Synthesis and Structure of the β-Carboline Derivatives and Their Binding Intensity with Cyclin-Dependent Kinase 2

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Series of 3-substituted of 6-aminosulfonyl-β-carbolines were designed and synthesized. In addition, the binding mode of these β-carboline derivatives with cyclin-dependent kinase 2 (CDK2) was studied by means of fluorescence measurements and molecular docking calculation. The results showed that replacement of 3-cyclohexylmethoxy group will increase the hydrophobic binding interaction with the deep hydrophobic pocket of CDK2 correlate to the higher binding intensity.

Key words cyclin-dependent kinase 2; β-carboline; synthesis; molecular docking; fluorescence

It is well known that the phases of the cell cycle are driven by cyclin-dependent kinases (CDKs). Upon complexation with its activating proteins, cyclin E or cyclin A, cyclin-dependent kinase 2 (CDK2) modulates the activity of many cellular substrates *via* phosphorylation on serine (Ser) and/or threonine (Thr) residues.[1–4] Abnormal CDK control of the cell cycle has been strongly linked to the molecular pathology of cancer.[5] The importance of CDK2 for cell cycle progression has led to an active pursuit of small molecule inhibitors of this enzyme as a possible treatment against cancer and other hyper-proliferative disorders.[6–9] All CDK inhibitors, identified so far, function by competing with ATP for binding to the catalytic site. Actually several CDK inhibitors have entered clinical evaluation for the treatment of cancer, including flavopiridol, amino-thiazole compound.[10] In our program to develop CDK2 inhibitors, we recently investigate the β-carboline alkaloids containing a planar tricyclic system as a large group of naturally-occurring and synthetic alkaloids,[11–14] which has a broad spectrum of biochemical effects and pharmaceutical properties. These compounds have been shown to intercalate into DNA, to inhibit CDK, topisomerase and monoamine oxidase, and to interact with benzodiazepine receptors (BZ), 5-hydroxy serotonin receptors (5-HT), dopamine (DA) and imidazoline receptors.[15–23]

Inspired by these results, our research group recently focused on first of all, using a structure-guided strategy based on CDK2 as appropriate means to generate potent CDK2 inhibitors; then to synthesize disubstituted β-carbolines and to complete their biological evaluation study. As a part of our systematic work, in this paper, structure modification of β-carboline based on increasing its hydrophobic nature has been adopted. Herein we reported the design and synthesis of series of 3-substituted of 6-aminosulfonyl-β-carbolines. Series of 3-substituted compounds with different hydrophobic groups (Chart 1) were synthesized and one of their structures was elucidated with X-ray crystal analysis. The new derivatives were prepared following the reaction sequences depicted in Charts 2–4. Their binding intensity with CDK2 was measured by fluorescence spectroscopy.

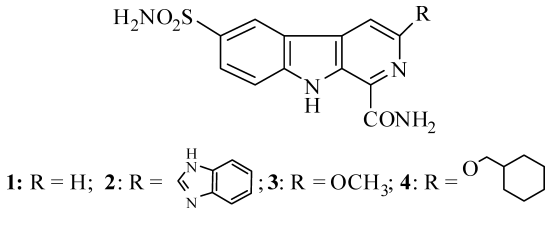
Experimental

Synthesis The target compounds were prepared using the reaction sequence. All the chemical structures of the synthesized compounds were confirmed by spectroscopic methods, and exact stereostructure of compound **5** has been determined by X-ray crystal structure analysis.

Materials Unless otherwise specified, reagents were purchased from commercial suppliers and used without further purification. CDK2/cyclin A was purchased from Carna Biosciencens Inc. and used without purification. All other chemicals were of analytical grade. Doubly distilled water was used throughout.

Apparatus Reaction progress was monitored using analytical thin layer chromatography (TLC) on percolated Merck silica gel Kiesegel 60 F254 plates, and the spots were detected under UV light (254 nm). Melting points were determined with a digital melting point apparatus and are reported uncorrected. ^{1}H-NMR spectra was recorded at 300 MHz on a Bruker ARX 300 spectrometers. IR spectra were measured on a Jasco FT/IR-430 spectrophotometer. Mass spectra were recorded on an a Quattro microMS Micromass UK mass spectrometer, and were recorded on an electrospray ionization mass spectrometer as the value *m*/*z*. The X-ray measurements were made on a Nonius Cad4 diffractometer with a graphite monochromatised Mo*Kα* radiation (λ=0.71069 Å) using ω scan mode. And all fluorescence spectra were recorded on RF-5301PC Spectrofluorimeter (Shimadzu, Japan) equipped with 0.2 cm quartz cells, the widths of both the excitation slit and the emission slit were set to 5.0 nm.

