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Synthesis, biological evaluation and molecular docking studies of 1,3,4-thiadiazole derivatives containing 1,4-benzodioxan as potential antitumor agents

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ABSTRACT

A series of 1,3,4-thiadiazole derivatives containing 1,4-benzodioxan (**2a–2s**) have been synthesized to screen for FAK inhibitory activity. Compound **2p** showed the most potent biological activity against HEPG2 cancer cell line (EC_{50} = 10.28 μg/mL for HEPG2 and EC_{50} = 10.79 μM for FAK), which was comparable to the positive control. Docking simulation was performed to position compound **2p** into the FAK structure active site to determine the probable binding model. The results of antiproliferative and Western-blot assay demonstrated that compound **2p** possessed good antiproliferative activity against HEPG2 cancer cell line. Therefore, compound **2p** with potent FAK inhibitory activity may be a potential anticancer agent against HEPG2 cancer cell.

Cancer, second cause of mortality in the world, is continuing to be a major health problem in developing as well as undeveloped countries.[1] Despite the progress achieved in medicine during century, the continued commitment to the laborious task of discovering new anticancer agents remains critically important. 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.[2]

Focal adhesion kinase is a 125 kDa protein that localizes to focal adhesions[3] and is activated and tyrosine phosphorylated in response to integrin clustering.[4] These result in signal transmission to the cell nucleus to trigger cell division and motility. FAK is involved in multiple cellular functions such as cell proliferation, survival, motility, invasion, metastasis, and angiogenesis.[5] Different approaches to inhibit FAK with FAK antisense oligonucleotides,[6] dominant-negative C-terminal domain of FAK, FAK-CD or FRNK[7,8] or FAK siRNA[9,10] caused decreased cellular viability, growth inhibition, or apoptosis. Recently, FAK was proposed to be a new potential therapeutic target in cancer.[11,12]

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. For example (Fig. 1), the mesylate salt of doxazosin (A) is an effective drug for treatment of hypertension.[13] The 6-position substituted 1,4-benzodioxan (B) is known as a non-steroidal anti-inflammatory drug.[14] WB 4104 (C) is recognized as a selective α-adrenoceptor antagonist.[15–19] In addition, 1,3,4-thiadiazole nucleus constitutes the active part of several biologically active compounds (D), including antitumor,[20–22] antimycotic[23,24] and anti-inflammatory agents.[25–27]

Recently, it was reported that a number of other compounds containing the 1,4-benzodioxan template showed potent antitumor activity.[28] However, to our knowledge, few reports have been dedicated to the synthesis and focal adhesion kinase structure inhibitory activity of 1,3,4-thiadiazole derivatives containing 1,4-benzodioxan. Herein, in continuation to extend our research on antitumor compounds with FAK structure inhibitory activity, we report in the present work the synthesis and structure–activity relationships of a series of 1,3,4-thiadiazole derivatives containing 1,4-benzodioxan as antitumor agents. Biological evaluation indicated that some of the synthesized compounds were potent inhibitors of FAK structure.

Nineteen 1,3,4-thiadiazole derivatives containing 1,4-benzodioxan were synthesized to screen for the antitumor activity. All of them were synthesized for the first time. The synthesis of compounds **2a–2s** followed the general pathway outlined in Scheme 1. They are prepared in two steps. Firstly, the 2,3-dihydrobenzo[b][1,4] dioxine-6-carboxylic acid on treatment with thiosemicarbazide in presence of phosphoryl chloride yielded *N*-(5-(2,3-dihydrobenzo[b][1,4] dioxin-6-yl)-1,3,4-thiadiazol-2-amine. Secondly, the coupling reaction between the obtained colorless solid and the different substituted phenyl acetic acid or

