Chemistry general

All the chemical solvents and reagents used in the current study were analytically pure without further purification. TLC was performed on Merck precoated silica GF254 plates. Melting points were determined on a XT4 MP apparatus (Taike Corp, Beijing, China). ^{1}H NMR spectra were recorded on a Bruker DPX 300 model Spectrometer in DMSO - d_6 and chemical shifts were reported in ppm (δ). Elemental analyses were performed using a CHN-O-Rapid instrument and were within 0.4% of the theoretical values. ESI-MS spectra were recorded on a Mariner System 5304 Mass spectrometer. Column chromatography was performed using silica gel (200-300 mesh) eluting with petroleum ether and ethyl acetate.

General method for synthesis of indole derivations containing sulfonamide scaffold

General procedure for Synthesis Scheme (A)

To a solution of indole-3-carboxylic acid (1.61 g, 10 mmol) in methanol (30 mL) was added dropwise sulfuric acid (2.5 mL) with methanol (20 mL) at 0 ˚C. Subsequently, the mixture was stirred and heated to reflux for 8 hours. After the reaction was completed by TLC (petroleum ether and ethyl acetate (2:1)), the mixture was extracted with EtOAc and water and dried over anhydrous Na_2SO_4 and evaporated to give a crude residue. The residue was recrystallized from the mixture of petroleum ether and ethanol to get pure compounds **2**.

A solution of NaH (0.24 g, 10 mmol) in THF (10 mL) was added slowly to a solution of compound **2** (0.7 g, 4 mmol) in THF (30 mL) at 0 ˚C. The slurry was warmed to room temperature, next was added dropwise iodomethane (150 μL, 1.2 mmol) and stirred for additional 12 h. After the reaction was completed by TLC (petroleum ether and ethyl acetate (2:1)), the mixture was extracted with CH_2Cl_2 and water. The combined organic extract was dried over anhydrous Na_2SO_4 and evaporated to give a crude residue. The compounds **3a-3d** were obtained in this manner.

A solution of compounds **3a** (0.66 g, 3.5 mmol) in a 1:1:1 mixture of THF: CH_3OH:H_2O (40 mL) was added 2N NaOH (20 mL), which was heated at reflux for 7

hours afterwards. Then the reaction was completed by TLC (petroleum ether and ethyl acetate (2:1)). The reaction mixture was partially evaporated to remove the THF, CH_3OH and the remaining part was extracted with EtOAc (100 mL) and water (200 mL), then the combined water layer was acidified to pH = 2 with 1N HCl. The precipitate was filtered and washed by water, then dried to give the title compound **4a-4d**, which was used directly in the next step.

General procedure for Synthesis Scheme (B)

Triethylamine (3327 μL, 24 mmol) was added slowly to a solution of piperazinein CH_2Cl_2 at 0 ˚C, then to this was added benzenesulfonyl chloride (1024 μL，8 mmol) and stirred for 2 hours. After the reaction was completed by TLC (petroleum ether and ethyl acetate (2:1)), the reaction was quenched with water and extracted with CH_2Cl_2. The combined organic layer was dried over anhydrous Na_2SO_4 and evaporated to give **6e**. The compounds **6e-6j** were obtained in this manner.

General procedure for Synthesis Scheme (C)

To a mixture of compound **4a** and CH_2Cl_2 was added EDC and HOBt, and stirred it at 35 ˚C subsequently. After the mixture stirred for 30 min, compounds **6e-6j** was added and stirred for an additional 24 h. After the reaction was completed by TLC (petroleum ether and ethyl acetate (2:1)), a saturated $KHSO_4$ solution was added to the reaction and extracted with CH_2Cl_2, then was washed with water. The combined organic layer was dried over anhydrous Na_2SO_4, concentrated under reduced pressure, and purified by column chromatography over silica gel (petroleum ether and ethyl acetate (8:1)) to afford compounds **7-30**.

1-(phenylsulfonyl)piperazine (6e)

White powder, yield: 88%, m.p. 108-109 ˚C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.71~7.45 (m, 5H, ArH), 3.52 (t, J = 4.93 Hz, 4H, CH_2), 2.76 (t, J = 4.93 Hz, 4H, CH_2), 1.16 (s, 1H, NH). MS (EI): 226.29 ($C_{10}H_{14}N_2O_2S$, $[M]^+$).

1-((4-bromophenyl)sulfonyl)piperazine (6f)

Yellow powder, yield: 79%, m.p. 112-113 ˚C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.72~7.64 (m, 4H, ArH), 3.51 (t, J = 4.80 Hz, 4H, CH_2), 2.78 (t, J = 4.80 Hz, 4H, CH_2), 1.15 (s, 1H, NH). MS (EI): 305.19 ($C_{10}H_{13}BrN_2O_2S$, $[M]^+$).

1-((4-chlorophenyl)sulfonyl)piperazine (6g)

Yellow powder, yield: 75%, m.p. 97-98 ˚C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.69~7.64 (m, 4H, ArH), 3.51 (t, J = 4.80 Hz, 4H, CH_2), 2.79 (t, J = 4.80 Hz, 4H, CH_2), 1.15 (s, 1H, NH). MS (EI): 260.74 ($C_{10}H_{13}ClN_2O_2S$, $[M]^+$).

1- tosylpiperazine (6h)

Yellow powder, yield: 83%, m.p. 110-111 ˚C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.65~7.46 (m, 4H, ArH), 3.51 (t, J = 5.07 Hz, 4H, CH_2), 2.73 (t, J = 4.80 Hz, 4H, CH_2), 2.42 (s, 3H, CH_3), 1.17 (s, 1H, NH). MS (EI): 240.32 ($C_{11}H_{16}N_2O_2S$, $[M]^+$).

4-(piperazin-1-ylsulfonyl)phenyl nitrate (6i)

Yellow powder, yield: 79%, m.p. 93-94 ˚C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.02 (d, J = 8.67 Hz, 2H, ArH), 7.70~7.64 (m, 2H, ArH), 3.53 (t, J = 4.67 Hz, 4H, CH_2), 2.86 (t, J = 4.40 Hz, 4H, CH_2), 1.15 (s, 1H, NH). MS (EI): 271.29 ($C_{10}H_{13}N_3O_4S$, $[M]^+$).

1-(mesitylsulfonyl)piperazine (6j)

Yellow powder, yield: 82%, m.p. 74-75 ˚C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.37~7.32 (m, 2H, ArH), 3.48 (t, J = 4.53 Hz, 4H, CH_2), 2.93 (t, J = 4.53 Hz, 4H, CH_2), 2.98~2.93 (m, 3H, CH), 1.21 (d, J = 6.80 Hz, 18H, CH_3), 1.15 (s, 1H, NH). MS (EI): 352.54 ($C_{19}H_{32}N_2O_2S$, $[M]^+$).

(*E*)-(1-Methyl-1H-indol-3-yl)(4-(phenylsulfonyl)piperazin-1-yl)methanone (7).

