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Synthesis and Biological Evaluation of a Benz[*cd*]indol-2(1*H*)-one Derivatives

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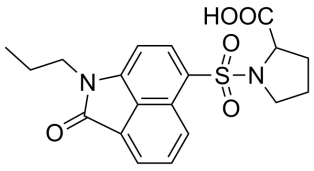
Virtual screening of a library of 6.4 million compounds *versus* the structure of Xenopus Laevis' Aurora B kinase identified 1-(*n*-propyl)-6-[2-(carboxyl)tetrahydropyrrol-1-yl]sulfonyl-benzo[cd]indol-2(1*H*)-one **1** as a possible lead compound. Then, a novel series of benz[cd]indol-2(1*H*)-one derivatives were synthesized and evaluated as Aurora B kinase inhibitors. The structures of the synthetic compounds were confirmed by ^{1}H NMR, IR, mass spectrometry and elemental analysis. These compounds were evaluated by *in vitro* enzyme assay using spectrophotometry. Among them, compound **7e** displayed potent antitumor activity against Aurora B kinase.

Keywords: Aurora B, Kinase inhibitor, Benz[*cd*]indol-2(1*H*)-one derivatives.

INTRODUCTION

Aurora kinase family of serine/threonine kinases are crucial regulators of mitosis and cytokinesis that are constantly overexpressed in human cancers. Among the three members of the kinase family in humans, Aurora A, Aurora B and Aurora C, that are distinctive localized and mediate different functions in cell division[1,2]. Aurora B kinase is a member of the Chromosomal Passenger Complex (CPC), widely expressed in the inner centromere from early mitosis to the spindle midzone, equatorial cortex and midbody in late mitosis and cytokinesis, which binds other chromosomal passenger proteins INCENP, survivin and borealin to form a chromosomal complex[3,4]. Aurora B functions associates with regulation of chromosome-microtubule interactions, cohesion, spindle stability and cytokinesis[5]. Given Aurora A and B pivotal roles in mitotic process, over expression in multiple human tumor types including breast, colourectal, prostate, ovarian, pancreas, thyroid and glioma as well as oncogenic signaling pathways, Aurora kinase inhibitors have emerged as promising antitumor agents[6-9]. Currently, there are a few of inhibitors in phase I/II clinical trials for cancer[10]. In preclinical experiments, some reports have demonstrated that the phenotype of pan-Aurora inhibitors is the same as Aurora B selective inhibitors, namely, the clinical reaction of pan-Aurora inhibitors will resemble compounds which selectively inhibit Aurora B[11]. Therefore, Aurora B has become a drug target in cancer treatment.

In this paper, we aim to discover inhibitors with novel scaffolds by virtual screening. Currently, the X-ray crystallized structure (3D-structure) of human Aurora B is unavailable, the alternative structure of Xenopus Laevis (PDB ID: 2VRX, Resolution: 1.86 Å) downloaded from the Protein Data Bank (http://www.pdb.org) was applied since the 3D sturcture of which was rather similar with human Aurora B (similarity more than 98 %)[12]. In this study, our screening database which consists of 6.4 million compounds were collected from the Internet and prepared for docking and screening. Finally, 1-(*n*-propyl)-6-[2-(carboxyl)tetrahydropyrrol-1-yl]sulfonyl-benzo[cd]indol-2(1*H*)-one **1** was obtained as non-peptide small-molecule inhibitor from virtual screening, which got an highest score in the results by Schrodinger Suite 2009 (Fig. 1).



1

Fig. 1. Structure of the compound **1**

In the early 1920s, benzo[*c*,*d*]indol-2(1*H*)-one derivatives were initially developed as dyes and then were used for electronic typing materials[13]. Many researches focused on optimizing its structure to broaden its application. Until recently, their

excellent bioactivities have been found. As shown in Fig. 2, Guez *et al.* reported that a series of 5-HT7R antagonists (a) showed satisfactory pharmacological properties for depression and IBS (irritable bowel syndrome)[14]. Liu *et al.*[15] reported that compound (b) showed good binding affinity for CDK2 in the major solid tumor types including colon, breast, prostate, ovarian carcinomas and non-small cell lung. The discovery of new compound (c) with selective antitumor activity for leukemia P388 cell line and lung cancer A549 cell line was reported in 2007[16]. It was reported by Chen *et al.*[17] that compound (d) was capable of blocking the mPTPB-mediated ERK1/2 inactivation and may become a novel class of anti-TB agents. Meanwhile, Talukdar *et al.*[18] showed compound (**e**) with moderately improved inhibitory activity against *M. tuberculosis lumazine* synthase.

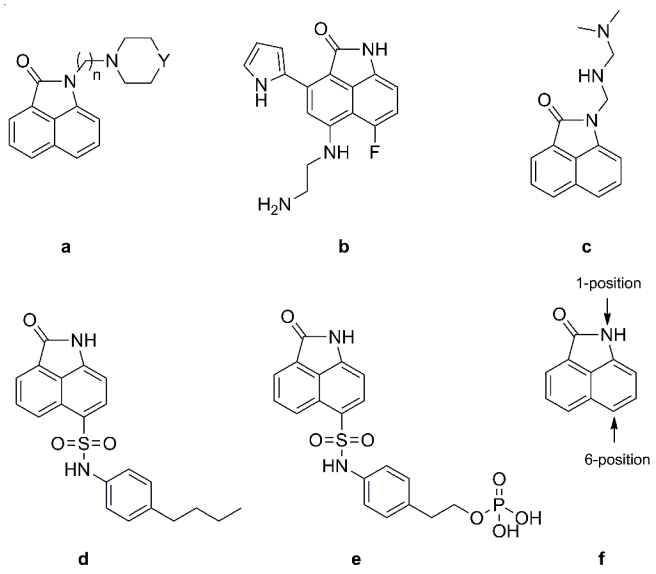


Fig. 2. Structures of some reported compounds **a-e**

Although the benzo[*c*,*d*]indol-2(1*H*)-one derivatives were widely used as protein inhibitors, their inhibition activity against Aurora B haven't been reported. Therefore, we focused on the modification of the 1- and 6-position of benzo[*c*,*d*]indol-2(1*H*)-one (f) to design and synthesis of novel benzo[*c*,*d*]indol-2(1*H*)-one derivatives. In this paper, we reported the design, synthesis and initial biological evaluation as Aurora B kinase inhibitors

EXPERIMENTAL

All solvents of reagent grade were obtained from commercial suppliers and were used without further purification. All commercial reagents were of the highest purity available. Tetrahydrofuran was distilled over sodium and benzophenone. Pyridine was distilled over KOH. Column chromatography was carried out on SHANGHAI SANPONT Gel (200-300 mesh). NMR spectra were recorded on a Bruker AM-400. All NMR spectra were recorded in $CDCl_3$, CD_3OD or DMSO at room temperature (20 °C). Chemical shifts for ^1H spectra are quoted in ppm downfield from TMS. Coupling constants are referred to as J values. ESI mass spectra were obtained using a Bruker ESQUIRELCTM ESI ion trap spectrometer. FT-IR spectra were determined at room temperature (20 °C) in the region of 4000-400 cm^{-1} with a Perkin Elmer spectrum 65 FT-IR spectrometer using KBr pellets. Melting points were determined using an SGW-X4B digital melting point apparatus. Elemental analysis of carbon, hydrgen and nitrogen were obtained with a Perkin Elmer 2400 Serie II CHN elemental analyzer.

Preparation of 1-(aliphatic group)-benzo[*cd*]indol-2(1*H*)-one (4, 5): 1,8-Naphthalic anhydride **2** (13 g, 0.065 mol) and hydroxylamine hydrochloride (4.6 g, 0.065 mol) were heated under reflux in dry pyridine (75 mL) for 1 h. *p*-Toluene sulfonyl chloride (27 g, 0.141 mol) was carefully added in portions to cause controlled boiling. Reflux was continued for a further 1 h. The mixture was poured onto water (300 mL) and the crystalline precipitate collected by filtration and washed with 0.5 N NaOH and water to remove N-hydroxy-naphthalimide. The crystals were stirred in refluxing water (150 mL) and ethanol (50 mL) containing NaOH (10 g) for 2 h. After this time ethanol was removed by distillation. The resulting mixture was cooled to room temperature, acidified with concentrated HCl (30 mL) and the crude product allowed to precipitate overnight. The solid was collected by filtration, washed with water and dried at 100 °C. Recrystallization from benzene gave 1,8-naphtholactam **3** (10 g, 91.7 %) as yellow needles. 1-Chlorobutane or ethyl chloroacetate (0.005 mol) was added to solution of compound 3 (0.005 mol) in DMF (20 mL), then K_2CO_3 (1.38 g) and a little of KI was added, the reaction mixture was stirred at 140 °C for 4 h. The mixture was cooled and filtered and extract with ethyl acetate, The crude product was separated on silica gel chromatography, eluting with ethyl acetate-petroleum ether (1:5) to give the product.

