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Synthesis and antitumor activity of 1,3,4-oxadiazole possessing 1,4-benzodioxan moiety as a novel class of potent methionine aminopeptidase type II inhibitors

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ABSTRACT

A series of 1,3,4-oxadiazole derivatives containing 1,4-benzodioxan moiety (**7a**–**7q**) have been designed, synthesized and evaluated for their antitumor activity. Most of the synthesized compounds were proved to have potent antitumor activity and low toxicity. Among them, compound **7a** showed the most potent biological activity against Human Umbilical Vein Endothelial cells, which was comparable to the positive control. The results of apoptosis and flow cytometry (FCM) demonstrated that compound **7a** induce cell apoptosis by the inhibition of MetAP2 pathway. Molecular docking was performed to position compound **7a** into MetAP2 binding site in order to explore the potential target.

Cancer, second cause of mortality in the world, increases with age in both rodents and humans.[1,2] Cancer continues to be a worldwide killer, despite the enormous amount of research and rapid developments during the past decade.[3] Cancer chemotherapy has entered a new field of molecularly targeted therapeutics, which is highly selective and not associated with the serious toxicities of conventional cytotoxic drugs.[4] Therefore, there is an increasing need for new therapies, especially those that are based on current knowledge of cancer biology as well as that taking advantage of the cancer cells phenotype, described by Hanahan and Weinberg.[5] Methionine aminopeptidase 2 (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.[6] Available reports also suggested that MetAP2 plays an important role in the growth of different types of tumors.[7]

Compounds containing a 1,4-benzodioxan template have received significant attention in chemical, medicinal and pharmaceutical research as this structural scaffold is found in a variety of drugs.[8] Some 1,4-benzodioxan derivatives are anti-psychotic agents and others are used by living species in their chemical communication systems. Recently, some reports claimed that a number of other 1,4-benzodioxan template-containing compounds also have ability as potential anticancer drugs with excellent bioavailability and low cytotoxicity.[9–12] For example, (*Z*)-*N*-(5-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-1,3,4-thiadiazol-2-yl)-3-phenylacrylamide, having a 1,4-benzodioxan structure was suggested to have strong antitumor activity.[13] Oxadiazole derivatives play a significant role in various pharmaceutical applications.[14–18] As an important class of heterocyclic compound, 1,3,4-oxadiazoles exhibit antitumor activities particularly.[19–21] 2-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-5-((2-methylbenzyl)thio)-1,3,4-oxadiazole, having a 1,3,4-oxadiazole structure was suggested to have strong antitumor activity.[21]

A series of 1,3,4-oxadiazole derivatives containing 1,4-benzodioxan template were firstly synthesized and antitumor activities were examined. Among them, the compounds **7a** displayed the most potent antitumor activity by inhibiting Human Umbilical Vein Endothelial Cells proliferation and had low toxicity. Preliminary study on the antitumor mechanism of the compounds found that compounds induced cell apoptosis by the inhibition of MetAP2 pathway. From the results, we can conclude that some of the synthesized compounds are potent antitumor agents.

Methyl 2,3-dihydrobenzo[b][1,4]dioxine-6-carboxylate **3** was prepared in two steps as shown in Scheme 1. It was prepared in two steps. Firstly, 3,4-dihydroxybenzoic acid **1** gave methyl 3,4-dihydroxybenzoate **2**, catalyzed by concentrated sulfuric acid in methanol. Secondly, compound **3** was prepared by treatment of **2** with dibromoethane in acetone.

Seventeen 1,3,4-oxadiazole derivatives (**7a**–**7q**) were prepared as shown in Scheme 1. Firstly, 2,3-dihydrobenzo[b][1,4]dioxine-6-carbohydrazide was prepared by treatment of **3** with hydrazine