White solid, yield 63%, m.p. 215~216 ˚C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.75 (t, J = 4.00 Hz, 3H, ArH, CHN), 7.71 (s, 1H, ArH), 7.68 (t, J = 7.46 Hz, 2H, ArH), 7.63 (t, J = 7.20 Hz, 1H, ArH), 7.47 (d, J = 8.27 Hz, 1H, ArH), 7.21 (t, J = 7.60 Hz,

1H, ArH), 7.11 (t, J = 7.46 Hz, 1H, ArH), 3.79 (s, 3H, CH_3), 3.71 (t, J = 4.93 Hz, 4H, CH_2), 2.96 (t, J = 4.93 Hz, 4H, CH_2). ^{13}C NMR (DMSO-d_6, 75.46 MHz) δ: 165.56，138.37, 136.63, 134.09, 131.45, 129.87, 127.54, 124.38, 122.87, 120.46, 110.25, 108.24, 46.32, 41.33, 18.32. ESI-MS: m/z 383.5 (M^+). Anal. Calcd for $C_{20}H_{21}N_3O_3S$: C, 62.64; H, 5.52; N, 10.96. Found: C, 63.23; H, 5.43; N, 8.32.

(*E*)-(4-((4-Bromophenyl)sulfonyl)piperazin-1-yl)(1-methyl-1H-indol-3-yl)methanone (8).

White solid, yield 67%, m.p. 205~208 ˚C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.88 (dd, J = 6.67 Hz, J = 1.50 Hz, 2H, ArH, CHN), 7.72 (s, 1H, ArH), 7.69 (t, J = 2.13 Hz, 1H, ArH), 7.67 (t, J = 2.27 Hz, 1H, ArH), 7.64 (d, J = 7.87 Hz, 1H, ArH), 7.48 (d, J = 8.27 Hz, 1H, ArH), 7.21 (t, J = 7.73 Hz, 1H, ArH), 7.11 (t, J = 7.60 Hz, 1H, ArH), 3.80 (s, 3H, CH_3), 3.71 (t, J = 4.80 Hz, 4H, CH_2), 2.98 (t, J = 4.80 Hz, 4H, CH_2). ^{13}C NMR (DMSO-d_6, 75.46 MHz) δ: 165.98，138.67, 136.65, 134.34, 131.65, 129.36, 127.65, 124.98, 122.32, 120.76, 110.49, 108.34, 46.36, 42.34, 17.33. ESI-MS: m/z 464.3 (M^+). Anal. Calcd for $C_{20}H_{20}BrN_3O_3S$: C, 51.95; H, 4.36; N, 9.09. Found: C, 62.63; H, 4.72; N, 8.54.

(*E*)-(4-((4-Chlorophenyl)sulfonyl)piperazin-1-yl)(1-methyl-1H-indol-3-yl)methanone (9).

White solid, yield 62%，m.p. 190~191 ˚C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.78~7.69 (m, 5H, ArH, CHN), 7.64 (d, J = 7.87 Hz, 1H, ArH), 7.48 (d, J = 8.13 Hz, 1H, ArH), 7.21 (t, J = 7.60 Hz, 1H, ArH), 7.12 (t, J = 7.73 Hz, 1H, ArH), 3.79 (s, 3H, CH_3), 3.71 (t, J = 4.80 Hz, 4H, CH_2), 2.99 (t, J = 4.80 Hz, 4H, CH_2). ^{13}C NMR (DMSO-d_6, 75.46 MHz) δ: 165.43，138.87, 136.05, 134.87, 131.23, 129.46, 127.62, 124.32, 122.22, 120.65, 110.79, 108.64, 46.86, 42.25, 18.93. ESI-MS: m/z 417.9 (M^+). Anal. Calcd for $C_{20}H_{20}ClN_3O_3S$: C, 57.48; H, 4.82; N, 10.06. Found: C, 68.21; H, 4.92; N, 8.87.

(*E*)-(1-Methyl-1H-indol-3-yl)(4-tosylpiperazin-1-yl)methanone (10).

White solid, yield 59%, m.p. 204~206 ˚C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.71 (s, 1H, ArH), 7.63 (d, J = 8.13 Hz, 3H, ArH, CHN), 7.46 (d, J = 6.67 Hz, 3H, ArH), 7.21 (t, J = 7.47 Hz, 1H, ArH), 7.11 (t, J = 7.47 Hz, 1H, ArH), 3.79 (s, 3H, CH_3), 3.71 (t, J = 5.07 Hz, 4H, CH_2), 2.93 (t, J = 4.80 Hz, 4H, CH_2), 2.41 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6, 75.46 MHz) δ: 165.76，138.65, 136.23, 134.43, 130.25, 129.86, 127.22, 124.53, 122.82, 120.32, 110.73, 108.65, 46.73, 42.28, 18.13. ESI-MS: m/z 397.5 (M^+). Anal. Calcd for $C_{21}H_{23}N_3O_3S$: C, 63.46; H, 5.83; N, 10.57. Found: C, 63.16; H, 5.21; N, 8.83.

(*E*)-(1-Methyl-1H-indol-3-yl)(4-((4-nitrophenyl)sulfonyl)piperazin-1-yl)methanone (11).

Light orange solid, yield 63%, m.p. 182~184 ˚C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.45 (d, J = 8.67 Hz, 2H, ArH, CHN), 8.02 (d, J = 8.67 Hz, 2H, ArH), 7.70 (s, 1H, ArH), 7.64 (d, J = 7.87 Hz, 1H, ArH), 7.48 (d, J = 8.13 Hz, 1H, ArH), 7.21 (t, J = 7.60 Hz, 1H, ArH), 7.11 (t, J = 7.47 Hz, 1H, ArH), 3.79 (s, 3H, CH_3), 3.73 (t, J = 4.67 Hz, 4H, CH_2), 3.06 (t, J = 4.40 Hz, 4H, CH_2). ^{13}C NMR (DMSO-d_6, 75.46 MHz) δ: 165.82, 138.65, 136.13, 134.87, 130.65, 129.42, 127.32, 124.65, 122.32, 120.67, 110.72, 108.23, 46.76, 42.98, 18.73. ESI-MS: m/z 428.5 (M^+). Anal. Calcd for $C_{20}H_{20}N_4O_5S$: C, 56.07; H, 4.71; N, 13.08. Found: C, 62.21; H, 4.89; N, 8.67.

(*E*)-(1-Methyl-1H-indol-3-yl)(4-((2,4,6-triisopropylphenyl)sulfonyl)piperazin-1-yl)methanone (12).