1-Carbamoyl-6-aminosulfonyl-β-carboline (1) For **1**,



1: R = H; **2:** R = ; **3:** R = OCH_3; **4:** R =

Chart 1. Chemical Structures of the Title Compounds

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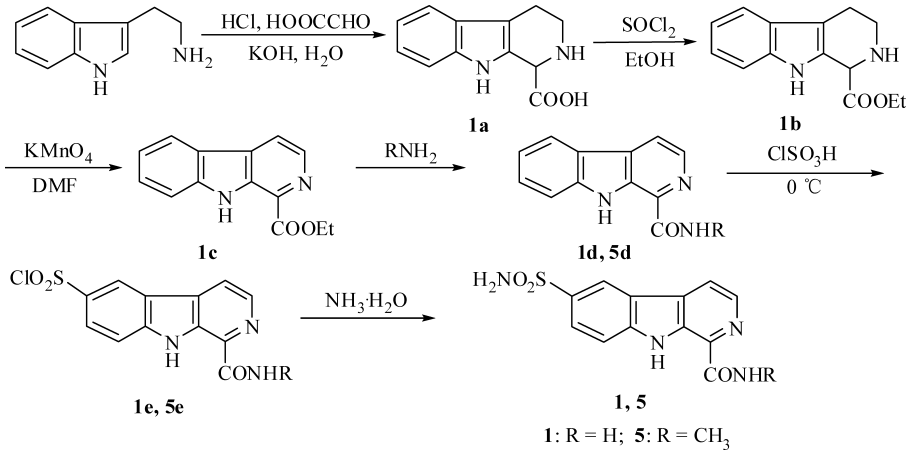

Chart 2. Synthesis of 6-Substituted *β*-Carboline Compounds **1** and **5**

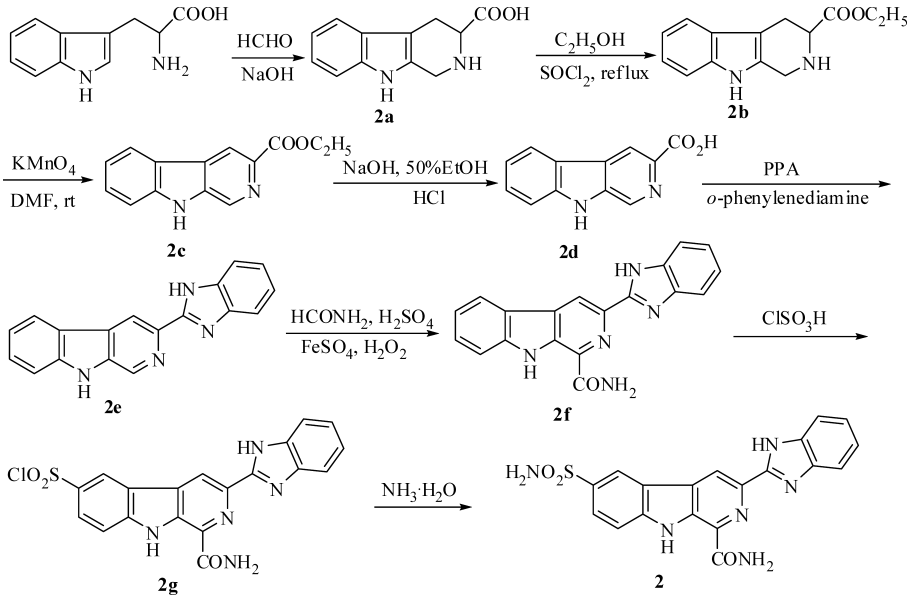

Chart 3. Synthesis of 3,6-Substituted *β*-Carboline Compound **2**

tryptamine was chosen as the starting material for the study, which was converted into 1,2,3,4-tetrahydro-*β*-carboline-1-carboxylic acid (**1a**) according to the Pictet–Spengler reaction in good yield.[24,25] And the treatment of **1a** with excess thionyl chloride in anhydrous EtOH at reflux for 6 h gave ethyl 1,2,3,4-tetrahydro-*β*-carboline-1-carboxylate (**1b**) as a white solid.[26] Then, ethyl *β*-carboline-1-carboxylate (**1c**) was carried out by dehydrogenation with $KMnO_4$ in *N*,*N*-dimethylformamide (DMF) at room temperature for 24 h.[27] 1-Amino-*β*-carboline (**1d**) was prepared by the aminolysis of (**1c**).[28] The key intermedium (**1d**) was converted into the target molecule (**1**) in 45–80% yield through chlorosulfonation in dry chlorosulfonic acid and subsequent aminolysis with amine solution[28] (Chart 2).

