Bioorganic & Medicinal Chemistry 20 (2012) 3615–3621

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

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

Synthesis, biological evaluation and molecular docking studies of novel 2-(1,3,4-oxadiazol-2-ylthio)-1-phenylethanone derivatives

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ARTICLE INFO

Article history:
Received 7 March 2012
Revised 27 March 2012
Accepted 27 March 2012
Available online 11 April 2012

Keywords:
Skin cancer
2-(1,3,4-Oxadiazol-2-ylthio)-1-phenylethanone derivatives
Focal adhesion kinase
MCF-7
A431
Molecular docking

ABSTRACT

In present study, a series of new 2-(1,3,4-oxadiazol-2-ylthio)-1-phenylethanone derivatives (**6a–6x**) as potential focal adhesion kinase (FAK) inhibitors were synthesized. The bioassay assays demonstrated that compound **6i** showed the most potent activity, which inhibited the growth of MCF-7 and A431 cell lines with IC_{50} values of 140 ± 10 nM and 10 ± 1 nM, respectively. Compound **6i** also exhibited significant FAK inhibitory activity (IC_{50} = 20 ± 1 nM). Docking simulation was performed to position compound **6i** into the active site of FAK to determine the probable binding model.

1. Introduction

Skin cancer is a deteriorating public health problem because its incidence has increased more than sixfold worldwide since the 1940s.[1,2] In the U.S.A. alone, more than three million cases of skin cancer are estimated to occur annually, with the total direct and indirect costs exceeding two billion dollars annually.[3] However, treatment options for skin cancer such as surgery, radiotherapy and chemotherapy have not been satisfactory. Therefore, it is important to identify new agents and novel targets for the treatment of skin cancer.

The non-receptor protein tyrosine kinase focal adhesion kinase (FAK) was discovered almost 15 years ago,[4] it is activated and tyrosine phosphorylated in response to integrin clustering,[5] which result in signal transmission to the cell nucleus to trigger cell division and motility.[6] FAK plays an important role in cell proliferation, survival, motility, invasion, metastasis, and angiogenesis.[7] In skin cells, FAK controls cytoskeletal dynamics and focal adhesion disassembly.[8] Elevated expression of FAK in human tumors has been correlated with increased malignancy and invasiveness.[9] Therefore, compounds that inhibit the activity of FAK are proposed to be a potential candidate against skin cancer.[10]

1,3,4-Oxadiazoles attract a lot of interest in the fields of medicinal chemistry and synthetic study. Primary research showed that compounds containing 1,3,4-oxadiazoles possessed a variety of biological activities, such as antitumor,[11] anti-inflammatory,[12] hypoglycemic,[13] antifungal[14] and antibacterial activities.[15,16] In addition, Usman Ghani reported some 1,3,4-oxadiazoles derivatives as the potent inhibitors of tyrosinase.[17]

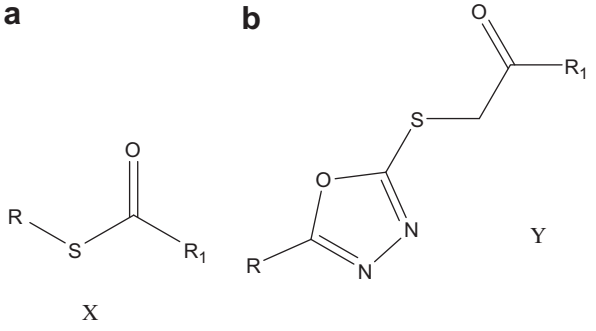

Figure 1. (a) Structure of thioester's functional group X. (b) Structure of 1,3,4-oxadiazol-2-ylthio)-1-phenylethanone group.

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http://dx.doi.org/10.1016/j.bmc.2012.03.061

Thioesters containing the functional group X (Fig. 1a) are widely used in the field of biochemistry as the common intermediates in many biosynthetic reactions. In addition, thioesters play important roles in the tagging of proteins with ubiquitin, which tags the protein for degradation.[18,19]

Based on the above, we designed a new series of compounds containing 1,3,4-oxadiazole ring and thioester analog Y (Fig. 1b) to expect that the oxadiazole ring could provide antitumor activity and thioester analog could increase the compounds' adhesive ability to the target protein.

In this paper, our main work are (1) to synthesize new novel 2-(1,3,4-oxadiazol-2-ylthio)-1-phenylethanone derivatives; (2) to evaluate their anticancer activities and FAK inhibitory activities; (3) to explore the preliminary mechanism of their role in cell apoptosis and (4) to investigate the inhibitor interaction with FAK by docking study.

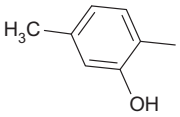
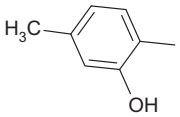
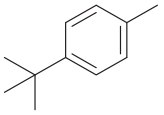
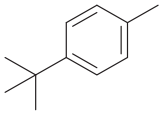
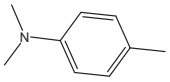
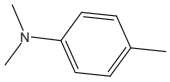
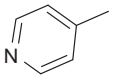
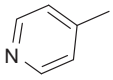
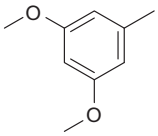
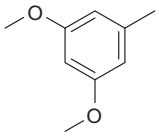
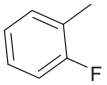
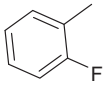
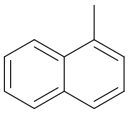
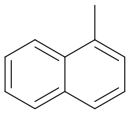
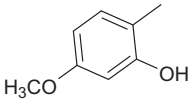
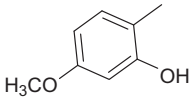
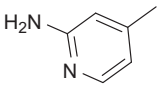
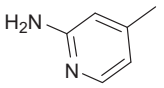
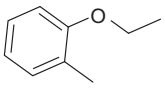
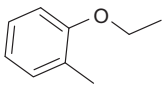
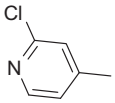
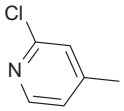
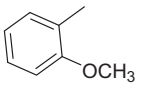
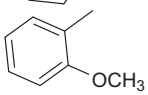
2. Results and discussion