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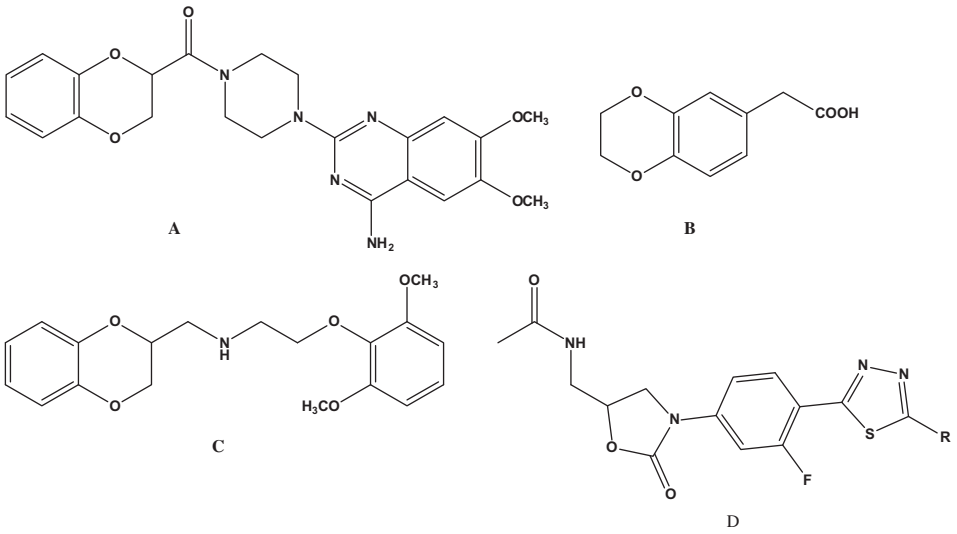
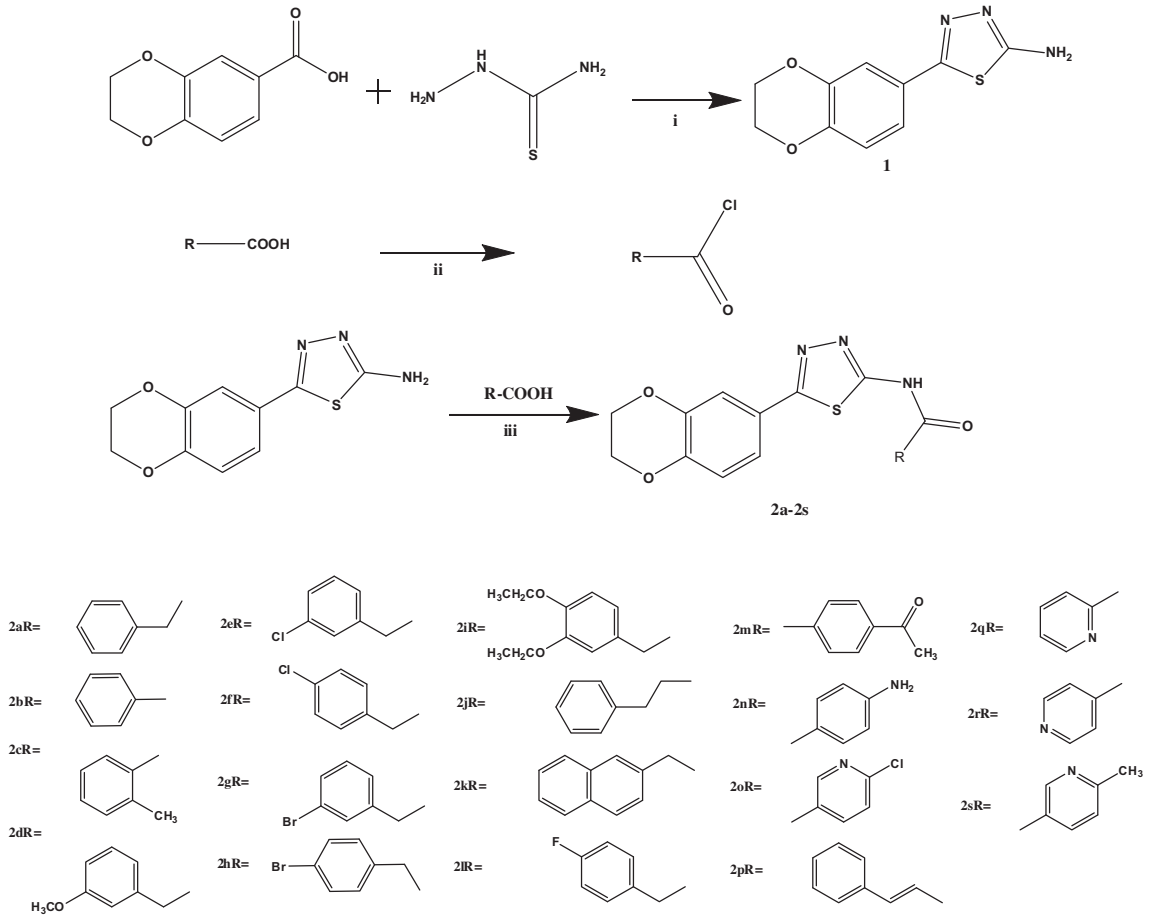


