Supporting Information

Synthesis and evaluation of novel N-3-benzimidazolephenylbisamide derivatives for antiproliferative and hedgehog pathway inhibitory activity

Chiyu Sun,[a] Yangsheng Li,[a] Ailong Shi,[a] Jingzhou Zhang,[a] Yafei Li,[a] Mingming Zhao,[a] Lijuan Zhang,[a] Huachuan Zheng,[b] Ying Meng,[c] Huaiwei Ding,*[a] and Hongrui Song**[a]

[a] *Key Laboratory of Structure-Based Drug Design and Discovery, Ministry of Education, Shenyang Pharmaceutical University, Shenyang 110016, PR China*

[b] *The first affiliated hospital of liaoning medical university, Renmin Street, Jinzhou 121001, PR China*

[c] *College of pharmacy, Liaoning University of Traditional Chinese Medicine, Dalian 116600, PR China*

4. Experimental Section

4.1 Materials and measurements

All chemicals were purchased from commercial sources and used without further purification unless otherwise noted. Melting points were obtained on a Büchi Melting Point B-540 apparatus (Büchi Labortechnik, Flawil, Switzerland) and were uncorrected. Reactions' time and purity of the products were monitored by TLC on FLUKA silica gel aluminum cards (0.2 mm thickness) with fluorescent indicator 254 nm. Mass spectra (MS) were taken in ESI mode on Agilent 1100 LC-MS (Agilent, Palo Alto, CA, USA.). ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker ARX-300, ARX-400 and ARX-600 (Bruker Bioscience, Billerica, MA, USA) with TMS as an internal standard. Elemental analysis was determined on a Carlo-Erba 1106 Elemental analysis instrument (Carlo Erba, Milan, Italy).

4.1.1 General procedure for preparation intermediate of 3-(1H-benzo[d]imidazol-2-yl)-4-substituted aniline (3a-b)

4.1.1.1 Procedure for preparation of N-(2-aminophenyl)-2-chloro-5-nitrobenzamide (1a)

To a solution of 2-chloro-5-nitrobenzoic acid (3.18 g,17.6 mmol) in dry tetrahydrofuran (70 mL) was added oxalyl chloride (4.19 g, 35.2 mmol), followed by one drop of pyridine. The reaction mixture was stirred at 35 °C for 4 hours. The solvent was evaporated in vacuum to give a yellow solid. The crude acyl chloride was added into an ice-cooled solution of 1,2-diaminobenzene (1.91 g, 17.6 mmol) and Et_3N (2.42 g, 23.8 mmol) in anhydrous tetrahydrofuran (40 mL). After stirring overnight at room temperature, the white triethylamine hydrochloride was removed by filtering, the filtrate was evaporated in vacuum and then mixed with ethyl acetate. The mixture was filtered and the filtrate was concentrated in vacuum to give orange viscous solid. The crude solid was washed with water, the precipitate was collected by filtration to give **1a** as a deep yellow solid. Yield 76%. m.p.170-171 °C. ESI-MS m/z: 292.0. ^{1}H NMR(400 MHz, DMSO) δ, 9.88 (s, 1H), 8.54 (d, J=2.7 Hz, 1H), 8.29 (dd, J=8.7 Hz, 2.7 Hz, 1H), 7.86 (d, J=8.7 Hz), 7.25 (dd, J=7.8 Hz, 1.5 Hz, 1H), 6.99 (dt, J = 7.8 Hz, 1.5 Hz,1H), 6.75 (dd, J = 7.8 Hz, 1.5 Hz, 1H), 6.59 (dt, J = 7.5 Hz, 1.5 Hz, 1H), 5.00 (s, 2H);

N-(2-Aminophenyl)-2-methyl-5-nitrobenzamide (1b) : Yield 75%. m.p. 173-174 °C. ESI-MS m/z: 272.0 $[M+H]^+$.

4.1.1.2 Procedure for preparation of 2-(2-chloro-5-nitrophenyl)-1H-benzo[d]imidazole (2a)

1a (0.84 g, 2.8 mmol) was dissolved in glacial acetic acid (40 mL) and refluxed at 110 °C for 3 hours. The mixture was poured into water (200 mL) and acidified with saturated sodium bicarbonate aqueous solution up to pH= 5-6. The precipitate was collected by filtration and purified by column chromatography (silica gel, petroleum

ether /ethyl acetate = 6~9:1) to give **2a** as a pale yellow solid. Yield 80%. m.p. 190-192 °C. ESI-MS m/z: 274.0 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO) δ, 8.75 (d, J = 2.7 Hz, 1H), 8.26 (dd, J = 8.7 Hz, 3.0 Hz, 1H), 7.89(d, J = 8.7 Hz, 1H), 7.64 (dd, J = 6.0 Hz, 3.3 Hz, 2H), 7.23 (dd, J = 6.0 Hz, 3.3 Hz, 2H).

2-(2-methyl-5-nitrophenyl)-1H-benzo[d]imidazole (2b): Yield 78%. m.p. 192-194 °C. ESI-MS m/z: 254.0 $[M+H]^+$.

4.1.1.3 Procedure for preparation of 3-(1 H-Benzo[d]imidazol-2-yl)-4-chloroaniline (3a)

To a solution of **2a** (0.76 g, 3.2 mmol) in aqueous ethanol (30 mL) was added stannous chloride dehydrate (2.6 g, 12.8 mmol). The mixture was heated to reflux at 80 °C and added hydrochloric acid (37%, 25 mL). The mixture was stirred at the same temperature for 8 hours. The solvent was evaporated in vacuum, neutralized to weak alkaline with sodium hydroxide solution (1mol/L) and then extracted with ethyl acetate (50 mL) two times. The combined organic phase was washed with saturated sodium chloride solution and then dried by sodium sulfate. The mixture was filtered and the filtrate was concentrated in vacuum to give **3a** as dark yellow solid. Yield 85%. m.p. 207-209 °C. ESI-MS m/z: 244.0 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO) δ, 12.50 (s, 1H), 7.54-7.63 (m, 2H), 7.19-7.22 (m, 3H), 7.11 (d, J = 3.0 Hz, 1H), 6.69 (dd, J = 8.7 Hz, 3.0 Hz, 1H), 5.47 (s, 2H).

3-(1H-benzo[d]imidazol-2-yl)-4-methylaniline (3b): The preparation of the key intermediate **3b** was same with the preparation of **3a**, so the synthesis method would not be listed here. Yield 80%. m.p. 204-206 °C. ESI-MS m/z: 224.0 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO) δ 7.57 (s, 2H), 7.19 (dd, J = 5.8, 3.1 Hz, 2H), 7.02 (d, J = 8.1 Hz, 1H), 6.96 (d, J = 1.9 Hz, 1H), 6.64 (dd, J = 8.0, 1.9 Hz, 1H), 2.39 (s, 3H).

4.1.2 General procedure for preparation intermediates of 4-benzyloxy-substituted benzoic acid (5a-l)

4.1.2.1 Procedure for preparation of 4-benzyloxy-substituted benzoic acid methyl ester (4a-l)

Methylparaben (5 g, 3.29 mmol) was dissolved in acetone (30 mL) and added potassium carbonate (1.3 g, 9.87 mmol), then warmed to 65 °C for 1 hour. The mixture was added benzyl bromide (0.84 g, 4.95 mmol) and heated under reflux for 5 hours at 80 °C. After cooling to room temperature, the mixture was filtered and the filtrate was concentrated in vacuum to give crude product. The residue was purified by washing with anhydrous ethanol and the solvent was removed under vacuum to give **4a-l** as white solids.

4.1.2.2 Procedure for preparation of 4-benzyloxy-substituted benzoic acid (5a-l)

A mixture of intermediates **4a-l** (5 g, 0.022 mol), sodium hydroxide (4.36 g, 0.110 mol), methanol (100 mL) and water (10 mL) was stirred at 75 °C for 2 hours. The solvent was evaporated in vacuum and the residue was poured into water. The mixture was neutralized to pH = 5 with 1mol/L HCl. The mixture was filtered to give white solids **5a-l**.

4-(benzyloxy)benzoic acid (5a): Yield 85%. m.p. 170-172 °C. ESI-MS m/z: 227.2 $[M-H]^-$. ^{1}H NMR (400 MHz, DMSO) δ, 5.11 (s, 2H), 6.99 (d, J = 8.9 Hz, 2H), 7.31-7.45 (m, 5H), 7.99 (d, J = 8.9 Hz, 2H);

4-(4-chlorobenzyloxy)benzoic acid (5b): Yield 78%. m.p. 199-201°C. ESI-MS m/z: 261.0 $[M-H]^-$.

4-(4-(trifluoromethyl)benzyloxy)benzoic acid (5c): Yield 76%. m.p. 196-198 °C. ESI-MS m/z: 295.2 $[M-H]^-$.

4-(3-(trifluoromethyl)benzyloxy)benzoic acid (5d): Yield 81%. m.p. 202-204 °C. ESI-MS m/z: 295.2 $[M-H]^-$.