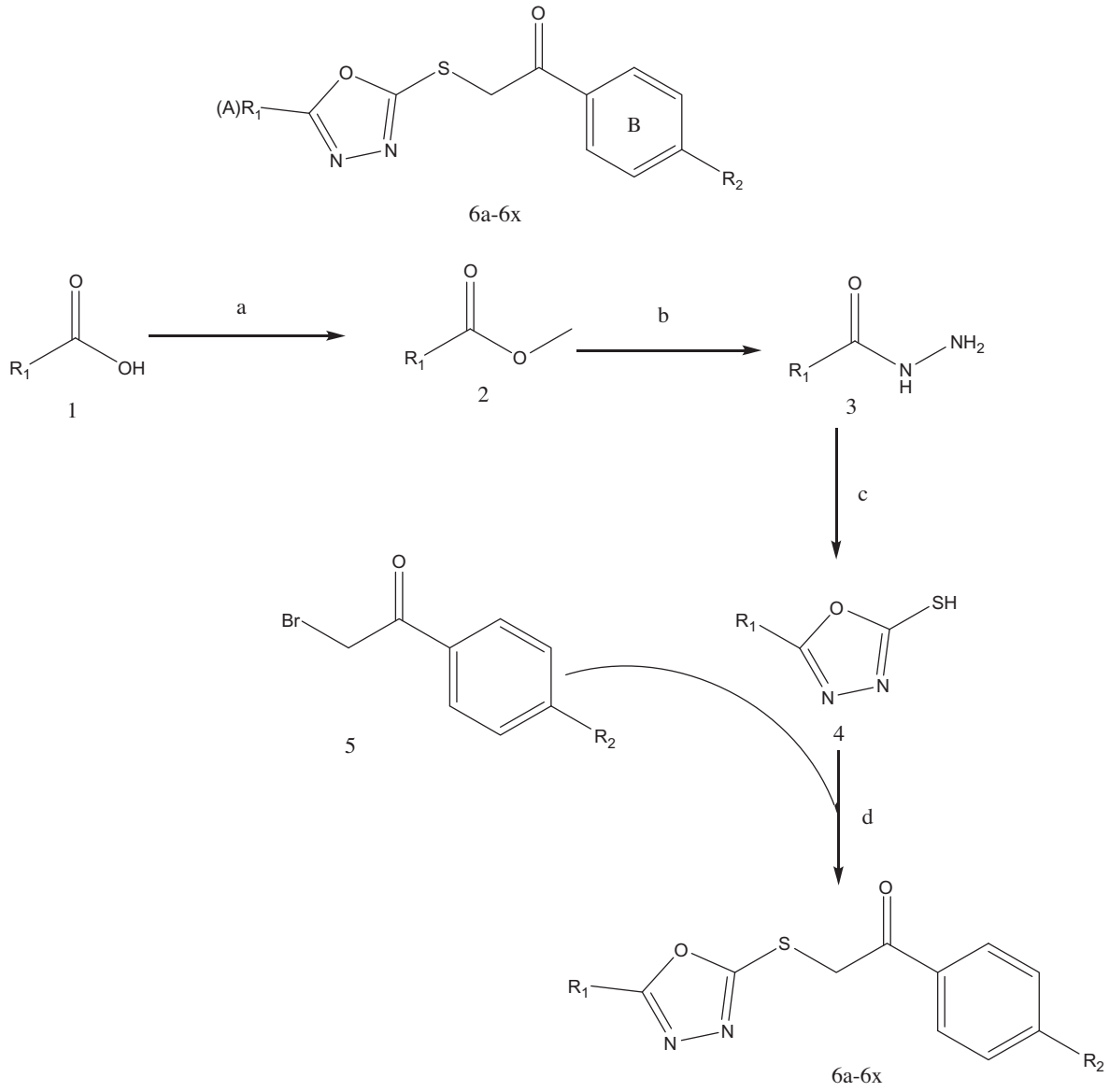
2.1. Chemistry

In this study, twenty-four 2-(1,3,4-oxadiazol-2-ylthio)-1-phenylethanone derivatives **6a–6x** (Table 1) were synthesized, and 10 of them were reported for the first time.

The synthetic route for the preparation of new 2-(1,3,4-oxadiazol-2-ylthio)-1-phenylethanone derivatives **6a–6x** was outlined in Scheme 1. The synthesis of these derivatives was started from 5-phenyl-1,3,4-oxadiazole-2-thiol derivatives **4** and 2-bromo-1-phenylethanone (or 2-bromo-1-(4-bromophenyl)ethanone) **5** and the key intermediate **4** was prepared in these three steps. Esterification of the carboxylic acids **1** with ethanol and concentrated sulfuric acid afforded the corresponding esters **2**. The aroyl hydrazide **3** was obtained by the reaction of the ester **2** with 85% hydrazine

Table 1
Structure of 2-(1,3,4-oxadiazol-2-ylthio)-1-phenylethanone derivatives **6a–6x**

Compound	R_1	R_2	Compound	R_1	R_2
6a		H	**6m**		Br
6b		H	**6n**		Br
6c		H	**6o**		Br
6d		H	**6p**		Br
6e		H	**6q**		Br
6f		H	**6r**		Br
6g		H	**6s**		Br
6h		H	**6t**		Br
6i		H	**6u**		Br
6j		H	**6v**		Br
6k		H	**6w**		Br
6l		H	**6x**		Br



Scheme 1. General synthesis of compounds (**6a–6x**). Reagents and conditions: (a) Methanol, concentrated sulfuric acid; reflux 8–12 h; (b) $NH_2NH_2{\cdot}H_2O$ (85%), ethanol; reflux 8–12 h; (c) (i) CS_2/KOH, ethanol (95%), reflux 24 h; (ii) HCl, pH 5–6; (d) NaOH (0.25 mol/L), ethanol, reflux 4–6 h.

monohydrate in ethanol. Treatment of the hydrazide **3** with carbon disulfide in the presence of KOH and 95% ethanol under reflux gave the key intermediates **4**.[20] The synthesis of compounds **6a–6x** was accomplished by reacting compounds **4** with 2-bromo-1-phenylethanone (or 2-bromo-1-(4-bromophenyl)ethanone) **5**, in the presence of NaOH aqueous solution in ethanol. All of the synthetic compounds were given satisfactory analytical and spectroscopic data, which were in full accordance with their depicted structures.

2.2. Biological activity and molecular modeling

The synthesized 2-(1,3,4-oxadiazol-2-ylthio)-1-phenylethanone derivatives **6a–6x** were evaluated for their antitumor activities against MCF-7 (human breast cancer cell line) and A431 (human epidermoid carcinoma cell line). The results were summarized in Table 2. As shown in Table 2, the active analogs showed a distinctive potential pattern of selectivity as well as antitumor activity. Compound **6i** (Fig. 2) displayed the most potent antitumor activity with IC_{50} of 140 ± 10 nM against MCF-7 cells comparable to the positive control staurosporine (IC_{50} = 180 ± 5 nM). For A431 cells, higher inhibitory efficiencies of all synthesized compounds were observed with IC_{50} values range of 10–60 nM compared with staurosporine (IC_{50} = 70 ± 2 nM). It was concluded that compounds **6a, 6e, 6i** and **6u** showed broad antitumor activity with IC_{50} concentration range of 10–200 nM against MCF-7 and A 431 cell lines. However, compounds **6j, 6k** and **6p** were proved to be less effective against the two cancer lines.