White solid, yield 65%, m.p. 155~157 ˚C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.81 (s, 1H, ArH), 7.71 (d, J = 7.87 Hz, 1H, CHN), 7.49 (d, J = 8.13 Hz, 1H, ArH), 7.32 (d, J = 7.60 Hz, 2H, ArH), 7.22 (t, J = 7.33 Hz, 1H, ArH), 7.14 (t, J = 7.47 Hz, 1H, ArH), 4.13~4.06 (m, 2H, CH), 3.82 (s, 3H, CH_3), 3.68 (t, J = 4.53 Hz, 4H, CH_2), 3.13 (t, J = 4.53 Hz, 4H, CH_2), 2.98~2.91 (m, 3H, CH), 1.22 (d, J = 6.80 Hz, 18H, CH_3). ^{13}C NMR (DMSO-d_6, 75.46 MHz) δ: 165.82, 153.84, 151.54, 136.73, 134.43, 130.91, 129.02, 127.42, 124.43, 122.02, 120.96, 110.75, 108.34, 46.53, 42.12, 33.83, 29.22, 25.42, 23.32, 18.73. ESI-MS: m/z 509.7 (M^+). Anal. Calcd for $C_{29}H_{39}N_3O_3S$: C, 68.34;

H, 7.71; N, 8.24. Found: C, 68.54; H, 7.82; N, 8.12.

(*E*)-(1-Ethyl-1H-indol-3-yl)(4-(phenylsulfonyl)piperazin-1-yl)methanone (13).

Orange solid, yield 63%, m.p. 112~115 ˚C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.77~7.62 (m, 7H, ArH, CHN), 7.48 (d, J = 7.20 Hz, 1H, ArH), 7.21 (t, J = 7.60 Hz, 1H, ArH), 7.11 (t, J = 7.40 Hz, 1H, ArH), 3.79 (s, 3H, CH_3), 3.72 (t, J = 5.00 Hz, 4H, CH_2), 2.96 (t, J = 8.80 Hz, 4H, CH_2), 2.51 (s, 2H, CH_2). ^{13}C NMR (DMSO-d_6, 75.46 MHz) δ: 165.98, 138.34, 135.52, 134.06, 131.14, 130.53, 129.33, 127.42, 122.24, 120.33, 110.43, 108.44, 45.23, 41.34, 15.32. ESI-MS: m/z 397.5 (M^+). Anal. Calcd for $C_{21}H_{23}N_3O_3S$: C, 63.46; H, 5.83; N, 10.57. Found: C, 63.01; H, 5.52; N, 8.76.

(*E*)-(4-((4-Bromophenyl)sulfonyl)piperazin-1-yl)(1-ethyl-1H-indol-3-yl)methanone (14).

White solid, yield 56%, m.p. 142~143 ˚C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.61 (d, J = 8.27 Hz, 2H, ArH, CHN), 7.46 (d, J = 8.13 Hz, 2H, ArH,), 7.28 (t, J = 7.20 Hz, 2H, ArH), 7.22 (t, J = 6.00 Hz, 3H, ArH), 7.64 (d, J = 7.87 Hz, 1H, ArH), 7.48 (d, J = 8.27 Hz, 1H, ArH), 7.21 (t, J = 7.73 Hz, 1H, ArH), 3.44 (s, 2H, CH_2), 2.84 (s, 4H, CH_2), 2.42 (s, 4H, CH_2). ^{13}C NMR (DMSO-d_6, 75.46 MHz) δ: 165.54, 138.36, 135.28, 134.45, 131.24, 130.16, 129.93, 127.93, 122.34, 120.37, 110.82, 108.24, 46.18, 41.54, 15.74. ESI-MS: m/z 476.4 (M^+). Anal. Calcd for $C_{21}H_{22}BrN_3O_3S$: C, 52.95; H, 4.66; N, 8.82. Found: C, 62.91; H, 4.32; N, 8.12.

(*E*)-(4-((4-Chlorophenyl)sulfonyl)piperazin-1-yl)(1-ethyl-1H-indol-3-yl)methanone (15).

White solid, yield 64%, m.p. 155~158 ˚C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.78~7.72 (m, 5H, ArH, CHN), 7.64 (d, J = 8.00 Hz, 1H, ArH), 7.53 (d, J = 8.67 Hz, 1H, ArH), 7.19 (t, J = 7.60 Hz, 1H, ArH), 7.11 (t, J = 7.60 Hz, 1H, ArH), 4.22 (q, J = 7.20 Hz, 2H, CH_2), 3.72 (t, J = 4.67 Hz, 4H, CH_2), 2.99 (t, J = 4.67 Hz, 4H, CH_2), 1.36 (t, J = 7.20 Hz, 3H, CH_3). ^{13}C NMR (DMSO-d_6, 75.46 MHz) δ: 165.86, 138.86, 135.68, 134.42, 131.44, 130.16, 129.93, 126.93, 122.44, 120.97, 110.80, 108.60,

46.48, 41.14, 15.77. ESI-MS: m/z 431.9 (M^+). Anal. Calcd for $C_{21}H_{22}ClN_3O_3S$: C, 58.40; H, 5.13; N, 9.73. Found: C, 68.87; H, 7.81; N, 8.01.

(*E*)-(1-Ethyl-1H-indol-3-yl)(4-tosylpiperazin-1-yl)methanone (16).

White solid, yield 65%, m.p. 182~185 ˚C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.76 (s, 1H, ArH), 7.63 (d, J = 8.00 Hz, 3H, ArH, CHN), 7.52 (d, J = 8.27 Hz, 1H, ArH), 7.46 (d, J = 8.00 Hz, 2H, ArH), 7.19 (t, J = 7.60 Hz, 1H, ArH), 7.10 (t, J = 7.60 Hz, 1H, ArH), 4.20 (q, J = 7.33 Hz, 2H, CH_2), 3.71 (t, J = 4.80 Hz, 4H, CH_2), 2.94 (t, J = 4.53 Hz, 4H, CH_2), 2.41 (s, 3H, CH_3), 1.35 (t, J = 7.20 Hz, 3H, CH_3). ^{13}C NMR (DMSO-d_6, 75.46 MHz) δ: 165.68, 138.54, 135.73, 134.36, 131.43, 129.91, 126.03, 122.63, 121.64, 120.36, 110.34, 108.53, 44.53, 41.35, 15.43. ESI-MS: m/z 411.5 (M^+). Anal. Calcd for $C_{22}H_{25}N_3O_3S$: C, 64.21; H, 6.12; N, 10.21. Found: C, 63.78; H, 5.43; N, 8.65.

(*E*)- (1-Ethyl-1H-indol-3-yl)(4-((4-nitrophenyl)sulfonyl)piperazin-1-yl)methanone (17).