Yield 48%. mp 217–219°C (MeOH). ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 12.03 (1H, s, indole), 8.78 (1H, s, ArH), 8.50 (1H, m, $-CONH_2$), 8.30 (1H, s, ArH), 8.02 (1H, d, J=7.7 Hz, ArH), 7.91 (1H, d, J=7.7 Hz, ArH), 7.80 (1H, s, ArH), 7.27 (2H, s, $-SO_2NH_2$). ^{13}C-NMR (300 MHz, DMSO-d_6) δ: 167.6 (s), 142.8 (s), 137.5 (s), 135.7 (d), 135.2 (s), 133.4 (s), 130.4 (s), 126.1 (d), 120.2 (d), 119.2 (s), 118.3 (d), 113.2 (d). IR (KBr) cm^{-1}: 3495, 3458, 3381, 3352, 3259, 1674. MS *m*/*z* 291 $(M+1)^+$. *Anal.* Calcd for $C_{12}H_{10}N_4O_3S$: C, 49.65; H, 3.47; N, 19.30; Found: C, 49.61; H, 3.53; N, 19.26.

6-Aminosulfonyl-1-carbamoyl-3-(1*H*-benzo[*d*]imidazol-2-yl)-*β*-carboline (2) Compound **2** was prepared following the reaction sequences depicted in Chart 3. (±)-Tryptophan was chosen as the starting material for the study, which was converted into 1,2,3,4-tetrahydro-*β*-carboline-3-carboxylic acid (**2a**) according to the Pictet–Spengler reaction in good yield.[24,25] And the treatment of **2a** with excess thionyl chloride in anhydrous EtOH for 6 h at reflux gave ethyl 1,2,3,4-tetrahydro-*β*-carboline-3-carboxylate (**2b**) as a white solid in 88% yield.[27] Then, ethyl *β*-carboline-3-carboxylate (**2c**) was dehydrogenated with $KMnO_4$ in DMF at room temperature for 24 h.[27] The *β*-carboline-3-carboxylic acid (**2d**) was obtained through both the hydrolysis of **2c** in a solution of EtOH/NaOH at reflux and followed by acidation with hydrochloric acid.[29] One of the most important procedures in our experiment was to synthesize 3-(1*H*-benzo[*d*]imidazol-2-yl)-*β*-carboline (**2e**), which was achieved by the condensation of *o*-phenylenediamine with *β*-carboline-3-carboxylic

