Supplementary Information

Synthesis and antitumor evaluation of 5-(benzo[*d*][1,3]dioxol-5-ylmethyl) -4-(*tert*-butyl)-*N*-arylthiazol-2-amines

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1. Materials and instrumentation

Reagents and solvents were of analytical grade and were used without further purification. Compound 1-(benzo[d][1,3]dioxol-5-yl)-2-bromo-4,4-dimethylpentan-3-one (**A**) was synthesized according to the methods described in our previous studies[1]. 1-Arylthioureas (**B1**-**B31**) were prepared according to reported procedures[2]. Melting points were measured on an X-4 electrothermal digital melting point apparatus (China) and were uncorrected. The ^{1}H and ^{13}C NMR were recorded on a Varian INOVA 400NB NMR spectrometer (USA), using tetramethylsilane (TMS) as internal standard. Elemental analyses were performed on a Vario EL III (Germany) instrument.

2. Synthesis

2.1 General procedure for the synthesis of compounds C1-C31

2.1.1 *5-(Benzo[d][1,3]dioxol-5-ylmethyl)-4-(tert-butyl)-N-phenylthiazol-2-amine (**C1**) hydrochloride*

A solution of 0.626 g (2.0 mmol) compound **A** and 0.304 g (2.0 mmol) compound **B1** in 15 mL acetone was stirred for 8 h under reflux. Aqueous ammonia was then added to adjust pH = 7-8. The solution was concentrated under reduced pressure to remove the solvent. The residue was then purified purified by column chromatography on silica gel eluted with petroleum ether/ethyl acetate (8:1 v/v) to afford compound **C1** as yellow oil in 82% yield. The oil was dissolved in diethyl ether and dry HCl was bubbled into the solution to afford a hydrochloride of **C1** as white solid, m.p. 99–101 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.45 (s, 9H, 3×CH_3), 4.08 (s, 2H, CH_2), 5.95 (s, 2H, OCH_2O), 6.65 (d, J = 8.0 Hz, 1H, C_6H_3 6-H), 6.67 (s, 1H, C_6H_3 2-H), 6.75 (d, J = 8.0 Hz, 1H, C_6H_3 5-H), 7.09 (t, J = 8.0 Hz, 1H, C_6H_5 4-H), 7.27 (d, J = 8.0 Hz, C_6H_5 2,6-H), 7.33 (t, J = 8.0 Hz, C_6H_5 3,5-H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 30.29, 31.50, 35.44, 101.20, 108.53, 108.91, 118.19, 119.72, 121.50, 124.47, 129.82, 133.73, 139.44, 146.18, 147.65, 162.58. Anal. Calcd. (%): C 62.60, H 5.75, N 6.95, S 7.96, found (%): C 62.41, H 5.64, N 6.88, S 7.84.

*5-(Benzo[d][1,3]dioxol-5-ylmethyl)-4-(tert-butyl)-N-(o-tolyl)thiazol-2-amine (**C2**)*

Compound **C2** was synthesized using a method similar to that of compound **C1** and was gained as yellow solid in 61% yield, m.p. 99–101 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.45 (s, 9H, 3×CH_3), 2.34 (s, 3H, CH_3), 4.06 (s, 2H, CH_2), 5.94 (s, 2H, OCH_2O), 6.64–6.67 (m, 2H, C_6H_3 6,2-H), 6.74 (d, J = 8.0 Hz, 1H, C_6H_3 5-H), 7.08 (d, J = 8.0 Hz, 1H, C_6H_4), 7.17–7.21 (m, 3H, C_6H_4). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.44, 30.56, 31.59, 35.56, 101.18, 108.50, 108.86, 115.57, 117.90, 119.13, 121.40, 123.99, 129.46, 134.18, 138.95, 140.01, 146.13, 147.67, 161.51. Anal. Calcd. (%): C 69.44, H 6.36, N 7.36, S 8.43, found (%): C 69.21, H 6.18, N 7.23, S 8.27.

*5-(Benzo[d][1,3]dioxol-5-ylmethyl)-4-(tert-butyl)-N-(m-tolyl)thiazol-2-amine (**C3**) hydrochloride*

Compound **C3** was synthesized using a method similar to that of compound **C1** and was gained as yellow oil in 66% yield. The hydrochloride was obtained as white solid, m.p. 155–157 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.45 (s, 9H, 3×CH_3), 2.33 (s, 3H, CH_3), 4.08 (s, 2H, CH_2), 5.96 (s, 2H, OCH_2O), 6.64–6.67 (m, 2H, C_6H_3 6,2-H), 6.75 (d, J = 8.0 Hz, 1H, C_6H_3 5-H), 6.91 (d, J = 7.6 Hz, 1H, C_6H_4 4-H), 7.07 (d, J = 7.6 Hz, 1H, C_6H_4 6-H), 7.08 (s, 1H, C_6H_4 2-H), 7.22 (t, J = 8.0 Hz, 1H, C_6H_4 5-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 30.99, 32.60, 35.98, 100.92, 108.16, 108.70, 114.71, 117.76, 120.04, 121.05, 121.50, 124.54, 129.18, 134.58, 146.16, 147.77, 150.30, 153.80, 158.23. Anal. Calcd. (%): 63.37, H 6.04, N 6.72, S 7.69, found (%): C 63.21, H 5.90, N 6.58, S 7.56.

*5-(Benzo[d][1,3]dioxol-5-ylmethyl)-4-(tert-butyl)-N-(2-fluorophenyl)thiazol-2-amine (**C4**)*

Compound **C4** was synthesized using a method similar to that of compound **C1** and was gained as yellow solid in 70% yield, m.p. 110–111 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.42 (s, 9H, 3×CH_3), 4.12 (s, 2H, CH_2), 5.94 (s, 2H, OCH_2O), 6.67 (d, J = 8.0 Hz, 1H, C_6H_3 6-H), 6.70 (s, 1H, C_6H_3 2-H), 6.75 (d, J = 8.0 Hz, 1H, C_6H_3 5-H), 6.90–6.95 (m, 1H, C_6H_4), 7.05–7.13 (m, 2H, C_6H_4), 8.02–8.05 (m, 1H, C_6H_4). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 30.99, 32.60, 35.98, 100.92, 108.16, 108.70, 114.71 (d, J_{FCC} = 19.0 Hz), 117.76, 120.04, 121.05, 121.50, 124.54, 129.18 (d, J_{FCC} = 10.0 Hz), 134.58, 146.16, 147.77, 150.30, 153.80 (d, J_{FC} = 219.0 Hz), 158.23. Anal. Calcd. (%): 66.64, H 6.57, N 6.76, S 7.74, found (%): C 66.39, H 6.44, N 6.51, S 7.61.

*5-(Benzo[d][1,3]dioxol-5-ylmethyl)-4-(tert-butyl)-N-(4-fluorophenyl)thiazol-2-amine (**C5**)*

Compound **C5** was synthesized using a method similar to that of compound **C1** and was gained as yellow solid in 57% yield, m.p. 122–124 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ:1.53 (s, 9H, 3×CH_3), 4.04 (s, 2H, CH_2), 5.98 (s, 2H, OCH_2O), 6.61–6.63 (m, 2H, C_6H_3 6,2-H), 6.78 (d, J = 8.0 Hz, 1H, C_6H_3 5-H), 7.09 (t, J = 8.8 Hz, 2H, C_6H_4 3,5-H), 7.23–7.26 (m, 2H, C_6H_4 2,6-H), 13.63 (s, 1H, NH).^{13}C NMR (100 MHz, DMSO) δ: 30.43, 31.75, 35.65, 101.49, 108.81, 109.17, 116.88 (d, J_{FCC} = 22.0 Hz, 2×CH), 118.47, 121.81, 123.12 (2×CH), 129.18, 133.76, 135.88, 146.49, 147.92, 153.25, 159.58 (d, J_{FC} = 241.0 Hz). Anal. Calcd. (%): C 66.64, H 6.57, N 6.76, S 7.74, found (%): C 66.41, H 6.47, N 6.55, S 7.63.

*5-(Benzo[d][1,3]dioxol-5-ylmethyl)-4-(tert-butyl)-N-(2-chlorophenyl)thiazol-2-amine (**C6**)*

Compound **C6** was synthesized using a method similar to that of compound **C1** and was gained as yellow solid in 53% yield, m.p. 108–110 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.44 (s, 9H, 3×CH_3), 4.11 (s, 2H, CH_2), 5.95 (s, 2H, OCH_2O), 6.65–6.69 (m, 2H, C_6H_3 6,2-H), 6.75 (d, J = 8.0 Hz, 1H, C_6H_3 5-H), 6.97–7.14 (m, 4H, C_6H_4), 7.99 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 31.10, 31.77, 35.92, 101.10, 108.43, 108.77, 115.03, 115.22, 119.46, 121.24, 121.58, 124.74, 129.61, 135.14, 145.91, 147.57, 150.39, 153.66, 158.64. Anal. Calcd. (%): C 62.91, H 5.28, N 6.99, S 8.00, found (%): C 62.68, H 5.05, N 6.79, S 7.86

*5-(Benzo[d][1,3]dioxol-5-ylmethyl)-4-(tert-butyl)-N-(3-chlorophenyl)thiazol-2-amine (**C7**)*

A solution of 0.626 g (2.0 mmol) compound **A** and 0.304 g (2.0 mmol) compound **B7** in 15 mL ethanol was stirred for 8 h under reflux. Aqueous ammonia was then added to adjust pH = 7-8. The solution was concentrated under reduced pressure to remove some of the solvent. The solution was transfered into the refrigerator and then the crystal of compound **C7** was slowly formed in the solution. After filtration, the compound **C7** was obtained as yellow solid in 52% yield, m.p.105–106 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.42 (s, 9H, 3×CH_3), 4.11 (s, 2H, CH_2), 5.95 (s, 2H, OCH_2O), 6.66 (d, J = 8.0 Hz, 1H, C_6H_3 6-H), 6.69 (s, 1H, C_6H_3 2-H), 6.75 (d, J = 8.0z, 1H, C_6H_3 5-H), 6.98 (d, J = 8.0z, 1H, C_6H_4 6-H), 7.16 (d, J = 8.0 Hz, 1H, C_6H_4 4-H), 7.22 (t, J = 8.0z, 1H, C_6H_4 5-H), 7.43 (s, 1H, C_6H_4 2-H).^{13}C NMR (100 MHz, DMSO-d_6) δ: 30.79, 31.56, 35.73, 38.97, 39.18, 39.39, 39.60, 39.81, 40.02, 40.23, 100.94, 108.26, 108.59, 115.22, 116.29, 119.10, 120.40, 121.07, 130.49, 133.37, 134.75, 142.66, 145.77, 147.40, 153.25, 158.36. Anal. Calcd. (%): C 62.91, H 5.28, N 6.99, S 8.00, found (%): C 62.72, H 5.11, N 6.83, S 7.88.

*5-(Benzo[d][1,3]dioxol-5-ylmethyl)-4-(tert-butyl)-N-(4-chlorophenyl)thiazol-2-amine (**C8**)*

Compound **C8** was synthesized using a method similar to that of compound **C7** and was gained as yellow solid in 75% yield, m.p. 121–123 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.40 (s, 9H, 3×CH_3), 4.10 (s, 2H, CH_2), 5.94 (s, 2H, OCH_2O), 6.66 (d, J = 8.0 Hz, 1H, C_6H_3 6-H), 6.69 (s, 1H, C_6H_3 2-H), 6.74 (d, J = 8.0 Hz, 1H, C_6H_3 5-H), 7.23–7.28 (m, 4H, C_6H_4). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 30.98, 32.64, 35.92, 100.96, 108.20, 108.70, 118.55, 119.58, 121.07, 126.93, 129.21, 134.49, 139.15, 146.21, 147.81, 159.09. Anal. Calcd. (%): C 62.91, H 5.28, N 6.99, S 8.00, found (%): C 62.75, H 5.12, N 6.86, S 7.89.

*5-(Benzo[d][1,3]dioxol-5-ylmethyl)-N-(2-bromophenyl)-4-(tert-butyl)thiazol-2-amine (**C9**)*

Compound **C9** was synthesized using a method similar to that of compound **C7** and was gained as reddish brown solid in 75% yield, m.p.83–84 °C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ:1.33 (s, 9H, 3×CH_3), 4.07 (s, 2H, CH_2), 5.98 (s, 2H, OCH_2O), 6.68 (d, J = 8.0 Hz, 1H, C_6H_3 6-H), 6.77 (s, 1H, C_6H_3 2-H), 6.85 (d, J = 8.0 Hz, 1H, C_6H_3 5-H), 6.92 (t, J = 7.2 Hz, 1H, C_6H_4 4-

H), 7.33 (t, J = 7.2 Hz, 1H, C_6H_4 5-H), 7.58 (d, J = 8.0 Hz, 1H, C_6H_4 6-H), 8.21 (d, J = 8.0 Hz, 1H, C_6H_4 3-H), 9.04 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 31.00, 32.69, 36.03, 100.97, 108.21, 108.75, 117.72, 117.81, 120.55, 121.13, 122.61, 128.44, 132.51, 134.51, 138.29, 146.25, 147.83, 158.34. Anal. Calcd. (%): C 56.63, H 4.75, N 6.29, S 7.20, found (%): C 56.55, H 4.68, N 6.15, S 7.08.

*5-(Benzo[d][1,3]dioxol-5-ylmethyl)-N-(3-bromophenyl)-4-(tert-butyl)thiazol-2-amine (**C10**) hydrochloride*

Compound **C10** was synthesized using a method similar to that of compound **C1** and was gained as yellow oil in 59% yield. The hydrochloride was obtained as white solid, m.p.102–104 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.41 (s, 9H, 3×CH_3), 4.11 (s, 2H, CH_2), 5.95 (s, 2H, OCH_2O), 6.66 (d, J = 8.0 Hz, 1H, C_6H_3 6-H), 6.69 (s, 1H, C_6H_3 2-H), 6.75 (d, J = 8.0 Hz, 1H, C_6H_3 5-H), 7.12–7.23 (m, 3H, C_6H_4 4,5,6-H), 7.60 (s, 1H, C_6H_4 2-H).^{13}C NMR (100 MHz, DMSO-d_6), δ: 30.95, 31.72, 35.89, 101.13, 108.45, 108.79, 115.79, 119.25, 119.41, 121.27, 122.14, 123.52, 130.99, 134.90, 142.90, 145.97, 147.59, 154.29, 158.65. Anal. Calcd. (%): C 52.35, H 4.60, N 5.81, S 6.65, found (%): C 52.24, H 4.48, N 5.69, S 6.49.

*5-(Benzo[d][1,3]dioxol-5-ylmethyl)-N-(4-bromophenyl)-4-(tert-butyl)thiazol-2-amine (**C11**)*

Compound **C11** was synthesized using a method similar to that of compound **C1** and was gained as yellow solid in 52% yield, m.p. 118–120 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.41 (s, 9H, 3×CH_3), 4.10 (s, 2H, CH_2), 5.95 (s, 2H, OCH_2O), 6.66 (d, J = 8.0 Hz, 1H, C_6H_3 6-H), 6.69 (s, 1H, C_6H_3 2-H), 6.75 (d, J = 8.0 Hz, 1H, C_6H_3 5-H), 7.22 (d, J = 8.8 Hz, 2H, C_6H_4 2,6-H), 7.40 (d, J = 8.8 Hz, 2H, C_6H_4 3,5-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 31.00, 32.66, 35.94, 100.96, 108.20, 108.71, 114.14, 118.80, 119.73, 121.08, 132.11, 134.55, 139.72, 146.20, 147.81, 158.82. Anal. Calcd. (%): C 56.63, H 4.75, N 6.29, S 7.20, found (%): C 56.53, H 4.77, N 6.17, S 7.11.

*5-(Benzo[d][1,3]dioxol-5-ylmethyl)-4-(tert-butyl)-N-(3-(trifluoromethyl)phenyl)thiazol-2-amine (**C12**)*

Compound **C12** was synthesized using a method similar to that of compound **C1** and was gained as yellow solid in 59% yield, m.p. 90–92 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.42 (s, 9H, 3×CH_3), 4.11 (s, 2H, CH_2), 5.94 (s, 2H, OCH_2O), 6.66 (d, J = 8.0 Hz, 1H, C_6H_3 6-H), 6.69 (s, 1H, C_6H_3 2-H), 6.75 (d, J = 8.0 Hz, 1H, C_6H_3 5-H), 7.24 (d, J = 8.0 Hz, 1H, C_6H_4 6-H), 7.39 (t, J = 8.0 Hz, 1H, C_6H_4 5-H), 7.46 (d, J = 8.0 Hz, 1H, C_6H_4 4-H), 7.83 (s, 1H, C_6H_4 2-H). ^{13}C NMR (100 MHz, DMSO-d_6), δ: 30.94, 31.74, 35.89, 101.12, 108.44, 108.78, 112.90, 116.96, 119.50, 120.31, 121.25, 124.57 (q, J_{FC} = 270.0 Hz), 129.59 (q, J_{FC} = 32.0 Hz), 130.07, 134.97, 142.17, 145.96, 147.59, 153.60, 158.41. Anal. Calcd. (%): C 60.82, H 4.87, N 6.45, S 7.38, found (%): C 60.64, H 4.80, N 6.34, S 7.32.