Figure 1. The structure of compounds A, B, C and D.



Scheme 1. General synthesis of compounds (**2a–2s**). Reagents and conditions: (i) $POCl_3$, reflux, 30 min, KOH; (ii) $SOCl_2$, 90 °C; (iii) EDC, HOBT, dichloromethane, rt.

benzoic acid was performed by using carbodiimide hydrochloride and *N*-hydroxybenzotriazole in anhydrous CH_2Cl_2, what afforded the corresponding target compounds *N*-(5-(2,3-dihydrobenzo [b][1,4]dioxin-6-yl)-1,3,4-thiadiazol-2-yl)-substituted-acetamide. Then compounds **2a–2s** were obtained by subsequent purification with recrystallisation. All of the synthetic compounds gave satisfactory analytical and spectroscopic data, which were full accordance with their depicted structures.

All the synthesized derivatives **2a–2s** 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

Table 1
Antiproliferative activity of the synthesized compounds (**2a–2s**)

Compound	EC_{50} (μg/mL)			
	HEPG2	HELA	SW1116	BGC823
2a	18.28	49.89	51.29	27.38
2b	34.78	>100	67.64	46.15
2c	>100	>100	>100	>100
2d	>100	>100	>100	>100
2e	18.43	34.86	47.62	>100
2f	13.78	26.76	40.69	>100
2g	45.78	54.22	>100	>100
2h	42.15	51.21	>100	>100
2i	>100	>100	>100	>100
2j	15.33	32.45	16.16	14.21
2k	>100	>100	43.57	67.87
2l	23.78	>100	>100	>100
2m	>100	>100	>100	>100
2n	>100	>100	41.21	>100
2o	15.35	>100	30.35	>100
2p	10.28	>100	60.17	45. 36
2q	>100	>100	>100	35.76
2r	>100	>100	>100	41.02
2s	>100	>100	>100	>100
5-Fluorouracil	23.31	31.02	28.52	17.37
Staurosporine	21.74	29.12	24.92	26.83

pattern of selectivity as well as broad-spectrum antitumor activity. Besides, compound **2p** showed the most potent biological activity against HEPG2 cancer cell line with EC_{50} value of 10.28 μg/mL.

The activity of the tested compounds could be correlated to structure variation and modifications. By investigating the variation in selectivity of the tested compounds over the four cell lines, it was revealed that among the tested compounds, compound **2j** was the most active members with EC_{50} values of 14.21–32.45 μg/mL. Besides, compound **2p** showed the most potent biological activity against HEPG2 cancer cell line with EC_{50} value of 10.28 μg/mL. Regarding the nineteen compounds, different acids substitutes led to different antitumor activity, and the potency order was phenylpropinic acid >phenylacetic acid >benzoic acid. As shown in Table 1, compounds **2e** and **2f** with substituted Cl group on benzene ring showed better antitumor activity with EC_{50} of 13.78–47.62 μg/mL than compounds **2g** and **2h** with substituted Br group with EC_{50} of 42.15–54.22 μg/mL against HEPG2 and HELA cancer cell lines. Among these compounds, compounds with *p*-substituted Cl group (**2f**) exhibited more potent activity than compounds with *m*-substituted Cl group (**2e**). Besides, there was the same rule with the compounds substituted by Br. Based on the data obtained, compounds **2q** and **2r** with pyridine ring were