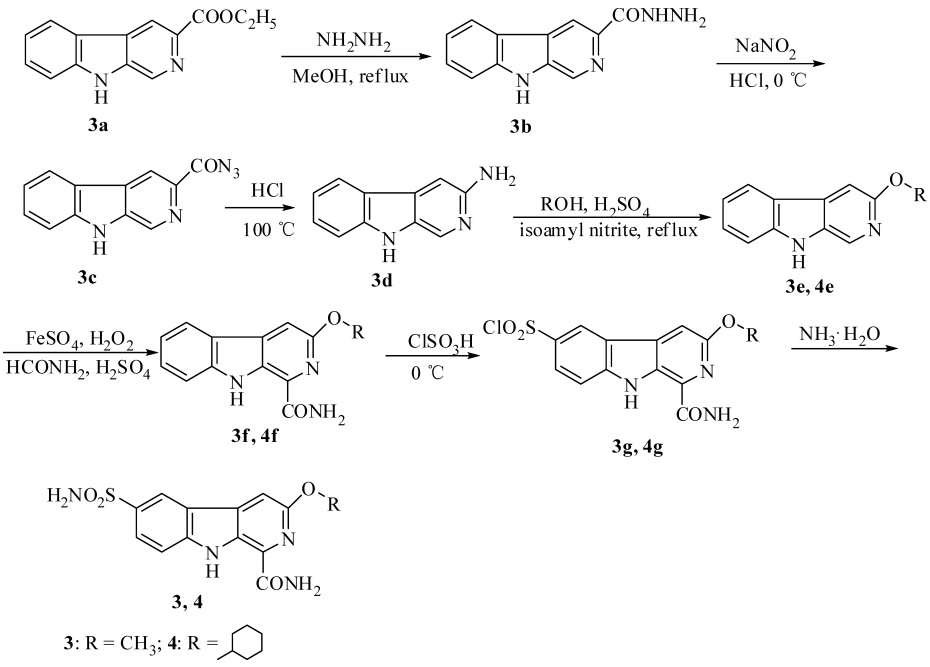

Chart 4. Synthesis of 3,6-Substituted *β*-Carboline Compounds **3** and **4**

acid in the presence of polyphosphoric acid (PPA) in 180°C for 6 h in 45% yield.[30] Subsequently, we treated **2e** with $FeSO_4$ and H_2O_2 in different reagents (including formamide or *N*-methylformamide), under mild conditions (10–15°C) *via* Minisci-Reaction, giving the expected products 1-carbamoyl-3-(1*H*-benzo[*d*]imidazol-2-yl)-*β*-carboline (**2f**) in 55% yield (Chart 3).[31–36] The title compound **2** was achieved from **2f** by the method analogus with **1f** prepared from **1d** mentioned above.

Yield 60%. mp 321–323°C (MeOH). ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 13.12 (1H, s, benzimidazole), 12.27 (1H, s, indole), 9.34 (1H, s, ArH), 9.06 (1H, s, ArH), 8.95 (1H, d, J=7.7 Hz, ArH), 8.07 (1H, d, J=7.7 Hz, ArH), 7.59–7.74 (2H, m, –$CONH_2$), 7.30 (2H, s, –SO_2NH_2), 7.29–7.24 (4H, m, ArH). ^{13}C-NMR (300 MHz, DMSO-d_6) δ: 167.2 (s), 151.4 (s), 144.2 (s), 143.4 (s), 137.1 (s), 136.2 (s), 135.4 (s), 134.6 (s), 132.3 (s), 131.7 (d), 126.5 (d), 122.8 (d), 121.7 (d), 120.6 (d), 119.5 (s), 119.1 (d), 115.6 (d), 113.5 (d), 111.3 (s). IR (KBr) cm^{-1}: 3415, 3280, 3124, 3111, 1725. MS *m*/*z*: 408 $(M+1)^+$. *Anal.* Calcd for $C_{19}H_{14}N_6O_3S$: C, 56.15; H, 3.47; N, 20.68; Found: C, 56.18; H, 3.36; N, 20.70.

6-Aminosulfonyl-1-carbamoyl-3-methoxy-*β*-carboline (3) For compound **3**, the same method with compound **4** as follow is used, only the cyclohexanol group was introduced from intermedium **3d**.[27]

Yield 57%. mp 165–167°C (MeOH). ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 11.68 (1H, s, indole), 8.72 (1H, s, ArH), 8.12 (1H, s, ArH), 7.96 (1H, d, J=7.8 Hz, ArH), 7.8 (2H, m, –$CONH_2$), 7.23 (2H, s, –SO_2NH_2), 7.1 (1H, d, J=7.8 Hz, ArH), 4.02 (3H, s, –OCH_3). ^{13}C-NMR (300 MHz, DMSO-d_6) δ: 167.2 (s), 155.6 (s), 144.5 (s), 135.2 (s), 134.9 (s), 132.2 (d), 128.1 (s), 126.6 (d), 120.7 (d), 118.8 (s), 112.8 (d), 104.1 (s), 53.8 (q). IR (KBr) cm^{-1}: 3470, 3350, 3304, 1672. MS *m*/*z*: 321 $(M+1)^+$. *Anal.* Calcd for $C_{13}H_{12}N_4O_4S$: C, 48.74; H, 3.78; N, 17.49; Found: C, 48.70; H, 3.84; N, 17.51.

6-Aminosulfonyl-1-carbamoyl-3-cyclohexylmethoxy-*β*-carboline (4) 3-Amino-*β*-carboline (**3d**) was prepared by a Curtius-Rearrangement reaction starting with ethyl *β*-carboline-3-carboxylate (**2c**). Thus, refluxing of **2c** with 85% hydrazine hydrate in MeOH gave the hydrazide **3b** which, after treatment with sodium nitrite, yielded the azide **3c**.[27] When **3c** was refluxed for several minutes in HCl solution, rearrangement to the amine **3d** occurred in good yield.[27] Treatment of **3d** with methanol (or cyclohexylmethanol) and H_2SO_4 in the presence of isoamyl nitrite provided **4e**.[27] Subsequently, we treated **4e** with $FeSO_4$ and H_2O_2 in the presence of H_2SO_4 in formamide under mild conditions (10–15°C) *via* Minisci-Reaction, giving the expected products 1-carbamoyl-3-cyclohexylmethoxy-*β*-carboline (**4f**).[37] The key intermediate **4f** was converted into the target molecules **4** in 45–80% yields through both the chlorosulfonylation in dry chlorosulfonic acid and subsequent aminolysis with amine solution (Chart 4).