General procedure A: Preparation of 1-(*n*-butyl)-6-substituted sulfonyl-benzo[*cd*]indol-2(1*H*)-one (7a-f): Chlorosulfonic acid (3.2 mL) was added slowly to compound **4** (5.9 mmol). The reaction mixture was stirred at 0 °C for 1 h at room temperature for 2 h. The mixture was then poured into ice water (20 mL) and extract with ethyl acetate (3 × 20 mL), then washed with brine, dried over anhydrous sodium sulfate overnight. The solvent was removed under vacuum and dried to give compound **6** as yellow solid (44 %). amines (0.84 mmol) was added to solution of compound **6** (0.56 mmol) in THF (5 mL) and the reaction mixture was stirred at room temperature for 12 h. THF was removed under vacuum and the residue was purified by silica gel column chromatography, eluting with ethyl acetate-petroleum ether (1:5) to give the product.

General procedure B: Preparation of 1-(*n*-butyl)-6-substituted sulfonyl-benzo[*cd*]indol-2(1*H*)-one (7g-v): Under nitrogen, amines (4.5 mmol) and diisopropylethylamine (6 mmol) was dissolved in 5 mL dried THF, a solution of compound **6** (4 mmol) in 15 mL THF was added dropwise at 0 °C. Then the reaction mixture was stirred at room temperature overninght. After removal of solvent and the residue was purified by silica gel column chromatography, eluting with ethyl acetate-petroleum ether (1:10) to give the product.

General procedure C: Preparation of 1-(*n*-butyl)-6-substituted sulfonyl-benzo[*cd*]indol-2(1*H*)-one (9a-e): Chlorosulfonic acid (3.2 mL) was added slowly to compound **5** (5.9 mmol). The reaction mixture was stirred at 0 °C for 1 h at room temperature for 2 h. The mixture was then poured

into ice water (20 mL) and extract with ethyl acetate (3 × 20 mL), then washed with brine, dried over anhydrous sodium sulfate overnight. The solvent was removed under vacuum and dried to give compound **8** as yellow solid (44 %). Under nitrogen, amines (4.5 mmol) and diisopropylethylamine (6 mmol) was dissolved in 5 mL dried THF, a solution of compound **8** (4 mmol) in 15 mL THF was added dropwise at 0 °C. Then the reaction mixture was stirred at room temperature overninght. After removal of solvent and the residue was purified by silica gel column chromatography, eluting with ethyl acetate-petroleum ether (1:20) to give the product.

1-(*n*-Butyl)-6-(morpholin-1-yl)sulfonyl-benzo[*cd*]-indol-2(1*H*)-one (7a): Compound **7a** was prepared according to standard procedure **A** by using compound **6** and morpholine, obtained a yellow solid in 87.2 % yield. m.p. 123.1-124.9 °C. IR (KBr, ν_{max}, cm^{-1}): 1705, 1626, 1496, 1344, 1160. ^1H NMR ($CDCl_3$, 400 MHz): 8.71 (d, *J* = 8.4 Hz, 1H), 8.12 (dd, *J* = 12 Hz, 7.2 Hz, 2H), 7.84 (dd, *J* = 8.4 Hz, 6.8 Hz, 1H), 6.97 (d, *J* = 7.2 Hz, 1H), 3.94 (t, *J* = 7.2 Hz, 2H), 3.73 (t, *J* = 4.8 Hz, 4H), 3.10 (t, *J* = 4.8 Hz, 4H), 1.78 (m, 2H), 1.45 (m, 2H), 0.99 (t, *J* = 7.2 Hz, 3H). MS [M + H^+]: *m/z* = 375.01. Anal. (%) Calcd. for $C_{19}H_{22}N_2O_4S$: C, 60.94; H, 5.92; N, 7.48. Found: C, 60.55; H, 5.81; N, 7.66.

1-(*n*-Butyl)-6-(piperidin-1-yl)sulfonyl-benzo[*cd*]indol-2(1*H*)-one (7b): Compound **7b** was prepared according to standard procedure **A** by using compound **6** and piperidine, obtained a yellow solid in 85.6 % yield. m.p. 111.3-112.8 °C. IR (KBr, ν_{max}, cm^{-1}): 1711, 1626, 1495, 1336, 1149. ^1H NMR ($CDCl_3$, 400 MHz): 8.72 (d, *J* = 8.4 Hz, 1H), 8.11 (t, *J* = 6.4 Hz, 2H), 7.82 (dd, *J* = 7.6 Hz, 8.4 Hz, 1H), 6.94 (d, *J* = 7.6 Hz, 1H), 3.93 (t, *J* = 7.2 Hz, 2H), 3.84 (d, *J* = 11.6 Hz, 2H), 3.39 (t, *J* = 11.6 Hz, 2H), 1.78 (m, 2H), 1.66 (d, *J* = 9.6 Hz, 2H), 1.45 (m, 2H), 1.29-1.26 (m, 4H), 0.98 (t, *J* = 7.2 Hz, 3H). MS [M + H^+]: *m/z* = 373.03. Anal. (%) Calcd. for $C_{20}H_{24}N_2O_3S$: C, 64.49; H, 6.49; N, 7.52. Found: C, 64.35; H, 6.36; N, 7.43.

1-(*n*-Butyl)-6-(4-methylpiperidin-1-yl)sulfonyl-benzo[*cd*]indol-2(1*H*)-one (7c): Compound **7c** was prepared according to standard procedure **A** by using compound **6** and 4-methyl piperidine, obtained a yellow solid in 88.1 % yield. m.p. 96.1-97.4 °C. IR (KBr, ν_{max}, cm^{-1}): 1702, 1627, 1495, 1336, 1151. ^1H NMR ($CDCl_3$, 400 MHz): 8.73 (d, *J* = 8.4 Hz, 1H), 8.11 (t, *J* = 7.2 Hz, 2H), 7.82 (dd, *J* = 8.4 Hz, 7.2 Hz, 1H), 6.94 (d, *J* = 7.6 Hz, 1H), 3.93 (t, *J* = 7.2 Hz, 2H), 3.10 (t, *J* = 5.6 Hz, 4H), 1.78 (m, 2H), 1.62 (m, 4H), 1.48-1.38 (m, 3H), 1.29 (d, *J* = 6.4 Hz, 3H), 0.99 (t, *J* = 7.2 Hz, 3H). MS [M + H^+]: *m/z* = 387.06, Anal. (%) Calcd. for $C_{21}H_{26}N_2O_3S$: C, 65.26; H, 6.78; N, 7.25. Found: C, 64.88; H, 6.64; N, 7.17.

1-(*n*-Butyl)-6-(2-methylpiperidin-1-yl)sulfonyl-benzo[*cd*]-indol-2(1*H*)-one (7d): Compound **7d** was prepared according to standard procedure **A** by using compound **6** and 2-methyl-piperidine, obtained a yellow oil in 87.4 % yield. IR (KBr, ν_{max}, cm^{-1}): 1715, 1630, 1494, 1321, 1143. ^1H NMR ($CDCl_3$, 400 MHz): 8.57 (d, *J* = 8.4 Hz, 1H), 8.17 (d, *J* = 7.6 Hz, 1H), 8.09 (d, *J* = 7.2 Hz, 1H), 7.82 (dd, *J* = 8.4 Hz, 7.2 Hz, 1H), 6.90 (d, *J* = 7.6 Hz, 1H), 4.32 (m, 1H), 3.92 (t, *J* = 7.2 Hz, 2H), 3.70 (dt, *J* = 13.6 Hz, 3.2 Hz, 1H), 3.05 (td, *J* = 13.2 Hz, 2.4 Hz, 1H), 1.77 (m, 2H), 1.60-1.24 (m, 8H), 1.15 (d, *J* = 6.8 Hz, 3H), 0.09 (t, *J* = 7.6 Hz, 3H). MS [M + H^+]: *m/z* = 387.06. Anal. (%) Calcd. for $C_{21}H_{26}N_2O_3S$: C, 65.26; H, 6.78; N, 7.25. Found: C, 65.0; H, 6.42; N, 7.21.

