cancer cell line with IC_{50} value of 0.81 μM. Structure–activity relationship (SAR) analysis indicated that compounds with electron-withdrawing group showed stronger activity than that with electron-donating group, with all the IC_{50} values below 50 μM against HEPG2. In further study of compounds with electron-withdrawing group, different group led to different antitumor activity, and the potency order was F (fluorine) > Cl (chlorine) > Br (bromine) > NO_2 (nitro-group). With regard to the F-substituted compounds, di-substituted compounds **5m** and **5n** have lower activity comparing with the mono-substituted compounds **5k** and **5l**. Substituents at different positions led to different antitumor activities and the potency order was *ortho-* > *meta-* > *para-*.



Scheme 1. Synthesis of compound **4**. Reagents and conditions: (a) methanol, concentrated sulfuric acid, reflux, 12 h; (b) hydrazine hydrate (85%), ethanol, reflux, 5 d; (c) phenyl isothiocyanate, ethanol, reflux, 30 min; (d) NaOH (2 N), reflux, 30 min.



5a $R_1 = R_2 = R_3 = R_4 = H$
5b $R_1 = NO_2$; $R_2 = R_3 = R_4 = H$
5c $R_1 = R_3 = R_4 = H$, $R_2 = NO_2$
5d $R_1 = R_2 = R_4 = H$, $R_3 = NO_2$
5e $R_1 = Cl$, $R_2 = R_3 = R_4 = H$
5f $R_1 = R_3 = R_4 = H$, $R_2 = Cl$
5g $R_1 = R_2 = R_4 = H$, $R_3 = Cl$;
5h $R_1 = R_3 = R_4 = H$, $R_2 = OCH_3$;
5i $R_1 = CH_3$; $R_2 = R_3 = R_4 = H$
5j $R_1 = R_2 = R_4 = H$, $R_3 = CH_3$
5k $R_1 = F$, $R_2 = R_3 = R_4 = H$
5l $R_1 = R_2 = R_4 = H$, $R_3 = F$
5m $R_1 = R_3 = F$, $R_2 = R_4 = H$
5n $R_1 = R_4 = F$, $R_2 = R_3 = H$
5o $R_1 = Br$, $R_2 = R_3 = R_4 = H$
5p $R_1 = R_3 = R_4 = H$, $R_2 = Br$
5q $R_1 = R_2 = R_4 = H$, $R_3 = Br$

Scheme 2. General synthesis of compounds (**5a**–**q**). Reagents and conditions: (e) reflux, acetonitrile.

Table 1
Antiproliferative activity of the synthesized compounds (**5a**–**5q**)

Compound	IC_{50} (μM)			
	HEPG2	HELA	SW1116	BGC823
4	60.01	51.92	38.36	47.04
5a	5.96	32.75	7.93	73.42
5b	12.02	69.78	8.30	26.45
5c	49.28	45.24	7.59	30.07
5d	13.77	36.18	13.84	48.59
5e	0.97	28.96	9.67	17.43
5f	2.39	15.03	10.64	51.24
5g	3.53	79.18	8.30	79.32
5h	35.12	>100	10.08	>100
5i	20.49	36.01	12.70	>100
5j	56.76	49.74	16.16	44.28
5k	0.81	24.25	13.57	18.57
5l	1.02	46.92	20.79	>100
5m	1.96	18.60	7.83	>100
5n	2.07	35.17	9.01	84.16
5o	4.73	>100	7.35	>100
5p	5.21	44.20	26.17	65. 64
5q	7.94	54.63	5.81	33.76
TNP-470	0.86	2.02	5.54	1.48

2.2.2. MetAP2 inhibitory assay

The MetAP2 inhibitory potency of the triazole derivatives containing 1,4-benzodioxan was examined and the results were summarized in Table 2. Most of the tested compounds displayed potent MetAP2 inhibiting activity. Among them, compound **5k** showed the most potent inhibitory with IC_{50} of 0.93 μM. The results of MetAP2 inhibitory activity of the tested compounds were in agreement to the structure relationships (SAR) of their antitumor activities. This agreement suggested that the potent antitumor activities of the synthetsized compounds were likely related to their MetAP2 inhibitory activities.