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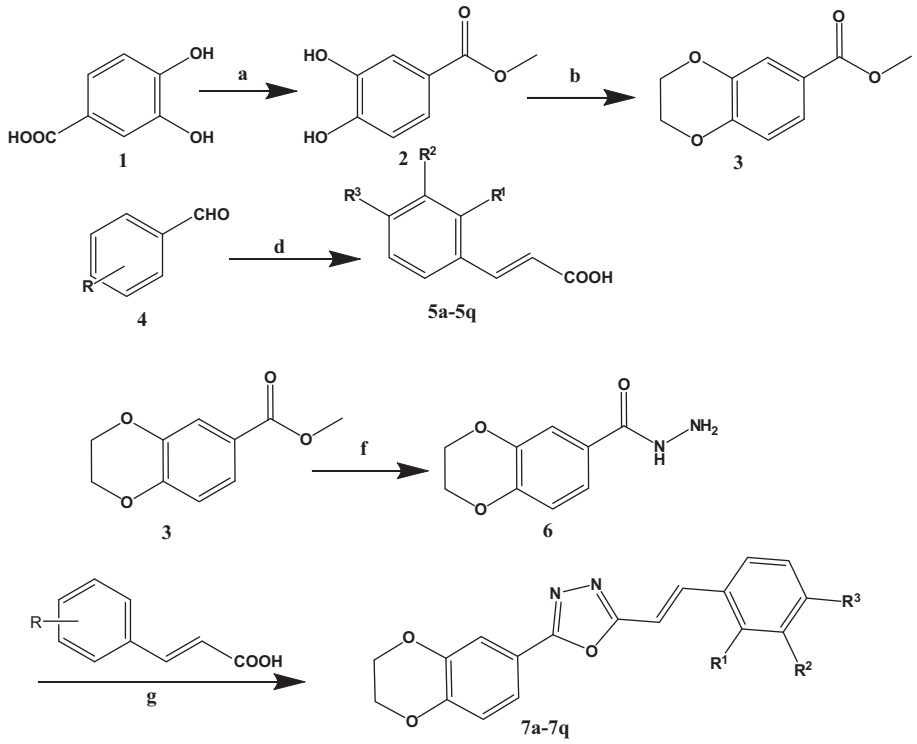

a R^1=F , R^2=H , R^3=H
b R^1=H , R^2= F, R^3=H
c R^1=H , R^2=H , R^3=F
d R^1=Cl , R^2=H , R^3=H
e R^1=H , R^2=H , R^3=Cl
f R^1=Br , R^2=H , R^3=H
g R^1=H , R^2=Br , R^3=H
h R^1=H , R^2=H , R^3=Br
i R^1=CH_3 , R^2= H, R^3=H
j R^1=H , R^2=CH_3 , R^3=H
k R^1=H , R^2=H , R^3=CH_3
l R^1=NO_2 , R^2=H , R^3=H
m R^1=H , R^2=NO_2 , R^3=H
n R^1=H , R^2=H , R^3=NO_2
o R^1=H , R^2=H , R^3=H
p R^1=H , R^2=CH_3O , R^3=H
q R^1=H , R^2=H , R^3=CH_3O

Scheme 1. Synthesis of compounds **3**, **5a**–**5q** and **7a**–**7q**. Reagents and conditions: (a) methanol, concentrated sulfuric acid, 90 °C, 8 h; (b) dibromoethane, potassium carbonate, acetone, 70 °C, 12 h; (d) pyridine, piperidine, 85 °C, 24 h; (f) hydrazine hydrate, ethanol, 90 °C, 4 h; (g) phosphorous oxychloride, 110 °C, 5 h.

Table 1
Cytotoxicity assay of the compounds on Human Umbilical Vein Endothelial Cells

Compounds	CC_{50} ± SD (μM)
6	200.18 ± 12.19
7a	402.23 ± 21.04
7b	295.27 ± 20.00
7c	325.17 ± 31.11
7d	367.82 ± 18.91
7e	312.00 ± 21.12
7f	287.45 ± 12.95
7g	321.95 ± 22.11
7h	491.23 ± 19.76
7i	356.23 ± 11.09
7j	200.98 ± 11.02
7k	265.26 ± 21.08
7l	323.15 ± 21.23
7m	309.13 ± 12.45
7n	298.77 ± 22.10
7o	209.22 ± 18.36
7p	332.18 ± 24.12
7q	312.23 ± 18.46

Table 2
Cell proliferation assay of the compounds on Human Umbilical Vein Endothelial Cells

Compounds	IC_{50} ± SD (μM)
6	>50
7a	1.16
7b	3.53
7c	4.96
7d	7.71
7e	8.03
7f	8.89
7g	13.53
7h	22.32
7i	40.49
7j	40.60
7k	45.21
7l	25.02
7m	30.13
7n	32.07
7o	>50
7p	>50
7q	>50
TNP-470	1.96

hydrate (85%) in ethanol. Then, compound **6** gave 1,3,4-oxadiazole derivatives by refluxing in anhydrous phosphorus oxychloride with different substituted cinnamic acids.

Generally speaking, the inhibitory activity of the compounds is due to cell apoptosis or toxic effect, so we firstly performed cytotoxicity test before detecting biological activity. The oxadiazole compounds were detected for their cyototoxicity on Human Umbilical Vein Endothelial cells (HUVEC). The pharmacological results of these compounds were summarized in Table 1. What we

Table 3
MetAP2 inhibitory activity of the selected compounds

Compounds	MetAP2 (IC_{50}, μM)
7a	2.08
7b	4.79
7c	6.33
7d	10.04
7e	7.92
7f	8.41
7g	10.71
7h	12.66
7l	22.03
7m	18.24
7n	13.95
TNP-470	1.32

can see from the data is that most of the compounds were low toxic.