1-(*n*-Butyl)-6-(3,5-dimethylpiperidin-1-yl)sulfonyl-benzo[*cd*]indol-2(1*H*)-one (7e): Compound **7e** was prepared according to standard procedure **A** by using compound **6** and 3,5-dimethylpiperidine, obtained a yellow solid in 83.3 % yield. m.p. 134.5-135.2 °C. IR (KBr, ν_{max}, cm^{-1}): 1702, 1628, 1496, 1340, 1154. ^1H NMR ($CDCl_3$, 400 MHz): 8.71 (d, *J* = 8.4 Hz, 1H), 8.14 (dd, *J* = 7.6 Hz, 4.8 Hz, 2H), 7.86 (dd, *J* = 7.2 Hz, 1H), 6.95 (d, *J* = 7.6 Hz, 1H), 3.93 (t, *J* = 7.2 Hz, 2H), 3.81 (d, *J* = 8.0 Hz, 2H), 1.86 (t, *J* = 11.2 Hz, 2H), 1.82-1.69 (m, 5H), 1.45 (m, 2H), 0.99 (t, *J* = 7.2 Hz, 3H), 0.83 (d, *J* = 6.4 Hz, 6H), 0.48 (q, *J* = 12 Hz, 1H). MS [M + H^+]: *m/z* = 401.08. Anal. (%) Calcd. for $C_{22}H_{28}N_2O_3S$: C, 65.97; H, 7.05; N, 6.99. Found: C, 66.19 H, 6.88; N, 6.58.

1-(*n*-Butyl)-6-(tetrahydropyrrole-1-yl)sulfonyl-benzo-[*cd*]indol-2(1*H*)-one (7f): Compound **7f** was prepared according to standard procedure **A** by using compound **6** and tetrahydropyrrole, obtained a yellow solid in 82.5 % yield. m.p. 76.6-78.5 °C. IR (KBr, ν_{max}, cm^{-1}): 1716, 1627, 1496, 1333, 1150. ^1H NMR ($CDCl_3$, 400 MHz): 8.79 (d, *J* = 8.4 Hz, 1H), 8.15 (d, *J* = 7.6 Hz, 1H), 8.12 (d, *J* = 7.2 Hz, 1H), 7.83 (dd, *J* = 8.4 Hz, 7.2 Hz, 1H), 6.95 (d, *J* = 7.6 Hz, 1H), 3.93 (t, *J* = 7.2 Hz, 2H), 3.32 (t, *J* = 6.8 Hz, 4H), 1.79 (m, 2H), 1.45 (m, 2H), 0.98 (t, *J* = 7.2 Hz, 3H). MS [M + H^+]: *m/z* = 359.02. Anal. (%) Calcd. for $C_{19}H_{22}N_2O_3S$: C, 63.66; H, 6.19; N, 7.82. Found: C, 63.55; H, 6.08; N, 7.71.

1-(*n*-Butyl)-6-(N-phenylpiperazin-1-yl)sulfonyl-benzo-[*cd*]indol-2(1*H*)-one (7g): Compound **7g** was prepared according to standard procedure **B** by using compound **6** and 1-phenylpiperazine, obtained a yellow solid in 78.5 % yield. m.p. 111.8-112.9 °C. IR (KBr, ν_{max}, cm^{-1}): 1709, 1628, 1599, 1495, 1347, 1157. ^1H NMR ($CDCl_3$, 400 MHz): 8.72 (d, *J* = 8.4 Hz, 1H), 8.16 (dd, *J* = 6.8 Hz, 1.2 Hz, 2H), 7.87 (dd, *J* = 8.4 Hz, 7.2 Hz, 1H), 7.29 (t, *J* = 8.0 Hz, 2H), 7.04-6.96 (m, 3H), 3.94 (t, *J* = 7.2 Hz, 2H), 3.41 (s, 4H), 3.31 (s, 4H), 1.76 (m, 2H), 1.44 (m, 2H), 0.99 (t, *J* = 7.2 Hz, 3H). MS [M + H^+]: *m/z* = 450.08. Anal. (%) Calcd. for $C_{25}H_{27}N_3O_3S$: C, 66.79; H, 6.05; N, 9.35. Found: C, 66.37; H, 5.96; N, 9.01.

1-(*n*-Butyl)-6-[4-(4-fluorophenyl)piperazin-1-yl]-sulfonyl-benzo[*cd*]indol-2(1*H*)-one (7h): Compound **7h** was prepared according to standard procedure **B** by using compound **6** and 1-(4-fluorophenyl)piperazine, obtained a yellow solid in 75.7 % yield. m.p. 143.5-144.6 °C. IR (KBr, ν_{max}, cm^{-1}): 1709, 1628, 1509, 1495, 1348, 1156. ^1H NMR ($CDCl_3$, 400 MHz): 8.74 (d, *J* = 8.4 Hz, 1H), 8.13 (dd, *J* = 7.2 Hz, 2.8 Hz, 2H), 7.85 (dd, *J* = 8.4 Hz, 7.2 Hz, 1H), 6.98-6.92 (m, 3H), 6.84 (s, 2H), 3.94 (t, *J* = 7.2 Hz, 2H), 3.30 (s, 4H), 3.18 (s, 4H), 1.78 (m, 2H), 1.44 (m, 2H), 0.99 (t, *J* = 7.2 Hz, 3H). MS [M + H^+]: *m/z* = 468.12. Anal. (%) Calcd. for $C_{25}H_{26}FN_3O_3S$: C, 64.22; H, 5.60; N, 8.99. Found: C, 64.13; H, 5.25; N, 8.69.

1-(*n*-Butyl)-6-[4-(2-fluorophenyl)piperazin-1-yl]-sulfonyl-benzo[*cd*]indol-2(1*H*)-one (7i): Compound **7i** was prepared according to standard procedure **B** by using compound **6** and 1-(2-fluorophenyl)piperazine, obtained a yellow solid in 73.5 % yield. m.p. 168.4-168.9 °C. IR (KBr, ν_{max}, cm^{-1}): 1718, 1626, 1492, 1348, 1155. ^1H NMR ($CDCl_3$, 400 MHz): 8.75 (d, *J* = 8.4 Hz, 1H), 8.13 (d, *J* = 6.8 Hz, 2H), 7.85 (dd, *J* = 6.8 Hz, 8 Hz, 1H), 7.06-6.97 (m, 5H), 3.95 (t, *J* = 7.2 Hz, 2H), 3.36 (s, 4H), 3.20 (s, 4H), 1.79 (m, 2H), 1.46 (m, 2H), 0.99 (t, *J* = 7.2 Hz, 3H). MS [M + H^+]: *m/z* = 468.03. Anal.

(%) Calcd. for $C_{25}H_{26}N_3O_3SF$: C, 64.22; H, 5.60; N, 8.99. Found: C, 64.43; H, 6.70; N, 9.14.

1-(*n*-Butyl)-6-(4-(4-methylphenyl)piperazin-1-yl)-sulfonyl-benzo[*cd*]indol-2(1*H*)-one (7j): Compound **7j** was prepared according to standard procedure **B** by using compound **6** and 1-(4-methylphenyl)piperazine, obtained a yellow solid in 79.3 % yield. m.p. 145.3-145.9 °C. IR (KBr, ν_{max}, cm^{-1}): 1709, 1628, 1495, 1347, 1157. ^1H NMR ($CDCl_3$, 400 MHz): 8.67 (d, *J* = 8.4 Hz, 1H), 8.13 (d, *J* = 7.2 Hz, 2H), 7.85 (dd, *J* = 8.4 Hz, 7.2 Hz, 1H), 7.15 (m, 4H), 6.96 (d, *J* = 7.6 Hz, 1H), 3.94 (t, *J* = 7.2 Hz, 2H), 3.56 (s, 4H), 3.36 (s, 4H), 1.78 (m, 2H), 1.45 (m, 2H), 0.99 (t, *J* = 7.2 Hz, 3H). MS [M + H^+]: *m/z* = 464.29. Anal. (%) Calcd. for $C_{26}H_{29}N_3O_3S$: C, 67.36; H, 6.31; N, 9.06. Found: C, 67.25; H, 6.20; N, 8.95.