Table 2
MetAP2 inhibitory activity of the selected compounds

Compound	MetAP2 (IC_{50}, μM)
5a	6.01
5b	11.78
5d	12.39
5e	1.84
5f	1.92
5g	3.45
5h	30.71
5i	17.66
5k	0.93
5l	2.24
5m	3.95
5n	4.80
5o	5.37
5p	5.91
5q	7.29
TNP-470	1.05

2.2.3. Apoptosis assay

Apoptosis is an essential mechanism used to eliminate activated HEPG2 cells during the shut-down process of excessive immune responses and maintain proper immune homeostasis, while deficient apoptosis of activated HEPG2 cell is associated with a wide variety of immune disorders. As a representative of these triazole derivatives, compound **5k** was studied in vitro. We detected the mechanism of compound **5k** inhibition activity by flow cytometry (FCM) (Fig. 3) and found that the compound could induce the apoptosis of activated HEPG2 cells in a dose-dependent manner. As shown in Figure 3, HEPG2 cells were treated with 0.5, 1.0, 2.0 and 4.0 μM of compound **5k** for 24 h. The compound increased the percentage of apoptosis by Annexin V-FITC/PI staining in a dose-dependent

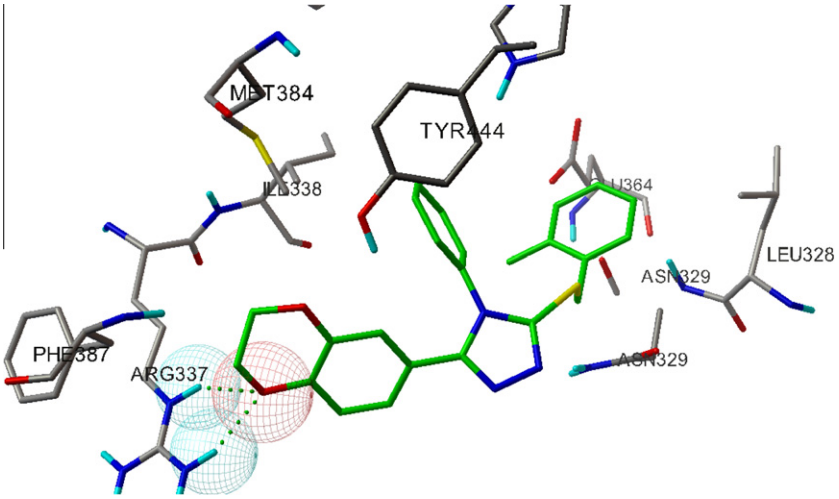


Figure 1. Molecular docking modeling of compound **5k** with MetAP2: compound **5k** is nicely bound to the MetAP2 with its oxygen atom of triazole ring project toward the amino hydrogen of ARG 337, forming two H-bond interactions, H–O···H: 2.100 Å, 148.9° and H–O···H: 2.139 Å, 145.7°, respectively.

manner. The result indicated that compound **5k** induced apoptosis of HEPG2 cells.

2.2.4. Western-blot assay

In an effort to study the preliminary mechanism of the compound with potent inhibitory activity, the western-blot experiment was performed to explore the effect of compound **5k**. The western-blot results were summarized in Figure 4, confirming compound **5k**'s inhibitory activity.

2.3. Binding model of compound 5k into MetAP2

In an effort to elucidate the possible mechanism by which the title compounds can induce anticancer activity in the four cells and guide further SAR studies, molecular docking of the potent inhibitor **5k** into ATP binding site of MetAP2 was performed on the binding model based on the MetAP2 (2EA4.pdb). The binding models of compound **5k** and MetAP2 were depicted in Figures 1

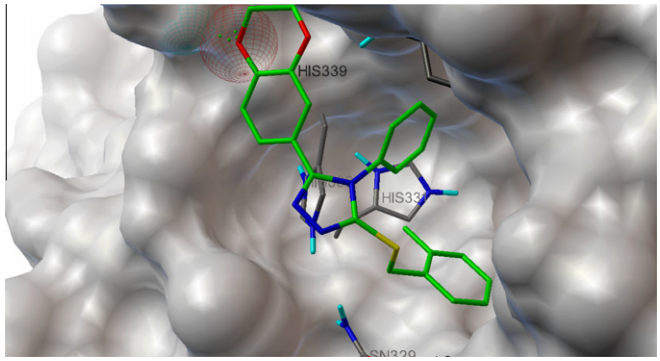


Figure 2. 3D model of the interaction between compound **5k** and MetAP2 binding site. MetAP2 is represented by molecular surface. Compound **5k** is depicted by sticks and balls.