*5-(Benzo[d][1,3]dioxol-5-ylmethyl)-4-(tert-butyl)-N-(4-(trifluoromethyl)phenyl)thiazol-2-amine (**C13**)*

Compound **C13** was synthesized using a method similar to that of compound **C1** and was gained as yellow solid in 75% yield, m.p. 96–98 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.43 (s, 9H, 3×CH_3), 4.12 (s, 2H, CH_2), 5.96 (s, 2H, OCH_2O), 6.67 (d, J = 8.0 Hz, 1H, C_6H_3 6-H), 6.69 (s, 1H, C_6H_3 2-H), 6.76 (d, J = 8.0 Hz, 1H, C_6H_3 5-H), 7.42 (d, J = 8.4 Hz, 2H, C_6H_4 2,6-H), 7.56 (d, J = 8.4 Hz, 2H, C_6H_4 3,5-H). ^{13}C NMR (100 MHz, DMSO-d_6), δ: 30.99, 31.77, 35.90, 101.13, 108.45, 108.80, 116.59, 119.83, 120.84 (q, J_{FC} = 33.0 Hz), 121.29 (2×CH), 124.91 (q, J_{FC} = 269.0 Hz), 126.38 (2CH), 134.87, 144.70, 145.99, 147.61, 153.30, 158.37. Anal. Calcd. (%): C 60.82, H 4.87, N 6.45, S 7.38, found (%): C 60.63, H 4.79, N 6.32, S 7.25.

*5-(Benzo[d][1,3]dioxol-5-ylmethyl)-4-(tert-butyl)-N-(4-methoxyphenyl)thiazol-2-amine (**C14**)*

Compound **C14** was synthesized using a method similar to that of compound **C7** and was gained as yellow solid in 47% yield, m.p.147.0–149.0 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ:1.34 (s, 9H, 3×CH_3), 3.73 (s, 3H, OCH_3), 4.05 (s, 2H, CH_2), 5.98 (s, 2H, OCH_2O), 6.67 (d, J = 8.0 Hz, 1H, C_6H_3 6-H), 6.75 (s, 1H, C_6H_3 2-H), 6.84 (d, J = 8.0 Hz, 1H, C_6H_3 5-H), 7.22 (d, J = 8.4 Hz, 2H, C_6H_4 3,5-H), 7.47 (d, J = 8.4 Hz, 2H, C_6H_4 2,6-H), 9.48 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 30.38, 31.77, 35.60, 55.87, 101.50, 108.84, 109.23, 114.41, 115.39, 117.99, 121.88, 123.76, 125.93, 132.26, 133.69, 146.52, 147.93, 157.57. Anal. Calcd. (%): C 66.64, H 6.10, N 7.07, S 8.09, found (%): C 66.38, H 6.13, N 7.00, S 8.01.

*5-(Benzo[d][1,3]dioxol-5-ylmethyl)-4-(tert-butyl)-N-(2-nitrophenyl)thiazol-2-amine (**C15**)*

Compound **C15** was synthesized using a method similar to that of compound **C7** and was gained as reddish brown solid in 57% yield, m.p.103.0–104.0 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.44 (s, 9H, 3×CH_3), 4.15 (s, 2H, CH_2), 5.95 (s, 2H, OCH_2O), 6.68 (d, J = 8.0 Hz, 1H, C_6H_3 6-H), 6.71 (s, 1H, C_6H_3 2-H), 6.76 (d, J = 8.0 Hz, 1H, C_6H_3 5-H), 6.97 (t, J = 7.2 Hz, 1H, C_6H_4 4-H), 7.60 (t, J = 7.2 Hz, 1H, C_6H_4 5-H), 8.23 (d, J = 8.8 Hz, 1H, C_6H_4 6-H), 8.75 (d, J = 8.8 Hz, 1H, C_6H_4 3-H), 10.52 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$), δ: 30.99, 32.69, 36.15, 101.01, 108.24, 108.74, 118.94, 119.85, 121.17, 123.57, 126.10, 133.55, 134.30, 136.24, 138.41, 146.33, 147.86, 155.67, 156.31. Anal. Calcd. (%): C 61.30, H 5.14, N 10.21, S 7.79, found (%): C 61.09, H 5.19, N 10.14, S 7.71.

*5-(Benzo[d][1,3]dioxol-5-ylmethyl)-4-(tert-butyl)-N-(4-nitrophenyl)thiazol-2-amine (**C16**)*

Compound **C16** was synthesized using a method similar to that of compound **C7** and was gained as reddish brown solid in 58% yield, m.p. 161–163 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.43 (s, 9H, 3×CH_3), 4.14 (s, 2H, CH_2), 5.96 (s, 2H, OCH_2O), 6.66–6.69 (m, 2H, C_6H_3 6,2-H), 6.76 (d, J = 8.0 Hz, 1H, C_6H_3 5-H), 7.48 (d, J = 8.8 Hz, 2H, C_6H_4 2,6-H), 8.20 (d, J = 8.8 Hz, 2H,

C_6H_4 3,5-H). ^{13}C NMR (100 MHz, $CDCl_3$), δ: 30.95, 32.68, 36.06, 101.05, 108.30, 108.71, 115.68, 121.17, 122.17, 125.72, 134.14, 141.39, 145.92, 146.38, 147.90, 156.84. Anal. Calcd. (%): C 61.30, H 5.14, N 10.21, S 7.79, found (%): C 61.06, H 5.17, N 10.13, S 7.68.

*Ethyl 4-((5-(benzo[d][1,3]dioxol-5-ylmethyl)-4-(tert-butyl)thiazol-2-yl)amino)benzoate (**C17**)*

Compound **C17** was synthesized using a method similar to that of compound **C7** and was gained as white solid in 76% yield, m.p. 177–180 °C. ^1H NMR (400 MHz, $CDCl_3$) δ:1.38 (t, J = 7.2 Hz, 3H, CH_3), 1.44 (s, 9H, 3×CH_3), 4.12 (s, 2H, CH_2), 4.35 (q, J = 7.2 Hz, 2H, OCH_2), 5.96 (s, 2H, OCH_2O), 6.67 (d, J = 8.0 Hz, 1H, C_6H_3 6-H), 6.69 (s, 1H, C_6H_3 2-H), 6.76 (d, J = 8.0 Hz, 1H, C_6H_3 5-H), 7.34 (d, J = 8.8 Hz, 2H, C_6H_4 2,6-H), 8.01 (d, J = 8.8 Hz, 2H, C_6H_4 3,5-H). ^{13}C NMR (100 MHz, $CDCl_3$), δ: 14.37, 30.95, 32.66, 35.94, 60.65, 101.00, 108.26, 108.72, 115.76, 120.63, 121.14, 123.53, 131.22, 134.28, 144.13, 146.30, 147.86, 157.95, 166.19. Anal. Calcd. (%): C 65.73, H 5.98, N 6.39, S 7.31, found (%): C 65.44, H 5.89, N 6.31, S 7.23.

*5-(Benzo[d][1,3]dioxol-5-ylmethyl)-4-(tert-butyl)-N-(2,4-dimethylphenyl)thiazol-2-amine (**C18**) hydrochloride*

Compound **C18** was synthesized using a method similar to that of compound **C1** and was gained as yellow oil in 67% yield. The hydrochloride was obtained as white solid, m.p.175–177 °C. ^1H NMR (400 MHz, $CDCl_3$) δ: 1.40 (s, 9H, 3×CH_3), 2.20 (s, 3H, CH_3), 2.23 (s, 3H, CH_3), 4.09 (s, 2H, CH_2), 5.94 (s, 2H, OCH_2O), 6.65 (d, J = 8.0 Hz, 1H, C_6H_3 6-H), 6.68 (s, 1H, C_6H_3 2-H), 6.68 (d, J = 8.0 Hz, 1H, C_6H_3 5-H), 7.00–7.07 (m, 3H, C_6H_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 19.40, 20.09, 30.38, 31.76, 35.60, 101.54, 108.87, 109.25, 118.13, 118.72, 121.89, 122.47, 131.08, 133.69, 136.88, 138.37, 145.87, 146.56, 147.98, 164.46. Anal. Calcd. (%): C 64.10, H 6.31, N 6.50, S 7.44, found (%): C 63.88, H 6.27, N 6.39, S 7.33.

*5-(Benzo[d][1,3]dioxol-5-ylmethyl)-4-(tert-butyl)-N-(2,6-dimethylphenyl)thiazol-2-amine (**C19**)*

Compound **C19** was synthesized using a method similar to that of compound **C7** and was gained as white solid in 62% yield, m.p.155–157 °C. ^1H NMR (400 MHz, $CDCl_3$) δ:1.37 (s, 9H, 3×CH_3), 2.30 (s, 6H, 2×CH_3), 4.00 (s, 2H, CH_2), 5.91 (s, 2H, OCH_2O), 6.40 (s, 1H, NH), 6.58 (d, J = 8.0 Hz, 1H, C_6H_3 6-H), 6.62 (s, 1H, C_6H_3 2-H), 6.69 (d, J = 8.0 Hz, 1H, C_6H_3 5-H), 7.09–7.12 (m, 3H, C_6H_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 18.33, 31.06, 32.77, 35.76, 100.83, 108.06, 108.64, 118.15, 120.94, 127.37, 128.72, 134.89, 136.65, 137.87, 145.92, 147.64, 155.13, 165.15. Anal. Calcd. (%): C 69.66, H 7.12, N 7.06, S 8.09, found (%): C 69.44, H 7.15, N 7.01, S 8.02.

*5-(Benzo[d][1,3]dioxol-5-ylmethyl)-4-(tert-butyl)-N-(3,4-dimethylphenyl)thiazol-2-amine (**C20**) hydrochloride*

Compound **C20** was synthesized using a method similar to that of compound **C1** and was gained as yellow oil in 71% yield. The hydrochloride was obtained as white solid, m.p.188–190 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.43 (s, 9H, 3×CH_3), 2.21 (s, 3H, CH_3), 2.23 (s, 3H, CH_3), 4.07 (s, 2H, CH_2), 5.95 (s, 2H, OCH_2O), 6.64 (d, J = 8.0 Hz, 1H, C_6H_3 6-H), 6.67 (s, 1H, C_6H_3 2-H), 6.74 (d, J = 8.0 Hz, 1H, C_6H_3 5-H), 7.01 (d, J = 8.0 Hz, C_6H_3), 7.04 (s, 1H, C_6H_3 2-H), 7.07 (d, J = 8.0 Hz, C_6H_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 19.20, 20.01, 30.48, 31.71, 35.60, 101.36, 101.47, 108.70, 109.06, 118.01, 120.39, 121.65, 130.76, 134.01, 137.54, 137.88, 146.33, 146.94, 147.82, 153.17, 163.38. Anal. Calcd. (%): C 64.10, H 6.31, N 6.50, S 7.44, found (%): C 63.87, H 6.28, N 6.44, S 7.37.

*5-(Benzo[d][1,3]dioxol-5-ylmethyl)-4-(tert-butyl)-N-(2,3-dichlorophenyl)thiazol-2-amine (**C21**) hydrochloride*

Compound **C21** was synthesized using a method similar to that of compound **C1** and was gained as yellow oil in 64% yield. The hydrochloride was obtained as white solid, m.p.159–161 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.42 (s, 9H, 3×CH_3), 4.13 (s, 2H, CH_2), 5.95 (s, 2H, OCH_2O), 6.68–6.70 (m, 2H, C_6H_3 6,2-H), 6.76 (d, J = 8.0 Hz, C_6H_3 5-H), 7.07 (d, J = 8.0 Hz, 1H, ClC_6H_3 4-H). 7.18 (t, J = 8.0 Hz, 1H, ClC_6H_3 5-H), 7.36 (d, J = 8.0 Hz, 1H, ClC_6H_3 6-H), 8.16 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 30.85, 31.91, 35.93, 101.44, 108.78, 109.17, 120.18, 120.86, 121.73, 122.05, 125.11, 129.05, 132.66, 134.52, 139.20, 146.35, 147.87, 150.39, 160.77. Anal. Calcd. (%): C 53.46, H 4.49, N 5.94, S 6.80, found (%): C 53.37, H 4.43, N 5.85, S 6.72.

*5-(Benzo[d][1,3]dioxol-5-ylmethyl)-4-(tert-butyl)-N-(3,4-dichlorophenyl)thiazol-2-amine (**C22**)*

Compound **C22** was synthesized using a method similar to that of compound **C1** and was gained as yellow solid in 69% yield, m.p. 124–126 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.41 (s, 9H, 3×CH_3), 4.11 (s, 2H, CH_2), 5.95 (s, 2H, OCH_2O), 6.66 (d, J = 8.0 Hz, 1H, C_6H_3 6-H), 6.68 (s, 1H, C_6H_3 2-H), 6.75 (d, J = 8.0 Hz, 1H, C_6H_3 5-H), 1.55 (dd, J_1 = 8.4 Hz, J_2 = 2.8 Hz, ClC_6H_3 6-H), 7.33 (d, J = 8.4 Hz, 1H, ClC_6H_3 5-H), 7.63 (d, J = 2.8 Hz, ClC_6H_3 2-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 30.96, 32.57, 35.97, 100.97, 108.20, 108.65, 116.34, 118.61, 120.33, 121.04, 124.57, 130.56, 132.82, 134.41, 140.07, 146.18, 147.77, 158.12. Anal. Calcd. (%): C 59.35, H 5.63, N 6.02, S 6.89 found (%): C 59.21, H 5.69, N 5.94, S 6.82.

*5-(Benzo[d][1,3]dioxol-5-ylmethyl)-4-(tert-butyl)-N-(3,5-dichlorophenyl)thiazol-2-amine (**C23**)*

Compound **C23** was synthesized using a method similar to that of compound **C1** and was gained as yellow solid in 69% yield, m.p. 99–101 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.41 (s, 9H, 3×CH_3), 4.12 (s, 2H, CH_2), 5.95 (s, 2H, OCH_2O), 6.66 (dd, J_1 = 8.0 Hz, J_2 = 1.6 Hz, 1H, C_6H_3 6-H), 6.69 (d, J = 1.6 Hz, 1H, C_6H_3 2-H), 6.75 (d, J = 8.0 Hz, 1H, C_6H_3 5-H), 6.96 (t, J = 1.6 Hz, 1H, ClC_6H_3 4-H), 7.34 (d, J = 1.6 Hz, 2H, ClC_6H_3 2,6-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 30.91, 32.51, 35.95, 100.95, 108.19, 108.62, 115.08, 120.65, 121.02, 121.47, 134.27, 135.27, 142.24, 146.16, 147.75, 154.73, 157.77. Anal. Calcd. (%): C 59.35, H 5.63, N 6.02, S 6.89 found (%): C 59.23, H 5.66, N 5.91, S 6.81.

*5-(Benzo[d][1,3]dioxol-5-ylmethyl)-4-(tert-butyl)-N-(3-chloro-4-fluorophenyl)thiazol-2-amine (**C24**)*

Compound **C24** was synthesized using a method similar to that of compound **C1** and was gained as yellow solid in 78% yield, m.p. 100–102 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ:1.51 (s, 9H, 3×CH_3), 4.07 (s, 2H, CH_2), 5.98 (s, 2H, OCH_2O), 6.62–6.64 (m, 2H, C_6H_3 6,2-H), 6.78 (d, J = 8.0 Hz, C_6H_3 5-H), 7.15–7.17 (m, 2H, FC_6H_3), 7.39 (d, J = 8.0 Hz, FC_6H_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 31.02, 31.95, 36.04, 101.43, 108.74, 109.06, 117.68 (d, J_{FCC} = 22.0 Hz), 118.34, 119.42, 119.49, 119.91 (d, J_{FCC} = 18.0 Hz), 121.59, 134.83, 138.39, 146.30, 147.88, 151.98, 152.77 (d, J_{FC} = 239.0 Hz), 160.06. Anal. Calcd. (%): C 60.21, H 4.81, N 6.69, S 7.65, found (%): C 60.04, H 4.85, N 6.59, S 7.58.