1-(*n*-Butyl)-6-(4-(4-ethylformate)piperidin-1-yl)-sulfonyl-benzo[*cd*]indol-2(1*H*)-one (7k): Compound **7k** was prepared according to standard procedure **B** by using compound **6** and ethyl 4-piperidinecarboxylate, obtained a yellow solid in 76.9 % yield. m.p. 131.4-132.6 °C, IR (KBr, ν_{max}, cm^{-1}): 1730, 1715, 1628, 1494, 1337, 1149. ^1H NMR ($CDCl_3$, 400 MHz): 8.69 (d, *J* = 8.4 Hz, 1H), 8.11 (dd, *J* = 7.2 Hz, 4.8 Hz, 2H), 7.83 (dd, *J* = 8.4 Hz, 7.2 Hz, 1H), 6.94 (d, *J* = 7.6 Hz, 1H), 4.08 (q, *J* = 7.2 Hz, 2H), 3.93 (t, *J* = 7.2 Hz, 2H), 3.73 (m, 2H), 2.64 (td, *J* = 2.8 Hz, 12.0 Hz, 2H), 2.25 (m, 1H), 1.97 (m, 2H), 1.83-1.74 (m, 4H), 1.45 (m, 2H), 1.19 (t, *J* = 7.2 Hz, 3H), 0.99 (t, *J* = 7.6 Hz, 3H). MS [M + H^+]: *m/z* = 445.09. Anal. (%) Calcd. for $C_{23}H_{28}N_2O_5S$: C, 62.14; H, 6.35; N, 6.30. Found: C, 61.99; H, 6.07; N, 6.11.

1-(*n*-Butyl)-6-(4-(3-trifluoromethylphenyl)piperazin-1-yl)sulfonyl-benzo[*cd*]indol-2(1*H*)-one (7l). Compound **7l** was prepared according to standard procedure **B** by using compound **6** and N-(3-trifluoromethylphenyl)piperazine, obtained a yellow solid in 72.3 % yield. m.p. 103.1-104.5 °C. IR (KBr, ν_{max}, cm^{-1}): 1709, 1628, 1495, 1359, 1158. ^1H NMR ($CDCl_3$, 400 MHz): 8.71 (d, *J* = 8.8 Hz, 1H), 8.14 (dd, *J* = 7.6 Hz, 4.4 Hz, 2H), 7.86 (t, *J* = 7.6 Hz, 1H), 7.38 (m, 1H), 7.21-7.16 (t, *J* = 7.2 Hz, 3H), 6.97 (d, *J* = 7.6 Hz, 1H), 3.94 (t, *J* = 7.2 Hz, 1H), 3.40 (s, 4H), 3.34 (s, 4H), 1.78 (t, *J* = 7.2 Hz, 2H), 1.46 (m, 2H), 0.99 (t, *J* = 7.2 Hz, 3H). MS [M + H^+]: *m/z* = 518.36. Anal. (%) Calcd. for $C_{26}H_{26}N_3O_3SF_3$: C, 60.34; H, 5.06; N, 8.12. Found: C, 60.11; H, 4.95; N, 8.08.

1-(*n*-Butyl)-6-(phenylethylamin-1-yl)sulfonyl-benzo-[*cd*]indol-2(1*H*)-one (7m): Compound **7m** was prepared according to standard procedure **B** by using compound **6** and phenylethylamine, obtained a yellow solid in 78.9 % yield. m.p. 120.0-121.4 °C. IR (KBr, ν_{max}, cm^{-1}): 3185, 1687, 1627, 1496, 1330, 1151. ^1H NMR (DMSO-d_6, 400 MHz): 8.63 (d, *J* = 8.4 Hz, 1H), 8.14 (d, *J* = 7.2 Hz, 1H), 8.05 (d, *J* = 7.6 Hz, 1H), 7.93-7.90 (m, 2H), 7.26 (d, *J* = 7.6 Hz, 1H), 7.11-7 (m, 5H), 3.90 (t, *J* = 7.2 Hz, 2H), 3.03 (dd, *J* = 7.2 Hz, 13.6 Hz, 2H), 2.61 (t, *J* = 7.2 Hz, 2H), 1.69 (m, 2H), 1.34 (m, 2H), 0.91 (t, *J* = 7.2 Hz, 3H). MS [M + H^+]: *m/z* = 409.24. Anal. (%) Calcd. For $C_{23}H_{24}N_2O_3S$: C, 67.62; H, 5.92; N, 6.86. Found: C, 67.41; H, 5.80; N, 6.77.

1-(*n*-Butyl)-6-(furfurylamin-1-yl)sulfonyl-benzo[*cd*]-indol-2(1*H*)-one (7n): Compound **7n** was prepared according to standard procedure **B** by using compound **6** and furfurylamine, obtained a yellow solid in 77.6 % yield. m.p. 111.4-112.9 °C. IR (KBr, ν_{max}, cm^{-1}): 3153, 1682, 1628, 1496, 1330, 1156. ^1H NMR (DMSO-d_6, 400 MHz): 8.66 (d, *J* = 8.4 Hz, 1H), 8.39 (t, *J* = 6.0 Hz, 1H), 8.14 (d, *J* = 7.2 Hz, 1H), 8.05 (d, *J* = 7.6 Hz, 1H), 7.92 (dd, *J* = 8.0 Hz, 7.2 Hz, 1H), 7.28-7.25 (m, 2H), 6.13 (t, *J* = 2.4 Hz, 1H), 5.99 (d, *J* = 3.2 Hz, 1H), 4.04 (d, *J* = 6.0 Hz, 2H), 3.91 (t, *J* = 7.2 Hz, 2H), 1.69 (m, 2H), 1.34 (m, 2H), 0.92 (t, *J* = 7.2 Hz, 3H). MS [M + H^+]: *m/z* = 385.01. Anal. (%) Calcd. for $C_{20}H_{20}N_2O_4S$: C, 62.48; H, 5.24; N, 7.29. Found: C, 62.37; H, 5.03; N, 7.18.

1-(*n*-Butyl)-6-(3-fluoroanilin-1-yl)sulfonyl-benzo[*cd*]-indol-2(1*H*)-one (7o): Compound **7o** was prepared according to standard procedure **B** by using compound **6** and 3-fluoroaniline, obtained a yellow solid in 74.9 % yield. m.p. 159.6-160.9 °C, IR (KBr, ν_{max}, cm^{-1}): 3160, 1691, 1629, 1493, 1340, 1159. ^1H NMR ($CDCl_3$, 400 MHz): 8.55 (d, *J* = 8.4 Hz, 1H), 8.16 (d, *J* = 7.6 Hz, 1H), 8.10 (d, *J* = 7.2 Hz, 1H), 7.83 (dd, *J* = 8.4 Hz, 7.2 Hz, 1H), 7.12 (m, 1H), 6.85-6.71 (m, 5H), 3.89 (t, *J* = 7.2 Hz, 2H), 1.74 (m, 2H), 1.39 (m, 2H), 0.98 (t, *J* = 7.2 Hz, 3H). MS [M + H^+]: *m/z* = 399.07. Anal. (%) Calcd. for $C_{21}H_{19}FN_2O_3S$: C, 63.30; H, 4.81; N, 7.03. Found: C, 63.09; H, 4.54; N, 6.92.

1-(*n*-Butyl)-6-(4-fluoroanilin-1-yl)sulfonyl-benzo[*cd*]-indol-2(1*H*)-one (7p): Compound **7p** was prepared according to standard procedure **B** by using compound **6** and 4-fluoroaniline, obtained a yellow solid in 73.7 % yield. m.p. 166.7-168.2 °C. IR (KBr, ν_{max}, cm^{-1}): 3121, 1684, 1630, 1509, 1495, 1334, 1153. ^1H NMR ($CDCl_3$, 400 MHz): 8.48 (d, *J* = 8.4 Hz, 1H), 8.10 (d, *J* = 6.8 Hz, 1H), 8.03 (d, *J* = 7.6 Hz, 1H), 7.79 (t, *J* = 7.6 Hz, 1H), 6.95-6.82 (m, 5H), 6.45 (s, 1H), 3.89 (t, *J* = 7.2 Hz, 2H), 1.74 (m, 2H), 1.35 (m, 2H), 0.97 (t, *J* = 7.2 Hz, 3H). MS [M + H^+]: *m/z* = 399.00. Anal. (%) Calcd. for $C_{21}H_{19}N_2O_3SF$: C, 63.30; H, 4.81; N, 7.03. Found: C, 63.59; H, 4.90; N, 7.31.

1-(*n*-Butyl)-6-(4-anisidin-1-yl)sulfonyl-benzo[*cd*]indol-2(1*H*)-one (7q): Compound **7q** was prepared according to standard procedure **B** by using compound **6** and *p*-anisidine, obtained a yellow solid in 73.4 % yield. m.p. 229.7-230 °C. IR (KBr, ν_{max}, cm^{-1}): 3139, 1684, 1630, 1511, 1496, 1335, 1151. ^1H NMR (DMSO-d_6, 400 MHz): 10.14 (s, 1H), 8.64 (d, *J* = 8.4 Hz, 1H), 8.13 (d, *J* = 7.2 Hz, 1H), 8.02 (d, *J* = 7.6 Hz, 1H), 7.92 (dd, *J* = 8.4 Hz, 7.2 Hz, 1H), 7.23 (d, *J* = 7.6 Hz, 1H), 6.91 (d, *J* = 8.8 Hz, 2H), 6.73 (d, *J* = 8.8 Hz, 2H), 3.86 (t, *J* = 7.2 Hz, 2H), 3.62 (s, 3H), 1.65 (m, 2H), 1.31 (m, 2H), 0.89 (t, *J* = 7.6 Hz, 3H). MS [M + H^+]: *m/z* = 411.02. Anal. (%) Calcd. For $C_{22}H_{22}N_2O_4S$: C, 64.37; H, 5.40; N, 6.82. Found: C, 64.16; H, 5.29; N, 6.71.