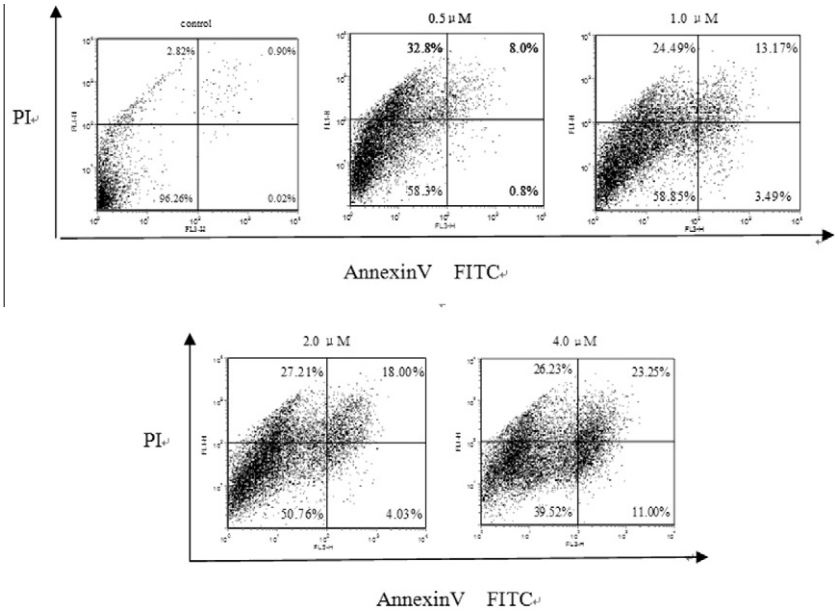


Figure 3. HEPG2 cells isolated from naïve mice were cultured with anticancer and various concentrations of **5k** for 24 h. Cells were stained by Annexin VeFITC/PI and apoptosis was analyzed by flow cytometry. Inhibition including early and late apoptosis.

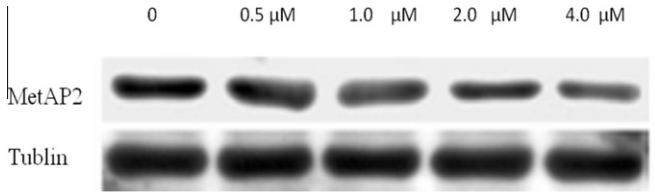


Figure 4. Compound **5k** was examined by western blotting. Data are representative of three independent experiments.

and 2. In the binding model, compound **5k** was nicely bound to the MetAP2 with its oxygen atom of triazole ring project toward the amino hydrogen of ARG 337, forming two H-bond interactions, H–O···H: 2.100 Å, 148.9° and H–O···H: 2.139 Å, 145.7°, respectively. The molecular docking results suggested that compound **5k** was a potential inhibitor of MetAP2.

3. Conclusions

A series of 1,2,4-triazole derivatives containing 1,4-benzodioxan have been synthesized and evaluated for their antitumor activities. Compound **5k** demonstrated the most potent inhibitory activity that inhibited the growth of HEPG2 cells with IC_{50} of 0.81 μM and inhibited the activity of MetAP2 with IC_{50} of 0.93 μM, which was comparable to the positive control TNP-470.

In order to gain deeper understanding of the structure–activity relationships observed at the MetAP2, molecular docking of the most potent inhibitor **5k** into the binding site of MetAP2 was performed on the binding model based on the MetAP2 complex structure. Analysis of the compound **5k**'s binding conformation demonstrated that compound **5k** was stabilized by hydrogen bonding interaction with ARG 337. Apoptosis assay and Western-blot results showed the compound **5k** was a potential antitumor agent.

4. Experimental section

4.1. Methods of synthesis

All chemicals used were purchased from Aldrich (USA). The eluates were monitored using TLC. Melting points (uncorrected) were determined on a XT4MP apparatus (Taike Corp., Beijing, China). ESI mass spectra were obtained on a Mariner System 5304 mass spectrometer, and ^{1}H NMR spectra were recorded on a DPX500 spectrometer at 25 °C with TMS and solvent signals allotted as internal standards, Chemical shifts are reported in ppm (δ). Elemental analyses were performed on a CHN-O-Rapid instrument and were within 0.4% of the theoretical values.

4.1.1. Synthesis of methyl 2,3-dihydrobenzo[b][1,4]dioxine-6-carboxylate (1)

2,3-Dihydrobenzo[b][1,4]dioxine-6-carboxylic acid (1 mmol) in methanol (30 ml) was treated with concentrated sulfuric acid (0.5 ml) under reflux overnight. The sovlent was removed in vacuo leaving oil which was dissovled in ethyl acetate (20 ml) and extracted with water (40 ml). After drying the organic layer with anhydrous Na_2SO_4 and evaporating the solvent under reduced pressure and a solid appeared. The solid was recyrstallized from ethanol to obtain the compound **1**. (Scheme 1)

4.1.2. Synthesis of 2,3-dihydrobenzo[b][1,4]dioxine-6-carbohydrazide (2)

A stirred solution of compound **1** (0.1 mol) in ethanol (50 mL) was treated with the hydrazine hydrate (85%), under reflux for 5 d The sovlent was removed leaving oil which was dissovled in chloroform (20 ml) and extracted with water (40 ml). After drying the organic layer with anhydrous Na_2SO_4 and evaporating the solvent under reduced pressure and a solid appeared. The solid was recyrstallized from ethanol to obtain the compound **2**. (Scheme 1)