Yield 65%. mp 304–306°C (MeOH). ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 11.64 (1H, s, indole), 8.70 (1H, s, ArH), 8.07 (1H, s, ArH), 7.97 (1H, d, J=7.9 Hz, ArH), 7.89 (1H, m, ArH), 7.69–7.81 (2H, m, –$CONH_2$), 7.22 (2H, s, –SO_2NH_2), 4.23 (2H, d, J=5.2 Hz, –CH_2O), 1.0–2.0 (11H, m, cyclohexyl). ^{13}C-NMR (300 MHz, DMSO-d_6) δ: 167.3 (s), 155.6 (s), 144.4 (s), 135.1 (s), 134.9 (s), 132.1 (d), 128.2 (s), 126.6 (d), 120.6 (d), 118.8 (s), 112.8 (d), 104.1 (s), 71.3 (t), 37.2 (d), 29.4 (t), 26.1 (t), 25.3 (t). IR (KBr) cm^{-1}: 3465, 3346, 3319, 1672. MS *m*/*z*: 402 $(M+1)^+$. *Anal.* Calcd for $C_{19}H_{22}N_4O_4S$: C, 56.70; H, 5.51; N, 13.92; Found: C, 56.67; H, 5.55; N, 13.90.

6-Aminosulfonyl-1-*N*-methylcarbamoyl-*β*-carboline (5) Compound **5** was obtained by the similar process as compound **1** mentioned above *via* **5d** and **5e**[37] (Chart 2).

Yield 68%. mp 256–257°C (MeOH). ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 12.09 (1H, s, indole), 8.90 (1H, d, J=4.8 Hz, ArH), 8.78 (1H, s, ArH), 8.50 (1H, m, ArH), 8.00 (1H, m, ArH), 7.90 (1H, d, J=8.4 Hz, –CONH–), 7.20 (2H, s, –SO_2NH–), 2.90 (3H, d, J=4.8 Hz, –$CONCH_3$). ^{13}C-NMR

(300 MHz, DMSO-d_6) δ: 167.2 (s), 142.8 (s), 137.5 (d), 135.7 (s), 135.0 (s), 133.4 (s), 130.4 (s), 126.1 (d), 120.2 (d), 119.2 (s), 118.3 (d), 113.2 (d). 30.5 (q). IR (KBr) cm^{-1}: 3495, 3458, 3381, 3352, 3259, 1674. MS *m/z*: 305 $(M+1)^+$. *Anal.* Calcd for $C_{13}H_{12}N_4O_3S$: C, 51.31; H, 3.97; N, 18.41; Found: C, 51.36; H, 3.89; N, 18.40.

Fluorescence Measurements β-Carbolines were obtained by the methods mentioned in the text. Each solution of the compound was prepared in methanol, respectively. On the basis of the results of the biological activity by calculation, in this study, four compounds with the different substituents at 3-position of β-carbolines (**1**–**4**) were investigated. CDK2/cycA2 solution which shows intrinsic fluorescence emission with the excitation nearby 280 nm was prepared in pH 7.5 Tris–HCl buffer solution (containing 50 nM Tris–HCl, 150 nM NaCl, pH=7.5). Titrations were done manually by using micro-injector. The fluorescence spectra were then measured (excitation at 282 nm and emission at 290–550 nm) at room temperature. All measurements were carried out keeping the concentration of CDK2 fixed at 1.0×10^{-7} M and that of β-carbolines varying from 1.25×10^{-10} M to 2.0×10^{-7} M for all systems. Five minutes later after adding the compounds, record the fluorescence intensity of the mixture, respectively. All the measurements were carried out at room temperature (25±0.5°C).

X-Ray Crystallography A suitable pale brown crystal of the compound **5** (0.20 mm×0.20 mm×0.20 mm) crystallized from methanol/DMF was selected and mounted on the top of a glass fiber. The data was collected by a Nonius cad4 diffractometer equipped with a graphite-monochromatized Mo$K\alpha$ radiation at 293 K. The structure was solved and refined with the SHELXS-97 and SHELXL-97 programs. The structure was refined on F^2 by successive full-matrix least-squares techniques. All hydrogen atoms were located in a difference Fourier map and their geometry was idealized, and refined by a riding model. (CCDC 740985 contains the supplementary crystallographic data of compound **5**. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre *via* http://www.ccdc.cam.ac.uk/data_request/cif.)[38]

Results and Discussion

Crystal Structure of Compound 5 The crystallographic data of **5** are summarized in Table 1. The basic scaffold of title compound **5** is nearly planar, with a largest deviation of 0.0815(3) Å from the carboline plane by N3 atom. The selected bond lengths, angles and torsion angles are given in Table 2. The ORTEP drawings of the compound **5** are illustrated in Fig. 1.