Yellow solid, yield 53%, m.p. 162~165 ˚C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.46 (d, J = 8.80 Hz, 2H, ArH, CHN), 8.03 (d, J = 9.07 Hz, 2H, ArH), 7.75 (s, 1H, ArH), 7.64 (d, J = 7.87 Hz, 1H, ArH), 7.53 (d, J = 8.40 Hz, 1H, ArH), 7.20 (t, J = 7.60 Hz, 1H, ArH), 7.10 (t, J = 7.60 Hz, 1H, ArH), 4.22 (q, J = 7.20 Hz, 2H, CH_2), 3.75 (t, J = 4.80 Hz, 4H, CH_2), 3.07 (t, J = 4.67 Hz, 4H, CH_2), 1.36 (t, J = 7.07 Hz, 3H, CH_3). ^{13}C NMR (DMSO-d_6, 75.46 MHz) δ: 165.34, 138.54, 135.12, 134.32, 131.65, 129.21, 126.23, 122.32, 121.62, 120.98, 110.36, 108.54, 44.65, 41.45, 15.67. ESI-MS: m/z 442.5 (M^+). Anal. Calcd for $C_{21}H_{22}N_4O_5S$: C, 57.00; H, 5.01; N, 12.66. Found: C, 68.20; H, 5.04; N, 8.03.

(*E*)-(1-Ethyl-1H-indol-3-yl)(4-((2,4,6-triisopropylphenyl)sulfonyl)piperazin-1-yl)methanone (18).

White solid, yield 53%, m.p. 192~193 ˚C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.85 (s, 1H, ArH), 7.72 (d, J = 7.47 Hz, 1H, CHN), 7.54 (d, J = 8.27 Hz, 1H, ArH), 7.31 (s,

2H, ArH), 7.21 (t, *J* = 7.60 Hz, 1H, ArH), 7.13 (t, *J* = 7.33 Hz, 1H, ArH), 4.24 (q, *J* = 7.07 Hz, 2H, CH_2), 4.14~4.07 (m, 2H, CH), 3.70 (t, *J* = 4.53 Hz, 4H, CH_2), 3.14 (t, *J* = 4.67 Hz, 4H, CH_2), 2.99~2.89 (m, 3H, CH), 1.38 (t, *J* = 7.20 Hz, 3H, CH_3), 1.22 (d, *J* = 8.00 Hz, 18H, CH_3). ^{13}C NMR (DMSO-d_6, 75.46 MHz) δ: 165.88, 153.81, 151.69, 135.74, 131.49, 129.91, 127.07, 124.44, 122.43, 121.05, 120.96, 110.79, 108.71, 44.56, 41.15, 33.86, 29.29, 25.05, 23.82, 15.76. ESI-MS: m/z 523.7 (M^+). Anal. Calcd for $C_{30}H_{41}N_3O_3S$: C, 68.80; H, 7.89; N, 8.02. Found: C, 68.62; H, 7.89; N, 8.04.

(*E*)-(4-(Phenylsulfonyl)piperazin-1-yl)(1-propyl-1H-indol-3-yl)methanone (19).

White solid, yield 63%, m.p. 182~185 ˚C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.98 (d, *J* = 8.80 Hz, 1H, ArH), 7.83 (s, 1H, CHN), 7.71~7.67 (m, 4H, ArH), 7.56 (d, *J* = 6.80 Hz, 2H, ArH), 7.22 (t, *J* = 7.20 Hz, 1H, ArH), 7.14 (t, *J* = 7.33 Hz, 1H, ArH), 4.17 (t, *J* = 7.33 Hz, 2H, CH_2), 3.76 (t, *J* = 5.07 Hz, 4H, CH_2), 3.07 (t, *J* = 4.80 Hz, 4H, CH_2), 1.87~1.78 (m, 2H, CH_2), 0.87 (t, *J* = 7.73 Hz, 3H, CH_3). ^{13}C NMR (DMSO-d_6, 75.46 MHz) δ: 165.58, 138.43, 136.03, 132.64, 130.32, 129.32, 127.24, 124.64, 122.27, 121.86, 120.82, 110.34, 108.65, 48.23, 45.52, 23.43, 11.24. ESI-MS: m/z 412.2 (M^+). Anal. Calcd for $C_{22}H_{25}N_3O_3S$: C, 64.21; H, 6.12; N, 10.21. Found: C, 64.68; H, 6.32; N, 10.43.

(*E*)-(4-((4-Bromophenyl)sulfonyl)piperazin-1-yl)(1-propyl-1H-indol-3-yl)methanone (20).

Light yellow solid, yield 63%, m.p. 189~191 ˚C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.88 (d, *J* = 8.40 Hz, 2H, ArH, CHN), 7.76 (s, 1H, ArH), 7.69~7.64 (m, 3H, ArH), 7.53 (d, *J* = 8.13 Hz, 1H, ArH), 7.19 (t, *J* = 7.60 Hz, 1H, ArH), 7.10 (t, *J* = 7.33 Hz, 1H, ArH), 4.15 (t, *J* = 7.07 Hz, 2H, CH_2), 3.72 (t, *J* = 4.67 Hz, 4H, CH_2), 3.00 (t, *J* = 4.80 Hz, 4H, CH_2), 1.82~1.72 (m, 2H, CH_2), 0.83 (t, *J* = 7.47 Hz, 3H, CH_3). ^{13}C NMR (DMSO-d_6, 75.46 MHz) δ: 165.98, 138.56, 136.33, 132.24, 130.76, 129.45, 127.34, 124.33, 122.87, 121.36, 120.87, 110.44, 108.87, 48.33, 44.32, 23.63, 11.27. ESI-MS: m/z 490.4 (M^+). Anal. Calcd for $C_{22}H_{24}BrN_3O_3S$: C, 53.88; H, 4.93; N, 8.57. Found: C, 68.02; H, 5.11; N, 8.61.

(*E*)-(4-((4-Chlorophenyl)sulfonyl)piperazin-1-yl)(1-propyl-1H-indol-3-yl)methanone (21).

White solid, yield 48%, m.p. 166~168 ˚C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.78~7.72 (m, 5H, ArH, CHN), 7.65 (d, J = 8.13 Hz, 1H, ArH), 7.53 (d, J = 8.27 Hz, 1H, ArH), 7.19 (t, J = 7.73 Hz, 1H, ArH), 7.10 (t, J = 7.47 Hz, 1H, ArH), 4.15 (t, J = 7.07 Hz, 2H, CH_2), 3.72 (t, J = 4.80 Hz, 4H, CH_2), 3.00 (t, J = 4.80 Hz, 4H, CH_2), 1.81~1.72 (m, 2H, CH_2), 0.83 (t, J = 7.47 Hz, 3H, CH_3). ^{13}C NMR (DMSO-d_6, 75.46 MHz) δ: 165.56, 138.74, 136.45, 132.64, 130.23, 129.98, 127.54, 124.35, 122.55, 121.34, 120.22, 110.54, 108.75, 48.63, 45.35, 23.64, 11.47. ESI-MS: m/z 445.9 (M^+). Anal. Calcd for $C_{22}H_{24}ClN_3O_3S$: C, 59.25; H, 5.42; N, 9.42. Found: C, 68.01; H, 5.05; N, 8.01.

(*E*)-(1-Propyl-1H-indol-3-yl)(4-tosylpiperazin-1-yl)methanone (22).