1-(*n*-Butyl)-6-(4-methylbenzylamin-1-yl)sulfonyl-benzo[*cd*]indol-2(1*H*)-one (7r): Compound **7r** was prepared according to standard procedure **B** by using compound **6** and 4-methylbenzylamine, obtained a yellow solid in 75.2 % yield. m.p. 147.5-148.0 °C. IR (KBr, ν_{max}, cm^{-1}): 3292, 1714, 1688, 1493, 1331, 1140. ^1H NMR ($CDCl_3$, 400 MHz): 8.63 (d, *J* = 8.4 Hz, 1H), 8.20 (d, *J* = 7.6 Hz, 1H), 8.14 (d, *J* = 7.2 Hz, 1H), 7.85 (dd, *J* = 8.4 Hz, 7.2 Hz, 1H), 6.98 (dd, *J* = 10.8 Hz, 8.4 Hz, 4H), 6.91 (d, *J* = 7.6 Hz, 1H), 4.71 (t, *J* = 6.0 Hz, 1H), 4.11 (d, *J* = 6.0 Hz, 2H), 3.95 (t, *J* = 7.2 Hz, 2H), 2.27 (s, 3H), 1.80 (m, 2H), 1.47 (m, 2H), 1.01 (t, *J* = 7.2 Hz, 3H). MS [M + H^+]: *m/z* = 409.10. Anal. (%) Calcd. for $C_{23}H_{24}N_2O_3S$: C, 67.62; H, 5.92; N, 6.86. Found: C, 67.31; H, 5.82; N, 6.55.

1-(*n*-Butyl)-6-(4-fluorobenzylamin-1-yl)sulfonyl-benzo[*cd*]indol-2(1*H*)-one (7s): Compound **7s** was prepared according to standard procedure **B** by using compound **6** and 4-fluorobenzylamine, obtained a yellow solid in 78.6 % yield. m.p. 136.5-136.9 °C. IR (KBr, ν_{max}, cm^{-1}): 3283, 1694, 1628, 1494, 1332, 1152. ^{1}H NMR ($CDCl_3$, 400 MHz): 8.62 (d, *J* = 8.4 Hz, 1H), 8.13 (t, *J* = 7.2 Hz, 2H), 7.84 (t, *J* = 7.2 Hz, 1H), 7.06 (dd, *J* = 8.4 Hz, 5.2 Hz, 2H), 6.88-6.83 (m, 3H), 4.79 (t, *J* = 6.0 Hz, 1H), 4.12 (d, *J* = 6.0 Hz, 2H), 3.93 (t, *J* = 7.2 Hz, 2H), 1.78 (m, 2H), 1.44 (m, 2H), 0.99 (t, *J* = 7.2 Hz, 3H). MS [M + H^+]: *m/z* = 413.02. Anal. (%) Calcd. for $C_{22}H_{21}N_2O_3SF$: C, 64.06; H, 5.13; N, 6.79. Found: C, 63.88; H, 4.97; N, 6.43.

1-(*n*-Butyl)-6-(4-methoxybenzylamin-1-yl)sulfonyl-benzo[*cd*]indol-2(1*H*)-one (7t): Compound **7t** was prepared according to standard procedure **B** by using compound **6** and 4-methoxybenzylamine, obtained a yellow solid in 79.3 % yield. m.p. 100.1-101.6 °C. IR (KBr, ν_{max}, cm^{-1}): 3239, 1717, 1627, 1492, 1251, 1143. ^{1}H NMR ($CDCl_3$, 400 MHz): 8.61 (d, *J* = 8.0 Hz, 1H), 8.17 (d, *J* = 7.6 Hz, 1H), 8.12 (d, *J* = 7.2 Hz, 1H), 7.85 (dd, *J* = 8.4 Hz, 7.2 Hz, 1H), 6.98 (d, *J* = 7.6 Hz, 2H), 6.89 (d, *J* = 7.6 Hz, 1H), 6.68 (d, *J* = 8.4 Hz, 2H), 4.71 (t, *J* = 6.0 Hz, 1H), 4.07 (d, *J* = 6.0 Hz, 2H), 3.93 (t, *J* = 7.2 Hz, 2H), 3.72 (s, 3H), 1.78 (m, 2H), 1.45 (m, 2H), 0.99 (t, *J* = 7.2 Hz, 3H). MS [M + H^+]: *m/z* = 425.04. Anal. (%) Calcd. for $C_{23}H_{24}N_2O_4S$: C, 65.07; H, 5.70; N, 6.60. Found: C, 64.96; H, 5.59; N, 6.49.

1-(*n*-Butyl)-6-(3-fluorobenzylamin-1-yl)sulfonyl-benzo[*cd*]indol-2(1*H*)-one (7u): Compound **7u** was prepared according to standard procedure **B** by using compound **6** and 3-fluorobenzylamine, obtained a yellow solid in 82.7 % yield. m.p. 113.2-114.8 °C. IR (KBr, ν_{max}, cm^{-1}): 3230, 1692, 1629, 1495, 1323, 1151. ^{1}H NMR ($CDCl_3$, 400 MHz): 8.61 (d, *J* = 8.4 Hz, 1H), 8.16 (d, *J* = 7.2 Hz, 1H), 8.12 (d, *J* = 6.8 Hz, 1H), 7.84 (d, *J* = 8.4 Hz, 7.2 Hz, 1H), 7.12-7.09 (m, 1H), 6.89-6.82 (m, 3H), 6.77 (d, *J* = 9.6 Hz, 1H), 4.85 (t, *J* = 6.4 Hz, 1H), 4.15 (d, *J* = 6.4 Hz, 2H), 3.93 (t, *J* = 7.2 Hz, 2H), 1.77 (m, 2H), 1.43 (m, 2H), 0.99 (t, *J* = 7.2 Hz, 3H). MS [M + H^+]: *m/z* = 413.02. Anal. (%) Calcd. for $C_{22}H_{21}FN_2O_3S$: C, 64.06; H, 5.13; N, 6.79. Found: C, 63.95; H, 5.02; N, 6.88.

1-(*n*-Butyl)-6-(2-fluorobenzylamin-1-yl)sulfonyl-benzo[*cd*]indol-2(1*H*)-one (7v): Compound **7v** was prepared according to standard procedure **B** by using compound **6** and 2-fluorobenzylamine, obtained a yellow solid in 83.4 % yield. m.p. 149.0-149.9 °C. IR (KBr, ν_{max}, cm^{-1}): 3270, 1690, 1629, 1495, 1332, 1151. ^{1}H NMR ($CDCl_3$, 400 MHz): 8.57 (d, *J* = 8.4 Hz, 1H), 8.15 (d, *J* = 7.6 Hz, 1H), 8.10 (d, *J* = 6.8 Hz, 1H), 7.83 (dd, *J* = 7.2 Hz, 8.4 Hz, 1H), 7.05 (m, 2H), 6.86-6.83 (m, 2H), 6.73 (dd, *J* = 8.4 Hz, 10.4 Hz, 1H), 4.98 (t, *J* = 6.4 Hz, 1H), 4.25 (d, *J* = 6.4 Hz, 2H), 3.93 (t, *J* = 7.2 Hz, 2H), 1.79 (m, 2H), 1.46 (m, 2H), 1.02 (t, *J* = 7.2 Hz, 3H). MS [M + H^+]: *m/z* = 412.98. Anal. (%) Calcd. for $C_{22}H_{21}FN_2O_3S$: C, 64.06; H, 5.13; N, 6.79. Found: C, 63.78; H, 5.43; N, 6.87.