4.1.3. Synthesis of 2-(2,3-dihydrobenzo[b][1,4]dioxine-6-carbonyl)-*N*-phenylhydrazinecarbothioamide (3)

To a solution of compound **2** (1 mmol) in ethanol (50 mL), phenyl isothiocyanate (1.5 mmol) was added and the solution was stirred under reflux for 30 min. The sovlent was removed under reduced pressure leaving a residue which was washed with water (60 ml) and a solid appeared. The solid was recyrstallized from ethanol to obtain the compound **3**. (Scheme 1)

4.1.4. Synthesis of 5-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-4-phenyl-4*H*-1,2,4- triazole-3-thiol (4)

The compound **3** (1 mmol) was added in NaOH (2 N, 50 mL), and the solution was stirred under reflux for 30 min. The resulting solution was cooled to room temperature and acidified to pH 3–4 with 37% HCl. The precipitate formed was filtered, washed with water to afford the desired compound **4**. (Scheme 1)

White powder, yield 96%, mp: 281–284 °C. ^{1}H NMR (500 MHz, $CDCl_3$): 10.65 (s, 1H), 7.49–7.58 (m, 3H), 7.32–7.35 (m, 2H), 6.89 (s, 1H), 6.72 (d, J = 8.0 Hz, 2H), 4.22–4.28 (m, 4H); MS (ESI): 312.36. ($C_{16}H_{14}N_3O_2S$, [M+H]+). Anal. Calcd for $C_{16}H_{13}N_3O_2S$: C, 61.72; H, 4.21; N, 13.50. Found: C, 61.49; H, 4.04; N, 13.88.

4.1.5. Synthesis of 3-(benzylthio)-5-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-4-phenyl-4*H*-1,2,4-triazole-substituted-benzyl bromide (5a–q)

To a solution of compound **4** (1 mmol) in acetonitrile, the corresponding benzyl bromide compounds (1 mmol) were added and the mixture was stirred under reflux for 4–8 h in the presence of NaOH (2 mmol). Then, the solvent was removed under reduced pressure and a solid obtained. The solid was recrystallized from acetonitrile to afford compounds **5a–q**. (Scheme 2)

4.1.5.1. 3-(Benzylthio)-5-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-4-phenyl-4*H*-1,2,4-triazole (5a). White powder, yield 87%, mp: 181–184 °C. ^{1}H NMR (500 MHz, $CDCl_3$): 7.45–7.50 (m, 3H), 7.35–7.36 (m, 2H), 7.30–7.32 (m, 1H), 7.27–7.28 (m, 2H), 7.10–7.11 (m, 2H), 6.99 (s, 1H), 6.89 (d, J = 8.5 Hz, 1H), 6.75 (d, J = 8.5 Hz, 1H), 4.50 (s, 2H), 4.21–4.26 (m, 4H). MS (ESI): 402.48 ($C_{23}H_{20}N_3O_2S$, [M+H]+). Anal. Calcd for $C_{23}H_{19}N_3O_2S$: C, 68.81; H, 4.77; N, 10.47. Found: C, 68.43; H, 4.94; N, 10.81.

4.1.5.2. 3-(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)-5-(2-nitrobenzylthio)-4-phenyl-4*H*-1,2,4-trazole (5b). Wheat power, yield 82%, mp: 179–182 °C. ^{1}H NMR (500 MHz, $CDCl_3$): 8.08 (d, J = 8.0 Hz, 1H), 7.95 (d, J = 8.0 Hz, 1H), 7.58–7.61 (m, 1H), 7.48–7.51 (m, 1H), 7.45–7.46 (m, 3H), 7.11 (d, J = 7.5 Hz, 2H), 6.96 (s, 1H), 6.84 (d, J = 8.5 Hz, 1H), 6.73 (d, J = 9.0 Hz, 1H), 4.86 (s, 2H), 4.20–4.25 (m, 4H). MS (ESI): 447.48 ($C_{23}H_{19}N_4O_4S$, [M+H]+). Anal. Calcd for $C_{23}H_{18}N_4O_4S$: C, 61.87; H, 4.06; N, 12.55. Found: C, 61.50; H, 4.33; N, 12.29.