Brown solid, yield 59%, m.p. 134~135 ˚C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.75 (s, 1H, ArH), 7.64 (d, J = 8.13 Hz, 3H, ArH, CHN), 7.52 (d, J = 8.13 Hz, 1H, ArH), 7.46 (d, J = 8.00 Hz, 2H, ArH), 7.18 (t, J = 7.60 Hz, 1H, ArH), 7.09 (t, J = 7.60 Hz, 1H, ArH), 4.14 (t, J = 7.07 Hz, 2H, CH_2), 3.71 (t, J = 4.80 Hz, 4H, CH_2), 2.94 (t, J = 4.40 Hz, 4H, CH_2), 2.41 (s, 3H, CH_3), 1.81~1.72 (m, 2H, CH_2), 0.82 (t, J = 7.47 Hz, 3H, CH_3). ^{13}C NMR (DMSO-d_6, 75.46 MHz) δ: 165.82, 138.34, 136.47, 132.23, 130.65, 129.64, 127.35, 124.45, 122.56, 121.53, 120.82, 110.32, 108.53, 48.83, 44.56, 23.23, 11.87. ESI-MS: m/z 425.6 (M^+). Anal. Calcd for $C_{23}H_{27}N_3O_3S$: C, 64.92; H, 6.40; N, 9.87. Found: C, 64.23; H, 6.56; N, 8.82.

(*E*)-(4-((4-Nitrophenyl)sulfonyl)piperazin-1-yl)(1-propyl-1H-indol-3-yl)methanone (23).

Orange solid, yield 58%, m.p. 121~123 ˚C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.45(d, J = 8.80 Hz, 2H, ArH, CHN), 8.03 (d, J = 8.80 Hz, 2H, ArH), 7.74 (s, 1H, ArH), 7.64 (d, J = 8.00 Hz, 1H, ArH), 7.53 (d, J = 8.13 Hz, 1H, ArH), 7.18 (t, J = 7.73 Hz, 1H, ArH), 7.09 (t, J = 7.47 Hz, 1H, ArH), 4.15 (t, J = 7.07 Hz, 2H, CH_2),

3.73 (t, J = 4.67 Hz, 4H, CH_2), 3.07 (t, J = 4.53 Hz, 4H, CH_2), 1.81~1.72 (m, 2H, CH_2), 0.82 (t, J = 7.33 Hz, 3H, CH_3). ^{13}C NMR (DMSO-d_6, 75.46 MHz) δ: 165.91, 138.21, 136.17, 132.63, 130.34, 129.61, 127.65, 124.45, 122.86, 121.41, 120.65, 110.42, 108.34, 48.84, 44.65, 23.43, 11.56. ESI-MS: m/z 456.5 (M^+). Anal. Calcd for $C_{22}H_{24}N_4O_5S$: C, 57.88; H, 5.30; N, 12.27. Found: C, 67.82; H, 5.02; N, 8.03.

(*E*)-(1-Propyl-1H-indol-3-yl)(4-((2,4,6-triisopropylphenyl)sulfonyl)piperazin-1-yl)methanone (24).

White solid, yield 54%, m.p. 168~169 ˚C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.84 (s, 1H, ArH), 7.71 (d, J = 7.87 Hz, 1H, CHN), 7.55 (d, J = 8.27 Hz, 1H, ArH), 7.31 (s, 2H, ArH), 7.20 (t, J = 7.60 Hz, 1H, ArH), 7.12 (t, J = 7.47 Hz, 1H, ArH), 4.17 (t, J = 7.07 Hz, 2H, CH_2), 4.13~4.06 (m, 2H, CH), 3.69 (t, J = 4.67 Hz, 4H, CH_2), 3.13 (t, J = 4.53 Hz, 4H, CH_2), 2.98~2.91 (m, 1H, CH), 1.83~1.74 (m, 2H, CH_2), 1.22 (d, J = 7.07 Hz, 18H, CH_3), 0.84(t, J = 7.47 Hz, 3H, CH_3). ^{13}C NMR (DMSO-d_6, 75.46 MHz) δ: 165.91, 153.80, 151.69, 136.10, 132.07, 129.91, 127.01, 124.44, 122.41, 121.01, 120.92, 110.92, 108.57, 47.80, 44.55, 33.86, 29.29, 25.05, 23.81, 23.43, 11.56. ESI-MS: m/z 537.8 (M^+). Anal. Calcd for $C_{31}H_{43}N_3O_3S$: C, 69.24; H, 8.06; N, 7.81. Found: C, 68.35; H, 8.01; N, 7.92.

(*E*)-(1-Benzyl-1H-indol-3-yl)(4-(phenylsulfonyl)piperazin-1-yl)methanone (25).

White solid, yield 73%, m.p. 185~188 ˚C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.46 (d, J = 8.80 Hz, 2H, ArH, CHN), 8.03 (d, J = 9.07 Hz, 2H, ArH), 7.75 (s, 1H, ArH), 7.64 (d, J = 7.87 Hz, 1H, ArH), 7.53 (d, J = 8.40 Hz, 1H, ArH), 7.20 (t, J = 7.60 Hz, 1H, ArH), 7.10 (t, J = 7.60 Hz, 1H, ArH), 4.22 (q, J = 7.20 Hz, 2H, CH_2), 3.75 (t, J = 4.80 Hz, 4H, CH_2), 3.07 (t, J = 4.67 Hz, 4H, CH_2), 1.36 (t, J = 7.07 Hz, 3H, CH_3). ^{13}C NMR (DMSO-d_6, 75.46 MHz) δ: 165.82, 138.86, 137.65, 136.23, 134.43, 132.22, 130.27, 129.43, 127.64, 127.43, 122.43, 121.54, 121.43, 111.65, 109.43, 49.38, 45.34. ESI-MS: m/z 442.5 (M^+). Anal. Calcd for $C_{26}H_{25}N_3O_3S$: C, 67.95; H, 5.48; N, 9.14. Found: C, 68.02; H, 5.44; N, 8.32.

(*E*)-(1-Benzyl-1H-indol-3-yl)(4-((4-bromophenyl)sulfonyl)piperazin-1-yl)methanone (26).

Light yellow solid, yield 53%, m.p. 170~173 ˚C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.94 (s, 1H, ArH), 7.88 (q, J = 8.53 Hz, 2H, ArH, CHN), 7.70~7.65 (m, 3H, ArH), 7.48 (d, J = 8.13 Hz, 1H, ArH), 7.33~7.23 (m, 5H, ArH), 7.17~7.08 (m, 2H, ArH), 5.44 (s, 2H, CH_2), 3.74 (t, J = 4.80 Hz, 4H, CH_2), 3.01 (t, J = 4.80 Hz, 4H, CH_2). ^{13}C NMR (DMSO-d_6, 75.46 MHz) δ: 165.42, 138.57, 137.43, 136.35, 134.45, 132.32, 130.07, 129.72, 127.66, 127.55, 122.45, 121.34, 121.23, 111.22, 109.33, 49.34, 46.34. ESI-MS: m/z 494.0 (M^+). Anal. Calcd for $C_{26}H_{24}BrN_3O_3S$: C, 58.00; H, 4.49; N, 7.80. Found: C, 63.98; H, 5.03; N, 7.92.

