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Synthesis, Molecular Modeling and Biological Evaluation of 7-Sulfanylflavone as Anticancer Agents

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Abstract

A series of novel flavonoids derivatives containing sulfhydryl groups have been designed, designing for potential FAK inhibitors. Docking simulation was performed to position these compounds into the FAK active site to determine the probable binding model. Simulation results showed that 5-hydroxy-3-(4-hydroxyphenyl)-7-mercapto-4H-chromen-4-one(**4a**),7-mercapto-3-(4-methoxyphenyl)-4H-chromen-4-one(**4b**) and 5-hydroxy-7-mercapto- 2-phenyl- 4H- chromen-4-one(**4c**) displayed the most potent biological activity. So the three compounds have been synthesized. Antiproliferative assay results demonstrated that the three compounds own fairly good antiproliferative activity .Therefore compounds **4a**, **4b** and **4c** would be potential anticancer agents.

Keywords: Flavonoids; Sulfydryl; FAK; Molecular docking; Anticancer

Introduction

Flavonoids are an extensive group of polyphenolic compounds. They are rich in seeds, citrus fruits, olive oil, tea, and red wine [1,2], which are low- molecular weight. These different flavonoids have been reported to possess a wide range of biological activities, such as anxiolytic [3,4], anti-inflammatory [2], phytoalexins [2,5], antiviral [6], antioxidant [2], inhibition of monoamine oxidase (MAO) [7-9]. The literatures have confirmed that 7-hydroxyl of flavonoids, widespread in the flavonoids [5], played a crucial role in its many biological activities [10] such as antidepressant [11]. 7-Hydroxyl could improves the water-solubility and lipid solubility [12]. Activity of flavonoids without 7-hydroxyl was significantly decreased [13]. Sulfhydryl groups , a known free radical scavenger, has the similar structure and nature of the hydroxyl [14], so we transformed 7-hydroxyl of flavonoids into 7-sulfydrylflavones , expecting to get new and better anticancer drugs.

Focal adhesion kinase (FAK) is a protein tyrosine kinase that is localized at contact points between cells and extracellular matrix (ECM) and is a point of convergence of a number of signaling pathways associated with cell adhesion, invasion, motility, and angiogenesis [15-18], FAK is found overexpressed in numerous cancers and constitutes an important target for the design of antitumor inhibitors [19]. Indeed, control of FAK signaling has been suggested as a potential anticancer therapy [20]. Compounds that inhibit the kinase activity of FAK are of potential interest as new therapeutic antitumor agents [21-24]. Flavonoids have many biological activities, however, to our knowledge, literatures about the synthesis and FAK activity of 7-sulfydrylflavone derivatives have been few reported. So we report the synthesis and structure–activity relationships of these 7-sulfydrylflavone derivatives, which could be anticancer agents. Biological evaluation indicated that some of the synthesized compounds are potent inhibitors of FAK. Docking simulations were performed using the X-ray crystallographic structure of the FAK in a complex with an inhibitor to explore the binding modes of these compounds at the active site.

Materials and Methods

General

All chemicals and reagents used in current study were analytical grade. All the 1H NMR spectra were recorded on a Bruker DPX 300 or DRX 500 model Spectrometer in DMSO–d6 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 glass-backed silica gel sheets (silica gel 60Å 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.

Synthesis

A mixture of flavonoids, dimethylthiocarbamoyl chloride, 1,4-diazabicyclo[2,2,2]octane, and anhydrous N,N-dimethylformamide (30ml) was stirred at room temperature for 2h, then poured into 5% hydrochloric acid(300ml). The precipitate was crystallized from MeOH to afford compound **2**. Compound **2** was dissolved in N,N-dimethylaniline (30ml) and refluxed for 1h, then poured into 10% hydrochloric acid(100ml), the precipitate was washed free of acid and crystallized from MeOH to obtain substance. Compound **3** was refluxed in 10% KOH, then the residue after the solvent evaporated was triturated with water and extracted with EtOAc, purified by column chromatography, EtOAc/petroleum ether 4:1 (Scheme 1).

5-hydroxy-3-(4-hydroxyphenyl)-7-mercapto-4H-chromen-4-one (4a): delta$_H$(300 MHz, DMSO): 6.81-6.84 (m, 2H), 6.99 (s, 1H), 7.28 (s, 1H), 7.37-7.40 (m, 2H), 8.46 (s, 1H), 9.60 (s, 1H). ESI-MS: 287.1($C_{15}H_{11}O_4S$, $[M+H]^+$). Anal. Calcd for $C_{15}H_{10}O_4S$: C 62.93, H 3.52. Found: C 62.71, H 3.49%.