4-(2-fluorobenzyloxy)benzoic acid (5e): Yield 80%. m.p. 177-179 °C. ESI-MS m/z: 245.0 $[M-H]^-$.

4-(2,3-dichlorobenzyloxy)benzoic acid (5f): Yield 83%. m.p. 220-222 °C. ESI-MS m/z: 295.0 $[M-H]^-$.

4-(3-methylbenzyloxy)benzoic acid (5g): Yield 79%. m.p. 215-217 °C. ESI-MS m/z: 241.0 $[M-H]^-$.

4-(3-fluorobenzyloxy)benzoic acid (5h):Yield 79%. m.p. 188-190 °C. ESI-MS m/z:245.0 $[M-H]^-$.

4-(2-chlorobenzyloxy)benzoic acid (5i): Yield 82%. m.p. 186-188 °C. ESI-MS m/z: 261.0 $[M-H]^-$.

4-(3,4-dichlorobenzyloxy)benzoic acid (5j): Yield 84%. m.p. 217-219 °C. ESI-MS m/z: 295.0 $[M-H]^-$.

4-(2,4-dichlorobenzyloxy)benzoic acid (5k): Yield 79%. m.p. 209-211 °C. ESI-MS m/z: 295.0 $[M-H]^-$.
4-(4-fluorobenzyloxy)benzoic acid (5l): Yield 81%. m.p. 213-215 °C. ESI-MS m/z: 245.0 $[M-H]^-$.

4.1.3 General procedure for Preparation of Target Compounds (6a-o)

To a solution of **5a-l** (0.3 g, 1.32 mmol) in anhydrous toluene (50 mL) was added thionyl chloride (0.79 g, 6.60 mmol), followed by one drop of pyridine. The mixture was stirred under reflux for 6 hours at 72 °C. The solvent was evaporated in vacuum to give a yellow solid, which was dissolved in moderate amount of acetonitrile for next step without further purification.

Potassium thiocyanate (0.90 g, 9.24 mmol) was dissolved in anhydrous acetonitrile (30 mL), before the crude acyl chloride was added. The reaction mixture was stirred at 80 °C for 5 hours. **3a-b** (0.22 g, 0.88 mmol) was added and refluxed at the same temperature for 10 hours. The mixture was poured into water, then extracted with ethyl acetate (100 mL). The organic phase was washed with saturated sodium chloride solution and dried by sodium sulfate. The mixture was concentrated in vacuum and the residue was purified by column chromatography (silica gel, cyclohexane/ethyl acetate = 2:1) to give **6a-o**.

N-(3-(1H-benzo[d]imidazol-2-yl)-4-methylphenylcarbamothioyl)-4-(benzyloxy)benzamide (6a): light yellow solid, 82% yield. m.p. 112-115 °C. ^{1}H NMR (600 MHz, DMSO) δ 12.65(s, 1H) ,8.04-7.98 (m, 3H), 7.77 (d, J = 8.0 Hz, 1H), 7.68 (d, J = 7.8 Hz, 1H), 7.52 (d, J = 7.7 Hz, 1H), 7.46 (d, J = 7.3 Hz, 2H), 7.41 (dd, J = 15.0, 7.8 Hz, 3H), 7.35 (d, J = 7.3 Hz, 1H), 7.21 (dt, J = 14.4, 7.0 Hz, 2H), 7.15 (d, J = 8.8 Hz, 2H), 5.21(s, 2H), 2.62(s, 3H). ESI-MS m/z: 493.1 $[M+H]^+$. Anal. calcd. for $C_{29}H_{24}N_4O_2S$ (%): C,70.71; H,4.91; N,11.37. Found (%): C, 70.65; H, 4.99; N, 11.35.

N-(3-(1H-benzo[d]imidazol-2-yl)-4-methylphenylcarbamothioyl)-4-(3-chlorobenzyloxy) benzamide (6b): light yellow solid, 79% yield. m.p. 94-95 °C. ^{1}H NMR (600 MHz, DMSO) δ 12.77 (s, 1H), 11.46 (s, 1H), 8.07 – 7.97 (m, 3H), 7.78 (d, J = 7.9 Hz, 1H), 7.62 (s, 2H), 7.54 (s, 1H), 7.45 – 7.41 (m, 4H), 7.23 (dd, J = 5.7, 2.9 Hz, 2H), 7.16 (d, J = 8.6 Hz, 2H), 5.24 (s, 2H), 2.62 (s, 3H). ESI-MS m/z: 527.1 $[M+H]^+$. Anal. calcd. for $C_{29}H_{23}ClN_4O_2S$ (%): C, 66.09; H, 4.40; N, 10.63. Found (%): C,66.14; H, 4.36; N, 10.66.

N-(3-(1H-benzo[d]imidazol-2-yl)-4-methylphenylcarbamothioyl)-4-(4-(trifluoromethyl)benzyloxy)benzamide (6c): light yellow solid, 81%. m.p. 110-113 °C. ^{1}H NMR (600 MHz, DMSO) δ 12.77 (s, 1H), 11.46 (s, 1H), 8.03 (d, J = 8.6 Hz,2H), 8.00 (s, 1H), 7.77 (d, J = 7.9 Hz, 3H), 7.69 (d, J = 7.9 Hz, 2H), 7.61 (s, 2H), 7.42 (d, J = 8.2 Hz,1H), 7.22 (dd, J = 5.6, 2.9 Hz, 2H), 7.16 (d, J = 8.6 Hz, 2H), 5.34 (s, 2H), 2.62 (s, 3H). ESI-MS m/z: 561.1 $[M+H]^+$. Anal. calcd. for $C_{30}H_{23}F_3N_4O_2S$ (%): C, 64.28; H, 4.14; N, 9.99. Found (%): C, 64.23; H, 4.18; N, 9.96.

N-(3-(1H-benzo[d]imidazol-2-yl)-4-methylphenylcarbamothioyl)-4-(3-(trifluoromethyl)benzyloxy)benzamide (6d): light yellow solid, 76% yield. m.p. 112-113 °C. ESI-MS m/z: 560.1. ^{1}H NMR (400 MHz, DMSO) δ 12.78 (s, 1H), 11.48 (s, 1H), 8.05 (d, J = 8.9 Hz, 2H), 8.01 (d, J = 2.0 Hz, 1H), 7.86 (s, 1H), 7.79 (dd, J = 9.7, 4.3 Hz, 2H), 7.74 (d, J = 7.8 Hz, 1H), 7.68 (d, J = 7.6 Hz, 1H), 7.63 (s, 2H), 7.44 (d, J = 8.4 Hz, 1H), 7.23 (dd, J = 6.0, 3.2 Hz, 2H), 7.19 (d, J = 8.9 Hz, 2H), 5.35 (s, 2H), 2.64 (s, 3H). ESI-MS m/z: 561.1 $[M+H]^+$. Anal. calcd. for $C_{30}H_{23}F_3N_4O_2S$ (%): C, 64.28; H, 4.14; N, 9.99. Found (%): C, 64.23; H, 4.18; N, 9.97.

N-(3-(1H-benzo[d]imidazol-2-yl)-4-methylphenylcarbamothioyl)-4-(2-fluorobenzyloxy)benzamide (6e): light yellow solid, 79% yield. m.p. 88-90 °C. ^{1}H NMR (400 MHz, DMSO) δ 12.79 (s, 1H), 11.48 (s, 1H), 8.05 (d, J = 8.9 Hz, 2H), 8.02 (d, J = 2.0 Hz, 1H), 7.80 (dd, J = 8.2, 1.9 Hz, 1H), 7.63 (dd, J = 5.4, 3.2 Hz, 2H), 7.59 (dd, J = 7.8, 1.5 Hz, 1H), 7.44 (d, J = 8.1 Hz, 2H), 7.29 – 7.26 (m, 2H), 7.24 (dd, J = 6.1, 3.1 Hz, 2H), 7.19 (d, J = 8.9 Hz, 2H), 5.27 (s, 2H), 2.64 (s, 3H). ESI-MS m/z: 511.1 $[M+H]^+$. Anal. calcd. for $C_{29}H_{23}FN_4O_2S$ (%): C, 64.22; H, 4.54; N,10.97. Found (%): C, 64.28; H, 4.52; N, 10.95.

N-(3-(1H-benzo[d]imidazol-2-yl)-4-methylphenylcarbamothioyl)-4-(2,3-dichlorobenzyloxy)benzamide (6f): light yellow solid, 80% yield. m.p. 185-186 °C. ^{1}H NMR (400 MHz, DMSO) δ 12.91 (s, 1H), 11.62 (s, 1H), 8.18 (d,

J = 8.7 Hz, 2H), 8.14 (s, 1H), 7.92 (d, J = 8.2 Hz, 1H), 7.81 (d, J = 7.8 Hz, 1H), 7.75 (t, J = 7.6 Hz, 3H), 7.56 (t, J = 6.8 Hz, 2H), 7.37 (dd, J = 6.0, 3.1 Hz, 2H), 7.32 (d, J = 8.8 Hz, 2H), 5.45 (s, 2H), 2.76 (s, 3H). ESI-MS m/z: 561.0 $[M+H]^+$. Anal. calcd. for $C_{29}H_{22}Cl_2N_4O_2S$ (%): C, 62.03; H, 3.95; N, 9.98. Found (%): C, 62.12; H, 3.89; N, 9.90.