All the synthesized derivatives (**7a**–**7q**) were tested in vitro for the inhibition activity on HUVEC. The results were summarized in Table 2. The table showed that most of the synthesized compounds exhibited potent inhibitory activity with the IC_{50} value at low micromolar.

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. 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) > CH_3 > CH_3O. Among these compounds, substituent at different positions led to different antitumor activity, and the potency order was *ortho-* > *meta-* > *para-*.

The MetAP2 inhibitory potency of the oxadiazole derivatives containing 1,4-benzodioxan was examined and the results were summarized in Table 3. Most of the tested compounds displayed potent MetAP2 inhibiting activity. Among them, compound **7a** showed the most potent inhibitory with IC_{50} of 2.08 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 antitumor activities of the synthesized compounds may be derived from the inhibition of MetAP2 enzymatic activities.

In order to study the preliminary antitumor mechanism of the compounds, we performed flow cytometry (FCM) (Fig. 1). As shown in Figure 1, HUVEC were treated with 0, 0.5, 1, 2 and 4 μM of compound **7a** for 24 h. The compound can increase the percentage of apoptosis in a dose-independent manner. The results indicated that compound **7a** can induce apoptosis of HUVEC.

In an effort to elucidate the possible mechanism by which the title compounds can induce anticancer activity and guide further SAR studies, molecular docking of the potent inhibitor **7a** into ATP binding site of MetAP2 was performed on the binding model based on the MetAP2 (2EA4.pdb). The binding models of compound **7a** and MetAP2 were depicted in Figure 2. In the binding model, compound **7a** was nicely bound to the MetAP2 via π–π interaction (between benzene ring and HIS 231) and one hydrogen bond (F atom on the benzene ring and amino hydrogen of ASN 329 form hydrogen bond: H–F···H).

In conclusion, a series of 1,3,4-oxadiazole derivatives containing 1,4-benzodioxan have been synthesized and evaluated for their antitumor activities. Compound **7a** demonstrated the most potent inhibitory activity that inhibited the growth of HUVEC cells with IC_{50} of 1.16 μM and inhibited the activity of MetAP2 with IC_{50} of 2.08 μM, which was comparable to the positive control TNP-470.