Fluorescence Quenching of CDK2 The binding intensity of CDK2 and β-carbolines were evaluated by the measurement of intrinsic fluorescence intensity of CDK2 before and after addition of compounds. The effect of β-carbolines on CDK2 fluorescence intensity was shown in Fig. 2. When the excitation was set at 282 nm, the intrinsic fluorescence of CDK2 at 332 nm was observed, whereas β-carbolines had no intrinsic fluorescence. With the addition of compound **1** into the CDK2 solution, a remarkable decrease of fluorescence intensity of CDK2 was observed, which indicated that **1** can interact with CDK2. Furthermore, with the concentration of compound **1** increasing to 10^{-7} M in the mixture, the fluorescence quenching tended to equilibrium. And the other three compounds exhibited the similar results. The maximum wavelength of intrinsic fluorescence of four compounds from **1**, **2**, **3** to **4** was 367 nm, 424 nm, 424 nm and 434 nm, respectively. As compari-

Table 1. Crystal and Experimental Data of Compound **5**

Empirical formula	$C_{13}H_{12}N_4O_3S$
Formula weight	304.33
Wavelength	0.71073
Crystal system	Monoclinic
Space group	P21/C
a (Å)	11.455 (2)
b (Å)	12.538 (3)
c (Å)	9.2690 (19)
β (°)	100.04 (3)
V (Å)	1310.9 (5)
Z	4
Crystal size (mm)	0.2×0.2×0.1
Shape	Block
Colour	Yellow
D calc (g/cm^{-3})	1.542
Reflections collected/unique	2494/2372
Unique reflections	1815
Absorption coefficient (mm^{-1})	0.264
F(000)	632
R ($F^2>2\sigma$ (F^2))	0.0456
wR (F^2)	0.1288
Goodness of fit	0.971
No. of variables	197
Program system	SHELXL 97
Structure determination	Direct method

Table 2. Selected Bond Distances (Å), Angles (°), and Torsion Angles (°) for **5**

Bond lengths	Bond angles	
N1–C2 1.325 (3)	N3–C12–C11 129.0 (3)	O3–S–O2 118.94 (13)
O1–C2 1.235 (3)	C6–C7–C8 134.2 (2)	C1–N1–C2 122.1 (3)
C9–S 1.772 (3)	C5–C6–C7 135.6 (3)	C8–C9–S 119.5 (2)
O2–S 1.437 (2)	C13–N13–C12 109.1 (2)	O3–S–N4 107.55 (14)
O3–S 1.426 (2)	N2–C3–C2 118.9 (2)	O3–S–C9 107.87 (13)
N4–S 1.618 (3)	C3–C2–O1 119.6 (2)	C3–N2–C4 118.9 (2)
N3–C13 1.369 (3)	N1–C2–O1 123.7 (3)	N4–S–O2 106.01 (14)

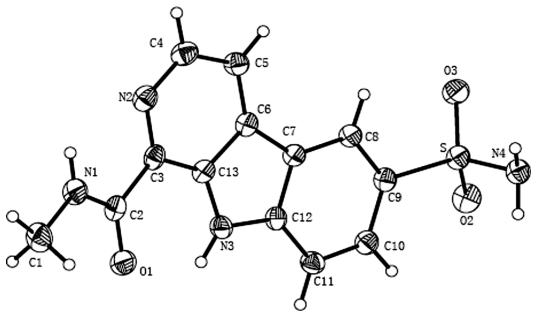

Fig. 1. ORTEP Drawing of the X-Ray Crystal Structure of Compound **5**

Displacement ellipsoids were drawn at 50% probability level.

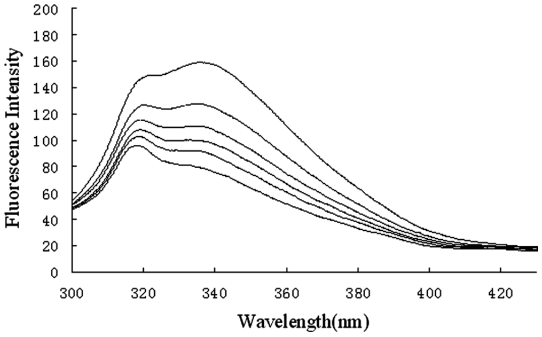

Fig. 2. The Effect of **1** on CDK2 Fluorescence Intensity

son, the red shift of maximum wavelength of compounds **2**, **3** and **4** mainly owed to their structure with higher conjugacy degree when inducing ether group in compound **3** and **4**, and the benzimidazoyl group in compound **2** at 3-position.