N-(3-(1H-benzo[d]imidazol-2-yl)-4-methylphenylcarbamothioyl)-4-(4-methylbenzyloxy)benzamide (6g):light yellow solid, 75% yield. m.p. 83-86 °C. ^{1}H NMR (400 MHz, DMSO) δ 12.72 (d, J = 56.2 Hz, 1H), 11.45 (s, 1H), 8.02 (d, J = 8.8 Hz, 3H), 7.79 (d, J = 7.9 Hz, 1H), 7.63 (s, 2H), 7.44 (d, J = 8.3 Hz, 1H), 7.36 (d, J = 7.3 Hz, 2H), 7.26 – 7.20 (m, 4H), 7.14 (d, J = 8.8 Hz, 2H), 5.18 (s, 2H), 2.64 (s, 3H), 2.31 (s, 3H). ESI-MS m/z: 507.1 $[M+H]^+$. Anal. calcd. for $C_{30}H_{26}N_4O_2S$ (%): C, 71.12; H, 5.17; N, 11.06. Found (%): C, 71.05;H,5.15;N,11.22.

N-(3-(1H-benzo[d]imidazol-2-yl)-4-methylphenylcarbamothioyl)-4-(3-fluorobenzyloxy)benzamide (6h): light yellow solid, 76% yield. m.p. 105-108 °C. ^{1}H NMR (400 MHz, DMSO) δ 12.79 (s, 1H), 11.48 (s, 1H), 8.05 (d, J = 8.9 Hz, 2H), 8.02 (d, J = 1.9 Hz, 1H), 7.80 (d, J = 8.2 Hz, 3H), 7.71 (d, J = 8.0 Hz, 2H), 7.63 (s, 2H), 7.44 (d, J = 8.4 Hz, 1H), 7.23 (dd, J = 6.0, 3.2 Hz, 2H), 7.18 (d, J = 8.9 Hz, 2H), 5.37 (s, 2H), 2.65 (s, 3H). ESI-MS m/z: 511.1 $[M+H]^+$. Anal. calcd. for $C_{29}H_{23}FN_4O_2S$ (%): C, 64.22 ; H, 4.54 ; N, 10.97. Found (%): C, 64.18; H, 4.50; N, 10.88.

N-(3-(1H-benzo[d]imidazol-2-yl)-4-methylphenylcarbamothioyl)-4-(2-chlorobenzyloxy)benzamide (6i): light yellow solid, 82% yield. m.p. 110-113 °C. ^{1}H NMR (600 MHz, DMSO) δ 12.80 (s, 1H), 11.49 (s, 1H), 8.04 (d, J = 8.0 Hz, 2H), 8.02 (s, 1H), 7.81 (d, J = 7.4 Hz, 1H), 7.64 (d, J = 13.0 Hz, 2H), 7.62 (d, J = 5.3 Hz, 1H), 7.52 (d, J = 6.3 Hz, 1H), 7.44 (d, J = 8.0 Hz, 2H), 7.40 (s, 1H), 7.27 (s, 2H), 7.18 (d, J = 8.2 Hz, 2H), 5.26 (s, 2H), 2.61 (s, 3H). ESI-MS m/z: 527.1 $[M+H]^+$. Anal. calcd. for $C_{29}H_{23}ClN_4O_2S$ (%): C, 66.09; H, 4.40; N, 10.63. Found (%): C, 66.14; H, 4.33; N, 10.59.

N-(3-(1H-benzo[d]imidazol-2-yl)-4-methylphenylcarbamothioyl)-4-(3,4-dichlorobenzyloxy)benzamide (6j): light yellow solid, 81% yield. m.p. 79-81 °C. ^{1}H NMR (400 MHz, DMSO) δ 12.79 (s, 1H), 11.48 (s, 1H), 8.05 (d, J = 8.7 Hz, 2H), 8.02 (s, 1H), 7.79 (d, J = 10.3 Hz, 2H), 7.70 (s, 1H), 7.68 (s, 1H), 7.63 (s, 1H), 7.49 (d, J = 6.7 Hz, 1H), 7.44 (d, J = 8.4 Hz, 1H), 7.23 (dd, J = 5.9, 3.1 Hz, 2H), 7.17 (d, J = 8.8 Hz, 2H), 5.26 (s, 2H), 2.65 (s, 3H). ESI-MS m/z: 561.0 $[M+H]^+$. Anal. calcd. for $C_{29}H_{22}Cl_2N_4O_2S$ (%): C, 62.03; H, 3.95; N, 9.98. Found (%): C, 62.15; H,3.87; N, 9.88.

N-(3-(1H-benzo[d]imidazol-2-yl)-4-methylphenylcarbamothioyl)-4-(2,4-dichlorobenzyloxy)benzamide (6k): light yellow solid, 77% yield. m.p. 198-201 °C. ^{1}H NMR (400 MHz, DMSO) δ 12.76 (d, J = 30.1 Hz, 1H), 11.50 (s, 1H), 8.06 (d, J = 8.9 Hz, 2H), 8.02 (d, J = 1.8 Hz, 1H), 7.80 (dd, J = 8.2, 1.8 Hz, 1H), 7.72 (t, J = 3.9 Hz, 1H), 7.65 (t, J = 8.7 Hz, 3H), 7.51 (dd, J = 8.3, 2.1 Hz, 1H), 7.44 (d, J = 8.4 Hz, 1H), 7.24 (dd, J = 6.0, 3.2 Hz, 2H), 7.19 (d, J = 8.9 Hz, 2H), 5.27 (s, 2H), 2.65 (s, 3H). ESI-MS m/z: 561.0 $[M+H]^+$. Anal. calcd. for $C_{29}H_{22}Cl_2N_4O_2S$ (%): C, 62.03; H, 3.95; N, 9.98. Found (%): C, 62.20; H, 3.89; N, 9.88.

N-(3-(1H-benzo[d]imidazol-2-yl)-4-methylphenylcarbamothioyl)-4-(4-fluorobenzyloxy)benzamide (6l): light yellow solid. 78% yield. m.p. 99-101 °C. ^{1}H NMR (400 MHz, DMSO) δ 12.80 (s, 1H), 11.47 (s, 1H), 8.04 (d, J = 6.7 Hz, 3H), 7.80 (s, 1H), 7.64 (s, 2H), 7.55 (d, J = 5.9 Hz, 2H), 7.44 (d, J = 7.2 Hz, 1H), 7.25 (d, J = 6.3 Hz, 4H), 7.16 (d, J =6.7Hz, 2H), 5.21 (s, 2H), 2.64 (s, 3H). ESI-MS m/z: 511.1 $[M+H]^+$. Anal. calcd. for $C_{29}H_{23}FN_4O_2S$ (%): C, 64.22; H, 4.54; N, 10.97. Found (%): C, 64.20; H, 4.49; N,10.92.

N-(3-(1H-benzo[d]imidazol-2-yl)-4-chlorophenylcarbamothioyl)-4-(4-(trifluoromethyl)benzyloxy)benzamide (6m): light yellow solid, 85% yield. m.p. 214-215 °C. ^{1}H NMR (400 MHz, DMSO) δ 11.10 (s, 1H), 9.89 (s, 1H), 9.51 (s, 1H), 8.53 (dd, J = 8.9, 2.6 Hz, 1H), 7.96 (d, J = 8.6 Hz, 3H), 7.79 (d, J = 8.1 Hz, 3H), 7.69 (d, J = 8.0 Hz, 3H), 7.13 (d, J = 8.7 Hz, 3H), 5.34 (s, 2H). ESI-MS m/z: 581.1 $[M+H]^+$. Anal. calcd. for $C_{29}H_{20}ClF_3N_4O_2S$ (%): C, 59.95; H, 3.47; N, 9.64. Found (%): C, 59.88; H, 3.40; N, 9.72.

N-(3-(1H-benzo[d]imidazol-2-yl)-4-chlorophenylcarbamothioyl)-4-(3,4-dichlorobenzyloxy)benzamide (6n): light yellow solid, 85% yield. m.p. 190-191 °C. ^{1}H NMR (400 MHz, DMSO) δ 12.78 (s, 1H), 11.55 (s, 1H), 8.27 (d, J = 2.3 Hz, 1H), 8.04 (d, J = 8.8 Hz, 2H), 7.91 (dd, J = 8.7, 2.3 Hz, 1H), 7.77 (d, J = 1.3 Hz, 1H), 7.72 – 7.66 (m,

4H), 7.48 (d, J = 8.0 Hz, 1H), 7.26 (dd, J = 5.9, 3.1 Hz, 2H), 7.17 (d, J = 8.8 Hz, 2H), 5.25 (s, 2H). ESI-MS m/z: 583.0 $[M+H]^+$. Anal. calcd. for $C_{28}H_{19}Cl_3N_4O_2S$ (%): C, 57.79; H, 3.29; N, 9.63. Found (%): C, 57.85; H, 3.21; N, 9.76.