*5-(Benzo[d][1,3]dioxol-5-ylmethyl)-4-(tert-butyl)-N-(2-fluoro-4-nitrophenyl)thiazol-2-amine (**C25**)*

Compound **C25** was synthesized using a method similar to that of compound **C7** and was gained as yellow solid in 72% yield, m.p. 147–148 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.44 (s, 9H, 3×CH_3), 4.15 (s, 2H, CH_2), 5.95 (s, 2H, OCH_2O), 6.68 (d, J = 8.0 Hz, 1H, C_6H_3 6-H), 6.70 (s, 1H, C_6H_3 2-H), 6.76 (d, J = 8.0 Hz, 1H, C_6H_3 5-H), 7.59 (s, 1H, NH), 8.19 (dd, J_1 = 8.0 Hz, J_2 = 2.4 Hz, 1H, FC_6H_3 5-H), 8.31 (d, J_1 = 8.0 Hz, J_2 = 2.4 Hz, 1H, FC_6H_3 3-H), 8.53 (d, J_1 = 8.0 Hz, J_2 = 2.4 Hz, 1H, FC_6H_3 6-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 31.00, 32.25, 35.66, 100.87, 108.11, 108.78, 116.26 (d, J_{FCC} = 26.0 Hz), 121.05, 121.46, 122.80, 123.01, 123.80, 137.82 (d, J_{FCC} = 30.0 Hz), 145.97, 146.56, 147.72, 152.75, 153.12 (d, J_{FC} = 269.0 Hz), 155.38. Anal. Calcd. (%): C 58.73, H 4.69, N 9.78, S 7.47, found (%): C 58.58, H 4.62, N 9.73, S 7.39.

*5-(Benzo[d][1,3]dioxol-5-ylmethyl)-4-(tert-butyl)-N-(2-chloro-4-nitrophenyl)thiazol-2-amine (**C26**)*

Compound **C26** was synthesized using a method similar to that of compound **C7** and was gained as yellow solid in 61% yield, m.p.143–145 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.44 (s, 9H, 3×CH_3), 4.15 (s, 2H, CH_2), 5.96 (s, 2H, OCH_2O), 6.68 (d, J = 8.0 Hz, 1H, C_6H_3 6-H), 6.70 (s, 1H, C_6H_3 2-H), 6.76 (d, J = 8.0 Hz, 1H, C_6H_3 5-H), 7.59 (s, 1H, NH), 8.18 (dd, J_1 = 9.2 Hz, J_2 = 2.8 Hz, 1H, ClC_6H_3 5-H), 8.29 (d, J = 2.8 Hz, 1H, ClC_6H_3 3-H), 8.55 (d, J = 9.2 Hz, 1H, ClC_6H_3 6-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 30.94, 32.71, 36.14, 101.03,

108.25, 108.71, 115.68, 119.58, 121.17, 123.76, 124.18, 124.93, 134.13, 140.45, 142.37, 146.36, 147.84, 155.57, 155.81. Anal. Calcd. (%): C 56.56, H 4.52, N 9.42, S 7.19, found (%): C 56.42, H 4.41, N 9.33, S 7.11.

*5-(Benzo[d][1,3]dioxol-5-ylmethyl)-N-(3,5-bis(trifluoromethyl)phenyl)-4-(tert-butyl)-thiazol-2-amine (**C27**) hydrochloride*

Compound **C27** was synthesized using a method similar to that of compound **C1** and was gained as yellow oil in 65% yield. The hydrochloride was obtained as white solid, m.p.148–150 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.48 (s, 9H, 3×CH_3), 4.12 (s, 2H, CH_2), 5.97 (s, 2H, OCH_2O), 6.64–6.66 (m, 2H, C_6H_3 6,2-H), 6.78 (d, J = 8.0 Hz, 1H, C_6H_3 5-H), 7.58 (s, 1H, $CF_3C_6H_3$ 4-H), 7.87 (s, 2H, $CF_3C_6H_3$ 2,6-H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 30.88, 31.76, 35.87, 101.14, 108.46, 108.80, 112.80, 116.29 (2×CH), 120.80, 121.29, 123.68 (q, J_{FC} = 271.0 Hz, 2×CF_3), 131.02 (q, J_{FCC} = 32.0 Hz, 2×C), 134.89, 143.11, 146.00, 147.61, 153.99, 157.73. Anal. Calcd. (%): C 51.26, H 3.93, N 5.20, S 5.95, found (%): C 51.04, H 3.85, N 5.11, S 5.87.

*5-(Benzo[d][1,3]dioxol-5-ylmethyl)-4-(tert-butyl)-N-(2,6-dibromo-4-(trifluoromethoxy)-phenyl)thiazol-2-amine (**C28**)*

Compound **C28** was synthesized using a method similar to that of compound **C7** and was gained as yellow solid in 67% yield, m.p. 126–128 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.38 (s, 9H, 3×CH_3), 4.02 (s, 2H, CH_2), 5.94 (s, 2H, OCH_2O), 6.62 (d, J = 8.0 Hz, 1H, C_6H_3 6-H), 6.65 (s, 1H, C_6H_3 2-H), 6.73 (d, J = 8.0 Hz, 1H, C_6H_3 5-H), 7.47 (s, 2H, C_6H_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 30.74, 32.87, 35.46, 100.93, 108.17, 108.66, 120.19 (q, J_{FC} = 258.0 Hz, 2×CF_3), 121.06, 121.61, 121.86, 125.30, 134.00, 140.28, 145.96, 146.14, 147.73, 150.93, 161.01. Anal. Calcd. (%): C 43.44, H 3.15, N 4.61, S 5.27, found (%): C 43.25, H 3.03, N 4.50, S 5.19.

*5-(Benzo[d][1,3]dioxol-5-ylmethyl)-4-(tert-butyl)-N-(naphthalen-1-yl)thiazol-2-amine (**C29**) hydrochloride*

Compound **C29** was synthesized using a method similar to that of compound **C1** and was gained as yellow oil in 86% yield. The hydrochloride was obtained as white solid, m.p.161–163 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.50 (s, 9H, 3×CH_3), 4.06 (s, 2H, CH_2), 5.93 (s, 2H, OCH_2O), 6.62 (d, J = 8.0 Hz, 1H, C_6H_3 6-H), 6.64 (s, 1H, C_6H_3 2-H), 6.72 (d, J = 8.0 Hz, 1H, C_6H_3 5-H), 7.43 (t, J = 8.0 Hz, 1H, $C_{10}H_7$), 7.52–7.71 (m, 5H, $C_{10}H_7$), 7.86 (d, J = 8.0 Hz, $C_{10}H_7$), 8.20 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 30.36, 31.76, 35.60, 101.54, 108.87, 109.31, 118.77, 121.12, 121.97, 122.41, 126.62, 127.54, 127.72, 129.06, 133.46, 134.58, 134.92, 146.60, 147.98, 166.28. Anal. Calcd. (%): C 66.28, H 5.56, N 6.18, S 7.08, found (%): C 66.08, H 5.48, N 6.11, S 6.99.

*5-(Benzo[d][1,3]dioxol-5-ylmethyl)-4-(tert-butyl)-N-(pyridin-2-yl)thiazol-2-amine (**C30**)*

Compound **C30** was synthesized using a method similar to that of compound **C1** and was gained as yellow solid in 61% yield, m.p. 147–149 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.40 (s, 9H, 3×CH_3), 4.16 (s, 2H, CH_2), 5.94 (s, 2H, OCH_2O), 6.68 (d, J = 8.0 Hz, 1H, C_6H_3 6-H), 6.71 (s, 1H, C_6H_3 2-H), 6.75 (d, J = 8.0 Hz, 1H, C_6H_3 5-H), 6.84–6.88 (m, 2H, pyridin ring), 7.58 (t, J = 8.0 Hz, 1H, pyridin ring), 8.27 (d, J = 4.8 Hz, 1H, pyridin ring). ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.07, 27.41, 30.73, 95.93, 103.19, 103.86, 105.11, 111.11, 115.96, 116.15, 130.02, 132.73, 141.04, 142.19, 142.80, 146.72, 148.20, 150.76. Anal. Calcd. (%): C 65.37, H 5.76, N 11.44, S 8.73, found (%): C 65.18, H 5.67, N 11.36, S 8.59.

*5-(Benzo[d][1,3]dioxol-5-ylmethyl)-4-(tert-butyl)-N-(2-methylquinolin-8-yl)thiazol-2-amine (**C31**)*

Compound **C31** was synthesized using a method similar to that of compound **C1** and was gained as yellow solid in 66% yield, m.p. 153–155 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.47 (s, 9H, 3×CH_3), 2.72 (s, 3H, CH_3), 4.16 (s, 2H, CH_2), 5.94 (s, 2H, OCH_2O), 6.70–6.77 (m, 3H, C_6H_3), 7.26–7.30 (m, 2H, quinolin ring), 7.44 (t, J = 8.0 Hz, 1H, quinolin ring), 8.00 (d, J = 8.4 Hz, 1H, quinolin ring), 8.38 (d, J = 7.2 Hz, 1H, quinolin ring), 9.55 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 25.06, 31.07, 32.65, 36.01, 100.88, 108.08, 108.76, 111.76, 118.20, 119.45, 121.06, 122.29, 126.09, 126.36, 134.94, 136.15, 137.05, 146.03, 147.68, 155.04, 156.49, 158.19. Anal. Calcd. (%): C 69.58, H 5.84, N 9.74, S 7.43, found (%): C 69.44, H 5.75, N 9.65, S 7.31.

3. X-ray structure determination

A light yellow crystal with dimensions of 0.41 mm×0.38 mm×0.37 mm of the title compound **C31** was obtained from ethanol solution by slowly evaporating for about 7 days at room temperature. X-ray intensity data were measured at 152(2) K on a Bruker AXS SMART 1000 CCD diffractometer (Germany) equipped with a graphite-monochromatic Mo$K\alpha$ (λ = 0.71073 Å) radiation. Corrections for incident and diffracted beam absorption effects were applied using SADABS[3]. The structure was solved by direct methods with SHELXS-97[4] and expanded by difference *Fourier* techniques. The non-hydrogen atoms were refined anisotropically, and hydrogen atoms were added according to theoretical models. The structure was refined by full-matrix least-squares techniques on F^2 with SHELXL-97[5]. A total of 12031 reflections were collected in the range of 2.99 to 26.00°, of which 4371 were independent (R_{int} = 0.0251) and 3649 were observed with $I > 2\sigma(I)$. The final R = 0.0430 and wR = 0.1074 ($w = 1/[\sigma^2(F_o^2) + (0.0530P)^2 + 5.2330P]$, where $P = (F_o^2 + 2F_c^2)/3$). S = 1.02, $(\Delta/\sigma)_{max}$ = 0.006, $(\Delta\rho)_{max}$ = 0.41 e Å^{-3} and $(\Delta\rho)_{min}$ = –0.32 e Å^{-3}. The selected bond lengths and bond angles are listed in Table 1S.

Table 1S. Selected bond lengths (Å) and bond angles (°) for compound **C31**.

Bond	Dist.	Bond	Dist.	Bond	Dist.
C(1)-O(2)	1.426(2)	C(8)-C(9)	1.508(2)	C(18)-C(19)	1.362(3)
C(1)-O(1)	1.433(2)	C(9)-C(10)	1.357(3)	C(19)-C(20)	1.413(3)
C(2)-C(3)	1.364(3)	C(9)-S(1)	1.7432(18)	C(20)-C(24)	1.412(3)
C(2)-O(2)	1.376(2)	C(10)-N(1)	1.394(2)	C(20)-C(21)	1.413(3)

C(2)-C(7)	1.383(3)	C(15)-N(1)	1.292(2)	C(21)-N(3)	1.367(2)
C(3)-C(4)	1.403(2)	C(15)-N(2)	1.371(2)	C(22)-N(3)	1.323(3)
C(4)-C(5)	1.386(3)	C(15)-S(1)	1.7386(17)	C(22)-C(23)	1.416(3)
C(4)-C(8)	1.513(2)	C(16)-C(17)	1.382(3)	C(22)-C(25)	1.497(3)
C(5)-C(6)	1.404(3)	C(16)-N(2)	1.389(2)	C(23)-C(24)	1.356(3)
C(6)-C(7)	1.362(3)	C(16)-C(21)	1.435(3)	N(2)-H(2)	0.85(2)
C(7)-O(1)	1.383(2)	C(17)-C(18)	1.413(3)		
Angle	(°)	Angle	(°)	Angle	(°)
O(2)-C(1)-O(1)	107.99(14)	C(9)-C(8)-H(8B)	108.1	N(2)-C(15)-S(1)	119.38(13)
O(2)-C(1)-H(1A)	110.1	C(4)-C(8)-H(8B)	108.1	C(17)-C(16)-N(2)	125.31(17)
O(1)-C(1)-H(1A)	110.1	H(8A)-C(8)-H(8B)	107.3	N(2)-C(16)-C(21)	115.30(16)
O(2)-C(1)-H(1B)	110.1	C(10)-C(9)-C(8)	133.86(17)	C(24)-C(20)-C(19)	123.80(18)
O(1)-C(1)-H(1B)	110.1	C(10)-C(9)-S(1)	109.44(14)	C(24)-C(20)-C(21)	116.45(19)
H(1A)-C(1)-H(1B)	108.4	C(8)-C(9)-S(1)	116.70(13)	N(3)-C(21)-C(20)	123.01(18)
C(3)-C(2)-O(2)	128.06(17)	C(9)-C(10)-N(1)	115.29(16)	N(3)-C(21)-C(16)	117.48(15)
C(3)-C(2)-C(7)	122.30(17)	C(9)-C(10)-C(11)	130.46(17)	N(3)-C(22)-C(23)	121.9(2)
O(2)-C(2)-C(7)	109.62(16)	N(1)-C(10)-C(11)	114.25(16)	N(3)-C(22)-C(25)	117.90(18)
C(2)-C(3)-C(4)	117.63(17)	C(13)-C(11)-C(10)	110.5(3)	C(15)-N(1)-C(10)	111.45(15)
C(5)-C(4)-C(8)	123.31(16)	C(13)-C(11)-C(14)	114.6(4)	C(15)-N(2)-C(16)	127.89(16)
C(3)-C(4)-C(8)	116.94(16)	C(10)-C(11)-C(14)	114.18(17)	C(15)-N(2)-H(2)	118.4(14)
C(7)-C(6)-C(5)	116.99(17)	C(13)-C(11)-C(12)	102.5(4)	C(16)-N(2)-H(2)	113.3(14)
C(6)-C(7)-O(1)	128.73(17)	C(10)-C(11)-C(12)	107.52(19)	C(22)-N(3)-C(21)	118.60(16)
O(1)-C(7)-C(2)	109.77(16)	C(14)-C(11)-C(12)	106.5(2)	C(7)-O(1)-C(1)	104.87(14)
C(9)-C(8)-C(4)	116.57(15)	N(1)-C(15)-N(2)	126.06(16)	C(2)-O(2)-C(1)	105.46(14)
C(9)-C(8)-H(8A)	108.1	N(1)-C(15)-S(1)	114.56(14)	C(15)-S(1)-C(9)	89.26(8)
C(4)-C(8)-H(8A)	108.1				

4. Biological assays

4.1. Cytotoxic assay in human cancer cell lines

The cytotoxicity was tested against HeLa (human cervical), A549 (human lung) and MCF-7 (human breast) cancer cell lines, obtained from Xiangya Medical College of South Central University, Changsha, China. Standard MTT method[6] was used to evaluate the antiproliferative activities of the synthesized compounds **C1-C31**. The HeLa, A549 and MCF-7 cell lines were seeded into 96-well plates at a density of 5×10^4 cells per well and then cultivated at 37 °C in a water-atmosphere (5% CO_2) for 24 h. The cells were then treated with different concentrations (0.1, 0.05, 0.01, 0.05 and 0.001 mM) of compounds **C**, 5-FU and Taxol for 48 h. Then 60 μL of MTT solution in phosphate buffered saline (PBS, 2.5 mg/mL) was added to each well, and the cells were incubated continually at 37 °C for 4 h. The medium was removed and 150 mL of

DMSO was added to dissolve the formazan crystals. Subsequently, the absorbance was recorded at 570 nm on a multiwall plate reader (Thermo Fisher Multiskan MK3, Waltham, MA, USA). The cytotoxicities were estimated based on the comparison with negative control cells. All experiments were performed in quadruplicate and each experiment was repeated at least three times.

4.2. Study the antiproliferative mechanism of compound C24 and C27 against HeLa cells

4.2.1 AO/EB staining

HeLa cells were plated in a glass-bottom dish (glass diameter 10 mm) at 1.0×10^4 cells/dish and incubated for 24 h at 37 °C in 5% humidified CO_2. After the culture medium was removed, 1 mL of fresh medium plus 10% fetal bovine serum and supplemented with compound **C24** and **C27** (5 and 10 μM) was added to the cells. After incubation for 24 h at 37 °C in 5% CO_2, 2 mL of AO/EB (Dojindo Laboratories) stain (20 μM) was added and the cells were washed twice with PBS. Cell morphology was examined by a fluorescence confocal microscope (OLYMPUS FV1000 TY1318, Japan) (For AO: Ex/Em = 488 nm/510–550 nm; For EB: Ex/Em = 543 nm/610–650 nm).