4.1.5.3. 3-(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)-5-(3-nitrobenzylthio)-4-phenyl-4*H*-1,2,4-triazole (5c). Brown power, yield 84%, mp: 164–167 °C. ^{1}H NMR (500 MHz, $CDCl_3$): 8.23 (s, 1H), 8.12 (d, J = 7.5 Hz, 1H), 7.81 (d, J = 7.5 Hz, 1H), 7.50–7.52 (m, 1H), 7.46–7.49 (m, 3H), 7.15 (d, J = 7.0 Hz, 2H), 6.97 (s, 1H), 6.84 (d, J = 8.5 Hz, 1H), 6.74 (d, J = 8.5 Hz, 1H), 4.55 (s, 2H), 4.21–4.25 (m, 4H). MS (ESI): 447.48 ($C_{23}H_{19}N_4O_4S$, [M+H]+). Anal. Calcd for ($C_{23}H_{18}N_4O_4S$: C, 61.87; H, 4.06; N, 12.55. Found: C, 61.50; H, 4.39; N, 12.22.

4.1.5.4. 3-(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)-5-(4-nitrobenzylthio)-4-phenyl-4*H*-1,2,4-triazole (5d). Yellow power, yield 85%, mp: 161–164 °C. ^{1}H NMR (500 MHz, $CDCl_3$): 8.16 (d, *J* = 8.5 Hz, 2H), 7.61 (d, *J* = 8.5 Hz, 2H), 7.47–7.53 (m, 3H), 7.14 (d, *J* = 8.5 Hz, 2H), 6.97 (s, 1H), 6.85 (d, *J* = 8.5 Hz, 1H), 6.74 (d, *J* = 8.5 Hz, 1H), 4.55 (s, 2H), 4.21–4.26 (m, 4H). MS (ESI): 447.48 ($C_{23}H_{19}N_4O_4S$, [M+H]+). Anal. Calcd for ($C_{23}H_{18}N_4O_4S$: C, 61.87; H, 4.06; N, 12.55. Found: C, 61.50; H, 4.43; N, 12.32.

4.1.5.5. 3-(2-Chlorobenzylthio)-5-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-4-phenyl-4*H*-1,2,4-triazole (5e). White powder, yield 94%, mp: 201–203 °C. ^{1}H NMR (500 MHz, $CDCl_3$): 7.57–7.58 (m, 1H), 7.46–7.49 (m, 3H), 7.35–7.36 (m, 1H), 7.20–7.23 (m, 2H), 7.09 (d, *J* = 7.5 Hz, 2H), 6.98 (s, 1H), 6.86 (d, *J* = 8 Hz, 1H), 6.75 (d, *J* = 8.5 Hz, 1H), 4.60 (s, 2H), 4.22–4.24 (m, 4H). MS (ESI): 436.93 ($C_{23}H_{19}ClN_3O_2S$, [M+H]+). Anal. Calcd for $C_{23}H_{18}ClN_3O_2S$: C, 63.37; H, 4.16; N, 9.64. Found: C, 63.08; H, 4.32; N, 9.30.

4.1.5.6. 3-(3-Chlorobenzylthio)-5-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-4-phenyl-4*H*-1,2,4-triazole(5f). White powder, yield 91%, mp: 123–125 °C. ^{1}H NMR (500 MHz, $CDCl_3$): 7.46–7.51 (m, 3H), 7.34 (s, 1H), 7.22–7.27 (m, 3H), 7.11 (d, *J* = 7.5 Hz, 2H), 6.98 (s, 1H), 6.86 (d, *J* = 8.5 Hz, 1H), 6.44 (d, *J* = 8.5 Hz, 1H), 4.44 (s, 2H), 4.22–4.25 (m, 4H). MS (ESI): 436.93 ($C_{23}H_{19}ClN_3O_2S$, [M+H]+). Anal. Calcd for $C_{23}H_{18}ClN_3O_2S$: C, 63.37; H, 4.16; N, 9.64. Found: C, 63.54; H, 4.53; N, 9.27.

4.1.5.7. 3-(4-Chlorobenzylthio)-5-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-4-phenyl-4*H*-1,2,4-triazole(5g). White powder, yield 92%, mp: 174–177 °C. ^{1}H NMR (500 MHz, $CDCl_3$): 7.47–7.48 (m, 3H), 7.31 (d, *J* = 7.5 Hz, 2H), 7.26 (d, *J* = 7.5 Hz, 2H), 7.11 (d, *J* = 6.5 Hz, 2H), 6.98 (s, 1H), 6.85 (d, *J* = 8.5 Hz, 1H), 6.74 (d, *J* = 8.5 Hz, 1H), 4.44 (s, 2H), 4.22–4.24 (m, 4H); MS (ESI): 436.93 ($C_{23}H_{19}ClN_3O_2S$, [M+H]+). Anal. Calcd for $C_{23}H_{18}ClN_3O_2S$: C, 63.37; H, 4.16; N, 9.64. Found: C, 63.09; H, 4.44; N, 9.28.