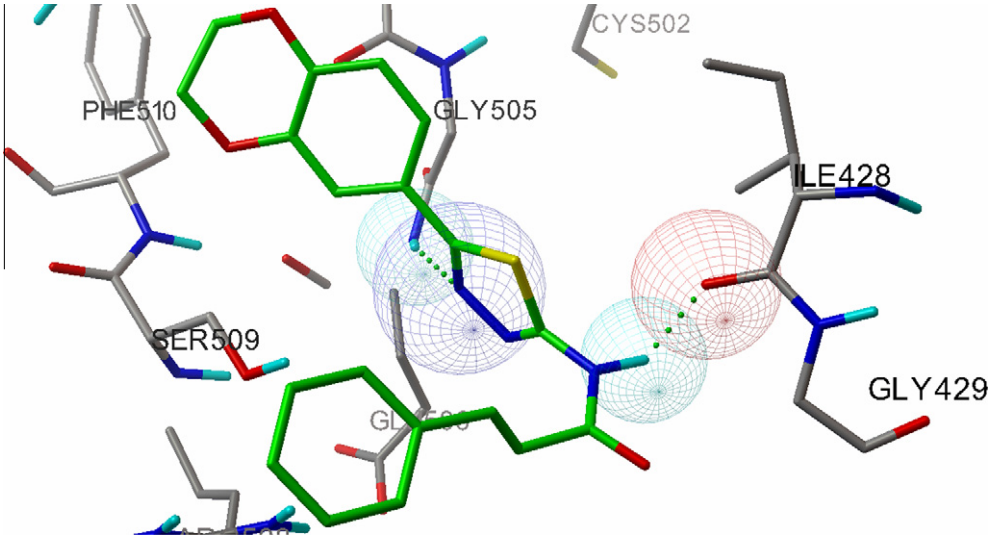


Figure 2. Molecular docking modeling of compound **2p** with FAK: compound **2p** is nicely bound to the FAK with its nitrogen atom of thiadiazole ring project toward the amino hydrogen of GLY 505, with the hydroxyl group forming a more optimal H-bond (H–N···H: 2.164 Å, 153.1°) interaction, and the hydrogen atom of imine group of **2p** also forms hydrogen bond (N–H···O: 2.010 Å, 121.8°) with oxygen atom of ILE428.

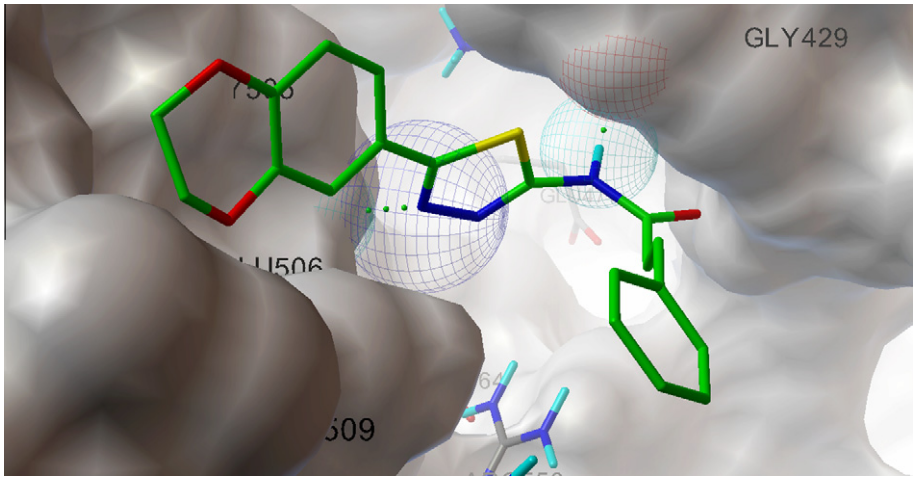


Figure 3. 3D model of the interaction between compound **2p** and FAK binding site. FAK is represented by molecular surface. Compound **2p** is depicted by sticks and balls.