4.2.2 Hoechst 33342 staining

HeLa cells were seeded in six-well plates at a density of 1×10^4 cells/mL and then cultivated at 37 °C for 24 h. The cells were cultured in RPMI-1640 supplemented with 10% of fetal bovine serum and incubated at 37 °C in 5% CO_2. The medium was removed and replaced with medium (final DMSO concentration, 0.05% v/v) containing the compounds **C24** and **C27** (5 μM and 10 μM) for 24 h. After being washed twice with PBS, the cells were stained with 1 mL of Hoechst 33324 (Dojindo Laboratories, Shanghai, China) for 10 min and then washed with PBS twice more. The stained cells were observed under an inverted fluorescence microscope (Nikon TE300, Japan) using ultraviolet excitation.

4.2.3 Apoptosis assessment by FACS analysis

The extent of apoptosis was quantitatively measured using Annexin V binding assay. HeLa cells were cultured in Minimum Essential Medium (MEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS). Cells were plated in a six-well culture dish at a density of 2.0×10^4 cells per well and cultured for 24 h at 37 °C in 5% CO_2. After the medium was removed, 1 mL of fresh MEM containing the indicated concentrations of compound **C24** and **C27** was added and the cells were cultured at 37 °C in 5% CO_2 for 24 h. Then, the cells floating in the supernatant were combined with the adherent fraction and washed with PBS thrice. The cells were incubated with Annexin V-FITC and PI for 15 min at 37 °C in the dark. The samples were immediately analyzed using a FACS Calibur flow cytometry system (Becton Dickinson, San Jose, CA, USA). The percentages of viable (Annexin V-/PI-), early apoptotic (Annexin V+/PI-), late apoptotic (Annexin V+/PI+), and necrotic (Annexin V-/PI+) cells were determined using FlowJo_V10 Software trial version (FlowJo, LLC, USA). A total of 2×10^4 cells were collected for each sample. All experiments were performed in triplicate.

4.2.4 Cell cycle assessment by FACS analysis

Cell cycle distributions in cells were determined through PI staining. In brief, after HeLa cells were treated with compound **C24** and **C27** at 5 and 10 μM, the suspension and adherent portions were collected into flow cytometry tubes and centrifuged at 1000 rpm for 5 min to obtain cell pellets. The cells were washed with PBS and then fixed with 70% ethanol (-20 °C) overnight. Fixed cells were washed with PBS and sequentially incubated with RNase A (0.1 mg/mL) for 30 min and PI (50 mg/mL) for 15 min in the dark. The distribution of the cell cycle was determined using novocyte flow cytometry (ACEA Biosciences). The data were analyzed using ModFit software trial version (Verity Software House, USA). All assays were conducted with three parallel samples, with 1×10^4 cells collected for each sample.

5. References

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6. Spectra of some selected compounds with high anticancer activities