1-(Ethyl-1-acetate)-6-(2-methylpiperidin-1-yl)sulfonyl-benzo[*cd*]indol-2(1*H*)-one (9a): Compound **9a** was prepared according to standard procedure **C** by using compound **8** and 2-methylpiperazine, obtained a yellow solid in 87.9 % yield. m.p. 99.3-100.2 °C. IR (KBr, ν_{max}, cm^{-1}): 1748, 1716, 1633, 1494, 1307, 1203, 1140. ^{1}H NMR ($CDCl_3$, 400 MHz): 8.62 (d, *J* = 8.4 Hz, 1H), 8.19 (dd, *J* = 11.2 Hz, 7.6 Hz, 2H), 7.85 (t, *J* = 7.6 Hz, 1H), 6.84 (d, *J* = 7.6 Hz, 1H), 4.68 (s, 2H), 4.34 (m, 1H), 4.26 (q, *J* = 6.8 Hz, 2H), 3.69 (d, *J* = 12.8 Hz, 1H), 3.08 (t, *J* = 12.8 Hz, 1H), 1.33-1.26 (m, 9H), 1.15 (d, *J* = 6.8 Hz, 3H). MS [M + H^+]: *m/z* = 417.46. Anal. (%) Calcd. for $C_{21}H_{24}N_2O_5S$: C, 60.56; H, 5.81; N, 6.73. Found: C, 60.45; H, 5.70; N, 6.62.

1-(Ethyl-1-acetate)-6-(morpholin-1-yl)sulfonyl-benzo-[*cd*]indol-2(1*H*)-one (9b): Compound **9b** was prepared according to standard procedure **C** by using compound **8** and morpholine, obtained a yellow solid in 92.7 % yield. m.p. 123.1-131.9 °C. IR (KBr, ν_{max}, cm^{-1}): 1748, 1720, 1628, 1498, 1344, 1213, 1157. ^{1}H NMR ($CDCl_3$, 400 MHz): 8.75 (d, *J* = 8.4 Hz, 1H), 8.18 (d, *J* = 7.2 Hz, 1H), 8.11 (d, *J* = 7.6 Hz, 1H), 7.88 (t, *J* = 7.6 Hz, 1H), 6.91 (d, *J* = 7.6 Hz, 1H), 4.70 (s, 2H), 4.27 (q, *J* = 7.2 Hz, 2H), 3.73 (t, *J* = 4.8 Hz, 4H), 3.10 (t, *J* = 4.8 Hz, 4H), 1.31 (t, *J* = 7.2 Hz, 3H). MS [M + H^+]: *m/z* = 405.21. Anal. (%) Calcd. for $C_{19}H_{20}N_2O_6S$: C, 56.42; H, 4.98; N, 6.93. Found: C, 56.21; H, 4.77; N, 6.82.

1-(Ethyl-1-acetate)-6-(4-methoxyanilin-1-yl)sulfonyl-benzo[*cd*]indol-2(1*H*)-one (9c): Compound **9c** was prepared according to standard procedure **C** by using compound **8** and 4-methoxyaniline, obtained a yellow solid in 90.6 % yield. m.p. 101.3-102.5 °C. IR (KBr, ν_{max}, cm^{-1}): 3178, 1746, 1696, 1632, 1512, 1496, 1339, 1152. ^{1}H NMR (DMSO-d_6, 400 MHz): 10.16 (s, 1H), 8.69 (d, *J* = 8.4 Hz, 1H), 8.20 (d, *J* = 7.2 Hz, 1H), 8.03 (d, *J* = 7.6 Hz, 1H), 7.96 (dd, *J* = 8.4 Hz, 7.6 Hz, 1H), 7.25 (d, *J* = 7.6 Hz, 1H), 6.91 (d, *J* = 8.8 Hz, 2H), 6.73 (d, *J* = 8.8 Hz, 2H), 4.79 (s, 2H), 4.16 (q, *J* = 7.2 Hz, 2H), 3.62 (s, 3H), 1.20 (t, *J* = 7.2 Hz, 3H). MS [M + H^+]: *m/z* = 441.00. Anal. (%) Calcd. for $C_{22}H_{20}N_2O_6S$: C, 59.99; H, 4.58; N, 6.36. Found: C, 59.76; H, 4.47; N, 6.15.

1-(Ethyl-1-acetate)-6-(furfurylamin-1-yl)sulfonyl-benzo[*cd*]indol-2(1*H*)-one (9d): Compound **9d** was prepared according to standard procedure **C** by using compound **8** and furfuryl amine, obtained a yellow solid in 88.9 % yield. m.p. 103.7-104.9 °C. IR (KBr, ν_{max}, cm^{-1}): 3253, 1749, 1727, 1631, 1495, 1322, 1214, 1148. ^{1}H NMR ($CDCl_3$, 400 MHz): 8.62 (d, *J* = 8.4 Hz, 1H), 8.15 (dd, *J* = 7.2 Hz, 4.8 Hz, 2H), 7.86 (t, *J* = 7.2 Hz, 1H), 7.01 (s, 1H), 6.83 (d, *J* = 7.2 Hz, 1H), 6.06 (d, *J* = 2.8 Hz, 1H), 5.93 (d, *J* = 2.8 Hz, 1H), 4.86 (t, *J* = 6.4 Hz, 1H), 4.69 (s, 2H), 4.26 (q, *J* = 7.2 Hz, 2H), 4.19 (d, *J* = 6.4 Hz, 2H), 1.30 (t, *J* = 7.2 Hz, 3H). MS [M + H^+]: *m/z* = 414.99. Anal. (%) Calcd. for $C_{20}H_{18}N_2O_6S$: C, 57.96; H, 4.38; N, 6.76. Found: C, 58.05; H, 4.67; N, 6.98.

1-(Ethyl-1-acetate)-6-(4-(4-ethylformate)piperidin-1-yl)sulfonyl-benzo[*cd*]indol-2(1*H*)-one (9e): Compound **9e** was prepared according to standard procedure **C** by using compound **8** and 4-piperidinecarboxylate, obtained a yellow solid in 86.9 % yield. m.p. 128.3-129.7 °C. IR (KBr, ν_{max}, cm^{-1}): 1749, 1725, 1630, 1498, 1336, 1219, 1148. ^{1}H NMR (CD_3OD, 400 MHz): 8.81 (d, *J* = 8.4 Hz, 1H), 8.19 (dd, *J* = 7.6 Hz, 2H), 7.98 (t, *J* = 7.6 Hz, 1H), 7.22 (d, *J* = 7.6 Hz, 1H), 4.82 (s, 2H), 4.27 (q, *J* = 7.2 Hz, 2H), 4.08 (q, *J* = 7.2 Hz, 2H), 3.76 (d, *J* = 12 Hz, 2H), 2.63 (t, *J* = 10.4 Hz, 2H), 2.33 (m, 1H), 1.95 (d, *J* = 10.4 Hz, 2H), 1.69 (m, 2H), 1.31 (t, *J* = 7.2 Hz, 3H), 1.19 (d, *J* = 7.2 Hz, 3H). MS [M + H^+]: *m/z* = 475.08. Anal. (%) Calcd. for $C_{23}H_{26}N_2O_7S$: C, 58.22; H, 5.52; N, 5.90. Found: C, 58.34; H, 5.41; N, 6.13.

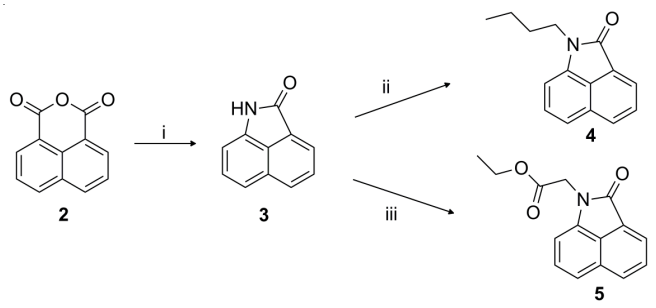
Preparation of active Aurora B: Recombinant human Aurora B was produced according to a procedure published

previously with minor modification[19]. Briefly, Aurora B was expressed and processed in Transetta (DE3) cells harboring a pET-28a plasmid that contained the cloned human Aurora B cDNA. Fermentation was performed at 37 °C for 4-5 h in 800 mL LB media containing 100 µg/mL Kanamycin until the OD600 reached 0.8-2. Then, the culture was transferred to 16 °C and protein expression was induced for 16 h with 1 mM isopropyl-β-D-1-thiogalactopyranoside (IPTG). Harvested cells were resuspended in lysis buffer containing HEPES 25 mM, NaCl 1 M, $MgCl_2$ 10 mM, imidazole 5 mM, glycerol 10 %, pH 7.5 and homogenized with a JN-3000 PLUS low temperature ultra-high pressure cell disrupter (JNBIO, Guangzhou). The lysate was centrifuged at 15,000 rpm for 25 min at 4 °C to remove cell debris. The supernatant was then loaded twice onto a Nickle-NTA agarose column pre-equilibrated with lysis buffer. The Aurora B was eluted with 50 and 500 mM imidazole in the above buffer respectively. Fractions containing Aurora B were pooled and concentrated with a Millipore Ultrafree filtration device and then the Aurora B was exchanged into a buffer of HEPES 25 mM, NaCl 1M, $MgCl_2$ 10 mM, glycerol 10 %, pH 7.5 . The concentrated Aurora B was loaded onto a Superdex 75 10/300 (GE Healthcare) equilibrated with above buffer. Fractions containing Aurora B were pooled and concentrated. The Aurora B obtained with this method showed 39 kD on 12 % SDS-PAGE and aliquots stored at -80 °C.