N-(3-(1H-benzo[d]imidazol-2-yl)-4-chlorophenylcarbamothioyl)-4-(2,4-dichlorobenzyloxy)benzamide (6o): light yellow solid, 83 % yield. m.p. 219-221°C. ^{1}H NMR (400 MHz, DMSO) δ 12.79 (s, 1H), 11.58 (s, 1H), 8.28 (d, J = 2.2 Hz, 1H), 8.05 (d, J = 8.8 Hz, 2H), 7.93 (dd, J = 8.6, 2.1 Hz, 1H), 7.71 (d, J = 8.9 Hz, 2H), 7.67 (dd, J = 8.5, 4.9 Hz, 3H), 7.51 (dd, J = 8.2, 1.8 Hz, 1H), 7.28 (dd, J = 6.0, 3.1 Hz, 2H), 7.19 (d, J = 8.8 Hz, 2H), 5.27 (s, 2H). ESI-MS m/z: 583.0 $[M+H]^+$. Anal. calcd. for $C_{28}H_{19}Cl_3N_4O_2S$ (%): C, 57.79; H, 3.29; N, 9.63. Found (%): C, 57.85; H, 3.21; N, 9.76.

4.1.4 General procedure for Preparation of Target Compounds (7a-o)

6a-o (0.051 g, 0.1 mmol) was dissolved in acetone solution (15 mL) and added saturation sodium solution (2 mL) and then dropped hydrogen peroxide (30%, 5 mL). The mixture was stirred overnight at room temperature. The mixture was neutralized to pH = 5 with hydrochloric acid (1 mol/L, 5 mL). The precipitate was collected by filtration to give **7a-o** as white solids.

N-(3-(1H-benzo[d]imidazol-2-yl)-4-methylphenylcarbamoyl)-4-(benzyloxy)benzamide (7a): white solid, 76% yield. m.p. 233-235 °C. ^{1}H NMR (600 MHz, DMSO) δ 12.65 (s, 1H), 11.04 (s, 1H), 10.92 (s, 1H), 8.05 (d, J = 8.8 Hz,2H), 7.93 (d, J = 1.9 Hz, 1H), 7.70 – 7.64 (m, 2H), 7.52 (d, J = 7.7 Hz, 1H), 7.46 (d, J = 7.4 Hz, 2H), 7.40 (t, J = 7.5 Hz, 2H), 7.35 (t, J = 7.4 Hz, 2H), 7.23 – 7.18 (m, 2H), 7.14 (d, J = 8.9 Hz, 2H), 5.21 (s, 2H), 2.57 (s, 3H). ESI-MS m/z: 477.1 $[M+H]^+$. Anal. calcd. for $C_{29}H_{24}N_4O_3$ (%): C,73.09; H, 5.08; N, 11.76. Found (%): C, 73.15; H, 5.22; N, 11.70.

N-(3-(1H-benzo[d]imidazol-2-yl)-4-methylphenylcarbamoyl)-4-(3-chlorobenzyloxy)benzamide (7b): white solid, 82% yield. m.p. 234-236 °C. ^{1}H NMR (400 MHz, DMSO) δ 12.79 (s, 1H), 11.17 (s, 1H), 11.06 (s, 1H), 8.20 (d, J = 8.7 Hz, 2H), 8.07 (d, J = 1.7 Hz, 1H), 7.81 (t, J = 7.3 Hz, 2H), 7.66 (d, J = 7.5 Hz, 2H), 7.56 (s, 3H), 7.49 (d, J = 8.4 Hz, 1H), 7.38 – 7.31 (m, 2H), 7.29 (d, J = 8.8 Hz, 2H), 5.37 (s, 2H), 2.71 (s, 3H). ESI-MS m/z: 511.1 $[M+H]^+$. Anal. calcd. for $C_{29}H_{23}ClN_4O_3$ (%): C, 68.17; H, 4.54; N, 10.96. Found (%): C, 68.09; H, 4.50; N, 10.88.

N-(3-(1H-benzo[d]imidazol-2-yl)-4-methylphenylcarbamoyl)-4-(4-(trifluoromethyl)benzyloxy)benzamide (7c): white solid, 79% yield. m.p. 234-236 °C. ^{1}H NMR (400 MHz, DMSO) δ 12.78 (s, 1H), 11.16 (s, 1H), 11.06 (s, 1H), 8.20 (d, J = 8.9 Hz, 2H), 8.06 (d, J = 2.1 Hz, 1H), 7.91 (d, J = 8.3 Hz, 2H), 7.82 (d, J = 8.1 Hz, 4H), 7.66 (d, J = 7.4 Hz, 1H), 7.49 (d, J = 8.5 Hz, 1H), 7.38 – 7.32 (m, 2H), 7.30 (d, J = 8.9 Hz, 2H), 5.48 (s, 2H), 2.70 (s, 3H). ESI-MS m/z: 545.1 $[M+H]^+$. Anal. calcd. for $C_{30}H_{23}F_3N_4O_3$ (%): C, 66.17; H, 4.26; N, 10.29. Found (%): C, 66.34; H, 4.19; N,10.22.

N-(3-(1H-benzo[d]imidazol-2-yl)-4-methylphenylcarbamoyl)-4-(3-(trifluoromethyl)benzyloxy)benzamide (7d): white solid, 83% yield. m.p.152-155 °C. ^{1}H NMR (400 MHz, DMSO) δ 11.10 (s, 1H), 10.99 (s, 1H), 8.09 (d, J = 8.7 Hz, 2H), 8.01 (s, 1H), 7.86 (s, 1H), 7.82 – 7.74 (m, 4H), 7.73 (s, 1H), 7.68 (d, J = 7.7 Hz, 1H), 7.45 (t, J = 7.7 Hz, 3H), 7.19 (d, J = 8.7 Hz, 2H), 5.34 (s, 2H), 2.55 (s, 3H). ESI-MS m/z: 545.1 $[M+H]^+$. Anal. calcd. for $C_{30}H_{23}F_3N_4O_3$ (%): C, 66.17; H, 4.26; N, 10.29. Found (%): C, 66.25; H, 4.19; N, 10.20.

N-(3-(1H-benzo[d]imidazol-2-yl)-4-methylphenylcarbamoyl)-4-(2-fluorobenzyloxy)benzamide (7e): white solid, 85% yield. m.p. 230-232 °C. ^{1}H NMR (400 MHz, DMSO) δ 11.08 (s, 1H), 10.97 (s, 1H), 8.09 (d, J = 8.6 Hz, 2H), 7.98 (s, 1H), 7.78 – 7.66 (m, 3H), 7.59 (t, J = 7.3 Hz, 1H), 7.44 (dd, J = 15.8, 7.7 Hz, 2H), 7.37 – 7.30 (m, 2H), 7.29 – 7.24 (m, 2H), 7.20 (d, J = 8.6 Hz, 2H), 5.26 (s, 2H), 2.57 (s, 3H). ESI-MS m/z: 495.1 $[M+H]^+$. Anal. calcd. for $C_{29}H_{23}FN_4O_3$ (%): C, 70.43; H, 4.69; N, 11.33. Found (%): C, 70.29; H, 4.86; N, 11.28.

N-(3-(1H-benzo[d]imidazol-2-yl)-4-methylphenylcarbamoyl)-4-(2,3-dichlorobenzyloxy)benzamide (7f): white solid,81% yield. m.p. 245-247 °C. ^{1}H NMR (400 MHz, DMSO) δ 11.12 (s, 1H), 8.09 (d, J = 8.6 Hz, 2H), 7.95 (s,

1H), 7.68 (d, J = 8.0 Hz, 2H), 7.61 (d, J = 7.4 Hz, 3H), 7.44 (t, J = 7.8 Hz, 1H), 7.37 (d, J = 8.3 Hz, 1H), 7.23 (dd, J = 5.7, 3.0 Hz, 2H), 7.19 (d, J = 8.7 Hz, 2H), 5.32 (s, 2H), 2.59 (s, 3H). ESI-MS m/z: 545.1 $[M+H]^+$. Anal. calcd. for $C_{29}H_{22}Cl_2N_4O_3$ (%): C, 63.86; H, 4.07; N,10.27. Found (%): C, 63.79; H, 4.12; N, 10.19.

N-(3-(1H-benzo[d]imidazol-2-yl)-4-methylphenylcarbamoyl)-4-(4-methylbenzyloxy)benzamide (7g): white solid, 73% yield. m.p. 246-248 °C. ^{1}H NMR (400 MHz, DMSO) δ 12.79 (s, 1H), 11.18 (s, 1H), 11.05 (s, 1H), 8.18 (d, J = 8.8 Hz, 2H), 8.07 (d, J = 1.9 Hz, 1H), 7.81 (dd, J = 12.2, 4.6 Hz, 2H), 7.66 (d, J = 7.5 Hz, 1H), 7.56 – 7.46 (m, 3H), 7.35 (t, J = 8.7 Hz, 4H), 7.26 (d, J = 8.9 Hz, 2H), 5.29 (s, 2H), 2.71 (s, 3H), 2.63 (s, 3H). ESI-MS m/z: 491.2. $[M+H]^+$. Anal. calcd. for $C_{30}H_{26}N_4O_3$ (%): C, 73.45; H, 5.34; N,11.42. Found (%): C, 73.39; H, 5.41; N, 11.38.