7-mercapto-3-(4-methoxyphenyl)-4H-chromen-4-one (4b): delta$_H$(300 MHz, DMSO): 3.79 (s, 3H), 4.18 (s, 1H), 6.86-6.89 (d, *J* 9.0, 2H), 6.93-6.96 (m, 1H), 7.06-7.07 (m, 1H), 7.15-7.17(d, *J* 6.0, 2H), 7.75-7.78(d, *J* 6.0, 1H),12.41 (s, 1H). ESI-MS: 285.3(C16H13O3S, [M+H]+). Anal. Calcd for $C_{16}H_{12}O_3S$: C 67.59, H 4.25 . Found: C 67.38, H 4.23%.

5-hydroxy-7-mercapto-2-phenyl-4H-chromen-4-one (4c): del-

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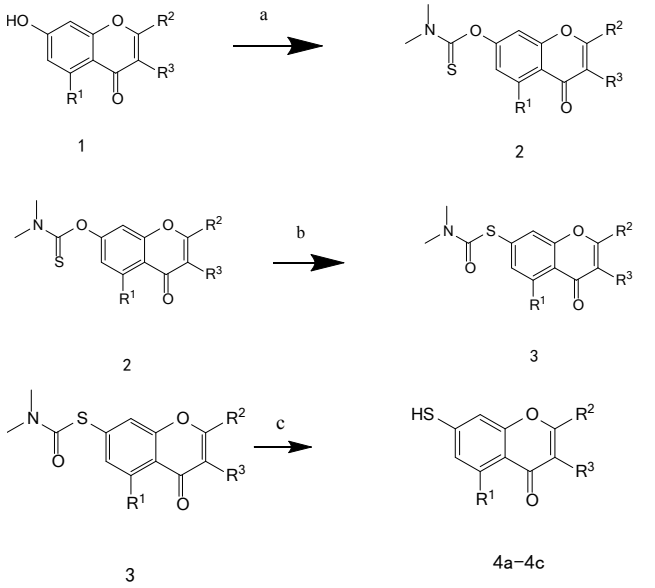
ta_H(300 MHz, DMSO): 6.27-6.30 (m, 1H), 6.81 (s, 1H), 7.08 (s, 1H), 7.18 (s, 1H), 7.59-7.66 (m, 3H), 8.09-8.10 (d, *J* 3.0, 2H), 12.73 (s, 1H). ESI-MS: 271.1($C_{15}H_{11}O_3S$, [M+H]+). Anal. Calcd for $C_{15}H_{10}O_3S$: C 66.65, H 3.73. Found: C 66.43, H 3.71%.

Results and Discussion

Biological activity

All the synthesized compounds **4a-4c** were evaluated for their ability to antiproliferative activities against B16-F10, U251 and HepG2 cell. The results are summarized in (Table 1). As shown in (Table 2), compounds **4a-4c** have shown fairly good inhibitory activity for three cancer cell lines and FAK, compound **4a** displayed good inhibitory activity with IC_{50} of 8.25±0.58μM for B16-F10, 12.36±0.68μM forU251, and 19.57±1.02μM for HepG2; Compound **4b**, with IC_{50} of 12.01±0.59μM for B16-F10, 18.32±0.85μM for U251, and 22.15±0.98μM for HepG2;



Reagents and conditions: (a) Dimethylthiocarbamoyl chloride, DABCO, DMF, rt; (b) N,N-Dimethylaniline, reflux; (c) KOH, MeOH, reflux.

Scheme 1: General synthesis of 7-sulfydrylflavones (4a-4c).

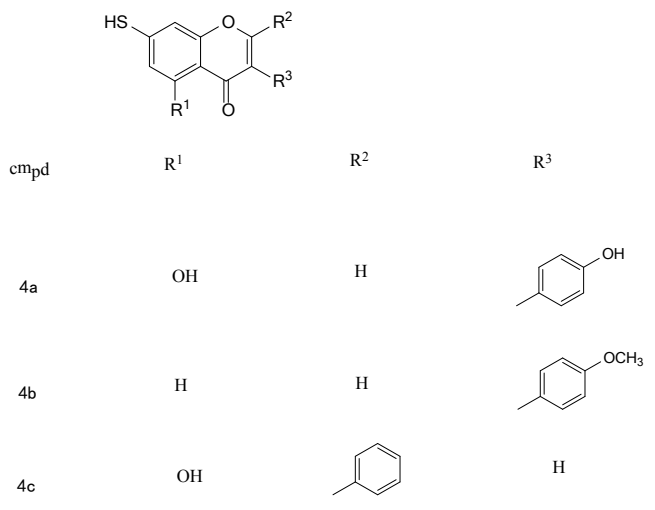


Table 1: Structures of 7-sulfydrylflavones (4a-4c).