The Comparison of the Four Compounds 1–4 Fluorescence quenching is an important method to study the interaction of small molecule with protein because of its accuracy, sensitivity, rapidity, and convenience of usage. It can reveal accessibility of quenchers to protein fluorophores, help to understand the binding mechanisms between small molecules and protein and provides clues to the nature of the binding phenomenon.[39–41] In our study, the quantitative analysis of the binding intensity of β-carbolines to CDK2 was carried out using the fluorescence quenching at 332 nm at various concentrations of the former (Fig. 3). The fluorescence intensity of system gradually decreased with the increase of β-carbolines concentration and with the further addition of β-carbolines, the fluorescence intensity of system decreased tardily in each titration curve which indicates the beginning of saturation of the CDK2 binding site. In Fig. 3, compound **4** show the strongest fluorescence quenching ability.

The ability of fluorescence quenching is: **4**>**1**>**3**>**2**. For the interaction between small molecule and protein mainly occurs in the small molecule and amino acid residue within the hydrophobic pocket, herein, the 3-cyclohexylmethoxy group in **4** may be extending to such hydrophobic field in the CDK2 structure. Although the benzimidazoyl group in compound **2** at 3-position is hydrophobic group, **2** exhibited lower ability of fluorescence quenching compared with compound **4**. The existence of benzimidazoyl group decreased the flexibility of branched chain, which lowered the ability to bind with the hydrophobic field. Owing to the absence of hydrophobic group of compound **1** at 3-position, the interaction between **1** and CDK2 was weaker than **4**. Otherwise, the methoxyl group in compound **3** had no influence in proving the intensity of fluorescence quenching.

Molecular Docking Studies and Binding Conformation Molecular docking acted as an additional tool for pharmacophore-based virtual screening, the concurrent use of which is believed to make the discovery of potent CDK2 kinase inhibitors more efficient. All dock runs were conducted using Glide with extra precision. The performance of it on CDK2 inhibitors was evaluated by re-docking co-crystallized ligand, showing a good reproducibility. The crystal complex structure of inhibitor CDK2 with a resolution of 1.95 was prepared using Protein Preparation Wizard workflow. The compound set to be docked was prepared with LigPre module. Glide

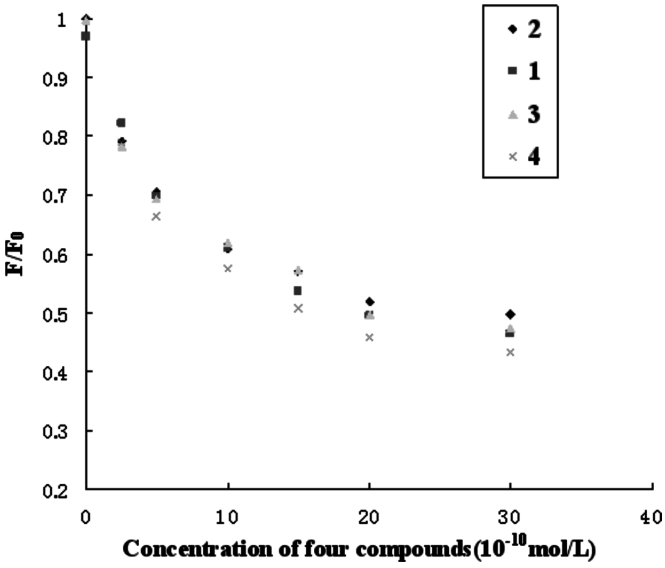

Fig. 3. Comparison of Fluorescence Quenching of Compounds **1**–**4**

Table 3. Docking Scour for Each Compound in the Test Set Mapped with Active Site of CDK2

Compound No.	Docking value (kcal/mol)	*C* log *P*
4	−8.507	3.53
1	−8.08	0.46
3	−6.36	0.88
2	−6.327	2.34

Score, which was composed by electrostatic, hydrogen bond, hydrophobic, and van der Waals *etc.*, was applied as the scoring function to prioritize the test set compounds. Then the compounds were all flexibly docked into the rigid binding pocket of the target protein.