Structure–activity relationship (SAR) study at cellular level for these 2-(1,3,4-oxadiazol-2-ylthio)-1-phenylethanone derivatives demonstrated that compounds with benzene ring showed less potent activities than those with pyridine ring in the A position (e.g., **6i, 6u, 6w**).When the substitute happened on the *ortho* position of pyridine ring, their antitumor activities against the two cancer cells increased with increasing the electron-withdrawing ability of substituent (the potency order was NH_2 > H > Cl). Meanwhile, comparison of the substitution on benzene ring was demonstrated as follows: the potent inhibitory activity order of the compounds with same electron-donating groups on the benzene ring (e.g., **6l, 6e, 6b**) was *ortho*-substituted > *meta*-substituted > *para*-substituted, the compounds with two substituents at 2,5-position (e.g., **6a**,

Table 2
Antitumor activities of the synthesized compounds (**6a**–**6x**)

Compounds	IC_{50} ± SD (nM)		
	MCF-7[a]	A431[a]	FAK[b]
6a	150 ± 10	60 ± 2	760 ± 23
6b	50 ± 1	170 ± 11	18600 ± 1250
6c	210 ± 15	160 ± 9	17500 ± 1010
6d	370 ± 20	1000 ± 10	98700 ± 8150
6e	170 ± 10	40 ± 1	650 ± 21
6f	260 ± 15	50 ± 1	1220 ± 90
6g	230 ± 12	130 ± 5	5800 ± 1340
6h	280 ± 13	180 ± 7	20500 ± 1590
6i	140 ± 10	10 ± 1	20 ± 1
6j	2670 ± 120	650 ± 23	59800 ± 4750
6k	1290 ± 90	3200 ± 130	302500 ± 16730
6l	2070 ± 130	20 ± 1	580 ± 14
6m	2070 ± 110	50 ± 2	2670 ± 130
6n	1950 ± 80	100 ± 1	9800 ± 570
6o	550 ± 30	200 ± 15	21500 ± 1740
6p	4230 ± 210	780 ± 41	76310 ± 6630
6q	270 ± 13	60 ± 2	1080 ± 15
6r	240 ± 11	200 ± 17	19800 ± 170
6s	260 ± 25	160 ± 1	17900 ± 1630
6t	260 ± 23	330 ± 12	35400 ± 2520
6u	200 ± 16	60 ± 1	1150 ± 90
6v	460 ± 23	360 ± 13	40500 ± 2530
6w	270 ± 17	50 ± 1	970 ± 35
6x	600 ± 25	80 ± 2	1350 ± 120
Staurosporine	180 ± 5	70 ± 2	30 ± 1

[a] Inhibition of the growth of tumor cell lines.
[b] Inhibition of FAK.

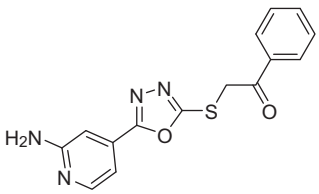


Figure 2. Structure of compound **6i**.

6h, **6m and 6t**) or 3,5-position (**6e, 6q**) showed poorer activities than these compounds with one substituent on the benzene ring. On the B-ring (Scheme 1), comparison of the *para* position substitutions on the B-ring demonstrated that a *meta* halogen (**6m–6x**) had slightly improved anticancer activity.

To help understand the SARs observed at the FAK, and guide further SAR studies, we proceeded to examine the interaction of compound **6i** with FAK (PDB: 2ETM). The molecular docking was performed by simulation of compound **6i** into the active site of FAK. All docking runs were applied LigandFit Dock protocol of Discovery Studio 3.1. The binding modes of compound **6i** and FAK were depicted in Figure 4. Compound **6i** was stabilized by hydrogen bonding interaction with Lys 454 (distance = 1.68528 Å)[21] and a π-cation interaction between benzene ring (ring B) and nitrogen atom of Lys 454 (distance = 4.32678 Å). The molecular docking results suggested that compound **6i** might be a potential inhibitor of FAK.

Apoptosis, or process of programmed cell death (PCD), is the normal pathway for clearance of defective or aged cells in the body, it provides a mechanism for the clearance of possibly dangerous material without causing harm to the body. It is an essential mechanism used to eliminate activated A431 cells during the shut-down process of excess immune responses and maintain proper immune homeostasis, while deficient apoptosis of activated A431 cell is associated with a variety of immune disorders. We detected the mechanism of compound 6i inhibition effects by flow cytometry (FCM) (Fig. 3). As shown in Figure 3, A431 cells were treated with 5, 10, 20 and40 nM of compound 6i for 24 h, the effect was observed in a dose-independent manner after treatment for 24 h with increasing dose of the compound **6i**. The percentages of cell apoptosis (26.24%, 29.70%, 44.18% and 60.16%) are corresponding to the presence of 5, 10, 20 and 40 nM of compound **6i**, respectively. These results indicated that compound **6i** induced apoptosis of antitumor stimulated A431 cells.

3. Conclusion

A series of new 2-(1,3,4-oxadiazol-2-ylthio)-1-phenylethanone derivatives were synthesized. Antitumor assay results indicated that most of compounds exhibited potent inhibitory activities against human breast cancer cell line MCF-7 and human epidermoid carcinoma cell line A431. Compounds such as **6a, 6e, 6i** and **6u** exhibited excellent antitumor activities compared to the positive control staurosporine. Compound 2-(5-(2-aminopyridin-4-yl)-1,3,4-oxadiazol-2-ylthio)-1-phenylethanone (**6i**) demonstrated the most potent inhibitory activity with IC_{50} of 140 ± 10 nM against MCF-7 cancer cells and IC_{50} of 10 ± 1 nM against A431 cancer cells. Compound **6i** also exhibited FAK inhibitory activity (IC_{50} = 20 ± 1 nM). Molecular docking study indicated that compound **6i** was nicely bound to the active site of FAK. Apoptosis assay also showed the compound **6i** was a potential antitumor agent.