Aurora B inhibition assay: The test procedure to assess the inhibition of recombinant Aurora B activity of selected compounds was adapted from previously reported procedures[20]. The reaction carried out in 100 µL system including HEPES pH 7.5, 60 mM NaCl, 10 mM $MgCl_2$, 0.3 mM NADH, 1 mM phosphoenolpyruvate (PEP), 1.5 U lactate dehydrogenase (LDH) (Sigma, USA), 1.5 U pyruvate kinase (PK) (Sigma), 1.2 µM Aurora B, 83 µM histones H3 substrate peptide and 0.2 mM ATP in the presence of 1 µL DMSO or 1 µL inhibitor in DMSO. The procedure was briefly described below. At 30 °C, the Aurora B, NADH, PEP, LDH, PK and histones H3 were incubated with inhibitors in 96-well plates for 5 min. Once added the substrate ATP, the reaction was started. As the absorptivity of NADH at 340 nm, with the reaction, NADH continuously transformed into NAD^+. Absorbance at 340 nm was continuously recorded for 10 min. Inhibition ratio was determined from the slope of the early linear part of the curve obtained by plotting decrements in absorbance at 340 nm with time (dB/dt). For IC_{50} determination, about 15 concentrations of inhibitor were freshly prepared by 2 fold gradient dilution with 95 % DMSO. After got the different initial rate according to this method, we used the GraphPad Prism 5 software to calculate the IC_{50} value of different inhibitors.

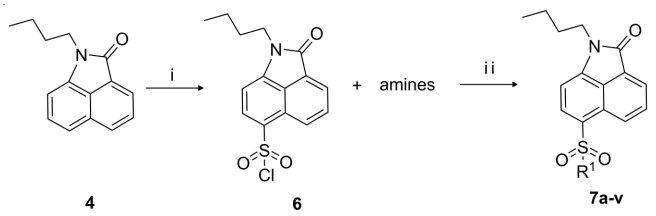
RESULTS AND DISCUSSION

The typical preparation of the benz(*cd*)indol-2(1*H*)-one derivatives was shown in **Scheme-I**. Firstly, compounds **3** was synthesized by a published method[21]. Briefly, the synthesis of compounds **3** was carried out from desymmetrization of commercially available 1, 8-naphthalic anhydride **2** *via* a Beckmann rearrangement. Then, compound **3** was reacted at 140 °C for 4 h with chlorobutane or ethyl chloroacetate, respectively, in presence of K_2CO_3 and KI as catalyst to give intermediates **4** and **5**.



Scheme-I: Synthetic routes of compounds **4** and **5**; Reagents and conditions: i. a) H_2N-OH, dry pyridine, reflux, 1 h; b) *p*-toluene sulfonyl-chloride, reflux, 1 h; c) water, ethanol, NaOH, reflux, 2 h. ii. chlorobutane, DMF, 140 °C, 4 h; iii. ethyl chloroacetate, DMF, 140 °C, 4 h.

A general approach to the preparation of derivatives **7a-v** were shown in **Scheme-II**, chlorosulfonic acid was added to the compound **4** drop-wise at 0 °C and stirring for 1 h, then at room temperature for 2 h to provide intermediate **6** in modest yield, there is a reaction with sulfonylation of the 6-position of benz(*cd*)indol-2(1*H*)-one[18]. Then **6** underwent different reaction conditions to give all the compounds **7a-v**.

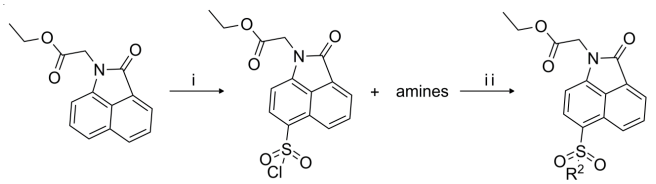


Scheme-II: Synthetic routes of compounds **7a-v**, Reagents and contions: (i) HSO_3Cl, 1 h, rt, 2 h; (ii) THF, rt, 12 h

The synthesis of derivatives **9a-e** was described in **Scheme-III**. The compound **8** was obtained according to the method of synthesis of compound **6**. Under nitrogen atmosphere, simply mixing compound **8** with amines in THF at room temperature provided the required compounds **9a-e** in quantitative yield. The structures of all these final products have been demonstrated by mass spectrometry (MS), infrared (IR), ^{1}H NMR and elemental analysis.

^{1}H NMR spectrum of compound **7a** was recorded in $CDCl_3$, Its ^{1}H NMR spectrum showed the signals of the aromatic protons corresponding to two phenyl groups in the range δ 6.97-8.71 ppm. Signals of the morpholine group in compound **7a** were found in the regions of 3.73 and 3.10 ppm. The *n*-butyl substitute gave rise to one triplet at 3.94 ppm for the N-CH_2, two multiplets at 1.45 ppm and 1.78 ppm for CH_2 and one triplet at 0.99 ppm for the CH_3. The IR spectra of **7a** showed absorption bands in the region of 3055, 2854, 1705, 1626, 1496, 1344 and 1160 cm^{-1}. Characteristic frequencies of the sulfonyl group were observed at 1344 and 1160 cm^{-1}. The strong absorptions at 1705 cm^{-1} were due to the presence of the carbonyl group.

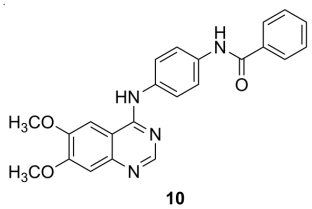
Biological evaluation: In the present study, benzo[*c*,*d*]-indol-2(1*H*)-one was confirmed as the scaffold compound, a series of novel derivatives have been synthesized to evaluate their potential biological activities as Aurora B inhibitors. Inhibition of recombinant human Aurora B by benzo[*c*,*d*]indol-2(1*H*)-one derivatives **7** and **9** was assessed using an enzyme-



5 8 9a-e

Scheme-III: Synthetic routes of compounds **9a-e**, Reagents and contions: (i) HSO_3Cl, rt, 2 h; (ii) THF, rt, overninght

coupled continuous spectrophotometric assay[22]. The Aurora Kinase Inhibitor II (CAS:331770-21-9) **10** was used as the positive control, which is a ATP-competitive inhibitor of Aurora B with IC_{50} values of 756 nM (Fig. 3). The inhibitors synthesized were examined for their ability to inhibit the activity of recombinant human effector Aurora B. The results were summarized in Tables 1 and 2.

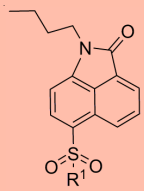


10

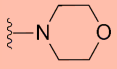
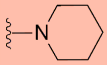
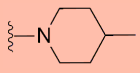
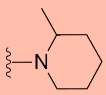
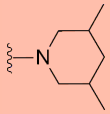
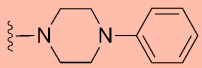
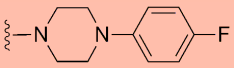
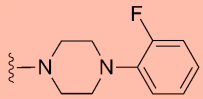
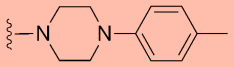
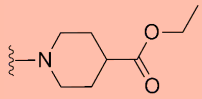
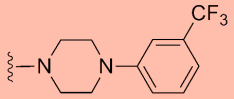
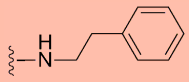
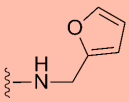
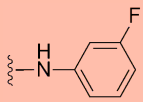
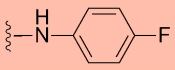
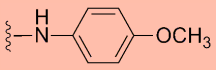
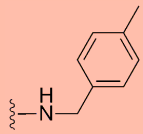
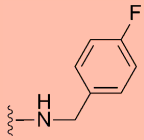
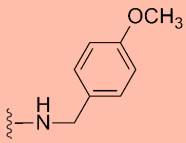
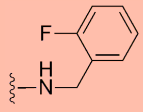
Fig. 3. Structure of the positive control

All 1-(*n*-butyl)-benzo[*cd*]indol-2(1*H*)-ones **7** were assessed for enzymatic activity against Aurora B kinase as shown in Table-1. Variation of the benzo[*c*,*d*]indol-2(1*H*)-one in 6-position revealed that substitution at this position could not increase in inhibitory potency, but provided only poor Aurora