The docking results suggested that all compounds make the same hydrogen binding interaction with LEU 83, GLU 81 and ASP 86, provided by the necessary group N–H, 1-carbamoyl group and 6-aminosulfonyl group, respectively, indicated by dashed line in Fig. 4. Moreover, replacement of 3-cyclohexylmethoxy group with the highest *C* log *P* value will increase the hydrophobic binding interaction with the deep hydrophobic pocket correlate to the higher docking scours (Table 3). From the docking scour in Table 3, the intensity of interaction between four compounds and CDK2 was: **4**>**1**>**3**>**2**. The result was consistent with our research result using fluorescence spectroscopy which induced the binding mode to be reasonable.

Conclusion

In conclusion, the desired 3-substituted of 6-aminosulfonyl-β-carbolines were prepared and their structures were characterized. From the data of binding intensity with CDK2 by means of fluorescence measurements and molecular docking studies, compound **4** with 3-cyclohexylmethoxy group showed the highest binding ability. To assess the potentials of these new compounds as possible CDK2 inhibitors, further antitumor evaluation studies are needed. The results obtained from this study can be used as guidelines for further development.

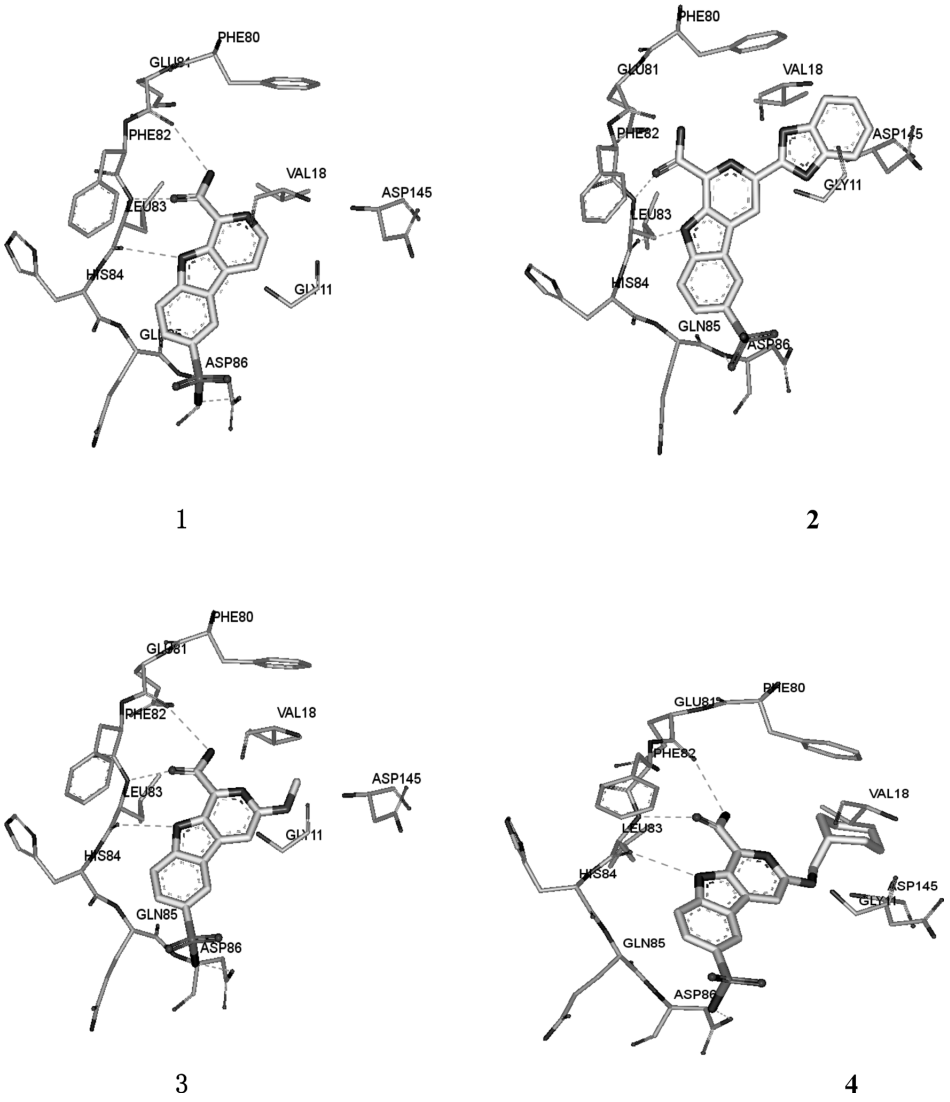

Fig. 4. The Docking Mode of Four Compounds with CDK2

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