4. Experiments

4.1. Materials and measurements

All chemicals and reagents used in current study were of analytical grade. All the ^{1}H NMR spectra were recorded on a Bruker DPX300 model Spectrometer in $CDCl_3$-d_6 and chemical shifts were reported in ppm (δ). ESI-MS spectra were recorded on a Mariner System 5304 Mass spectrometer. Elemental analyses were performed on a CHN-O-Rapid instrument. TLC was performed on the glassbacked silica gel sheets (Silica Gel 60 GF254) and visualized in UV light (254 nm). Column chromatography was performed using silica gel (200–300 mesh) eluting with ethyl acetate and petroleum ether.

4.2. General procedure for synthesis of the target compounds

A mixture of **4** (1 mmol) and 2-bromo-1-phenylethanone (or 2-bromo-1-(4-bromo phenyl)ethanone) **5** (1 mmol) were dissolved in ethanol (**5** mL, 80%). Aqueous sodium hydroxide solution (4 mL, 0.25 mol/L) was then slowly added dropwise to the reaction flask via a self-equalizing addition funnel, and the reaction solution was allowed to stir at room temperature for approximately 4–6 h. Then the solution was poured into ice-water. The precipitate was filtered and crystallized from methanol to gain the target compounds.

4.2.1. 2-(5-(2-Hydroxy-4-methylphenyl)-1,3,4-oxadiazol-2-ylthio)-1-phenylethanone (6a)

White powder, yield 83%, mp: 196–198 °C, ^{1}H NMR (300 MHz, $CDCl_3$-d_6) δ: 2.39 (d, J = 6.03 Hz, 3H); 4.98 (s, 2H); 6.82 (d, J = 8.07 Hz,1H); 6.93 (t, J = 6.00 Hz, 1H); 7.51–7.59 (m, 3H); 7.65 (t, J = 7.50 Hz, 1H); 8.05 (t, J = 4.50 Hz, 2H); 9.73 (s, 1H); MS (ESI): 327.37 ($C_{17}H_{15}N_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{14}N_2O_3S$: C, 62.56; H, 4.32; N, 8.58; Found: C, 62.48; H, 4.31; N, 8.62.

4.2.2. 2-(5-(4-*tert*-Butylphenyl)-1,3,4-oxadiazol-2-ylthio)-1-phenylethanone (6b)

White powder, yield 87%, mp: 136 °C, ^{1}H NMR(300 MHz, $CDCl_3$-d_6) δ: 1.36 (s, 9H); 4.98 (s, 2H); 7.53 (t, J = 8.55 Hz, 4H); 7.65 (d,

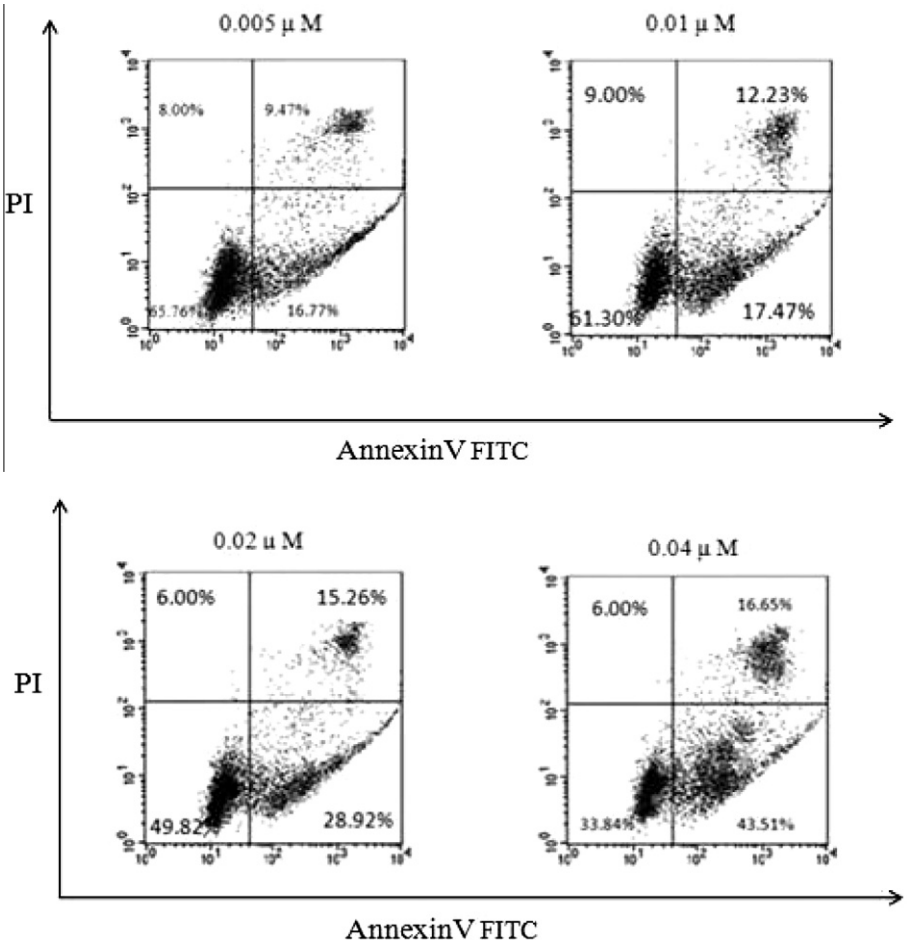


Figure 3. A431 cells were cultured with anticancer and various concentrations of **6i** for 24 h. Cells were stained by Annexin VeFITC/PI andapoptosis was analyzed by flow cytometry. As shown in the Figure 3, the lower left quadrants showed the vital cells; the upper right quadrants showed the dead cells (containing the secondary necrotic cells and apoptotic cells); and the lower right quadrants showed the apoptotic cells.

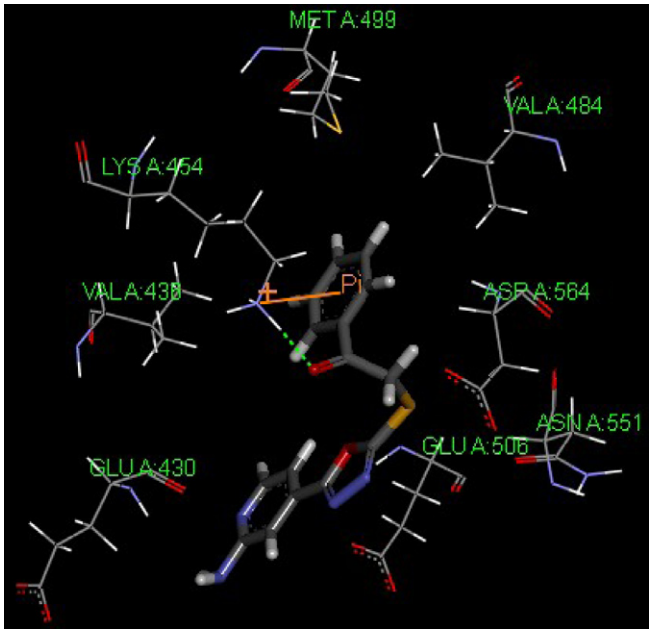


Figure 4. Docking of compound **6i** (carbon atoms are gray, nitrogen atoms are blue and hydrogen atoms are white) with FAK shows a hydrogen bond with Lys 454 and a π-cation interaction between **6i**'s benzene ring (ring A) and nitrogen atom of Lys 454.