Compound	B16-F10 IC_{50} ± SD(μM)	U251 IC_{50} ± SD(μM)	HepG2 IC_{50} ± SD(μM)	FAK inhibition IC_{50}(μM)
4a	8.25±0.58	12.36±0.68	19.57±1.02	19.12
4b	12.01±0.59	18.32±0.85	22.15±0.98	20.48
4c	13.56±0.89	21±0.92	25.65±1.05	23.27
staurosporine	-	-	-	11.32

Table 2: The antiproliferative effects of the compounds of 4a-4c.

Compound **4c**, with IC_{50} of 13.56±0.89μM for B16-F10, 21±0.92μM for U251, and 25.65±1.05μM for HepG2. Moreover, compounds **4a-4c** also have good inhibitory activity with IC_{50} of 19.12μM, 20.48μM, and 23.27μM for FAK, which was comparable to the positive control staurosporine.

The molecular docking study

Molecular docking of the most potent inhibitors **4a**, **4b,**and **4c** into ATP binding site of FAK are depicted in Figure 1~3). The binding affinity was evaluated by the hydrogen bonding and binding free energies. It can be concluded that H-bond plays an important effect in the FAK inhibitory. In the binding model of the interaction between compound **4a** and FAK (Figure 1), with binding free energy (△Gb, kcal/mol) of -6.11kcal/mol and inhibition constant(IC) unit= nM, three hydrogen bonds, the hydrogen atom of sulfydryl forms hydrogen bond with oxygen atom of LEU504(distance: 2.087Å, angle: 138.363°); the hydrogen atom of hydroxyl on A-ring forms hydrogen bond with oxygen atom of THR503 (distance: 1.839Å, angle:159.866°); the hydrogen atom of hydroxyl on B-ring forms hydrogen bond with oxygen atom of CYS427 (distance:2.034Å, angle:157.235°). In the binding model of the interaction between compound **4b** and FAK (Figure 2), △Gb=-4.33kcal/mol, IC unit= nM, two hydrogen bonds, the hydrogen atom of sulfydryl forms hydrogen bond with oxygen atom of GLU506 (distance: 2.006Å, angle: 147.832°); Oxygen atom of carbonyl forms hydrogen bond with the hydrogen atom of SER509 (distance: 2.164Å, angle: 146.338°). In the binding model of the interaction between compound **4c** and FAK (Figure 3), △Gb=-8.59kcal/mol, IC unit = nM, two hydrogen bonds, the hydrogen atom of sulfydryl forms hydrogen bond with oxygen atom of THR503(distance: 2.016Å, angle:123.338°); Oxygen atom of carbonyl forms hydrogen bond with amino hydrogen of ARG514 (distance:1.819Å, angle:145.442°).

In order to show contrast effects, we simulated forty compounds of similar structures by Auto-Dock 4.0[26], docking results of twenty compounds among them are enumerated. 7-sulfydrylapigenin, △Gb=-3.76kcal/mol, IC unit=μM, ARG514 N-H...S(distance:1.705Å, angle:148.855°), ARG426 N-H...O(distance:1.615Å, angle:140.207°); 7-sulfydrylluteolin, △Gb=-3.45kcal/mol, IC unit=μM, GLN438 N-H...S(distance:2.093Å,angle:139.34°), ARG514 N-H...O(distance:1.839Å,angle:147.23°); 7-sulfydrylkaempferide, △Gb=-3.43kcal/mol, IC unit=mM, ARG514 N-H...S(distance:1.884Å, angle:165.689°); 7-sulfydrylquercetin, △Gb=-3.83kcal/mol, IC unit=mM, GLY505 N-H...S(distance:2.141Å, angle:143.775°), ARG426 N-H...O(distance:1.642Å,angle:160.878°); 7-sulfydrylhesperetin, △Gb=-2.52kcal/mol, IC unit=mM, ARG514 N-H...S(distance:1.774Å, angle:135.691°), ARG514 N-H...S(distance:2.019Å, angle:143.034°); 7-sulfydrylcatechin, △Gb=-3.74kcal/mol, IC unit=mM, ARG514 N-H...S(distance :1.940Å,angle:148.702°), GLN438 N-H...O(distance:1.956Å,angle:144.438°); 7-sulfydrylliquirtigeninl, △Gb=-4.28kcal/mol, IC unit=mM, GLY505 N-H...S(distance:1.969Å, angle:144.883°); 7-sulfydryldaidzein, △Gb=-3.09kcal/mol, IC unit=mM, ARG514 N-H...S(distance:2.21Å, angle:161.302°); 7-sulfydrylleucocyanidin, △Gb=-3.68kcal/mol, *IC* unit=mM, GLY505 N-H...S(distance: 2.109Å, angle:162.375°), ARG426