TABLE-1
INHIBITORY ACTIVITY OF COMPOUNDS **7** AGAINST AURORA B KINASE

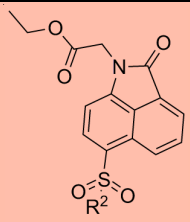


7a-v

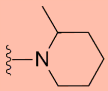
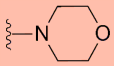
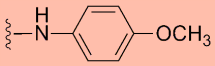
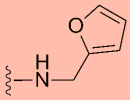
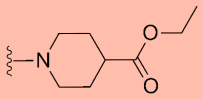
Compound	R^1	IC_{50}[a] or Inhibition ratio (%) in 50 mM
10		99.1; 756 nM
7a		45.3
7b		10.8
7c		49.6
7d		47.3
7e		87.3; 143.28 µM
7f		57.9
7g		26.3
7h		25.8
7i		27.8
7j		25.7
7k		15.7
7l		24.9
7m		10.7
7n		65.2
7o		34.7
7p		35.3
7q		32.6
7r		46.8
7s		34.3
7t		32.2
7u		35.2
7v		34.6

[a]IC_{50} value of compounds was tested when its inhibition ratio exceeded 80 %

TABLE-2
INHIBITORY ACTIVITY OF COMPOUNDS **9** AGAINST AURORA B KINASE



9a-e

Compound	R^2	Inhibition ratio (0.5 mM) (%)
10		99.1
9a		25.3
9b		40.8
9c		43.6
9d		41.3
9e		33.7

B inhibitory activity. Introduction of the single ring into 6-position of benzo[*cd*]indol-2(1*H*)-one **7a-7f** was more effective in increasing the inhibitory potency than compounds having double ring subsitituents **7g-7l**. Compound **7e** showed potential activity with IC_{50} values of 143.28 μM. To further improve the inhibitory potency, we synthesized compounds **7m-v** by introduction of aromatic ring in place of nitrogen-containing heterocycles. The inhibition ratio of compounds with substituted phenyl groups **7o-q** is basically consistent with compounds with substituted benzyl groups **7r-v**, but the inhibition ratio of compound with substituted phenethyl group **7m** is obvious reduced, which was similar with **7g-7l**. It illustrated that the chain length of substituent groups at 6-position showed the obvious influence on inhibitory potency, namely, extending the distance led the inhibitory activity to downtrend.

In addition, to increase the biological potency against Aurora B, a goal of this study was to design several compounds with better pharmaceutical properties by improving its aqueous solubility, which is a considerable molecular property that has been correlated with drug-likeness[23]. Therefore, to improve the aqueous solubility of these compounds, the compounds **9a-e** have been synthesized. Compared the inhibition ratio of compound **9d** with compound **7n**, the replacement at 1-position of compound **7n** with an ethyl-1-acetate group didn't increase in inhibitory potency as shown in Table-2.

Conclusion

In summary, we have completed the synthesis and *in vitro* evaluation of a series of benzo[*c*,*d*]indol-2(1*H*)-one derivatives. The biological activity results suggest that the modification of 1- and 6-position to synthesis of novel benzo[*c*,*d*]indol-2(1*H*)-one derivatives had a poor potency for inhibiting human Aurora B kinase *in vitro*. In this research, benzo[*c*,*d*]indol-2(1*H*)-one derivative **1** was not synthesized and tested inhibitory activity against recombinant human Aurora B kinase. According to experimental result above, presence of carboxyl group in compound **1** may be necessary. Thus, our further work is paid more attention to optimize the structures of benzo-[*c*,*d*]indol-2(1*H*)-one derivative **1** retaining carboxyl group. Meanwhile, we will carry out to modify the NH group of benzo[*c*,*d*]indol-2(1*H*)-one to improve potency and selectivity *in vitro* against human Aurora B kinase.

ACKNOWLEDGEMENTS

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REFERENCES

1. R. Giet, C. Petretti and C. Prigent, *Trends Cell Biol*., **15**, 241 (2005).
2. V.M. Bolanos-Garcia, *Int. J. Biochem. Cell Biol*., **37**, 1572 (2005).
3. M. Carmena, S. Ruchaud and W.C. Earnshaw, *Curr. Opin. Cell Biol*., **21**, 796 (2009).
4. M. Dennis, M. Davies, S. Oliver, R. D'Souza, L. Pike and P. Stockman, *Cancer Chemother. Pharmacol*., **70**, 461 (2012).
5. M. Schmidt and H. Bastians, *Drug Resist. Updat*., **10**, 162 (2007).
6. C.P. Gully, F.M. Zhang, J. Chen, J.A. Yeung, G. Velazquez-Torres, E. Wang, S.C. Yeung and M.H. Lee, *Mol. Cancer*, **9**, 42 (2010).
7. J.R. Bischoff, L. Anderson, Y.G. Zhu, K. Mossie, L. Nq, B. Souza, B. Schryver, P. Flanaqan, F. Clairvoyant, C. Ginther, C.S. Chan, M. Novotny, D.J. Slamon and G.D. Plowman, *EMBO J*., **17**, 3052 (1998).
8. H. Katayama and S. Sen, *Biochim. Biophys. Acta*, **1799**, 829 (2010).
9. T. Ikezoe, *Cancer Lett*., **262**, 1 (2008).
10. C.H.A. Cheung, M.S. Coumar, J.Y. Chang and H.P. Hsieh, *Expert. Opin. Ther. Patents*., **21**, 857 (2011).
11. M.L. Curtin, H. Robin Heyman, R.R. Frey, P.A. Marcotte, K.B. Glaser, J.R. Jankowski, T.J. Magoc, D.H. Albert, A.M. Olson, D.R. Reuter, J.J. Bouska, D.A. Montgomery, J.P. Palma, C.K. Donawho, K.D. Stewart, C. Tse and M.R. Michaelides, *Bioorg. Med. Chem. Lett*., **22**, 4750 (2012).
12. S. Sakkiah, S. Thangapandian, S. John and K.W. Lee, *J. Mol. Struct*., **985**, 14 (2011).
13. X. Li, Q. Wang, Y. Qing, Y. Lin, Y. Zhang, X. Qian and J. Cui, *Bioorg. Med. Chem*., **18**, 3279 (2010).
14. M.L. López-Rodríguez, E. Porras, M.J. Morcillo, B. Benhamu, L.J. Soto, J.L. Lavandera, J.A. Ramos, M. Olivella, M. Capillo and L. Pardo, *J. Med. Chem*., **46**, 5638 (2003).
15. J.J. Liu, A. Dermatakis, C. Lukacs, F. Konzelmann, Y. Chen, U. Kammlott, W. Depinto, H. Yang, X. Yin, Y. Chen, A. Schutt, M.E. Simcox and K.C. Luk, *Bioorg. Med. Chem. Lett*., **13**, 2465 (2003).
16. H. Yin, Y.F. Xu and X.H. Qian, *Bioorg. Med. Chem*., **15**, 1356 (2007).
17. L. Chen, B. Zhou, S. Zhang, L. Wu, Y. Wang, S.G. Franzblau and Z.Y. Zhang, *ACS. Med. Chem. Lett*., **1**, 355 (2010).
18. A. Talukdar, E. Morgunova, J. Duan, W. Meining, N. Foloppe, L. Nilsson, A. Bacher, B. Illarionov, M. Fischer, R. Ladenstein and M. Cushman, *Bioorg. Med. Chem*., **18**, 3518 (2010).
19. F. Sessa, M. Mapelli, C. Ciferri, C. Tarricone, L.B. Areces, T.R. Schneider, P.T. Stukenberg and A. Musacchio, *Mol. Cell*, **18**, 379 (2005).
20. C. Bernal, E. Mendez, J. Terencio, A. Boronat and S. Imperial, *Anal. Biochem*., **340**, 245 (2005).
21. A.N. Cammidge and O. Ozturk, *J. Org. Chem*., **67**, 7457 (2002).
22. G.M.T. Cheetham, R.M.A. Knegtel, J.T. Coll, S.B. Renwick, L. Swenson, P. Weber, J.A. Lippke and D.A. Austen, *J. Biol. Chem*., **277**, 42419 (2002).
23. J.-Y. Le Brazidec, A. Pasis, B. Tam, C. Boykin, C. Black, D. Wang, G. Claassen, J.-H. Chong, J. Chao, J. Fan, K. Nguyen, L. Silvian, L. Ling, L. Zhang, M. Choi, M. Teng, N. Pathan, S. Zhao, T. Li and A. Taveras, *Bioorg. Med. Chem. Lett*., **22**, 2070 (2012).