J = 6.87 Hz, 1H); 7.90 (d, *J* = 4.20 Hz, 2H); 8.06 (d, *J* = 3.66 Hz, 2H); MS (ESI): 353.45 ($C_{20}H_{21}N_2O_2S$, $[M+H]^+$). Anal. Calcd for $C_{20}H_{20}N_2O_2S$: C, 68.16; H, 5.72; N, 7.95; Found: C, 68.08; H, 5.73; N, 7.98.

4.2.3. 2-(5-(4-(Dimethylamino)phenyl)-1,3,4-oxadiazol-2-ylthio)-1-phenylethanon (6c)

Light yellow solid, yield 88%, mp: 203–204 °C, ^{1}H NMR (300 MHz, $CDCl_3$-d_6) δ: 4.98 (s, 2H); 5.32 (s, 6H); 7.55 (t, *J* = 7.50 Hz, 4H); 7.67 (t, *J* = 7.32 Hz, 1H); 7.91 (d, *J* = 8.61 Hz, 2H); 8.05 (d, *J* = 7.68 Hz, 2H). MS (ESI): 340.41 ($C_{18}H_{18}N_3O_2S$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{17}N_3O_2S$: C, 63.70; H, 5.05; N, 12.38. Found: C, 63.64; H, 5.03; N, 12.44.

4.2.4. 1-Phenyl-2-(5-(pyridin-4-yl)-1,3,4-oxadiazol-2-ylthio)ethanone (6d)

Pink powder, yield 89%, mp: 145 °C, ^{1}H NMR(300 MHz, $CDCl_3$-d_6) δ: 5.02 (s, 2H); 7.55 (t, *J* = 7.86 Hz,2H); 7.67 (t, *J* = 7.38 Hz, 1H); 8.00–8.07 (m, 4H); 8.83 (s, 2H); MS (ESI): 298.03 ($C_{15}H_{12}N_3O_2S$, $[M+H]^+$). Anal. Calcd for $C_{15}H_{11}N_3O_2S$: C, 60.59; H, 3.73; N, 14.13. Found: C, 60.54; H, 3.72; N, 14.16.

4.2.5. 2-(5-(3,5-Dimethoxyphenyl)-1,3,4-oxadiazol-2-ylthio)-1-phenylethanone (6e)

White powder, yield 88%, mp: 162–163 °C, ^{1}H NMR(300 MHz, $CDCl_3$-d_6) δ: 3.65 (t, *J* = 12.3 Hz, 6H); 4.98 (s, 2H); 6.60 (t,

J = 2.19 Hz, 1H); 7.15 (d, *J* = 2.37 Hz, 2H); 7.53 (t, *J* = 7.50 Hz, 2H); 7.60–7.67 (m, 1H); 8.06 (d, *J* = 3.75 Hz, 2H); MS (ESI): 357.40 ($C_{18}H_{17}N_2O_4S$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{16}N_2O_4S$: C, 60.66; H, 4.53; N, 7.86. Found: C, 60.58; H, 4.54; N, 7.88.

4.2.6. 2-(5-(2-Fluorophenyl)-1,3,4-oxadiazol-2-ylthio)-1-phenylethanone (6f)

White powder, yield 83%, mp: 152 °C, ^{1}H NMR(300 MHz, $CDCl_3$-d_6) δ: 4.98 (s, 2H); 7.22–7.30 (m, 3H); 7.52 (t, *J* = 4.58 Hz, 3H); 7.63 (d, *J* = 4.5 Hz,1H); 8.05 (d, *J* = 4.5 Hz, 2H); MS (ESI):315.33 ($C_{16}H_{12}FN_2O_2S$, $[M+H]^+$). Anal. Calcd for $C_{16}H_{11}FN_2O_2S$: C, 61.14; H, 3.53; N, 8.91.Found: C, 61.09; H, 3.52; N, 8.95.

4.2.7. 2-(5-(Naphthalen-1-yl)-1,3,4-oxadiazol-2-ylthio)-1-phenylethanone (6g)

White powder, yield 83%, mp: 134 °C, ^{1}H NMR(300 MHz, $CDCl_3$-d_6) δ: 5.03 (s, 2H); 7.52–7.70 (m, 6H); 7.93 (d, *J* = 3.93 Hz, 1H); 8.02–8.15 (m, 4H); 9.18 (d, *J* = 4.2 Hz, 1H). MS (ESI):347.40 ($C_{20}H_{15}N_2O_2S$, $[M+H]^+$). Anal. Calcd for $C_{20}H_{14}N_2O_2S$: C, 69.35; H, 4.07; N, 8.09. Found: C, 69.31; H, 4.08; N, 8.11.

4.2.8. 2-(5-(2-Hydroxy-4-methoxyphenyl)-1,3,4-oxadiazol-2-ylthio)-1-phenylethanone (6h)

white powder, yield 83%, mp: 195 °C, ^{1}H NMR(300 MHz, CDCl3-d_6) δ: 3.89 (d, *J* = 13.5 Hz; 3H); 4.96 (s, 2H); 5.35 (s, 1H); 6.57 (d, *J* = 2.61 Hz, 1H); 6.61 (s, 1H),7.54 (t, *J* = 4.62 Hz,2H); 7.59 (d, *J* = 2.61 Hz; 1H); 7.66 (t, *J* = 4.44 Hz; 1H); 8.05 (d, *J* = 2.39 Hz; 2H). MS (ESI): 343.37 (C17H15N2O4S, [M+H]+). Anal. Calcd for C17H14N2O4S: C, 59.74; H, 4.22; N, 8.18. Found: C, 59.66; H, 4.23; N, 8.21.