N-(3-(1H-benzo[d]imidazol-2-yl)-4-methylphenylcarbamoyl)-4-(3-fluorobenzyloxy)benzamide (7h): white solid, 76% yield. m.p. 155-158 °C. ^{1}H NMR (400 MHz, DMSO) δ 11.23 (s, 1H), 11.11 (s, 1H), 8.19 (d, J = 8.9 Hz, 2H), 8.14 (d, J = 2.1 Hz, 1H), 7.92 (dd, J = 6.0, 3.1 Hz, 3H), 7.64 – 7.58 (m, 3H), 7.57 (d, J = 8.9 Hz, 1H), 7.44 (d, J = 7.8 Hz, 2H), 7.29 (d, J = 9.0 Hz, 3H), 5.38 (s, 2H), 2.66 (s, 3H). ESI-MS m/z:495.1 $[M+H]^+$. Anal. calcd. for $C_{29}H_{23}FN_4O_3$ (%): C,70.43; H, 4.69; N, 11.33. Found (%): C, 70.48; H, 6.62; N, 11.29.

N-(3-(1H-benzo[d]imidazol-2-yl)-4-methylphenylcarbamoyl)-4-(2-chlorobenzyloxy)benzamide (7i): white solid, 84% yield. m.p. 230-232 °C. ^{1}H NMR (400 MHz, DMSO) δ 11.17 (s, 1H), 8.21 (d, J = 8.9 Hz, 2H), 8.07 (d, J = 2.2 Hz, 1H), 7.80 (dd, J = 8.2, 2.3 Hz, 2H), 7.75 (dd, J = 6.6, 2.7 Hz, 1H), 7.69 – 7.64 (m, 2H), 7.56 – 7.52 (m, 2H), 7.49 (d, J = 8.5 Hz, 1H), 7.35 (s, 2H), 7.31 (d, J = 9.0 Hz, 2H), 5.40 (s, 2H), 2.70 (s, 3H). ESI-MS m/z: 511.1 $[M+H]^+$. Anal. calcd. for $C_{29}H_{23}ClN_4O_3$ (%): C, 68.17; H, 4.54; N, 10.96. Found (%): C, 68.25; H, 4.59; N, 10.88.

N-(3-(1H-benzo[d]imidazol-2-yl)-4-methylphenylcarbamoyl)-4-(3,4-dichlorobenzyloxy)benzamide(7j): white solid, 81% yield. m.p. 178-180 °C. ^{1}H NMR (400 MHz, DMSO) δ 11.08 (s, 1H), 8.08 (d, J = 8.9 Hz, 2H), 7.95 (d, J = 2.2 Hz, 1H), 7.76 (d, J = 1.8 Hz, 1H), 7.68 (d, J = 8.3 Hz, 2H), 7.62 (s, 2H), 7.47 (dd, J = 8.3, 1.9 Hz, 1H), 7.37 (d, J = 8.5 Hz, 1H), 7.23 (dd, J = 6.0, 3.1 Hz, 2H), 7.16 (d, J = 8.9 Hz, 2H), 5.25 (s, 2H), 2.59 (s, 3H). ESI-MS m/z: 545.1 $[M+H]^+$. Anal. calcd. for $C_{29}H_{22}Cl_2N_4O_3$ (%): C, 63.86; H, 4.07; N, 10.27. Found (%): C, 63.89; H, 4.24; N, 10.34.

N-(3-(1H-benzo[d]imidazol-2-yl)-4-methylphenylcarbamoyl)-4-(2,4-dichlorobenzyloxy)benzamide (7k): white solid, 80% yield. m.p. 241-242 °C. ^{1}H NMR (400 MHz, DMSO) δ 11.17 (s, 1H), 11.08 (s, 1H), 8.20 (d, J = 8.6 Hz, 2H), 8.07 (s, 1H), 7.84 (s, 1H), 7.79 (dd, J = 13.4, 8.4 Hz, 4H), 7.63 (d, J = 8.4 Hz, 1H), 7.50 (d, J = 8.5 Hz, 1H), 7.37 (dd, J = 5.5, 3.1 Hz, 2H), 7.31 (d, J = 8.8 Hz, 2H), 5.38 (s, 2H), 2.70 (s, 3H). ESI-MS m/z: 545.1 $[M+H]^+$. Anal. calcd. for $C_{29}H_{22}Cl_2N_4O_3$ (%):C, 63.86; H, 4.07; N, 10.27. Found (%): C, 63.88; H, 4.24; N, 10.33.

N-(3-(1H-benzo[d]imidazol-2-yl)-4-methylphenylcarbamoyl)-4-(4-fluorobenzyloxy)benzamide (7l): white solid, 81% yield. m.p. 176-178°C. ^{1}H NMR (400 MHz, DMSO) δ 11.14 (s, 1H), 11.00 (s, 1H), 8.07 (d, J = 8.9 Hz, 2H), 8.04 (d, J = 2.2 Hz, 1H), 7.86 (dd, J = 6.1, 3.1 Hz, 2H), 7.83 (d, J = 2.3 Hz, 1H), 7.59 – 7.54 (m, 2H), 7.52 (dd, J = 12.2, 6.3 Hz, 3H), 7.23 (d, J = 8.9 Hz, 2H), 7.16 (d, J = 9.0 Hz, 2H), 5.21 (s, 2H), 2.54 (s, 3H).ESI-MS m/z: 495.1 $[M+H]^+$.Anal. calcd. for $C_{29}H_{23}FN_4O_3$ (%): C,70.43; H, 4.69; N, 11.33. Found (%): C,70.55 ; H, 4.64; N, 11.39.

N-(3-(1H-benzo[d]imidazol-2-yl)-4-chlorophenylcarbamoyl)-4-(2-fluorobenzyloxy)benzamide (7m) : white solid, 79% yield. m.p. 230-232°C. ^{1}H NMR (400 MHz, DMSO) δ 11.18 (s, 1H), 11.05 (s, 1H), 8.23 (d, J = 2.6 Hz, 1H), 8.08 (d, J = 8.9 Hz, 2H), 7.86 (dd, J = 8.8, 2.6 Hz, 1H), 7.75 (dd, J = 6.1, 3.1 Hz, 2H), 7.70 (d, J = 8.8 Hz, 1H), 7.59 (dd, J = 8.3, 6.8 Hz, 1H), 7.45 (dt, J = 9.4, 3.7 Hz, 1H), 7.40 (dd, J = 6.1, 3.1 Hz, 2H), 7.27 (dd, J = 15.9, 8.0 Hz, 2H), 7.19 (d, J = 9.0 Hz, 2H), 5.26 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ 180.11, 167.91, 162.65, 159.72, 148.96 (2C), 137.77, 131.58 (2C), 131.34, 131.30, 131.09 (2C), 130.36, 128.95, 127.98, 127.37, 125.07, 124.67, 123.75, 123.61, 122.85, 116.04, 115.83, 114.96 (2C), 64.42. ESI-MS m/z: 515.1 $[M+H]^+$. Anal. calcd. for $C_{28}H_{20}ClFN_4O_3$ (%): C, 65.31; H, 3.91; N, 10.88. Found (%): C, 65.27; H, 3.88; N, 10.74.

N-(3-(1H-benzo[d]imidazol-2-yl)-4-chlorophenylcarbamoyl)-4-(4-methylbenzyloxy)benzamide (7n): white solid, 83% yield. m.p. 270-272°C. ^{1}H NMR (400 MHz, DMSO) δ 12.76 (s, 1H), 11.13 (s, 1H), 11.00 (s, 1H), 8.20 (d, J = 2.7 Hz, 1H), 8.06 (d, J = 8.9 Hz, 2H), 7.87-7.70 (m, 3H), 7.61 (dd, J = 17.1, 7.9 Hz, 2H), 7.36 (d, J = 8.0 Hz, 2H), 7.23 (t, J = 5.7 Hz, 3H), 7.15 (d, J = 9.0 Hz, 2H), 5.18 (s, 2H), 2.32 (s, 3H). ESI-MS m/z: 511.1 $[M+H]^+$. Anal. calcd. for $C_{29}H_{23}ClN_4O_3$ (%): C,68.17; H, 4.54; N, 10.96. Found (%): C, 68.25; H, 4.47; N, 10.89.