4.1.5.8. 3-(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)-5-(3-methoxybenzylthio)-4-phenyl-4*H*-1,2,4-triazole (5h). White powder, yield 93%, mp: 115–118 °C. ^{1}H NMR (500 MHz, $CDCl_3$): 7.43–7.49 (m, 3H), 7.19–7.22 (m, 1H), 7.08 (d, *J* = 8.0 Hz, 2H), 6.97 (s, 1H), 6.92 (d, *J* = 7.5 Hz, 1H), 6.88 (s, 1H), 6.85 (d, *J* = 8.0 Hz, 1H), 6.80–6.82 (m, 1H), 6.74 (d, *J* = 8.5 Hz, 1H), 4.45 (s, 2H), 4.21–4.26 (m, 4H), 3.78 (s, 3H). MS (ESI): 432.51 ($C_{24}H_{22}N_3O_3S$, [M+H]+). Anal. Calcd for $C_{24}H_{21}N_3O_3S$: C, 66.80; H, 4.91; N, 9.74. Found: C, 66.53; H, 4.63; N, 9.91.

4.1.5.9. 3-(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)-5-(2-methylbenzylthio)-4-phenyl-4*H*-1,2,4-triazole (5i). White powder, yield 90%, mp: 166–168 °C. ^{1}H NMR (500 MHz, $CDCl_3$): 7.42–7.49 (m, 3H), 7.27 (d, *J* = 7.5 Hz, 1H), 7.17 (m, 1H), 7.14 (d, *J* = 8 Hz, 1H), 7.10 (m, 1H), 7.06 (d, *J* = 7.5 Hz, 2H), 6.98 (s, 1H), 6.86 (d, *J* = 8 Hz, 1H), 6.74 (d, *J* = 8.5 Hz, 1H), 4.49 (s, 2H), 4.21–4.25 (m, 4H), 2.33 (s, 3H). MS (ESI): 416.51 ($C_{24}H_{22}N_3O_2S$, [M+H]+). Anal. Calcd for $C_{24}H_{21}N_3O_2S$: C, 69.37; H, 5.09; N, 10.11. Found: C, 69.75; H, 5.32; N, 10.44.

4.1.5.10. 3-(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)-5-(4-methylbenzylthio)-4-phenyl-4*H*-1,2,4-triazole (5j). White powder, yield 91%, mp: 175–178 °C. ^{1}H NMR (500 MHz, $CDCl_3$): 7.44–7.50 (m, 3H), 7.24–7.25 (m, 2H), 7.10–7.13 (m, 4H), 6.99 (s, 1H), 6.87 (d, *J* = 8.5 Hz, 1H), 6.75 (d, *J* = 8.5 Hz, 1H), 4.47 (s, 2H), 4.22–4.25 (m, 4H), 2.33 (s, 3H). MS (ESI): 416.51 ($C_{24}H_{22}N_3O_2S$, [M+H]+). Anal. Calcd for $C_{24}H_{21}N_3O_2S$: C, 69.37; H, 5.09; N, 10.11. Found: C, 69.05; H, 5.45; N, 10.43.

4.1.5.11. 3-(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)-5-(2-fluorobenzylthio)-4-phenyl-4*H*-1,2,4-triazole (5k). White powder, yield 85%, mp: 173–175 °C. ^{1}H NMR (500 MHz, $CDCl_3$): 7.48–7.51 (m, 3H), 7.46 (d, *J* = 6.0 Hz, 1H), 7.26 (d, *J* = 7.0 Hz, 1H), 7.12 (d, *J* = 7.5 Hz, 2H), 7.06–7.09 (m, 1H), 7.01–7.05 (m, 1H), 6.98 (s, 1H), 6.87 (d, *J* = 8.5 Hz, 1H), 6.77 (d, *J* = 8.0 Hz, 1H), 4.52 (s, 2H), 4.21–4.26 (m, 4H). MS (ESI): 420.47 ($C_{23}H_{19}FN_3O_2S$, [M+H]+). Anal. Calcd for $C_{23}H_{18}FN_3O_2S$: C, 65.86; H, 4.33; N, 10.02. Found: C, 65.53; H, 4.50; N, 10.25.