Table 2
FAK inhibitory activity of selected compounds

Compound	FAK (EC_{50}, μM)
2a	17.74
2b	21.04
2e	18.11
2f	12.15
2g	26.15
2h	23.73
2j	13.98
2l	19.02
2o	13.69
2p	10.79
Staurosporine	11.32

Table 3
The docking calculation of the synthesized compounds (**2a**–**2s**)

Compound	Binding energy	IC units
2a	−8.58	nM
2b	−7.84	nM
2c	−8.31	μM
2d	−8.37	μM
2e	−7.39	nM
2f	−8.52	nM
2g	−6.97	nM
2h	−6.53	nM
2i	−8.16	μM
2j	−7.73	nM
2k	−8.27	μM
2l	−9.56	nM
2m	−7.11	μM
2n	−7.43	μM
2o	−8.08	nM
2p	−8.67	nM
2q	−6.45	μM
2r	−8.72	μM
2s	−7.58	μM

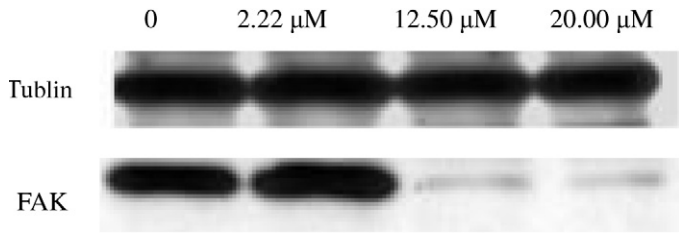


Figure 5. Compound **2p** was examined by Western blotting. Data are representative of three independent experiments.

found to be inactive against HEPG2, HELA and SW1116 cancer cell lines. However, introduction of NH_2 or Cl moieties, afforded **2n** and **2o** with a remarkable increase in the antitumor activity against SW1116 and HEPG2 cancer cells, EC_{50} values, 41.21 and 15.35 μg/mL, respectively. Interestingly, compounds **2q** and **2r** with pyridine ring showed moderate activity toward BGC823 cancer cell line with EC_{50} values, 35.76 and 41.02 μg/mL, respectively. The addition of CH_3 group (**2s**), however, led to decrease in cytotoxic activity against all cell lines.

In an effort to elucidate the possible mechanism by which the title compounds can induce antitumor activity in the four cells and guide further SAR studies, molecular docking of the potent inhibitor **2p** into ATP binding site of FAK were performed on the binding model based on the FAK structure (2ETM.pdb). The binding models of compound **2p** and FAK were depicted in Figures 2 and 3. In the binding model, compound **2p** is nicely bound to the FAK with its nitrogen atom of thiadiazole ring project toward the amino hydrogen of GLY 505, with the hydroxyl group forming a more optimal H-bond (H–N···H: 2.164 Å, 153.1°) interaction, and the hydrogen atom of imine group of **2p** also forms hydrogen bond (N–H···O: 2.010 Å, 121.8°) with oxygen atom of ILE428. The molecular docking results suggested that compound **2p** was a potential