N-(3-(1H-benzo[d]imidazol-2-yl)-4-chlorophenylcarbamoyl)-4-(4-fluorobenzyloxy)benzamide (7o): white solid, 80% yield. m.p. 270-272°C. ^{1}H NMR (400 MHz, DMSO) δ 12.75 (s, 1H), 11.13 (s, 1H), 11.01 (s, 1H), 8.20 (s, 1H), 8.07 (d, J = 8.5 Hz, 2H), 7.75 (dd, J = 15.8, 8.3 Hz, 2H), 7.61 (dd, J = 15.2, 8.2 Hz, 2H), 7.56-7.50 (m, 2H), 7.29-7.22 (m, 4H), 7.16 (d, J = 8.6 Hz, 2H), 5.21 (s, 2H). ^{13}C NMR (101MHz, DMSO) δ 180.05, 167.83, 162.72, 161.14, 148.96(2C), 137.76, 133.12, 133.09, 131.52(2C), 131.07, 130.65(2C), 130.56(2C), 130.39, 128.94, 127.92,127.29, 124.55, 115.90(2C),115.69(2C), 115.06(2C), 69.33. ESI-MS m/z: 515.9 $[M+H]^+$. Anal. calcd. for $C_{28}H_{20}ClFN_4O_3$ (%): C,65.31; H,3.91; N, 10.88. Found (%): C, 65.52; H, 3.99; N, 10.89.

4.2 Biology

4.2.1 Cell proliferation assay

The antiproliferative activities of compounds **6a-o** and **7a-o** were evaluated against SW620, HT29, MGC803 and MKN45 cells by MTT assay *in vitro*, with vismodegib and MRT-10 as references. The cancer cells were cultured in minimum essential medium (MEM) supplemented with 10 % fetal bovine serum (FBS). Approximately 4×10^3 cells, suspended in MEM medium, were plated onto each well of a 96-well plate and incubated in 5% CO_2 at 37 °C for 24 h. The test compounds were added to the culture medium at the indicated final concentrations and the cell cultures were continued for 48 h. Fresh MTT was added to each well at a final concentration of 5 mg/mL and incubated with cells at 37 °C for 4 h. The formazan crystals were dissolved in 100 μL DMSO per each well, and the absorbency at 492 nm (for the absorbance of MTT formazan) and 630 nm (for the reference wavelength) was measured with the ELISA reader. All of the compounds were tested three times in each of the cell lines. The results expressed as IC_{50} (inhibitory concentration of 50%) were the averages of three determinations and calculated by using the Bacus Laboratories Incorporated Slide Scanner (Bliss) software.

4.2.2 NIH3T3-Gli-Luciferase reporter assay

Compounds were evaluated using a luciferase reporter assay in NIH3T3 cells carrying a stably transfected Gli-reporter construct (Gli-luc reporter cell line). NIH3T3-Gli-luc cells were treated with DMEM + 10 % FBS + 1 g/mL Puromycin. The cells were seeded onto 96-well plates at 2×10^4 cells/well and cultured in the condition of 5% CO_2 and 37 °C overnight. After incubation, all test compounds (including vismodegib, as one test compound for internal standard control) diluted in a serial 8 × solution (0.05–300 nM) containing 0.5% FBS and 0.7 g/mL Sonic Hedgehog agonist (Shh-N) were added to each well (n = 4 wells per concentration). The cells were incubated for an additional 48 h. To determine the assay window, cells were incubated in media containing 0.1% DMSO with or without Sonic Hedgehog (0% or 100% inhibition control) respectively. Cells were then harvested and lysed in reporter lysis buffer, and luciferase activities were measured using a Dual-Luciferase Reporter Assay System (Promega E1910). The activity of the Gli reporter was defined as the ratio of Firefly/Renilla luciferase activities.

4.2.3 Differentiation of C3H10T1/2 cells assay

C3H10T1/2 cells are plated into 96-well plates at a concentration of 5000 cells/well in DMEM/10% FBS. The following day the media is changed to 20% CM (low serum media DMEM/2% FBS + SAG). Compounds are solubilized in 100% DMSO to a concentration of 10 mM and then serially diluted three fold in 100% DMSO. The highest concentration in the cell plate is 30 μM and the lowest is 3 nM. The compounds are then added to the cells.

Cell plates are incubated with compound for 72 h and then assayed for alkaline phosphatase production using pNp as a substrate. Briefly, after 72 h of incubation the media is aspirated from the cells and washed with 30 μL of PBS. PBS is aspirated off the cells and 15 μL of 1× RIPA cell lysis buffer is added on to the cells. The cell plates are then incubated at -78℃ for 30 min to insure proper cell lysis. The plates are then thawed back to room temperature. The substrate solution containing pNp at 1 mg/mL in diethanolamine buffer pH 9.8 is then added onto the lysed cells. The plates are incubated at 30 ℃ overnight for color development and read at absorbance of 405 nm.

4.2.4 hSMO-BC binding assay

In brief, HEK-hSmo cells were fixed in paraformaldehyde 4% and incubated with 5 nM of BODIPY-cyclopamine and various concentrations of the target compounds for 4h at 37 °C. After incubation, cells were washed with PBS, and then the fluorescence images were automatically captured and analyzed by a high content fluorescence imaging system (Array Scan VTI, Thermo). Data were expressed as % of fluorescence intensity observed with BC alone. IC_{50} values were calculated with GraphPad Prism software using the sigmoidal dose-response function.

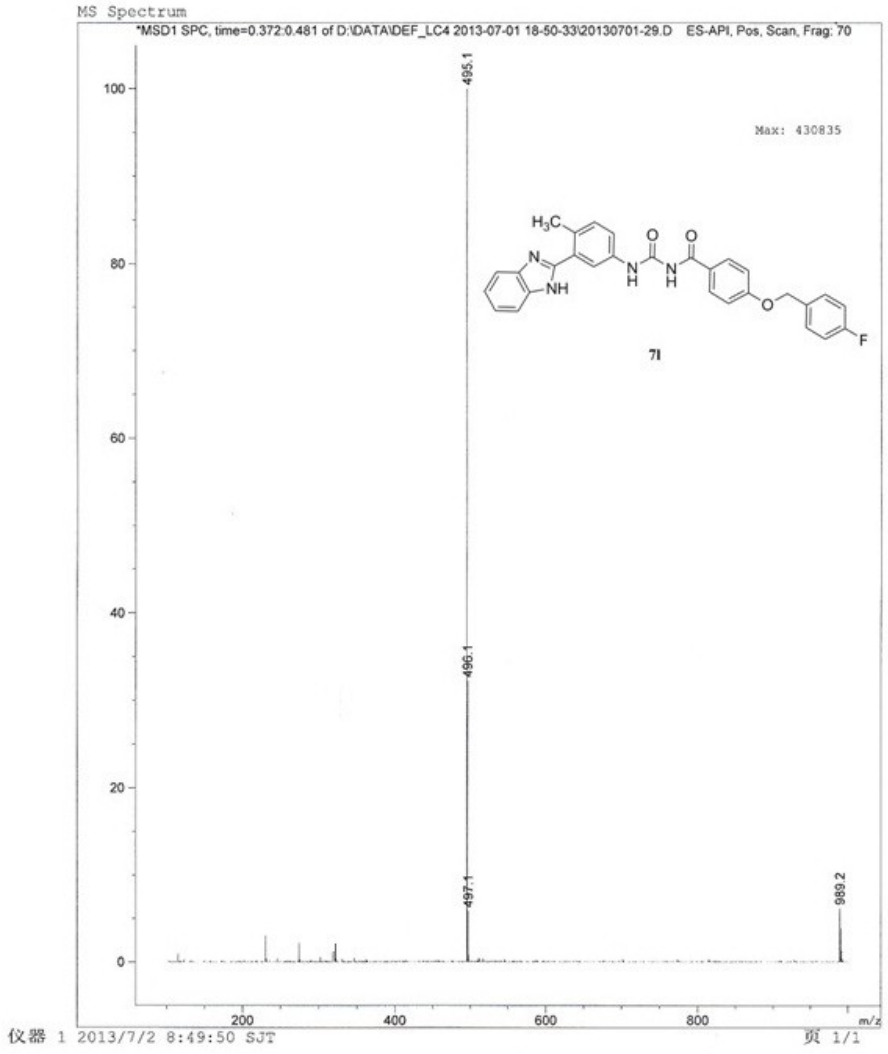
4.3 Molecular modeling

The crystal structure of Smo receptor was retrieved from the RCSB Protein Data Bank (http://www.rcsb.org/pdb) with the corresponding entry code 4KJV. Molecular docking was performed with Discovery Studio 3.0 software package. In the docking process, the protein protocol was prepared via several operations, including the standardization of atom names, insertion of missing atoms in residues and removal of alternate conformations, insertion of missing loop regions based on SEQRES data, optimization of short and medium sized loop regions with the Looper Algorithm, minimization of remaining loop regions, calculation of pK, removing all water molecules, and protonation of the structure. The receptor model was then typed with the CHARMm forcefield and a binding sphere with radius of 10.0Å was defined with the original ligand (taladegib) as the binding site. The compound **7m**, vismodegib and MRT-10 were drawn with Chemdraw and fully minimized using the CHARMm force field. Finally, they were docked into the binding site using the CDOCKER protocol with the default settings. The schematic diagrams of interactions between Smo receptor and docked poses were analyzed by Discovery Studio software package.