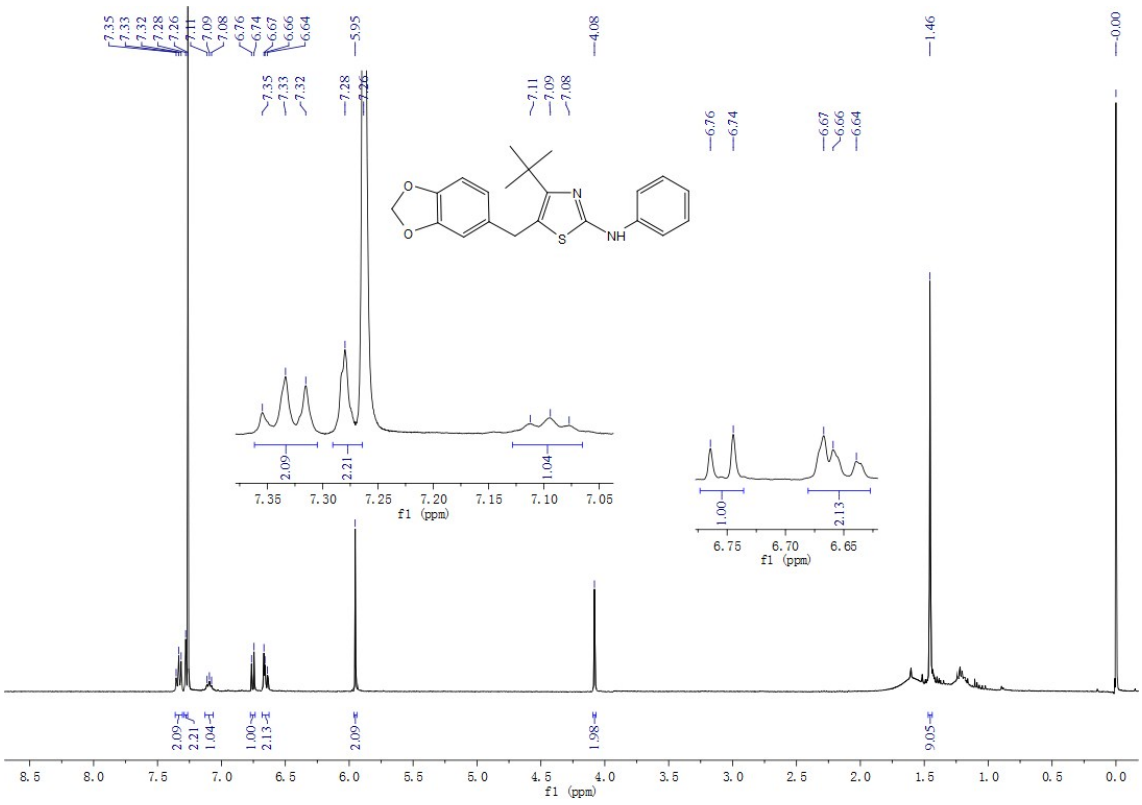

Fig. S1 ^{1}H NMR spectra (400 MHz, $CDCl_3$) of compound **C1**

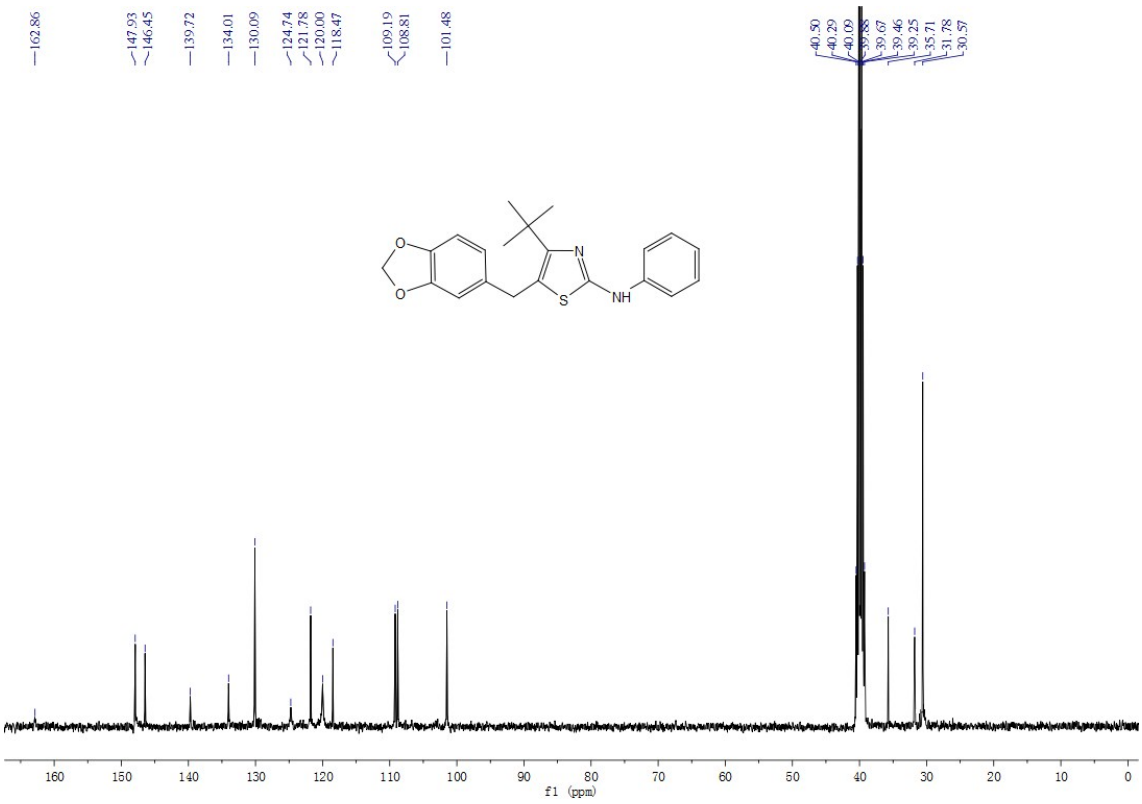

Fig. S2 ^{13}C NMR spectra (100 MHz, DMSO-d_6) of compound **C1**

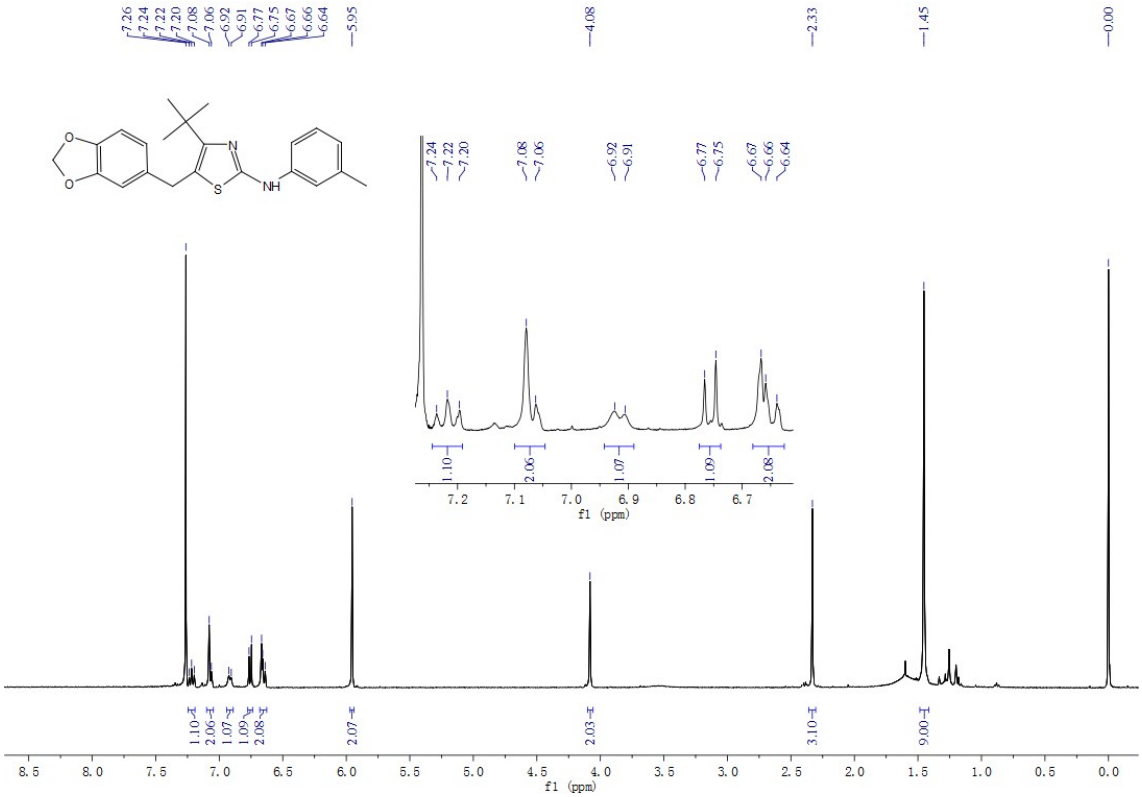

Fig. S3 ^{1}H NMR spectra (400 MHz, $CDCl_3$) of compound **C3**

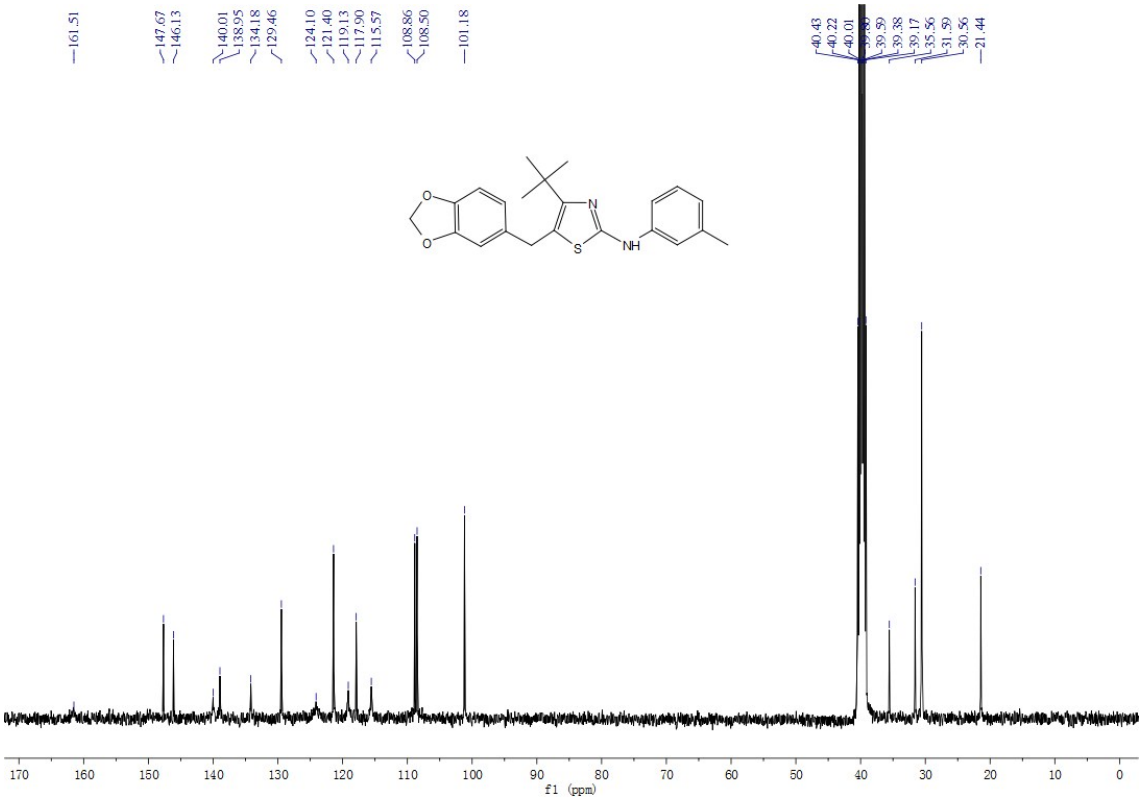

Fig. S4 ^{13}C NMR spectra (100 MHz, DMSO-d_6) of compound **C3**

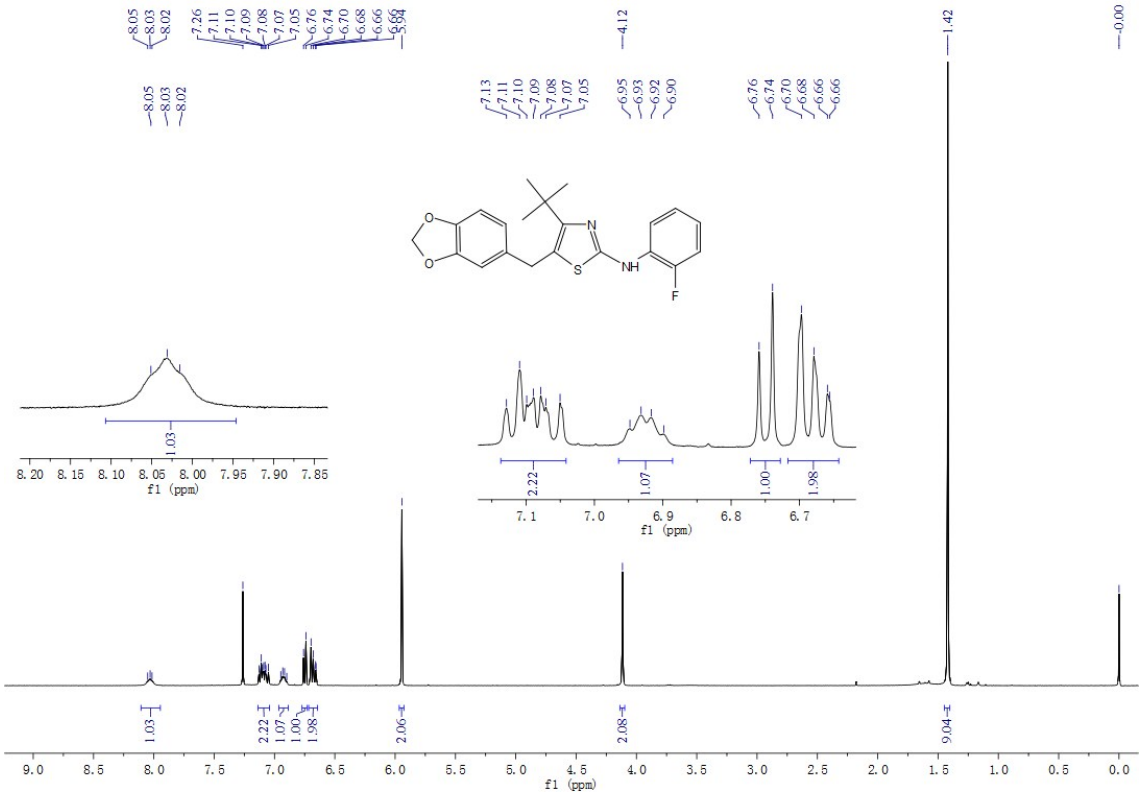

Fig. S5 ^{1}H NMR spectra (400 MHz, $CDCl_3$) of compound **C4**

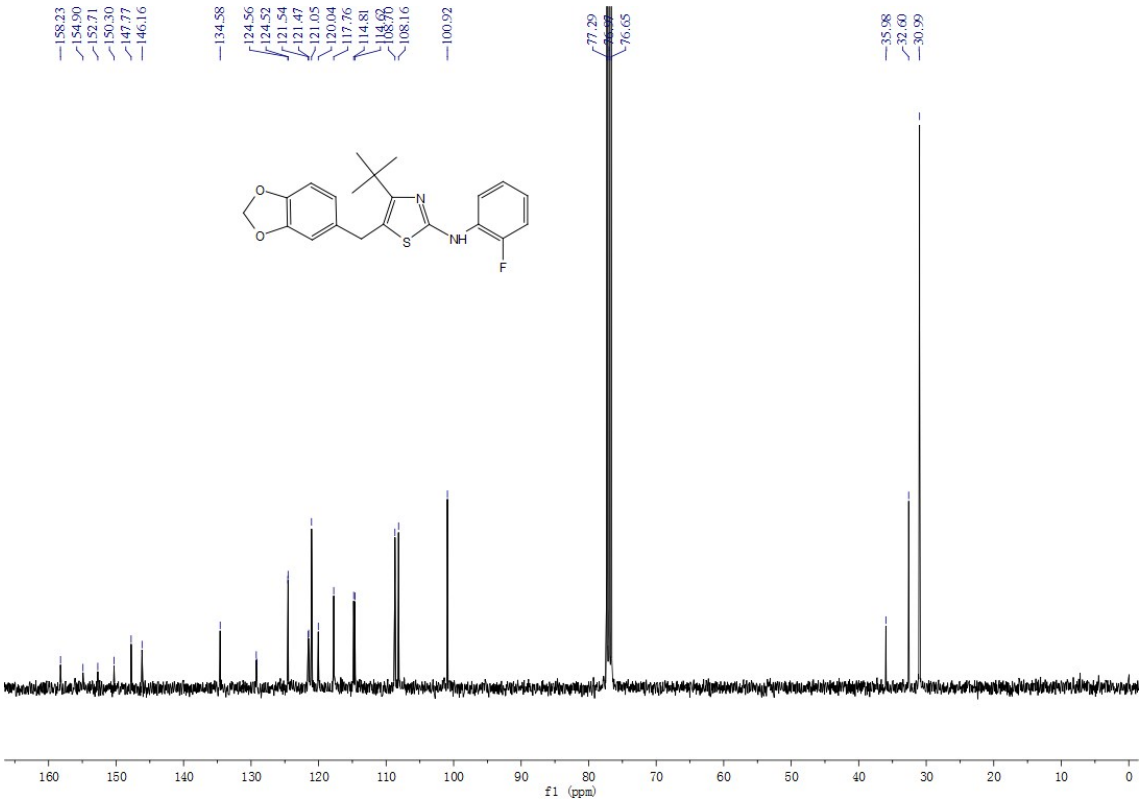

Fig. S6 ^{13}C NMR spectra (100 MHz, $CDCl_3$) of compound **C4**

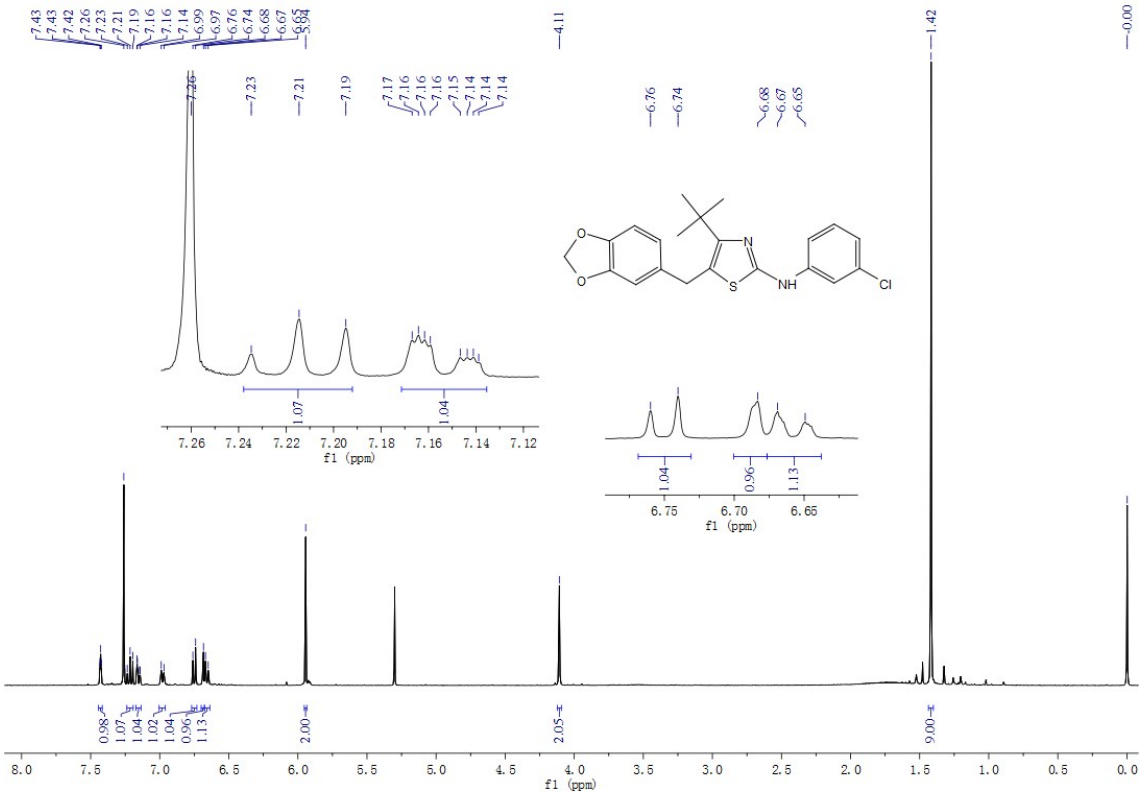

Fig. S7 ^{1}H NMR spectra (400 MHz, $CDCl_3$) of compound **C7**