4.2.9. 2-(5-(2-Aminopyridin-4-yl)-1,3,4-oxadiazol-2-ylthio)-1-phenylethanone (6i)

Light yellow powder, yield 83%, mp: 208 °C, ^{1}H NMR(300 MHz, $CDCl_3$-d_6) δ: 5.01 (s, 2H); 7.12 (s, 1H); 7.23 (d, *J* = 5.76 Hz, 1H); 7.54 (t, *J* = 4.62 Hz, 3H); 7.66 (s, 2H); 8.05 (d, *J* = 2.11 Hz; 3H). MS (ESI):313.25 ($C_{15}H_{13}N_4O_2S$, $[M+H]^+$). Anal. Calcd for $C_{15}H_{12}N_4O_2S$: C, 57.68; H, 3.87; N, 17.94. Found: C, 57.59; H, 3.88; N, 17.98.

4.2.10. 2-(5-(2-Ethoxyphenyl)-1,3,4-oxadiazol-2-ylthio)-1-phenylethanone (6j)

White powder, yield 87%, mp: 102 °C, ^{1}H NMR(300 MHz, $CDCl_3$-d_6) δ: 1.56 (s, 3H); 4.13–4.20 (m, 2H); 5.01 (s, 2H); 7.00–7.06 (m, 2H); 7.44–7.55 (m, 3H); 7.65 (t, *J* = 7.41 Hz, 1H); 7.87–7.90 (m, 1H); 8.06 (d, *J* = 7.32 Hz; 2H). MS (ESI): 341.40 ($C_{18}H_{17}N_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{16}N_2O_3S$: C, 63.51; H, 4.74; N, 8.23. Found: C, 63.54; H, 4.75; N, 8.21.

4.2.11. 2-(5-(2-Chloropyridin-4-yl)-1,3,4-oxadiazol-2-ylthio)-1-phenylethanone (6k)

White powder, yield 81%, mp: 165 °C, ^{1}H NMR(300 MHz, $CDCl_3$-d_6) δ:5.01 (s, 2H); 7.54 (t, *J* = 4.62 Hz, 2H); 7.67 (t, *J* = 4.44 Hz, 1H); 7.81 (s, 1H); 7.90 (s, 1H); 8.06 (d, *J* = 4.50 Hz; 2H); 8.57 (s, 1H). MS (ESI): 332.78 ($C_{15}H_{11}ClN_3O_2S$, $[M+H]^+$). Anal. Calcd for $C_{15}H_{10}ClN_3O_2S$: C, 54.40; H, 3.04; N, 12.67; Found: C, 54.36; H, 3.05; N, 12.69.

4.2.12. 2-(5-(2-Methoxyphenyl)-1,3,4-oxadiazol-2-ylthio)-1-phenylethanone (6l)

White powder, yield 89%, mp: 153 °C, ^{1}H NMR(300 MHz, $CDCl_3$-d_6) δ: 3.94 (s, 3H); 4.98 (s, 2H); 7.05 (t, *J* = 8.60 Hz, 2H); 7.47–7.55 (m, 3H); 7.64 (t, *J* = 9.60 Hz, 1H); 7.87 (d, *J* = 7.68 Hz, 1H); 8.06 (d, *J* = 7.5 Hz, 2H). MS (ESI): 327.37 ($C_{17}H_{15}N_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{14}N_2O_3S$: C, 62.56; H, 4.32; N, 8.58.Found: C, 62.52; H, 4.30; N, 8.61.

4.2.13. 1-(4-Bromophenyl)-2-(5-(2-hydroxy-4-methylphenyl)-1,3,4-oxadiazol-2-ylthio) ethanone (6m)

White powder, yield 84%, mp: 237–238 °C, ^{1}H NMR(300 MHz, $CDCl_3$-d_6) δ: 2.38 (s, 3H); 4.91 (s, 2H); 6.82 (d, *J* = 8.22 Hz, 1H); 6.92 (s, 1H); 7.58 (d, *J* = 8.04 Hz, 1H); 7.60 (d, *J* = 8.40 Hz, 2H); 7.92 (d, *J* = 8.40 Hz, 2H); 9.70 (s, 1H); MS (ESI): 406.27 ($C_{17}H_{14}BrN_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{13}BrN_2O_3S$: C, 50.38; H, 3.23; N, 6.91; Found: C, 50.43; H, 3.22; N, 6.89.

4.2.14. 1-(4-Bromophenyl)-2-(5-(4-*tert*-butylphenyl)-1,3,4-oxadiazol-2-ylthio) ethanone (6n)

White powder, yield 82%, mp: 145 °C, ^{1}H NMR (300 MHz, $CDCl_3$-d_6) δ: 1.35 (s, 9H); 4.97 (s, 2H); 6.61 (d, *J* = 8.97 Hz, 1H); 7.66 (t, *J* = 11.9 Hz, 3H); 7.93 (d, *J* = 8.43 Hz, 2H); 8.04 (d, *J* = 6.75 Hz, 1H); 8.67 (s, 1H). MS (ESI): 432.35 ($C_{20}H_{20}BrN_2O_2S$, $[M+H]^+$). Anal. Calcd for $C_{20}H_{19}BrN_2O_2S$: C, 51.68; H, 3.86; N, 10.05. Found: C, 51.59; H, 3.85; N, 10.09.

4.2.15. 1-(4-Bromophenyl)-2-(5-(4-(dimethylamino)phenyl)-1,3,4-oxadiazol-2-ylthio) ethanone (6o)

White powder, yield 85%, mp: 197–198 °C, ^{1}H NMR (300 MHz, $CDCl_3$-d_6) δ: 3.55 (s, 6H); 4.97 (s, 2H); 7.51 (d, *J* = 8.22 Hz, 2H); 7.67 (d, *J* = 8.40 Hz, 2H); 7.92 (d, *J* = 8.43 Hz, 4H). MS (ESI): 419.31 ($C_{18}H_{17}BrN_3O_2S$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{16}BrN_3O_2S$: C, 55.69; H, 4.44; N, 6.49. Found: C, 55.65; H, 4.45; N, 6.45.

4.2.16. 1-(4-Bromophenyl)-2-(5-(pyridin-4-yl)-1,3,4-oxadiazol-2-ylthio)ethanone (6p)

White powder, yield 88%, mp: 162–163 °C, ^{1}H NMR (300 MHz, $CDCl_3$-d_6) δ: 4.96 (s, 2H); 7.68 (d, *J* = 8.40 Hz, 2H); 7.90–7.98 (m, 4H); 8.82 (d, 2H). MS (ESI): 377.23 ($C_{15}H_{11}BrN_3O_2S$, $[M+H]^+$). Anal. Calcd for $C_{15}H_{10}BrN_3O_2S$: C, 47.89; H, 2.68; N, 11.17. Found: C, 47.83; H, 2.67; N, 11.19.