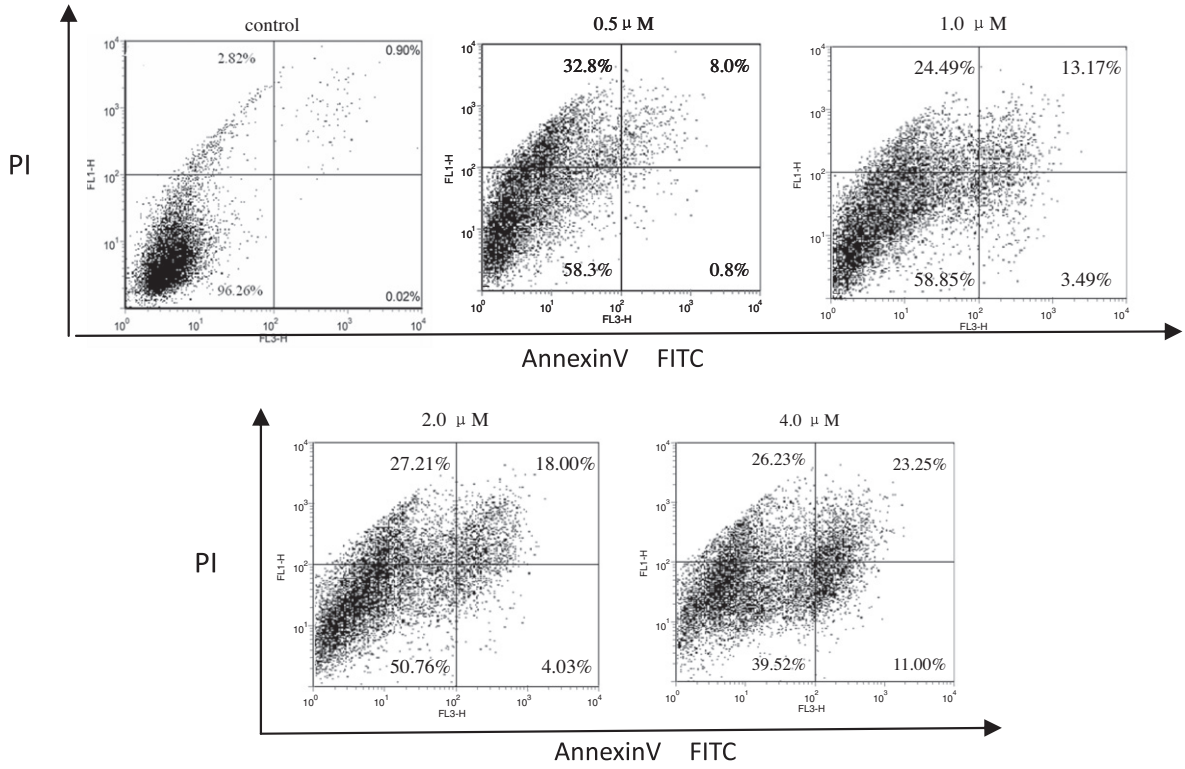


Figure 4. HEPG2 cells isolated from naïve mice were cultured with anticancer and various concentrations of **2p** for 24 h. Cells were stained by Annexin VeFITC/PI and apoptosis was analyzed by flow cytometry.