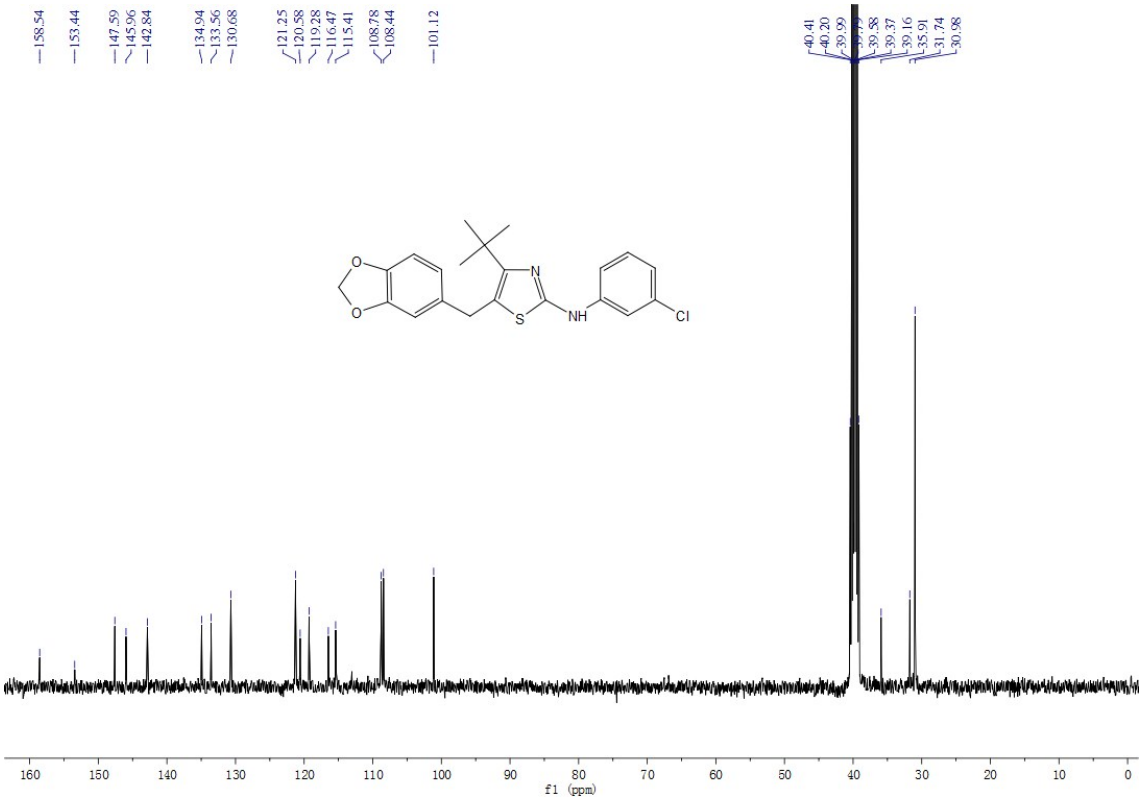

Fig. S8 ^{13}C NMR spectra (100 MHz, DMSO-d_6) of compound **C7**

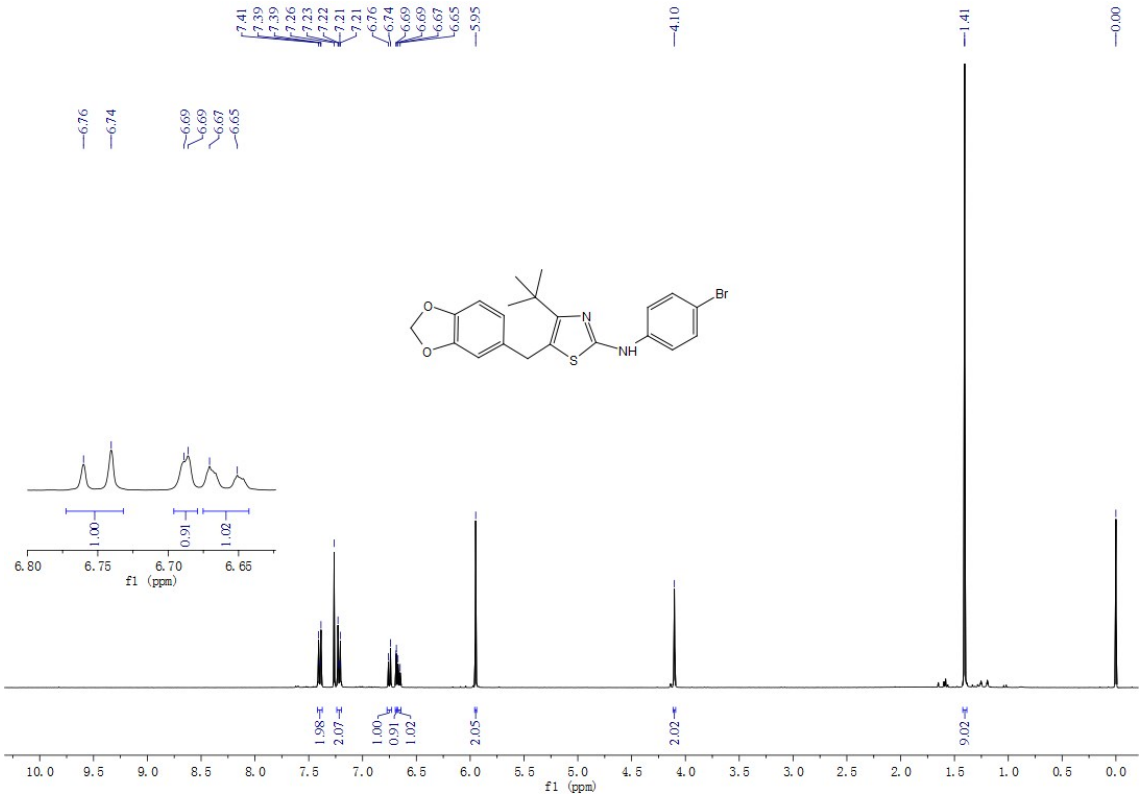

Fig. S9 ^{1}H NMR spectra (400 MHz, $CDCl_3$) of compound **C11**

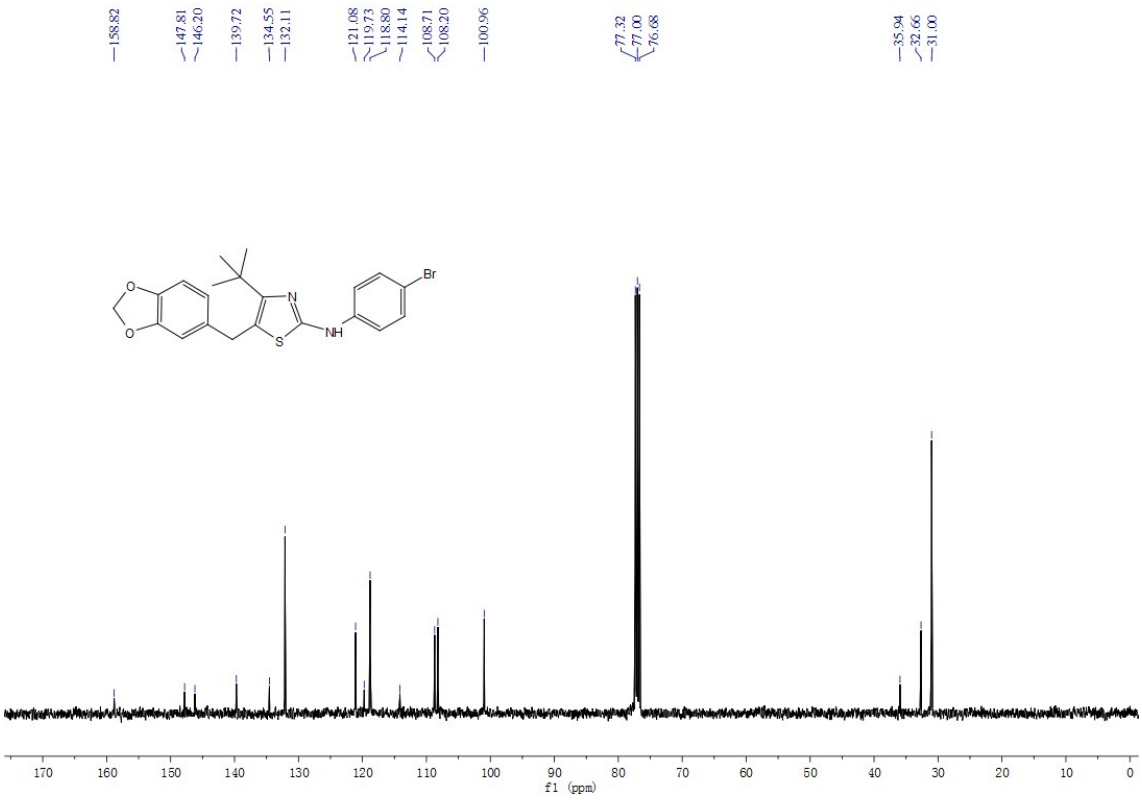

Fig. S10 ^{13}C NMR spectra (100 MHz, $CDCl_3$) of compound **C11**

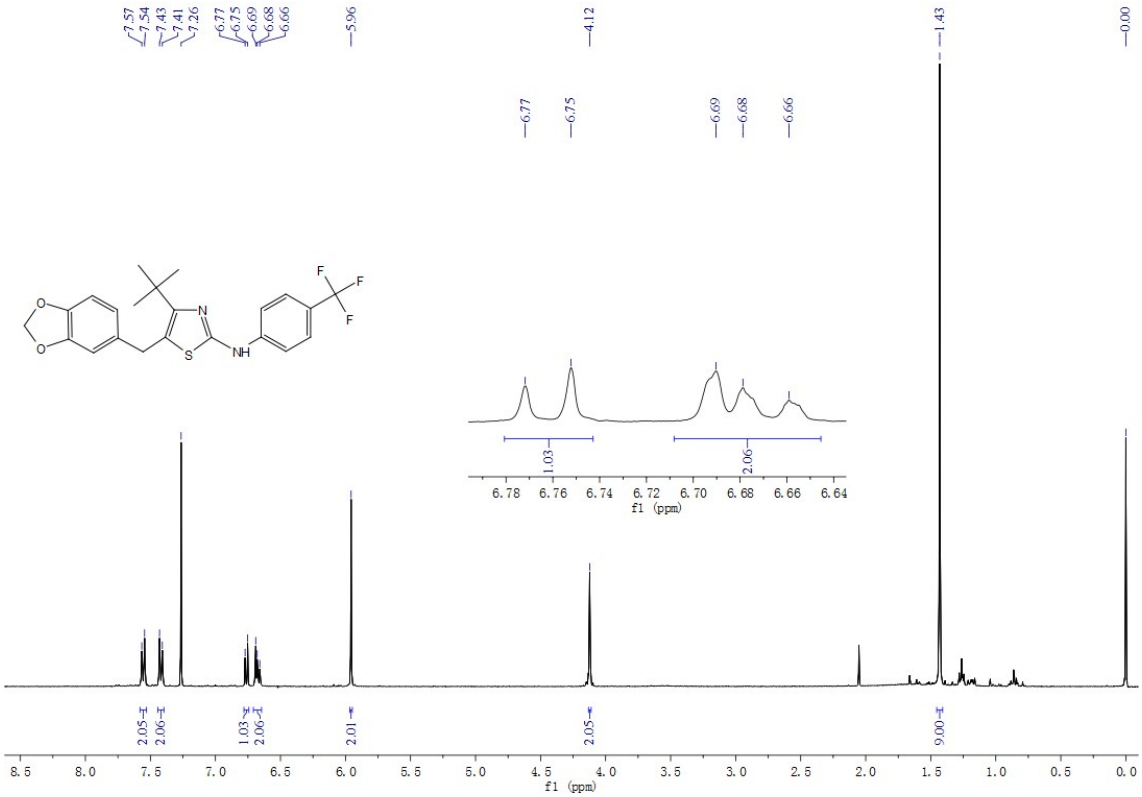

Fig. S11 ^{1}H NMR spectra (400 MHz, $CDCl_3$) of compound **C13**

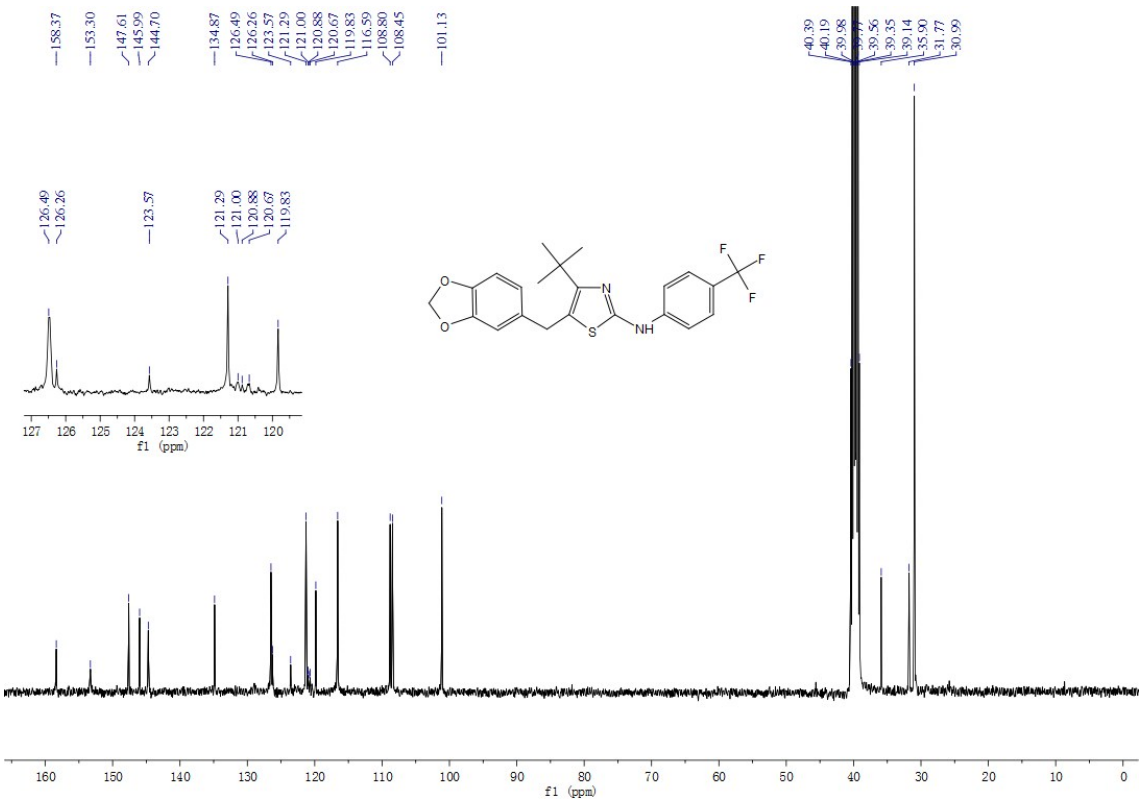

Fig. S12 ^{13}C NMR spectra (100 MHz, DMSO-d_6) of compound **C13**

Fig. S13 ^{1}H NMR spectra (400 MHz, DMSO-d_6) of compound **C14**

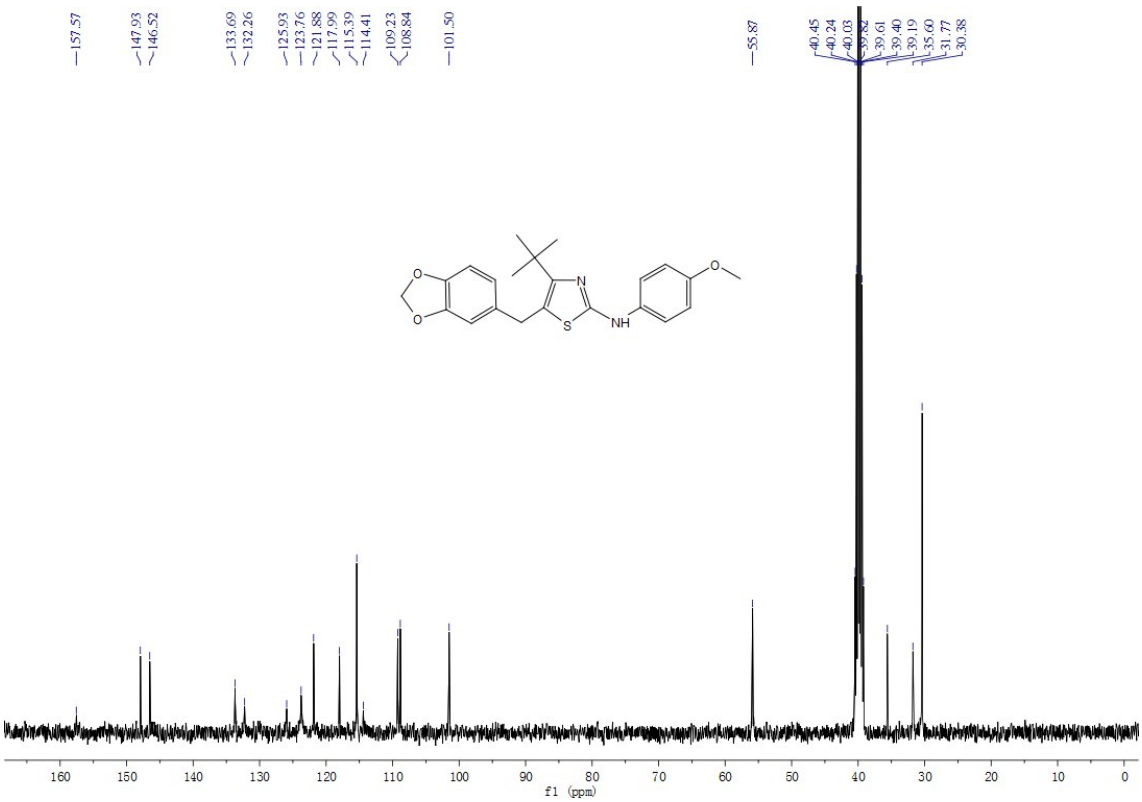

Fig. S14 ^{13}C NMR spectra (100 MHz, DMSO-d_6) of compound **C14**

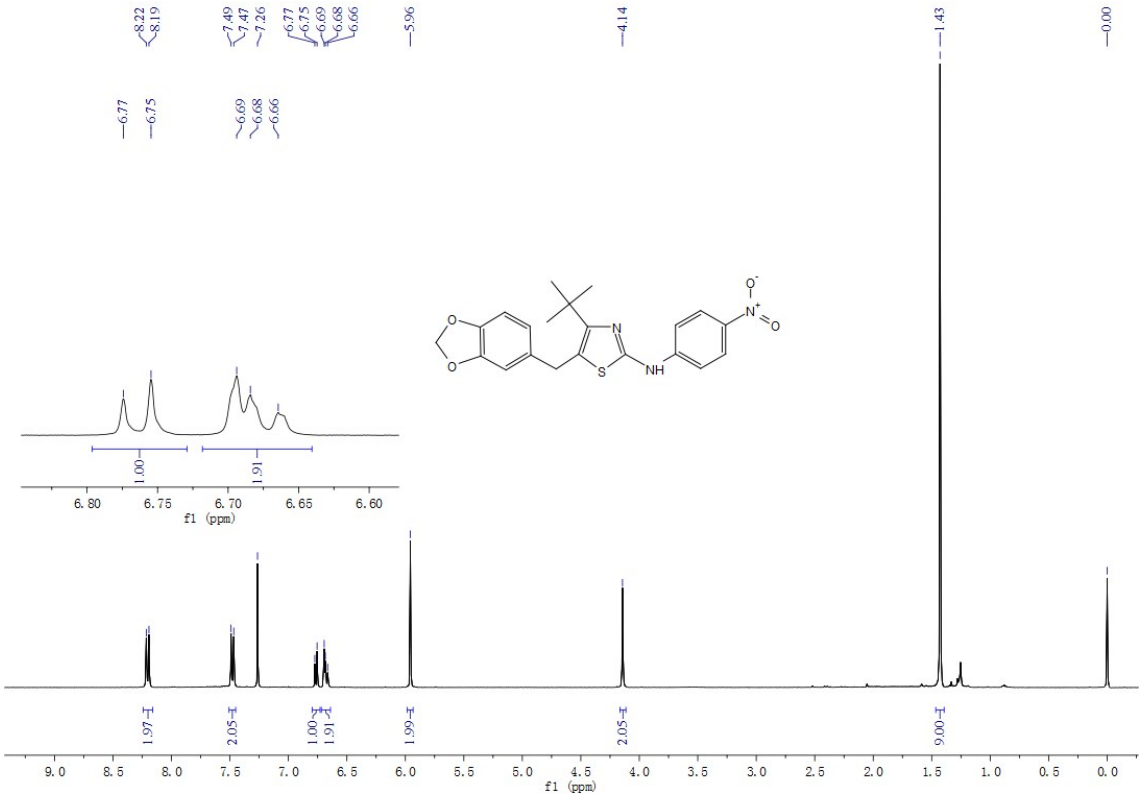