N-H...O(distance:2.117Å, angle:142.262°); 7-sulfydrylaureusidin, ▵Gb=-3.39kcal/mol, IC unit=mM, ARG514 N-H...O(distance:1.268Å, angle:146.19°); 7-sulfydrylmorin,▵Gb=-2.99kcal/mol, IC unit=mM, GLY505 N-H...S(distance:2.013Å, angle:148.201°), ARG426 N-H...O(distance:1.309Å, angle:139.44°); 7-sulfydrylgalangin, ▵Gb=-3.69kcal/mol, IC unit=mM, GLY505 N-H...O(distance:2.221Å, angle:123.543°); 7-sulfydrylnaringenin, ▵Gb=-3.93kcal/mol, IC unit=mM, CYS502 N-H...O(distance:1.906Å, angle:152.983°); 7-sulfydrylisorhamnetin, ▵Gb=-2.84kcal/mol, IC unit=mM, GLY429 N-H...S(distance:2.214Å, angle:153.781°), ARG508 N-H...O(distance: 2.006Å, angle: 126.227°); 7-sulfydrylacacetin, ▵Gb=-3.58kcal/mol, *IC* unit=mM, GLY429 N-H...S(distance: 1.888Å, angle:144.556°), ARG550 N-H...O(distance:1.162Å,angle:123.321°), ARG550 N-H...O(distance: 2.119Å, angle:130.431°); 7-sulfydrylalpinetinl, ▵Gb=-2.61kcal/mol, IC unit=mM, GLN438 N-H...S(distance:2.19Å, angle:139.468°), ARG426 N-H...O(distance:1.668Å; angle:124.665°); 7-sulfydryltaxifolin, ▵Gb=-1.87kcal/mol, IC unit=mM, GLY429 N-H...S(distance:2.195Å, angle:136.735°); 7-sulfydrylermanine, ▵Gb=-3.25kcal/mol, IC unit=mM, GLY505 N-H...S (distance:2.167Å, angle:174.156°), GLU506 O...H (distance:1.974Å, angle:140.118°); 7-sulfydrylhyperin, 7-sulfydrylisoquercitrin have no effect on FAK.

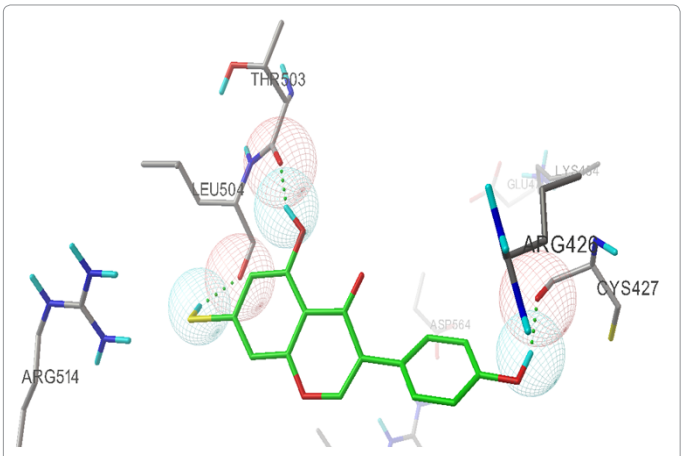


Figure 1: The binding model of compound **4a** and FAK (Carbon atoms are green, nitrogen atoms are dark blue, hydrogen atoms are light blue and sulfur atoms are yellow. The H-bond is displayed as green dotted line).