4.2.17. 1-(4-Bromophenyl)-2-(5-(3,5-dimethoxyphenyl)-1,3,4-oxadiazol-2-ylthio) ethanone (6q)

White powder, yield 88%, mp: 169 °C, ^{1}H NMR (300 MHz, $CDCl_3$-d_6) δ: 3.88 (d, *J* = 9.70 Hz, 6H); 4.92 (s, 2H); 6.61 (s, 1H); 7.13 (s, 2H); 7.67 (d, *J* = 8.43 Hz, 2H); 7.92 (d, *J* = 8.43 Hz, 2H). MS (ESI): 436.29 ($C_{18}H_{16}BrN_2O_4S$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{15}BrN_2O_4S$: C, 49.67; H, 3.47; N, 6.44. Found: C, 49.63; H, 3.49; N, 6.40.

4.2.18. 1-(4-Bromophenyl)-2-(5-(2-fluorophenyl)-1,3,4-oxadiazol-2-ylthio)ethanone (6r)

White powder, yield 88%, mp: 147 °C, ^{1}H NMR (300 MHz, $CDCl_3$-d_6) δ: 4.98 (s, 2H); 7.20–7.31 (m, 2H); 7.52 (t, *J* = 5.85 Hz,1H); 7.67 (d, *J* = 8.43 Hz, 2H); 7.92 (d, *J* = 8.43 Hz, 2H); 7.99 (t, *J* = 7.32 Hz, 1H). MS (ESI): 394.23 ($C_{16}H_{11}BrFN_2O_2S$, $[M+H]^+$). Anal. Calcd for $C_{16}H_{10}BrFN_2O_2S$: C, 48.87; H, 2.56; N, 7.12. Found: C, 48.80; H, 2.57; N, 7.17.

4.2.19. 1-(4-Bromophenyl)-2-(5-(naphthalen-1-yl)-1,3,4-oxadiazol-2-ylthio)ethanone (6s)

White powder, yield 85%, mp: 153–154 °C, ^{1}H NMR (300 MHz, $CDCl_3$-d_6) δ: 4.98 (s, 2H); 7.56–7.71 (m, 5H); 7.95 (t, *J* = 4.57 Hz, 3H); 8.05 (d, *J* = 8.25 Hz, 1H); 8.14 (d, *J* = 7.11 Hz, 1H); 9.18 (d, *J* = 8.61 Hz, 1H). MS (ESI): 426.30 ($C_{20}H_{14}BrN_2O_2S$, $[M+H]^+$). Anal. Calcd for $C_{20}H_{13}BrN_2O_2S$: C, 56.48; H, 3.08; N, 6.59. Found: C, 56.44; H, 3.07; N, 6.61.

4.2.20. 1-(4-Bromophenyl)-2-(5-(2-hydroxy-4-methoxyphenyl)-1,3,4-oxadiazol-2-ylthio)ethanone (6t)

White powder, yield 86%, mp: 229–230 °C, ^{1}H NMR (300 MHz, $CDCl_3$-d_6) δ: 3.89 (d, *J* = 7.64 Hz, 3H); 4.98 (s, 2H); 5.35 (s, 1H);

6.58 (t, J = 6.40 Hz, 2H); 7.59 (d, J = 8.61 Hz, 1H); 7.68 (d, J = 8.58 Hz, 2H); 7.92 (d, J = 8.61 Hz, 2H). MS (ESI): 422.27 ($C_{17}H_{14}BrN_2O_4S$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{13}BrN_2O_4S$: C, 48.47; H, 3.11; N, 6.65. Found: C, 48.42; H, 3.12; N, 6.61.

4.2.21. 2-(5-(2-Aminopyridin-4-yl)-1,3,4-oxadiazol-2-ylthio)-1-(4-bromophenyl) ethanone (6u)

Yellow powder, yield 87%, mp: 200 °C, ^{1}H NMR (300 MHz, $CDCl_3$-d_6) δ: 4.98 (s, 2H); 7.18 (d, J = 9.15 Hz, 1H); 7.35 (d, J = 8.80 Hz, 1H); 7.59 (d, J = 8.61 Hz, 2H); 7.68 (d, J = 8.43 Hz, 1H); 7.76 (d, J = 8.58 Hz, 1H); 7.92 (d, J = 8.61 Hz, 2H); 8.14 (d, J = 5.67 Hz, 1H). MS (ESI): 392.24 ($C_{15}H_{12}BrN_4O_2S$, $[M+H]^+$). Anal. Calcd for $C_{15}H_{11}BrN_4O_2S$: C, 46.05; H, 2.83; N, 14.32. Found: C, 46.01; H, 2.82; N, 14.37.

4.2.22. 1-(4-Bromophenyl)-2-(5-(2-ethoxyphenyl)-1,3,4-oxadiazol-2-ylthio)ethanone (6v)

White powder, yield 83%, mp: 110 °C, ^{1}H NMR (300 MHz, $CDCl_3$-d_6) δ: 1.25–1.55 (m, 3H); 4.13–4.20 (m, 2H); 4.91 (s, 2H); 7.03 (t, J = 8.87 Hz, 2H); 7.47 (t, J = 7.86 Hz, 1H); 7.67 (d, J = 8.22 Hz, 2H); 7.86–7.94 (m, 3H). MS (ESI): 429.29 ($C_{18}H_{16}BrN_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{15}BrN_2O_3S$: C, 51.56; H, 3.61; N, 6.68. Found: C, 51.58; H, 3.63; N, 6.65.

4.2.23. 1-(4-Bromophenyl)-2-(5-(2-chloropyridin-4-yl)-1,3,4-oxadiazol-2-ylthio) ethanone (6w)

White powder, yield 79%, mp: 177 °C, ^{1}H NMR (300 MHz, $CDCl_3$-d_6) δ: 5.01 (s, 2H); 7.54 (t, J = 4.62 Hz, 2H); 7.67 (t, J = 4.44 Hz, 2H); 7.90 (s, 1H); 8.06 (d, J = 4.5 Hz, 1H); 8.57 (s, 1H). MS (ESI): 411.67 ($C_{15}H_{10}BrClN_3O_2S$, $[M+H]^+$). Anal. Calcd for $C_{15}H_9BrClN_3O_2S$: C, 43.87; H, 2.21; N, 10.23. Found: C, 43.76; H, 2.22; N, 10.27.