(*E*)-(1-Benzyl-1H-indol-3-yl)(4-((4-chlorophenyl)sulfonyl)piperazin-1-yl)methanone (27).

White solid, yield 52%, m.p. 188~191 ˚C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.92 (s, 1H, ArH), 7.75 (q, J = 8.53 Hz, 4H, ArH, CHN), 7.66 (d, J = 7.87 Hz, 1H, ArH), 7.47 (d, J = 8.13 Hz, 1H, ArH), 7.32~7.23 (m, 5H, ArH), 7.17~7.7 (m, 2H, ArH), 5.44 (s, 2H, CH_2), 3.74 (t, J = 4.53 Hz, 4H, CH_2), 3.02 (t, J = 4.67 Hz, 4H, CH_2). ^{13}C NMR (DMSO-d_6, 75.46 MHz) δ: 165.72, 138.87, 137.88, 136.01, 134.46, 132.30, 130.17, 129.92, 127.64, 127.14, 122.65, 121.15, 121.03, 111.29, 109.03, 49.84, 46.46. ESI-MS: m/z 494.0 (M^+). Anal. Calcd for $C_{26}H_{24}ClN_3O_3S$: C, 63.22; H, 4.90; N, 8.51. Found: C, 63.00; H, 5.23; N, 8.40.

(*E*)-(2-Benzyl-1H-indol-3-yl)(4-tosylpiperazin-1-yl)methanone (28).

Orange solid, yield 47%, m.p. 189~191 ˚C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.46 (d, J = 8.80 Hz, 2H, ArH, CHN), 8.03 (d, J = 9.07 Hz, 2H, ArH), 7.75 (s, 1H, ArH), 7.64 (d, J = 7.87 Hz, 1H, ArH), 7.53 (d, J = 8.40 Hz, 1H, ArH), 7.20 (t, J = 7.60 Hz, 1H, ArH), 7.10 (t, J = 7.60 Hz, 1H, ArH), 4.22 (q, J = 7.20 Hz, 2H, CH_2), 3.75 (t, J = 4.80 Hz, 4H, CH_2), 3.07 (t, J = 4.67 Hz, 4H, CH_2), 1.36 (t, J = 7.07 Hz, 3H, CH_3). ^{13}C NMR (DMSO-d_6, 75.46 MHz) δ: 165.58, 138.44, 137.43, 136.64, 134.64, 132.52, 130.56, 129.64, 129.54, 128.43, 127.53, 127.57, 122.53, 121.43, 121.14, 111.24,

109.34, 49.37, 46.43. ESI-MS: m/z 442.5 (M^+). Anal. Calcd for $C_{27}H_{27}N_3O_3S$: C, 68.48; H, 5.75; N, 8.87. Found: C, 68.12; H, 5.82; N, 8.67.

(*E*)-(1-Benzyl-1H-indol-3-yl)(4-((4-nitrophenyl)sulfonyl)piperazin-1-yl)methanone (29)

Yellow solid, yield 47%, m.p. 197~199 ˚C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.46 (d, J = 8.67 Hz, 2H, ArH, CHN), 8.03 (d, J = 8.80 Hz, 2H, ArH), 7.91 (s, 1H, ArH), 7.65 (d, J = 7.73 Hz, 1H, ArH), 7.48 (d, J = 7.87 Hz, 1H, ArH), 7.33~7.23 (m, 5H, ArH), 7.17~7.07(m, 2H, ArH), 5.44 (s, 2H, CH_2), 3.75 (t, J = 4.80 Hz, 4H, CH_2), 3.09 (t, J = 4.67 Hz, 4H, CH_2). ^{13}C NMR (DMSO-d_6, 75.46 MHz) δ: 165.78, 138.43, 137.73, 136.24, 134.43, 132.92, 130.26, 129.21, 129.41, 128.21, 127.55, 127.61, 122.13, 121.33, 121.12, 111.29, 109.64, 49.27, 46.76. ESI-MS: m/z 504.6 (M^+). Anal. Calcd for $C_{26}H_{24}N_4O_5S$: C, 61.89; H, 4.79; N, 11.10. Found: C, 61.17; H, 4.92; N, 10.89.

(*E*)-(1-Benzyl-1H-indol-3-yl)(4-((2,4,6-triisopropylphenyl)sulfonyl)piperazin-1-yl)methanone (30).

White solid, yield 64%, m.p. 160~161 ˚C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.02 (s, 1H, ArH), 7.72 (q, J = 7.87 Hz, 1H, CHN), 7.49 (d, J = 7.87 Hz, 1H, ArH), 7.33~7.24 (m, 7H, ArH), 7.18~7.09 (m, 2H, ArH), 5.45 (s, 2H, CH_2), 4.14~4.07 (m, 2H, CH), 3.70 (t, J = 4.27 Hz, 4H, CH_2), 3.15 (t, J = 4.67 Hz, 4H, CH_2), 2.98~2.91 (m, 3H, CH), 1.22 (d, J = 4.27 Hz, 18H, CH_3). ^{13}C NMR (DMSO-d_6, 75.46 MHz) δ: 165.78, 153.82, 151.71, 137.90, 136.04, 132.40, 129.90, 129.06, 128.01, 127.71, 127.22, 124.45, 122.63, 121.15, 121.06, 111.29, 109.15, 49.87, 44.56, 33.87, 29.30, 25.06, 23.82. ESI-MS: m/z 585.8 (M^+). Anal. Calcd for $C_{35}H_{43}N_3O_3S$: C, 71.76; H, 7.40; N, 7.17. Found: C, 71.03; H, 7.43; N, 7.35.

Biological assays

Antiproliferation assays

Human cervical carcinoma cell (Hela), human breast cancer cell (MCF-7),

human liver cancer cell (HepG-2), human lung adenocarcinoma epithelial cell line (A549), and human renal epithelial cells (293T) incubated in DMEM/10% (V/V) fetal bovine serum, in the humidified atmosphere of 5% CO_2 at 37 ^oC. The cells were seeded into the 96-well plate (100 μL well^{-1}) giving 1000 cells/well respectively and incubated overnight. The synthesized compounds were initially prepared at 20 μM in DMSO and diluted at various concentrations. Each dilution (100 μL) were added into the wells and incubated for 24 h. Then 15 μL MTT was added into each well and incubated for 4 h. The MTT formazan precipitate was dissolved in 150 mL of DMSO. The optional absorbance was measured at a wavelength of 570 nm. Each assay was replicated for at least three times. The results were summarized in **Table 3.**

Effects on tubulin polymerization and on colchicine binding to tubulin

Bovine brain tubulin was purified as described previously.[27] To assess the effect of the compounds on tubulin assembly *in vitro*, varying concentrations of compounds were preincubated with 10 μM bovine brain tubulin in glutamate buffer at 30 ^oC and cooled to 0 ^oC subsequently. [27, 28]After being added 0.4 mM GTP, the mixtures were transferred to 0 ^oC cuvettes in a recording spectrophotometer and warmed up to 30 ^oC. Afterwards, tubulin polymerization was observed turbidimetrically at 350 nm. The IC_{50} was defined as the compound concentration that inhibited the extent of assembly by 50% after 20 min incubation.