Fig. S15 ^{1}H NMR spectra (400 MHz, $CDCl_3$) of compound **C16**

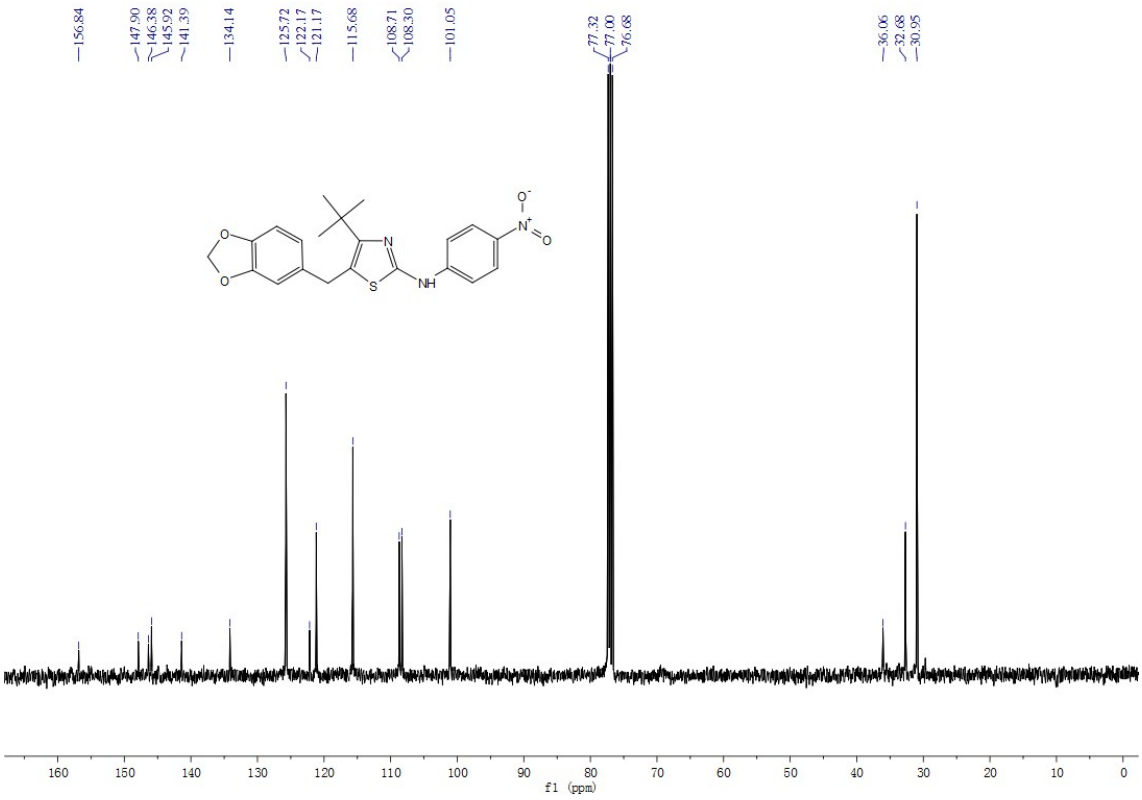

Fig. S16 ^{13}C NMR spectra (100 MHz, $CDCl_3$) of compound **C16**

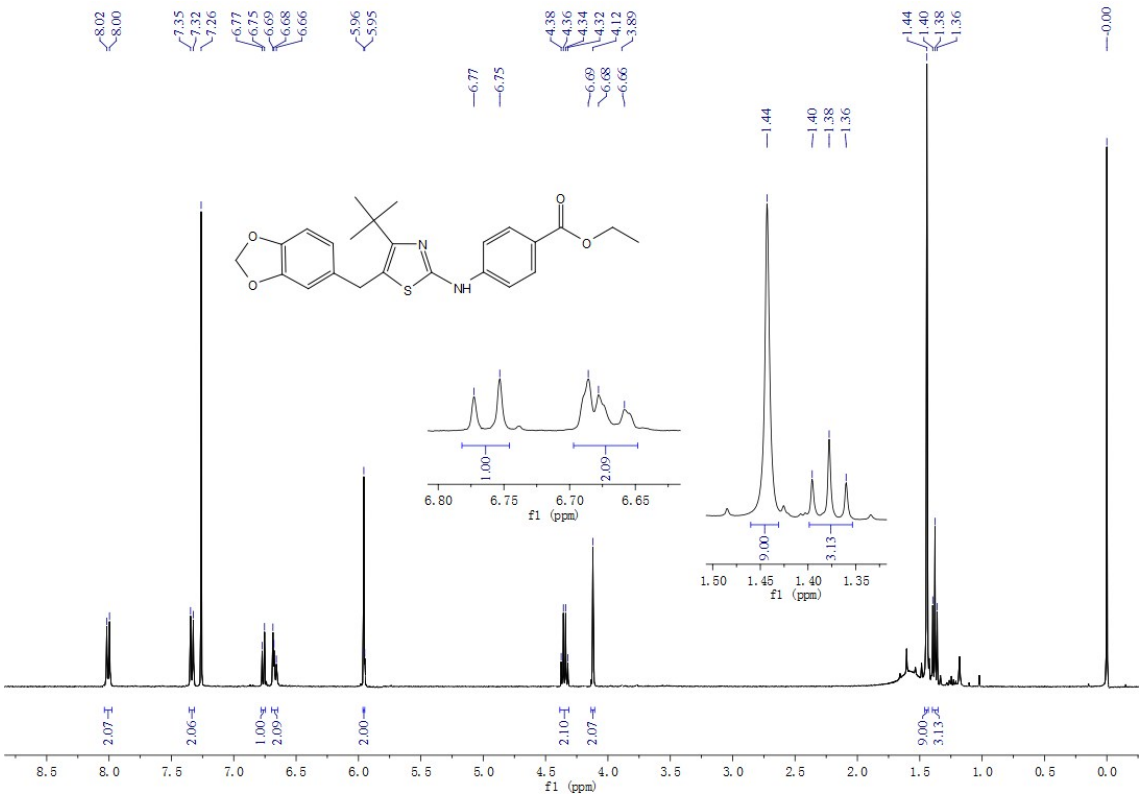

Fig. S17 ^{1}H NMR spectra (400 MHz, $CDCl_3$) of compound **C17**

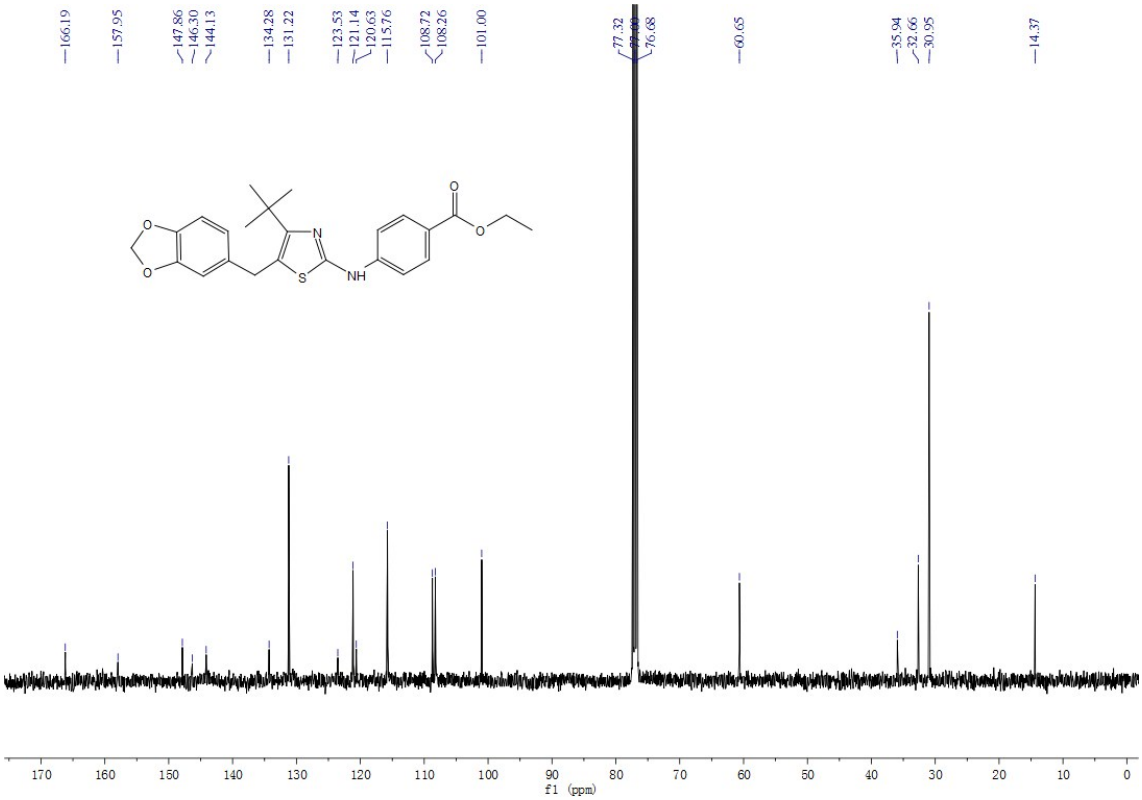

Fig. S18 ^{13}C NMR spectra (100 MHz, $CDCl_3$) of compound **C17**

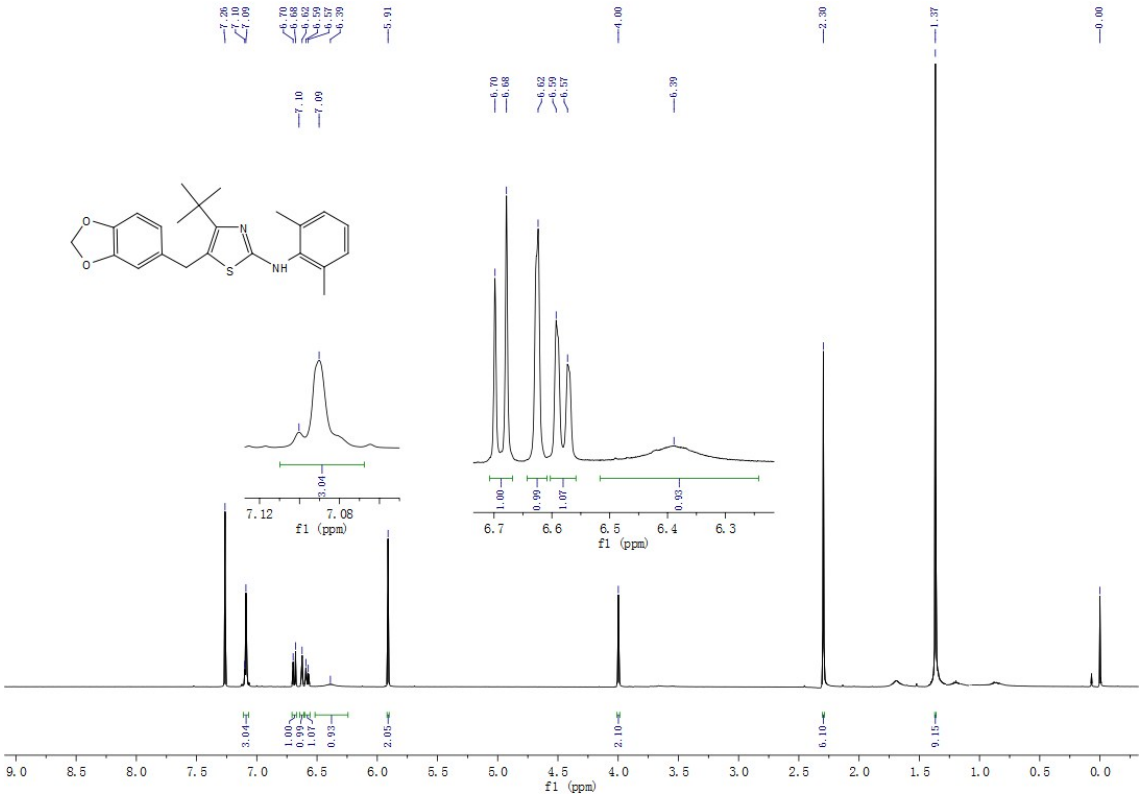

Fig. S19 ^{1}H NMR spectra (400 MHz, $CDCl_3$) of compound **C19**

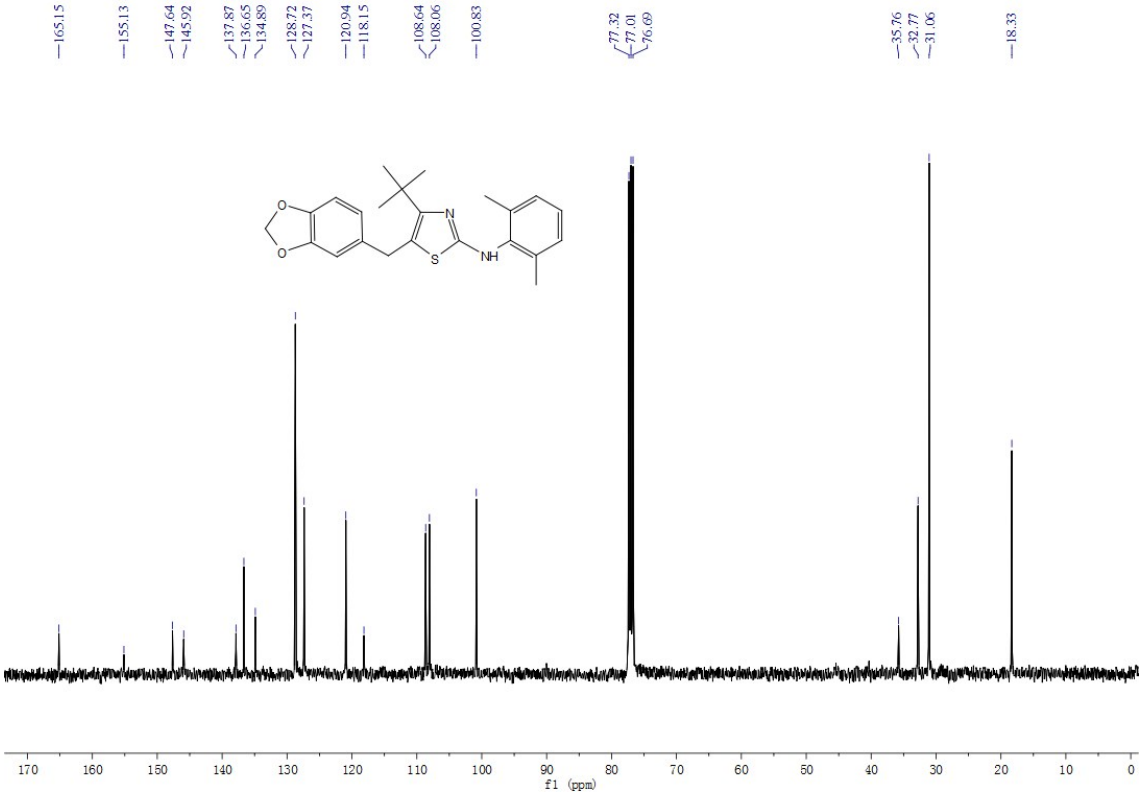

Fig. S20 ^{13}C NMR spectra (100 MHz, $CDCl_3$) of compound **C19**

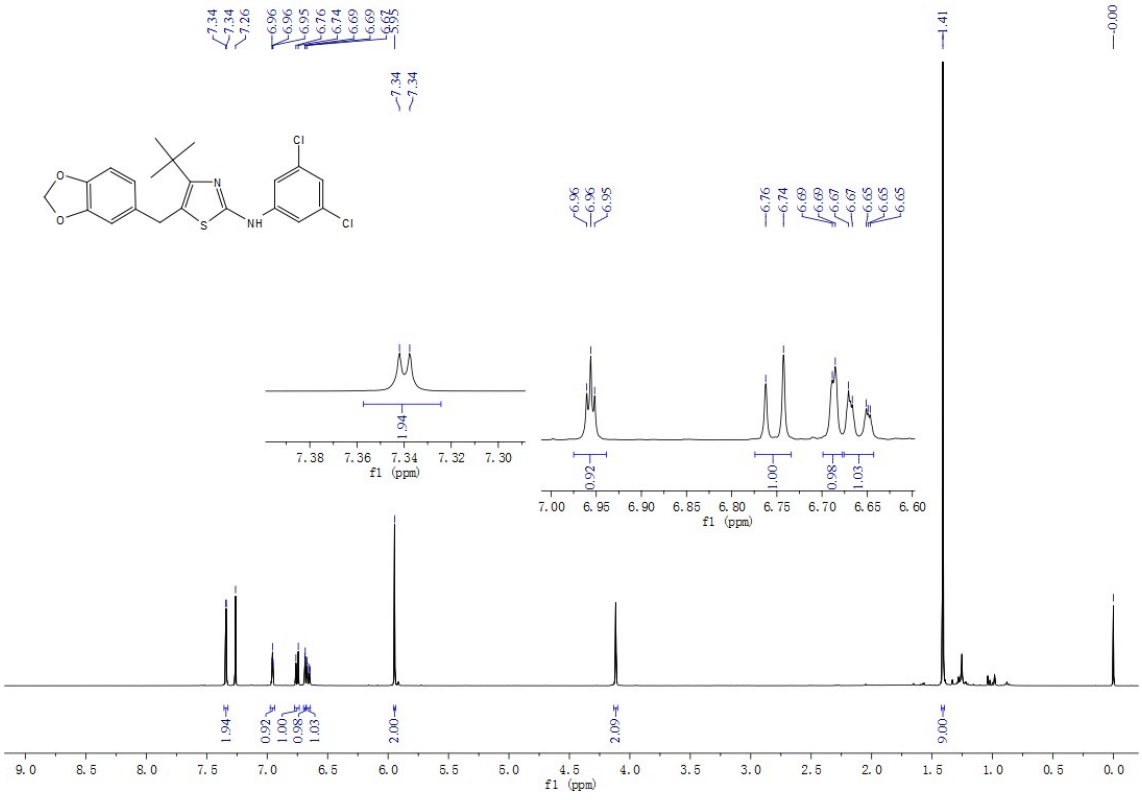

Fig. S21 ^{1}H NMR spectra (400 MHz, $CDCl_3$) of compound **C23**