4.1.5.12. 3-(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)-5-(4-fluorobenzylthio)-4-phenyl-4*H*-1,2,4-triazole (5l). White powder, yield 85%, mp: 157–159 °C. ^{1}H NMR (500 MHz, $CDCl_3$): 7.46–7.52 (m, 3H), 7.33–7.36 (m, 2H), 7.12 (d, *J* = 7.5 Hz, 2H), 6.99 (d, *J* = 8.0 Hz, 1H), 6.98 (s, 1H), 6.97 (d, *J* = 9.0 Hz, 1H), 6.87 (d, *J* = 9.0 Hz, 1H), 6.75 (d, *J* = 8.0 Hz, 1H), 4.46 (s, 2H), 4.22–4.25 (m, 4H). MS (ESI): 420.47 ($C_{23}H_{19}FN_3O_2S$, [M+H]+). Anal. Calcd for $C_{23}H_{18}FN_3O_2S$: C, 65.86; H, 4.33; N, 10.02. Found: C, 65.58; H, 4.06; N, 9.79.

4.1.5.13. 3-(2,4-Difluorobenzylthio)-5-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-4-phenyl-4*H*-1,2,4-triazole (5m). White powder, yield 87%, mp: 133–135 °C. ^{1}H NMR (500 MHz, $CDCl_3$): 7.47–7.54 (m, 4H), 7.15 (d, *J* = 6.5 Hz,2H), 6.99 (s, 1H), 6.87 (d, *J* = 8.5 Hz, 1H), 6.79–6.82 (m, 2H), 6.75 (d, *J* = 8.5 Hz, 1H), 4.48 (s, 2H), 4.21–4.26 (m, 4H). MS (ESI): 438.46 ($C_{23}H_{18}F_2N_3O_2S$, [M+H]+). Anal. Calcd for $C_{23}H_{17}F_2N_3O_2S$: C, 63.15; H, 3.92; N, 9.61. Found: C, 63.42; H, 3.69; N, 9.38.

4.1.5.14. 3-(2,6-Difluorobenzylthio)-5-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-4-phenyl-4*H*-1,2,4-triazole (5n). White powder, yield 89%, mp: 194–196 °C. ^{1}H NMR (500 MHz, $CDCl_3$): 7.49–7.50 (m, 3H), 7.23–7.24 (m, 1H), 7.21 (d, *J* = 8.5 Hz,2H), 7.01 (s, 1H), 6.86–6.89 (m, 3H), 6.76 (d, *J* = 8.5 Hz, 1H), 4.51 (s, 2H), 4.22–4.27 (m, 4H). MS (ESI): 438.46 ($C_{23}H_{18}F_2N_3O_2S$, [M+H]+). Anal. Calcd for $C_{23}H_{17}F_2N_3O_2S$: C, 63.15; H, 3.92; N, 9.61. Found: C, 63.42; H, 3.59; N, 9.24.

4.1.5.15. 3-(2-Bromobenzylthio)-5-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-4-phenyl-4*H*-1,2,4-triazole (5o). White powder, yield 89%, mp: 192–194 °C. ^{1}H NMR (500 MHz, $CDCl_3$): 7.60 (d, *J* = 7.0 Hz, 1H), 7.55 (d, *J* = 7.5 Hz, 1H), 7.45–7.50 (m, 3H), 7.24–7.27 (m, 1H), 7.13–7.16 (m, 1H), 7.09 (d, *J* = 7.5 Hz, 2H), 6.98 (s, 1H), 6.87 (d, *J* = 8.5 Hz, 1H), 6.75 (d, *J* = 8.5 Hz, 1H), 4.62 (s, 2H), 4.21–4.26 (m, 4H). MS (ESI): 481.38 ($C_{23}H_{19}BrN_3O_2S$, [M+H]+) Calcd for $C_{23}H_{18}BrN_3O_2S$: C, 57.51; H, 3.78; N, 8.75. Found: C, 57.24; H, 3.95; N, 8.48.

4.1.5.16. 3-(3-Bromobenzylthio)-5-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-4-phenyl-4*H*-1,2,4-triazole (5p). White powder, yield 92%, mp: 121–124 °C. ^{1}H NMR (500 MHz, $CDCl_3$): 7.46–7.51 (m, 4H), 7.39 (d, *J* = 7.5 Hz, 1H), 7.30 (d, *J* = 7.5 Hz, 1H), 7.14–7.17 (m, 1H), 7.09 (d, *J* = 8.5 Hz, 2H), 6.97 (s, 1H), 6.85 (d, *J* = 8.5 Hz, 1H), 6.741 (d, *J* = 8 Hz, 1H), 4.42 (s, 2H), 4.21–4.25 (m, 4H). MS (ESI): 481.38 ($C_{23}H_{19}BrN_3O_2S$, [M+H]+) Calcd for $C_{23}H_{18}BrN_3O_2S$: C, 57.51; H, 3.78; N, 8.75. Found: C, 57.24; H, 3.43; N, 8.42.