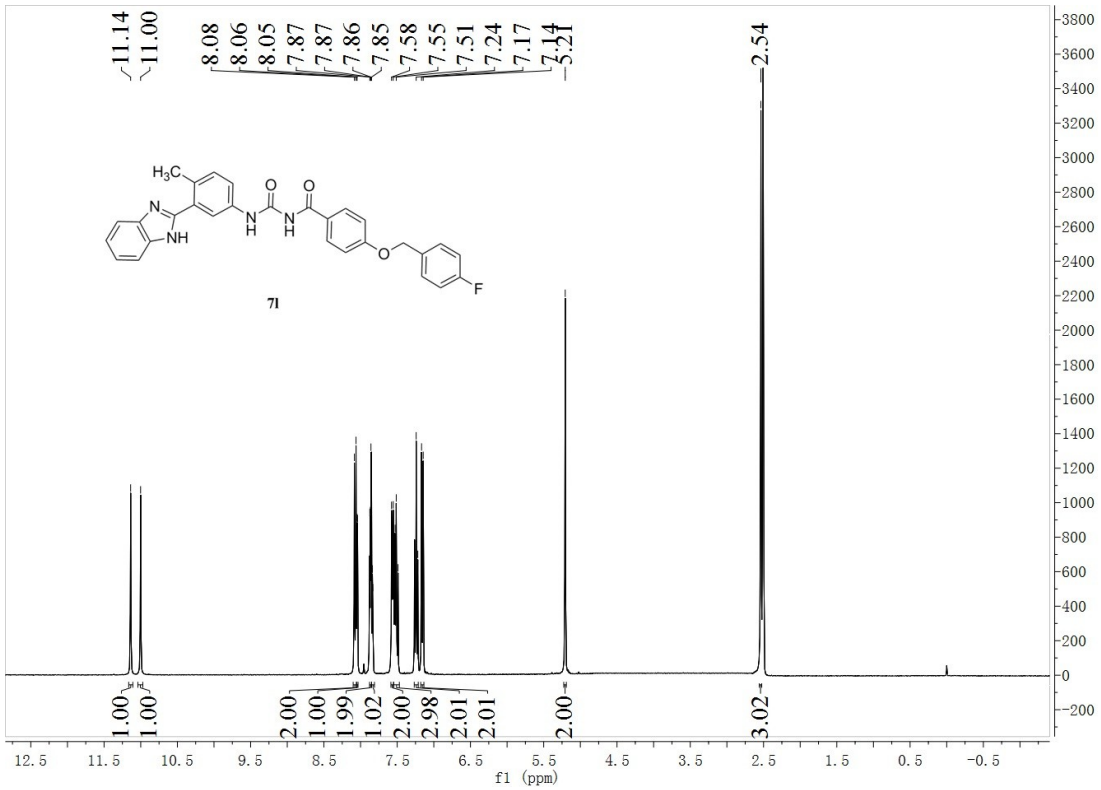
Spectrum of intermediates and the target compounds

MS spectrum of compound **7l**

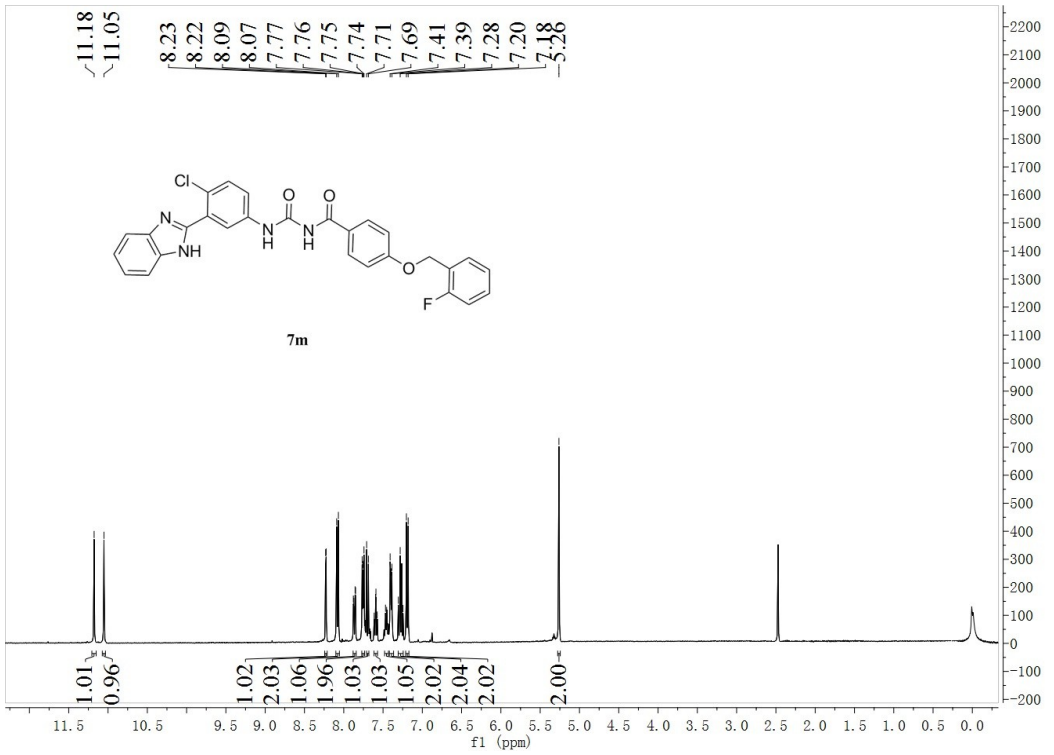
```
打印窗口 80: MS Spectrum
数据文件:  : D:\DATA\DEF_LC4 2013-07-01 18-50-33\20130701-29.D
样品名称       : TS12
==========================================================================
操作者          : SJT                                    序列行 :  32
仪器            : 仪器 1                                   位置 : 样品瓶 39
进样日期        : 2013/7/1 21:41:29                    进样次数 :   1
                                                         进样量 : 5.000 µl
采集方法        : D:\DATA\DEF_LC4 2013-07-01 18-50-33\ESI-B-LAMPOFF-2MIN.M
最后修改        : 2013/3/21 20:23:37 :  JH
分析方法        : D:\METHODS\ESI-B-LAMPOFF-2MIN.M
最后修改        : 2013/3/21 20:23:37 :  JH
方法信息        : ESI-B-LAMPOFF-2MIN
```