Cell cycle assay

The synchronization was performed by incubating Hela cells (10^5 cells/well) in 12 well-plates for 12 h. Then cells were incubated with different concentrations of compound **18** (0, 0.1, 0.2, 0.4, 0.8 μM) for 24 h. The control cells were treated with DMSO. Meanwhile, Hela cells were treated with compound **18** (0.5 μM) at 37 ^oC for 0, 12, 24, 36 h in the time-dependent assay. After incubation, cells were centrifuged, washed with cold PBS and fixed with 70% ethanol at 4^{o}C overnight. The fixed cells were washed with PBS, stained with 100 μg mL^{-1} of RNase A and 50 μg mL^{-1} of propidium iodide (PI), and then subjected to flow cytometric analysis.[28]

Cell apoptosis assay

Approximately 10^5 cells/well of Hela cells were dispensed into a 12 well-plate and incubated for 12 h. Then the medium was replaced with fresh culture medium containing compound **18** at final concentrations of 0, 0.1, 0.2, 0.4 and 0.8 μM for 24 h, and treated cells with compound **18** (0.5 μM) for 0, 12, 24, 36 h. Then cells were harvested after 24 h. They were trypsinized, washed with PBS and centrifuged at 2800 rpm for 5 min. The cells were then resuspended in 500 μL of staining solution (containing 5 μL Annexin V-PE and 5 μL PI in Binding Buffer), mixed gently and incubated for 15 min at room temperature in dark. [29] The samples were then read in a BD Accuri C6 Flow Cytometer (BD, USA). Statistical analysis was performed by the Flowjo 7.6.1 software.

Confocal microscopy assay

Hela cells were incubated on cover slips in the 6-well plates to 70% confluence and treated with 0 μM, 0.2 μM or 0.4 μM compound **18**, 0.2 μM paclitaxel and 0.2 μM colchicine for 24 h, respectively. After incubating for 24 h, Hela cells were washed with PBS for three times and fixed with 4% paraformaldehyde for 20 min, permeabilized with cold methanol for another 10 min. After that, the cells were blocked with 5% BSA for 1 h. Subsequently, the cells were washed with PBS, and incubated with anti-tubulin antibody in 3% BSA (1:200) overnight at 4 ^{o}C. After being washed with PBS for three times, each cover slip was added 200 μL of Cy3-labeled goat anti-mouse IgG (H+L) in 3% BSA (1:500) and incubated for 1 h at room temperature. At last, Hela cells was stained with 200 μL of DAPI for 5 min and observed under an Olympus confocal microscope.[30, 31]

Molecular Docking

Tubulin was chosen as the target receptor, of which the crystal structure was obtained from RCSB Protein Data Bank (PDB code: 1SA0). The three-dimensional X-ray structures of the synthesized compounds were constructed using Chem. 3D

ultra 12.0 software [Chemical Structure Drawing Standard; Cambridge Soft Corporation, USA (2010)]. The molecular docking procedure was performed by using CDOCKER protocol for receptor-ligand interactions section of Discovery Studio 3.5 (Accelrys Software ware Inc, Organic & Biomolecular Chemistry Page 20 of 3419 San Diego, CA40). For ligand preparation, the 3D structures of compounds were energetically minimized by using MMFF94 with 5000 iterations and minimum RMS gradient of 0.10. For protein preparation, all bound water and ligands were eliminated and the polar hydrogen was added. The whole tubulin was defined as a receptor and the site sphere was selected based on the ligand binding location of colchicine, then the molecule was removed and the synthesized compounds was placed during the molecular docking procedure. Types of interactions of the docked protein with ligand were analyzed after the end of molecular docking.

3 D-QSAR

To estimate the synthesized compounds as tubulin inhibitors systematically and explore more potent inhibitors, twenty-four compounds with definite IC_{50} values against tubulin were selected as the model dataset by using the Create 3D-QSAR protocol of Discovery Studio 3.5. 3D-QSAR model was built by using the corresponding pIC_{50} values which were converted from the obtained IC_{50} (μM) values of tubulin inhibition and performed by built-in QSAR software of DS 3.5 (Discovery Studio 3.5, Accelrys, Co. Ltd). The way of this transformation originated from an online calculator developed from an Indian medicinal chemistry lab (http://www.sanjeevslab.org/tools- IC_{50}.html).[24] The test and training set were chosen by the Diverse Molecules method in Discovery Studio. Considering a good alignment is important for the analysis of molecular fields, we applied CDOCKER protocol to explore each molecule with lowest energy before alignment conformation. Indole was selected as substructure to build alignment conformation before building the QSAR model. The test and training set were divided by the random diverse molecules method of DS 3.5, in which the test set accounts for 80% while the training set was set to 20%. The training set composes 19 agents and 5 agents were composed of the

relative test set, which had been listed in **Table 5**. The main purpose to create 3D-QSAR model was to choose activity conformation of the designed molecular and reasonably assessed the designed molecules. The success of this model depended on the reliability of previous study about activities data and docking study.[25]

In default situation, the alignment conformation of each molecule was the one that possessed the lowest CDOCKER_INTERACTION_ENENGY among the twenty-four docked poses. The 3D-QSAR model generated from DS 3.5, defined the critical regions (steric or electrostatic) affecting the binding affinity. It was a PLS model set up 400 independent variables (conventional R^2 = 0.9691). The predicted and observed values, corresponding residual values for the test set and training set molecules in 3D-QSAR model, were presented in **Table 5**. The well agreement between experimental pIC_{50} value and predicted pIC_{50} value for both test sets and training sets were shown in **Fig. 7.** Also the molecules aligned with the iso-surfaces of the 3D-QSAR model coefficients on van der Waals grids **(Fig. 8A)** and electrostatic potential grids **(Fig. 8B)** were listed. Electrostatic map demonstrated that red contours around regions where high electron density (negative charge) is expected to increase activity, and blue contours represented areas where low electron density (partial positive charge) is expected to increase activity. Similarly, steric map indicates areas where steric bulk is predicted to increase (green) or decrease (yellow) activity. It was widely acceptable that a better inhibitor in the 3D-QSAR model should have strong van der Waals attraction in the green areas and a polar group in the blue electrostatic potential areas (which were dominant close to the skeleton). As for the indole moiety and benzenesulfonyl group, they both are in appropriate size, the only point for further modification is that one of the substituent on the benzene ring of benzenesulfonyl group can introduce electron- donating substitute. Meanwhile, the size of Carbon chain on the N atom of indole ring should be shorter. The 3D QSAR model agrees with the inhibitory activity well and provide us the direction of further modification.