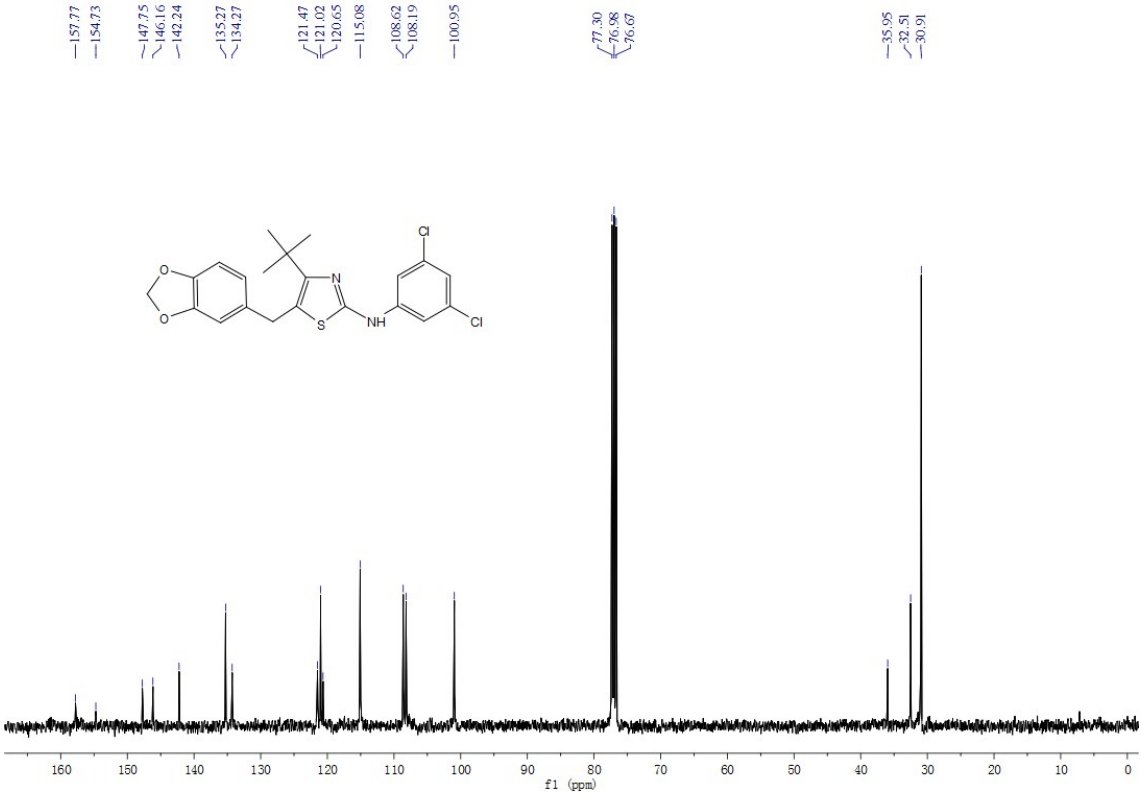

Fig. S22 ^{13}C NMR spectra (100 MHz, $CDCl_3$) of compound **C23**

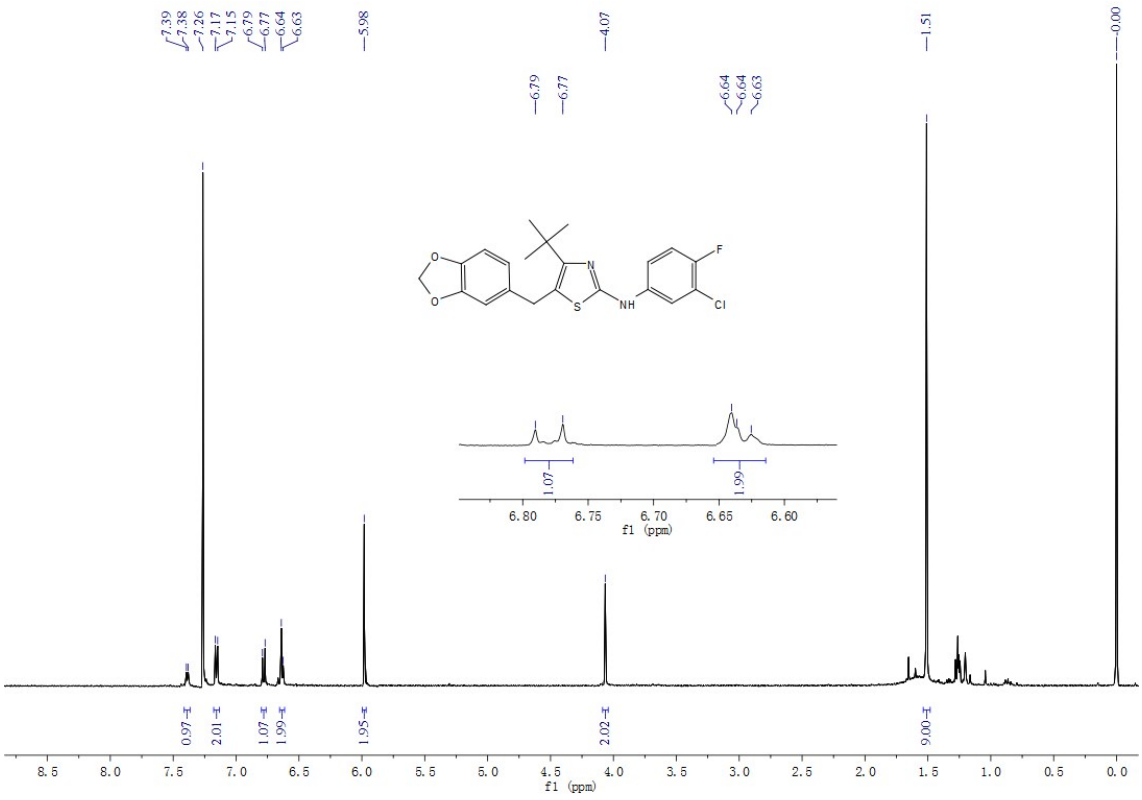

Fig. S23 ^{1}H NMR spectra (400 MHz, $CDCl_3$) of compound **C24**

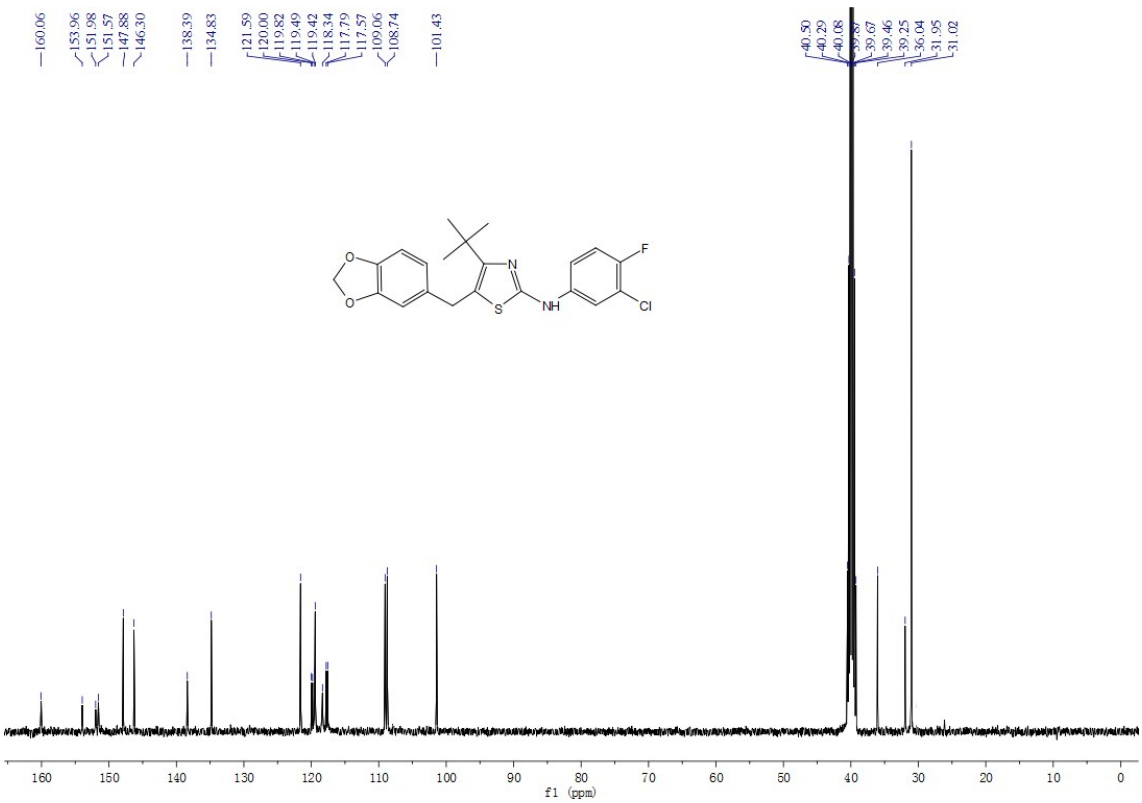

Fig. S24 ^{13}C NMR spectra (100 MHz, DMSO-d_6) of compound **C24**

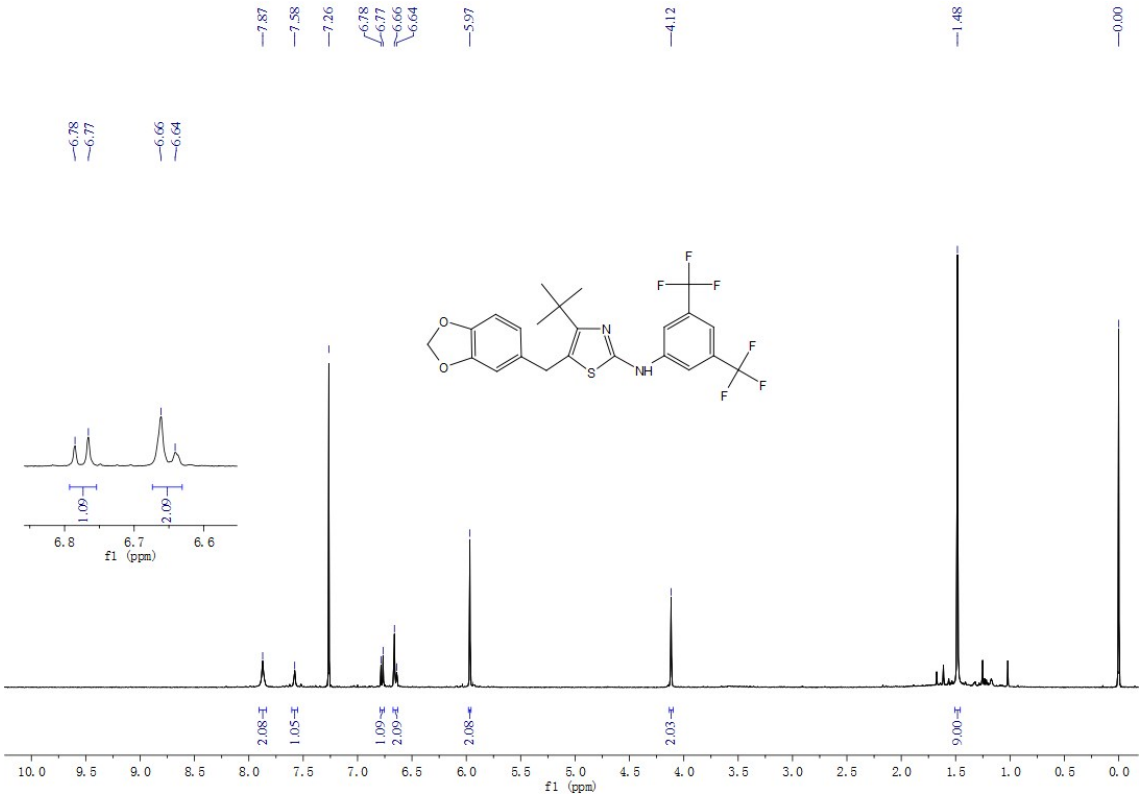

Fig. S25 ^{1}H NMR spectra (400 MHz, $CDCl_3$) of compound **C27**

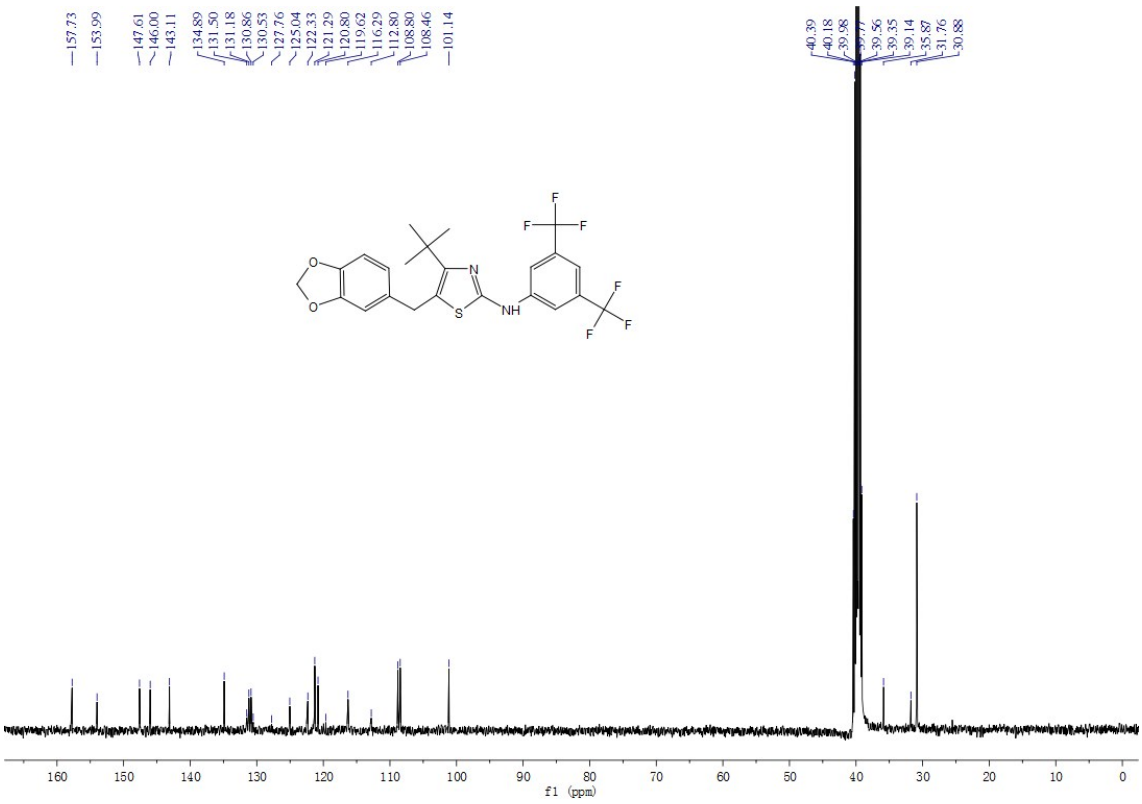

Fig. S26 ^{13}C NMR spectra (100 MHz, DMSO-d_6) of compound **C27**

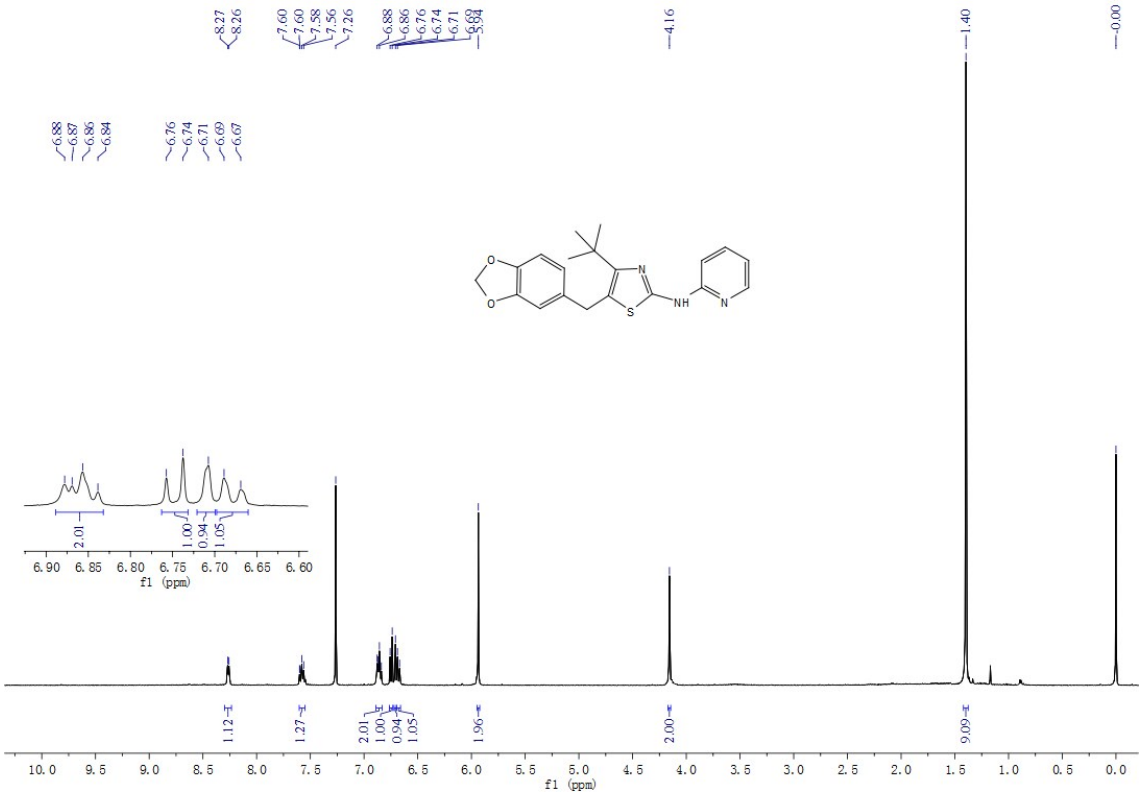

Fig. S27 ^{1}H NMR spectra (400 MHz, $CDCl_3$) of compound **C30**

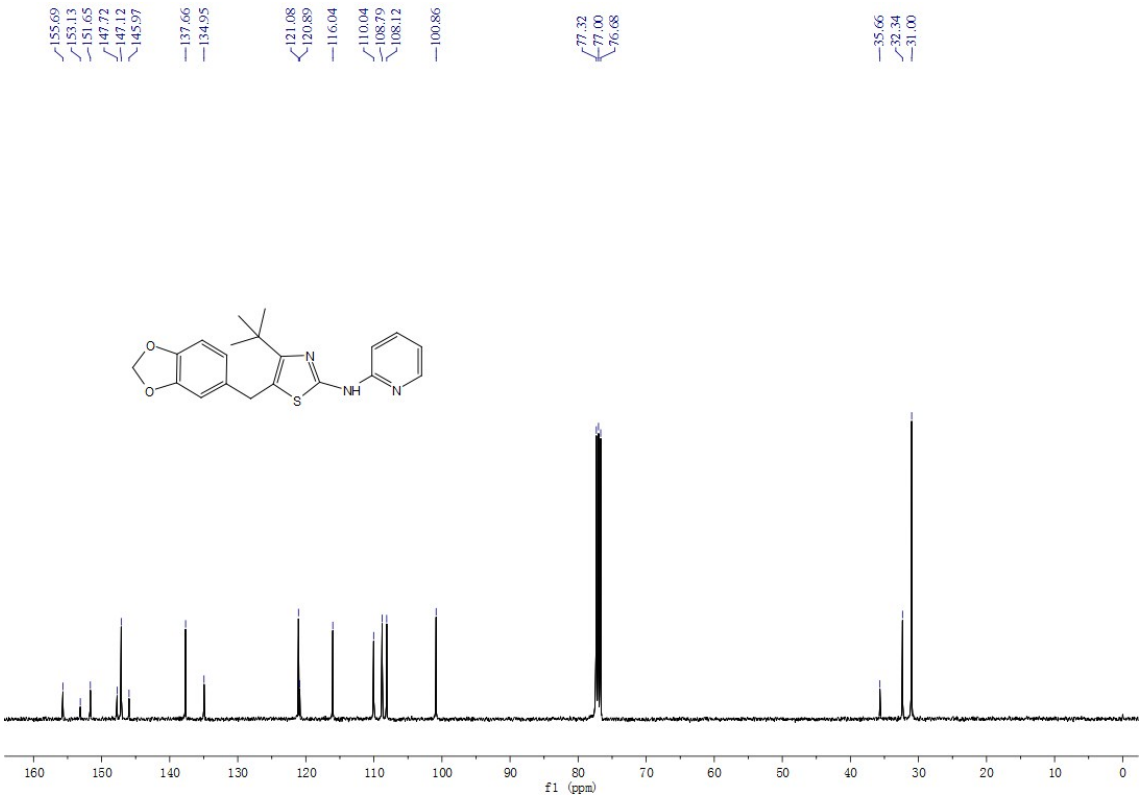

Fig. S28 ^{13}C NMR spectra (100 MHz, $CDCl_3$) of compound **C30**