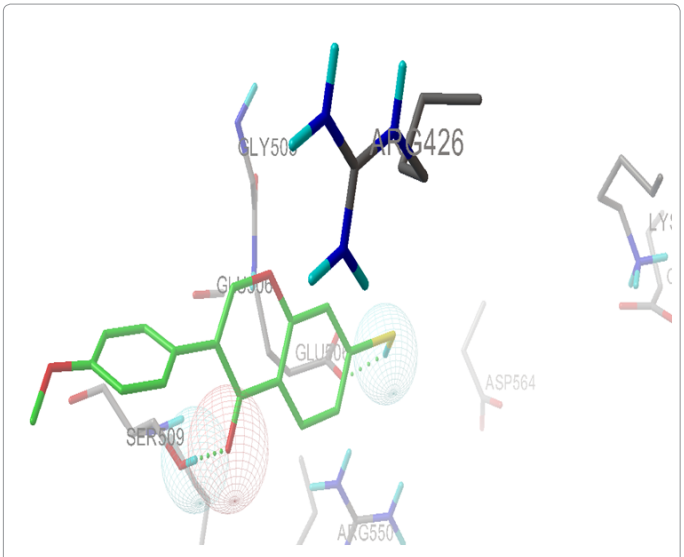


Figure 2: The binding model of compound **4b** and FAK (Carbon atoms are green, nitrogen atoms are dark blue, hydrogen atoms are light blue and sulfur atoms are yellow. The H-bond is displayed as green dotted line).

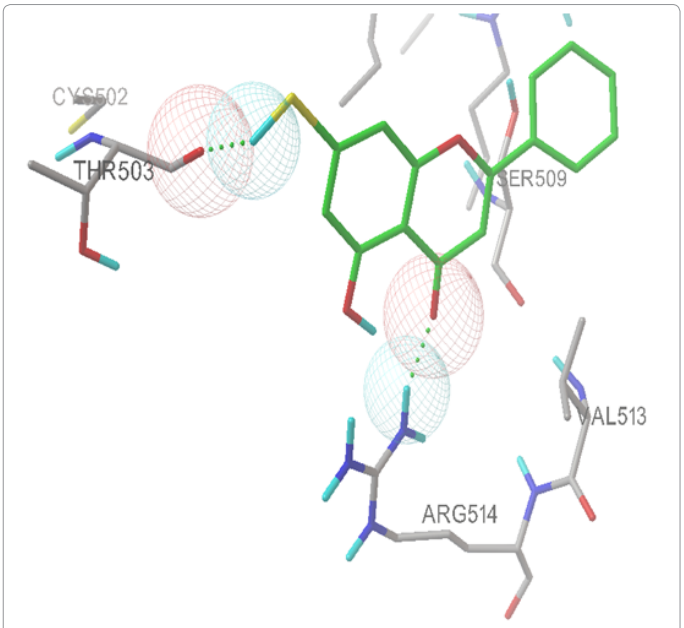


Figure 3: The binding model of compound **4c** and FAK (Carbon atoms are green, nitrogen atoms are dark blue, hydrogen atoms are light blue and sulfur atoms are yellow. The H-bond is displayed as green dotted line).

FAK inhibitory assay

Nighteen 1,3,4-thiadiazole derivatives containing 1,4-benzodioxan were tested in a search for small molecule inhibitors of FAK. In a typical study, human recombinant full-length FAK was incubated in kinase buffer containing ATP and the substrate for 4 h at room temperature with or without the presence of the thiadiazole derivatives, the final concentration of drug as 60, 20, 6.67, 2.22, 0.74, 0.25 and 0.082 μg/mL. The remaining ATP in solution was then quantified utilizing the Kinase-Glo-luminescence kit (Promega).

Antiproliferative activities assay

The antiproliferative activities of compounds **4a**-**4c** were determined using a standard (MTT)-based colorimetric assay (Sigma). Briefly, cell lines were seeded at a density of 7×10 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 40 mg/mL. After 48 h, cell survival was determined by the addition of an MTT solution (20 uL of 5 mg/mL MTT in PBS). After 6 h, 100 mL of 10% SDS in 0.01 N HCl was added, and the plates were incubated at 37℃ for a further 4 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. IC50 values were determined from replicates of 6 wells from at least two independent experiments.

Molecular docking modeling

The automated docking studies were carried out using AutoDock 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. The cubic grid box of 60 Å size (x, y, z) with a spacing of 0.375Å and grid maps were

created representing 17 the catalytic active target site region where the native ligand was embedded. 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 energeticallyminimized by using MOPAC with 100 iterations and minimum RMS gradient of 0.10. The crystal structures of FAK 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.

Conclusions

7-Sulfydrylflavone derivatives may function as inhibitors of FAK. The three synthesized compounds also displayed good FAK inhibitory and their biological activities were also evaluated as potent anticancer inhibitors. Compounds **4a**-**4c** demonstrated the most potent inhibitory activity that inhibited the growth of B16-F10, U251,and Hep G2 cells.

Furthermore, the intermolecular hydrogen bonds of compounds **4a**-**4c** were also investigated in order to find useful information for drug design. This molecular docking result, along with the biological assay data, suggesting that compounds **4a**-**4c** are potential inhibitors of FAK. Sulfydrylflavones, or the series of compounds with similar structures, have pretty good inhibitory activity of FAK.

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