4.2.24. 1-(4-Bromophenyl)-2-(5-(2-methoxyphenyl)-1,3,4-oxadiazol-2-ylthio)ethanone (6x)

White powder, yield 89%, mp: 163 °C, ^{1}H NMR (300 MHz, $CDCl_3$-d_6) δ: 3.94 (s, 3H); 4.98 (s, 2H); 7.04 (d, J = 8.22 Hz, 2H); 7.50 (t, J = 8.52 Hz, 1H); 7.66 (d, J = 7.86 Hz, 2H); 7.86 (d, J = 7.50 Hz, 1H); 7.92 (d, J = 8.04 Hz, 2H). MS (ESI): 406.27 ($C_{17}H_{14}BrN_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{13}BrN_2O_3S$: C, 50.38; H, 3.23; N, 6.91. Found: C, 50.41; H, 3.24; N, 6.87.

4.3. Antitumor assay

The antitumor activities of the title compounds **6a–6x** against the two cell lines MCF-7 and A431, were evaluated using a standard MTT-based colorimetric assay (Sigma). Briefly, cell lines were seeded at a density of 7×10^3 cells/well in 96-well microtiter plates (Costar). After 24 h, exponentially growing cells were exposed to the indicated compounds at final concentrations ranging from 0.1 to 100 μM, the indicated compounds was solved in PBS and DMSO (DMSO accounted for 0.4% in the solvent system), Negative control is also used the PBS and DMSO solvent system. After 24 h, cell survival was determined by the addition of an MTT solution (10 μL of 5 mg/mL MTT in PBS). After 4 h, 100 μL of 10% SDS in 0.01 N HCl was added, and the plates were incubated at 37 °C for a further 18 h; optical absorbance was measured at 570 nm on an LX300 Epson Diagnostic microplate reader. Survival ratios are expressed in percentages with respect to untreated cells. IC_{50} values were determined from replicates of six wells from at least three independent experiments.

4.4. FAK inhibitory assay

Bovine brain FAK was purified as described previously.[22] To evaluate the effect of the compounds on FAK assembly in vitro,[23] varying concentrations were preincubated with 10 μM FAK in glutamate buffer at 30 °C and then cooled to 0 °C. After addition of GTP, the mixtures were transferred to 0 °C cuvettes in a recording spectrophotometer and warmed up to 30 °C and the assembly of FAK was observed turbid metrically. The IC_{50} was defined as the compound concentration that inhibited the extent of assembly by 50% after 20 min incubation.

4.5. Docking simulations

Molecular docking of compounds **6i** into the FAK (PDB: 2ETM) was carried out using LigandFit Dock protocol of Discovery Studio 3.1.[24]

Acknowledgments

The work was financed by Natural Science Foundation of China (No. J1103512).

References and notes

1. Chang, N.-B.; Feng, R.; Gao, Z.; Gao, W. *Int. J. Hyg. Environ. Health* **2010**, *213*, 359.
2. Osterlind, A.; Hjalgrim, H.; Kulinsky, B.; Frentz, G. *Br. J. Dermatol.* **1991**, *125*, 580.
3. Rangwala, S.; Tsai, K. Y. *Br. J. Dermatol.* **2011**, *165*, 953.
4. van Nimwegen, M. J.; van de Water, B. *Biochem. Pharmacol.* **2007**, *73*, 597.
5. Hildebrand, J. D.; Schaller, M. D.; Parsons, J. T. *J. Cell Biol.* **1993**, *123*, 993.
6. Golubovskaya, V. M.; Cance, W. G. *Int. Rev. Cytol.* **2007**, *263*, 103.
7. Hiscox, S.; Barnfather, P.; Hayes, E.; Bramble, P.; Christensen, J.; Nicholson, R. I.; Barrett-Lee, P. *Breast Cancer Res. Treat.* **2011**, *125*, 659.
8. Semini, G.; Hildmann, A.; Reissig, H. U.; Reutter, W.; Danker, K. *Biochem. Pharmacol.* **2011**, *81*, 985.
9. Schaller, M. D. *Biochim. Biophys. Acta* **2001**, *1540*, 1.
10. Gabarra-Niecko, V.; Schaller, M. D.; Dunty, J. M. *Cancer Metastasis Rev.* **2003**, *22*, 359.
11. Bondock, S.; Adel, S.; Etman, H. A.; Badria, F. A. *Eur. J. Med. Chem.* **2012**, *48*, 192.
12. Jakubkiene, V.; Burbuliene, M. M.; Udrenaite, E.; Garaliene, V.; Vainilavicius, P. *Pharmazie* **2002**, *57*, 610.
13. Rosen, H.; Blumenthal, A.; Beckfield, W. J.; Agersborg, H. P. K., Jr. *Toxicol. Appl. Pharmacol.* **1966**, *8*, 13.
14. Liu, F.; Luo, X.-Q.; Song, B.-A.; Bhadury, P. S.; Yang, S.; Jin, L.-H.; Xue, W.; Hu, D.-Y. *Bioorg. Med. Chem.* **2008**, *16*, 3632.
15. Sahin, G.; Palaska, E.; Ekizoglu, M.; Ozalp, M. *Farmaco* **2002**, *57*, 539.
16. Khan, S. A.; Kumar, P.; Joshi, R.; Iqbal, P. F.; Saleem, K. *Eur. J. Med. Chem.* **2008**, *43*, 2029.
17. Ghani, U.; Ullah, N. *Bioorg. Med. Chem.* **2010**, *18*, 4042.
18. Fredslund, F.; Jenner, L.; Husted, L. B.; Nyborg, J.; Andersen, G. R.; Sottrup-Jensen, L. *J. Mol. Biol.* **2006**, *361*, 115.
19. Pangburn, M. K.; Muller-Eberhard, H. J. *J. Exp. Med.* **1980**, *152*, 1102.
20. Zhang, X.-M.; Qiu, M.; Sun, J.; Zhang, Y.-B.; Yang, Y.-S.; Wang, X.-L.; Tang, J.-F.; Zhu, H.-L. *Bioorg. Med. Chem.* **2011**, *19*, 6518.
21. Mao, W.-J.; Lv, P.-C.; Shi, L.; Li, H.-Q.; Zhu, H.-L. *Bioorg. Med. Chem.* **2009**, *17*, 7531.
22. Hamel, E.; Lin, C. M. *Biochem. J.* **1984**, *23*, 4173.
23. Hamel, E. *Cell Biochem. Biophys.* **2003**, *38*, 1.
24. Abagyan, R.; Totrov, M. *J. Mol. Biol.* **1994**, *235*, 983.