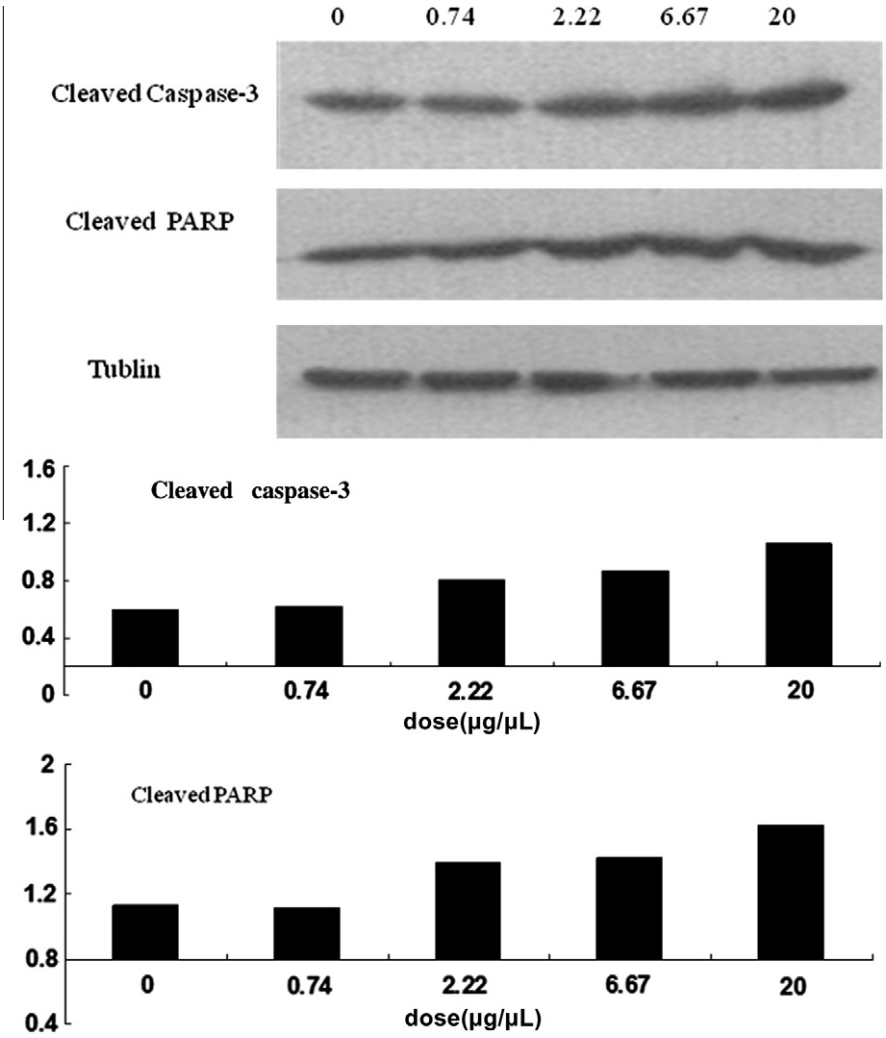


Figure 6. Compound **2p** was examined by Western blotting. Data are representative of three independent experiments.

inhibitor of FAK. The docking calculation of the other compounds were also depicted in Table 3.

In addition, we also selected the top 10 compounds which had better antiproliferative activity to test their FAK inhibitory activity against HEPG2 cell line. The results were summarized in Table 2. Most of the tested compounds displayed potent FAK inhibitory. Among them, compound **2p** showed the most potent inhibitory with EC_{50} of 10.79 μM. The results of FAK inhibitory activity of the tested compounds were corresponding to the structure relationships (SAR) of their antitumor activities. This demonstrated that the potent antitumor activities of the synthetic compounds were probably correlated to their FAK inhibitory activities.

Apoptosis is an essential mechanism used to eliminate activated HEPG2 cells during the shut down process of excess immune responses and maintain proper immune homeostasis, while deficient apoptosis of activated HEPG2 cell is associated with a wide variety of immune disorders. We detected the mechanism of compound **2p** inhibition effects by flow cytometry (FCM) (Fig. 4), and found that the compound could induce the apoptosis of activated HEPG2 cells in a dose dependent manner. The result indicated that compound **2p** induced apoptosis of antitumor stimulated HEPG2 cells.

In an effort to study the preliminary mechanism of the compound with potent inhibitory activity, the Western-blot experiment was performed to assay the effect of compound **2p**. The Western-blot results were summarized in Figures 5 and 6. The result indicated that compound **2p** showed excellent inhibitory activity.

In conclusion, a series of *N*-(5-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-1,3,4-thiadiazol-2-yl)-substituted-acetamide substituted derivatives have been synthesized and evaluated for their antitumor activities. Compound **2p** demonstrated the most potent inhibitory activity that inhibited the growth of HEPG2 cells with EC_{50} of 10.28 μM and inhibited the activity of FAK kinase with EC_{50} of 10.79 μM, which was comparable to the positive control staurosporine. Molecular docking study indicated that compound **2p** was nicely bound to the FAK with two hydrogen bonds. Apoptosis assay and Western-blot results also showed the compound **2p** was a potential antitumor agent.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.08.039.

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