Ligand-based 3D-QSAR approach was performed by QSAR software of DS 3.5 (Discovery Studio 3.5, Accelrys, Co. Ltd). The training sets consisted of inhibitors with the corresponding pIC_{50} values which were transformed from the obtained IC_{50} (μM), and test sets comprised compounds of data sets as list in **Table 5**. All the definition of the descriptors can be seen in the "Help" of DS 3.5 software and they were calculated by QSAR protocol of DS 3.5. The alignment conformation of each molecule was the one with lowest interaction energy in the docked results of CDOCKER. The predictive ability of 3D-QSAR modeling can be evaluated based on the cross-validated correlation coefficient, which qualities the predictive ability of the models. Scrambled test (Y scrambling) was performed to investigate the risk of chance correlations. The inhibitory potencies of compounds were randomly reordered for 30 times and subject to leave-one-out validation test, respectively. The models were also validated by test sets, in which the compounds were not included in the training sets. Usually, one can believe that the modeling is reliable, when the R^2 for test sets is larger than 0.6.

Table 5. Experimental, predicted inhibitory activity of compounds **7–30** by 3D-QSAR models based upon active conformation achieved by molecular docking.

	Tubulin assembly[a]		
Compounds	Actual pIC_{50}	Predicted pIC_{50}	Residual error
7	5.559	5.477	0.082
8	5.402	5.552	-0.15
9	5.476	5.497	-0.021
10	5.668	5.563	0.105
11	5.449	5.496	-0.047
12	5.706	5.609	0.097
13	5.426	5.411	0.015

14	5.312	5.44	-0.128
15	4.348	4.551	-0.203
16	5.426	5.495	-0.069
17	5.258	5.306	-0.048
18	5.739	5.671	0.068
19	4.311	4.371	-0.06
20	4.561	4.67	-0.109
21	4.343	4.279	0.064
22	5.501	5.476	0.025
23	4.349	4.41	-0.061
24	5.542	5.561	-0.019
25	5.249	5.268	-0.019
26	5.171	5.14	0.031
27	5.221	5.205	0.016
28	5.519	5.566	-0.047
29	5.165	5.099	0.066
30	5.439	5.473	-0.034

[a] Italicised compounds were selected as the test sets, while the rest ones were in the training sets.

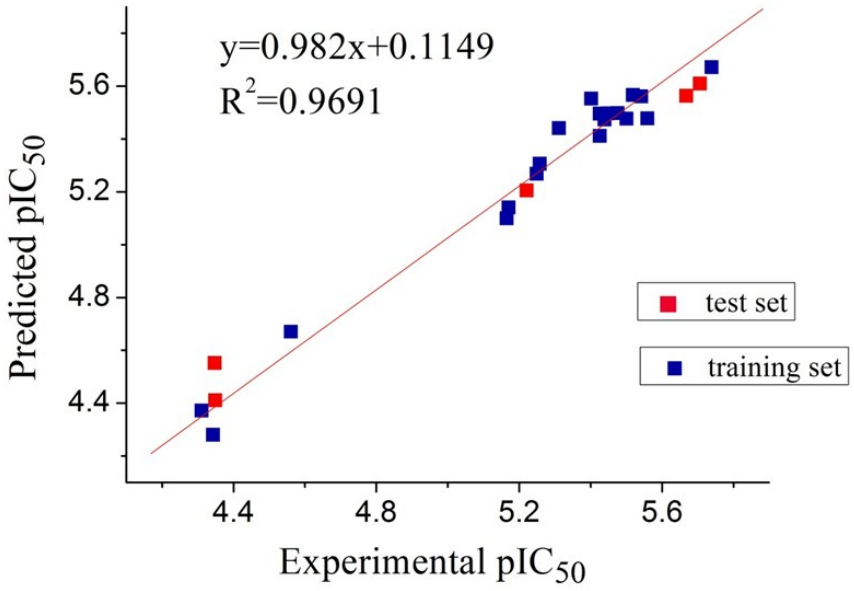

Fig. 7. Using linear fitting curve to compare the predicted pIC_{50} value (tubulin inhibitory activities) with that of experimental pIC_{50}.

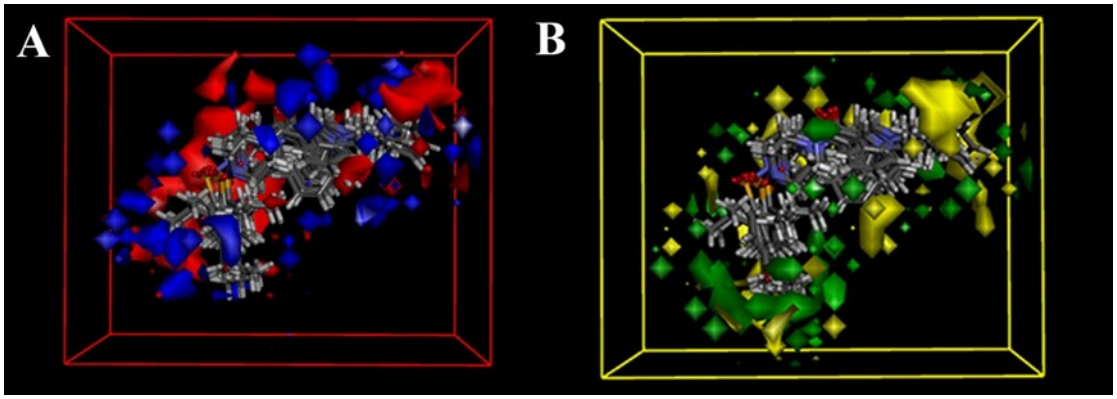

Fig. 8. (A) 3D-QSAR model coefficients on electrostatic potential grids. Blue represents positive coefficients; red represents negative coefficients. **(B)** 3D-QSAR model coefficients on van der Waals grids. Green represents positive coefficients; yellow represents negative coefficients.

Reference

27. J. R. Williams, J. C. Lee, *Methods Enzymology*, **1982**, *85*, 376-381.

28. E. Hamel, *Cell biochem. and biophy.* **2003**, *38*, 1-21.

29. Y. Luo, K.-M. Qiu, X. Lu, K. Liu, J. Fu, H.-L. Zhu, *Bioorg. & med. chem.* **2011**, *19*, 4730-4738.

30. S.-L. Zheng, Q. Zhong, M. Mottamal, Q. Zhang, C.-D. Zhang, E. LeMelle, H. McFerrin, G.-D. Wang, *J. Med. Chem..* **2014***, 57,* 3369−3381.

31. D. Simoni, R. Romagnoli, R. Baruchello, R. Rondanin, M. Rizzi, M.-G. Pavani, D. Alloatti, G. Giannini, M. Marcellini, T. Riccioni, M. Castorina, M.-B. Guglielmi, F. Bucci, P. Carminati, C. Pisano, *J. Med. Chem*. **2006**, *49*, 3143-3152.