4.1.5.17. 3-(4-Bromobenzylthio)-5-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-4-phenyl-4*H*-1,2,4-triazole (5q). White powder, yield 92%, mp: 189–191 °C. ^{1}H NMR (500 MHz, $CDCl_3$): 7.46–7.51 (m, 3H), 7.42 (d, *J* = 8.5 Hz, 2H), 7.26 (d, *J* = 8.0 Hz, 2H), 7.12 (d, *J* = 7.0 Hz, 2H), 6.98 (s, 1H), 6.87 (d, *J* = 8.5 Hz, 1H), 6.75 (d, *J* = 8.5 Hz, 1H), 4.43 (s, 2H), 4.21–4.26 (m, 4H). MS (ESI): 481.38 ($C_{23}H_{19}BrN_3O_2S$, [M+H]+) Calcd for $C_{23}H_{18}BrN_3O_2S$: C, 57.51; H, 3.78; N, 8.75. Found: C, 57.79; H, 3.42; N, 8.47.

4.2. Cell proliferation assay

The antitumor activities of compounds **5a–5q** were determined using a standard (MTT)-based colorimetric assay (Sigma). Seed 10^4 cells per well into 96-well plates, incubate at 37 °C, 5% CO_2 for 24 h. Then add 100 μL a series concentration of drug-containing medium into wells to maintain the final concentration of drug as 40, 20, 6.67, 2.22, 0.74, 0.25 and 0.082 μg/mL. One concentration should be triplicated. And TNP-470 was used for the positive control. After 48 h, cell survival was determined by the addition of an MTT solution (25 μL of 5 mg/mL MTT in PBS). After 4 h, discard the medium and add 100 μL DMSO; the plates were votexed for 10 min to make completely dissolution. Optical absorbance was measured at 490 nm.

4.3. MetAP2. inhibitory assay

Seventeen 1,2,4-triazole derivatives containing 1,4-benzodioxan were tested in a search for small molecule inhibitors of MetAP2, which was purchased from R&D Systems (Minneapolis, MN). In a typical study, MetAP2 was incubated for 4 h at room temperature with or without the presence of the triazole derivatives, the final concentration of drug as 40, 20, 6.67, 2.22, 0.74, 0.25 and 0.082 μM. The results were reported in Table 2.

4.4. Apoptosis assay

HEPG2 cells were treated with various concentrations of compound **5k** for 24 h and then stained with both Annexin V-FITC (fluorescein isothiocyanate) and propidium iodide (PI). Then samples were analyzed by FACSCalibur flow cytometer (Becton Dickinson, SanJose, CA).

4.5. Western-blot analysis

After incubation, cells were washed with PBS and lysed using lysis buffer (30 mm Tris, pH 7.5, 150 mm NaCl, 1 mm phenylmethylsulfonyl fluoride, 1 mm Na_3VO_4, 1% Nonidet P-40, 10% glycerol, and phosphatase and protease inhibitors). After centrifugation at 10,000*g* for 10 min, the protein content of the supernatant was determined by a BCATM protein assay kit (Pierce, Rockford, IL, USA). The protein lysates were separated by 10% SDS–PAGE and subsequently electrotransferred onto a polyvinylidene difluoride membrane (Millipore, Bedford, MA, USA). The membrane was blocked with 5% nonfat milk for 2 h at room temperature. The blocked membrane was probed with the indicated primary antibodies overnight at 4 °C, and then incubated with a horse radish peroxidase (HRP)-coupled secondary antibody. Detection was performed using a LumiGLO chemiluminescent substrate system (KPL, Guildford, UK).

4.6. Experimental protocol of docking study

The automated docking studies were carried out using Auto Dock version 4.0. First, AutoGrid component of the program precalculates a three-dimensional grid of interaction energies based on the macromolecular target using the AMBER force field. Then automated docking studies were carried out to evaluate the binding free energy of the inhibitors within the macromolecules.

The three-dimensional structures of the aforementioned compounds were constructed using Chem. 3D ultra 11.0 software [Chemical Structure Drawing Standard; Cambridge Soft corporation, USA (2009)], then they were energetically minimized by using MOPAC with 100 iterations and minimum RMS gradient of 0.10. The Gasteiger-Hückel charges of ligands were assigned. The crystal structures of MetAP2 (PDB code: 2EA4) complex were retrieved from the RCSB Protein Data Bank (http://www.rcsb.org/pdb/home/home.do). All bound waters and ligands were eliminated from the protein and the polar hydrogens and the Kollman-united charges were added to the proteins.

Acknowledgments

This work was supported by Jiangsu National Science Foundation (No. BK2009239), the Fundamental Research Funds for the Central Universities (No. 1092020804, 1106020824 and 1096020802), and the Six Kinds of Professional Elite Foundation of Jiangsu Province (J.Z.).

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