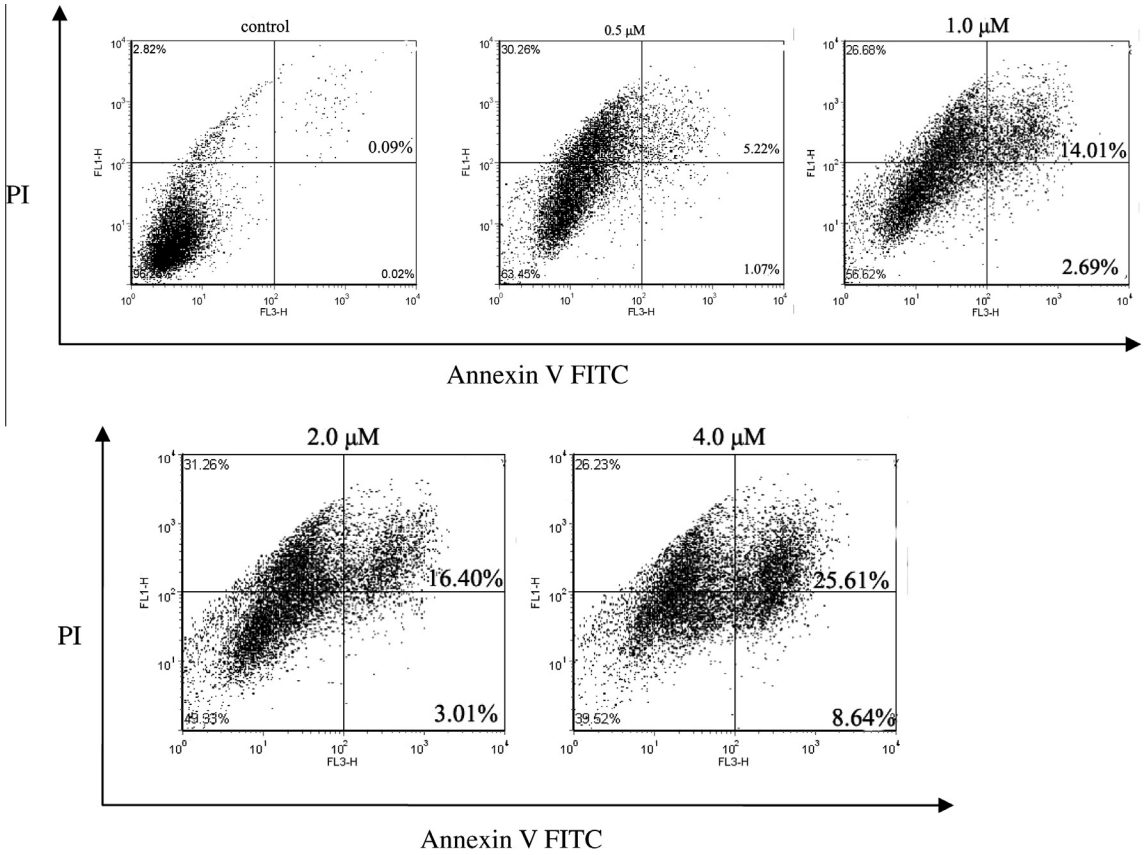

Figure 1. HUVEC were cultured with anticancer and various concentrations of **7a** for 24 h. Cells were stained by Annexin VeFITC/PI and apoptosis was analyzed by flow cytometry. Inhibition including early and late apoptosis.

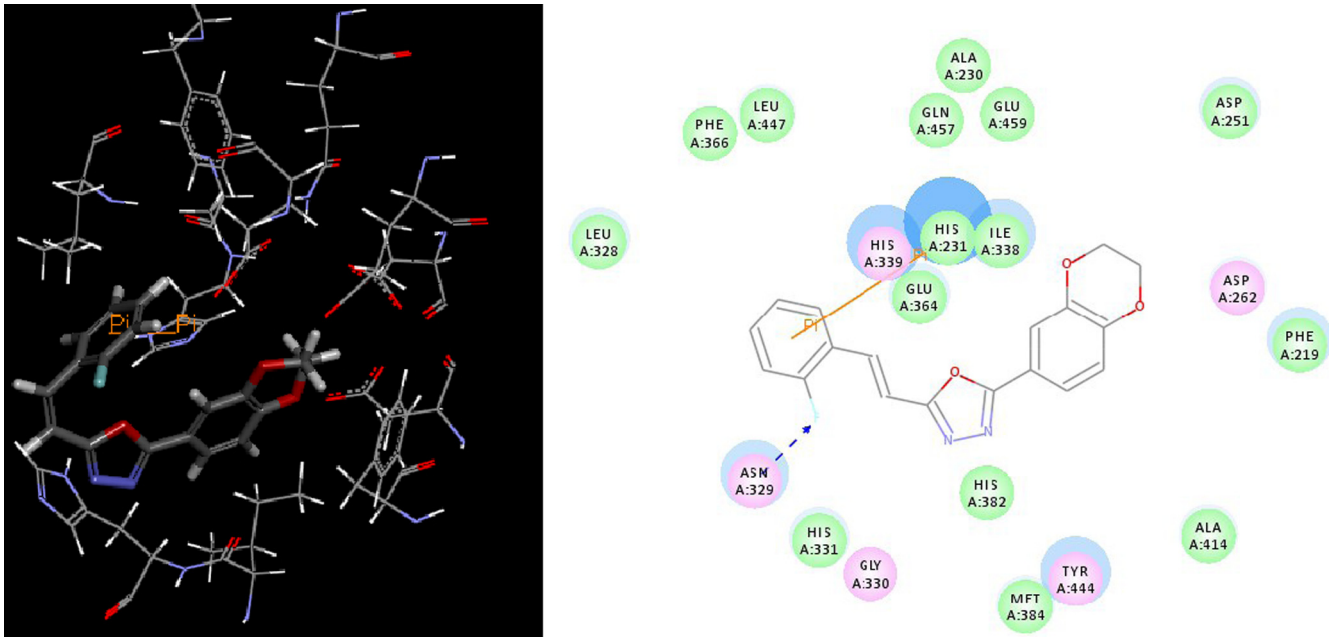

Figure 2. Molecular docking modeling of compound **7a** with MetAP2: for clarity, only interacting residues are displayed. Left: 3D model of the interaction between compound **7a** and the MetAP2 binding site. Right: 2D model of the interaction between compound **7a** and the MetAP2 binding site.

In order to gain deeper understanding of the structure–activity relationships observed at the MetAP2, molecular docking of the most potent inhibitor **7a** into the binding site of MetAP2 was performed on the binding model based on the MetAP2 complex structure. Analysis of the compound **7a**'s binding conformation demonstrated that compound **7a** was stabilized by π–π interaction and hydrogen bond. Apoptosis assay results showed the compound **7a** was a potential antitumor agent.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.03.068.

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