^{1}H-NMR spectrum of compound **7l**



^{1}H-NMR spectrum of compound **7m**

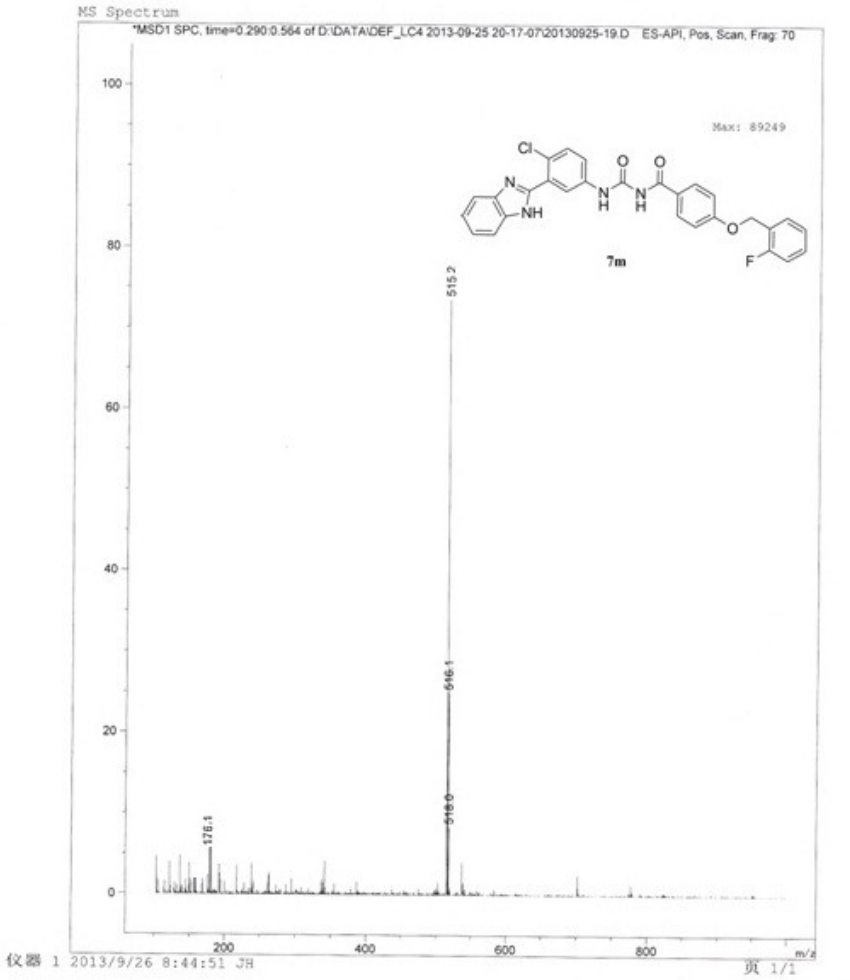


MS spectrum of compound **7m**

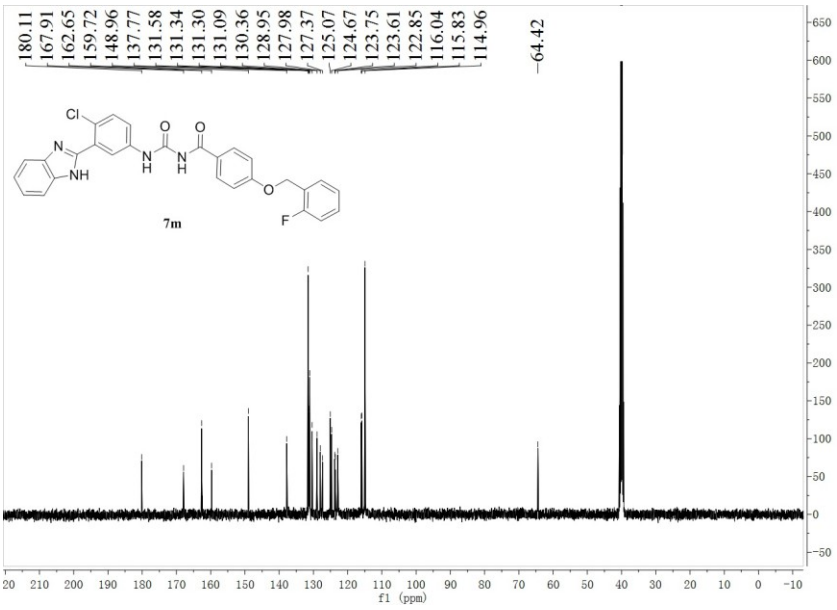
打印窗口 80: MS Spectrum
数据文件: : D:\DATA\DEF_LC4 2013-09-25 20-17-07\20130925-19.D
样品名称 : TS17

操作者 : JH
仪器 : 仪器 1
进样日期 : 2013/9/25 22:02:53
序列行 : 20
位置 : 样品瓶 29
进样次数 : 1
进样量 : 5.000 µl

采集方法 : D:\DATA\DEF_LC4 2013-09-25 20-17-07\ESI-B-LAMPOFF-2MIN.M
最后修改 : 2013/3/21 20:23:37 : JH
分析方法 : D:\METHODS\ESI-B-LAMPOFF-2MIN.M
最后修改 : 2013/3/21 20:23:37 : JH
方法信息 : ESI-B-LAMPOFF-2MIN



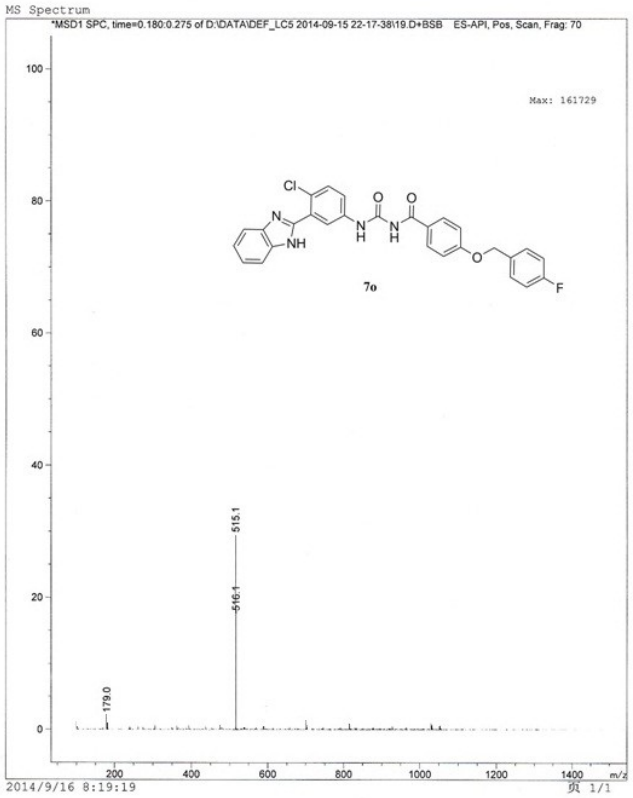
^{13}C-NMR spectrum of compound **7m**



MS spectrum of compound **7o**

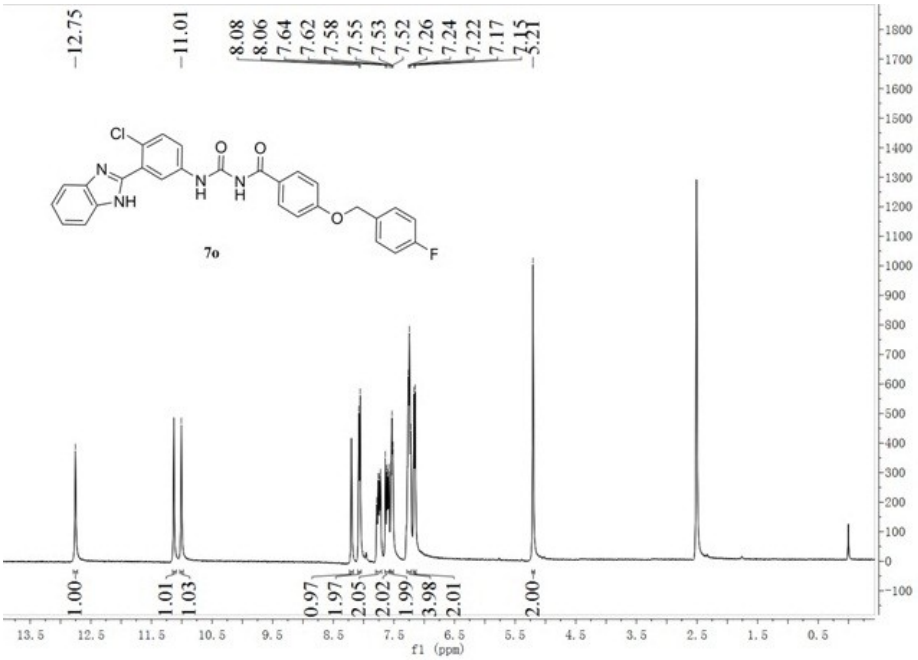
打印窗口 80: MS Spectrum
数据文件: : D:\DATA\DEF_LC5 2014-09-15 22-17-38\19.D
样品名称 : TS24

操作者	: [BSB1]	序列行	: 19
仪器	: 仪器 1	位置	: 样品瓶 29
进样日期	: 2014/9/16 0:08:13	进样次数	: 1
		进样量	: 5.000 µl
采集方法	: D:\DATA\DEF_LC5 2014-09-15 22-17-38\ESI-B-POS-100-1499.		
最后修改	: 2014/6/4 14:43:30		
分析方法	: D:\METHODS\ESI-B-LAMPOFF-2MIN+1----MOSHI.M		
最后修改	: 2014/9/16 8:18:52 (调用后修改)		
方法信息	: ESI-B-LAMPOFF-2MIN		

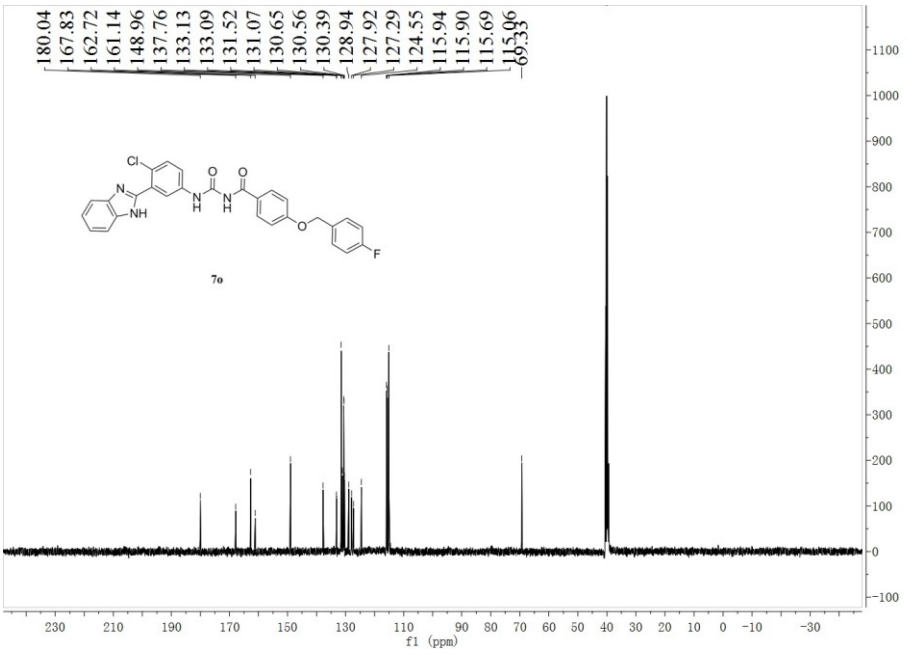


仪器 1 2014/9/16 8:19:19 页 1/1

^{1}H-NMR spectrum of compound **7o**



^{13}C-NMR spectrum of compound **7o**

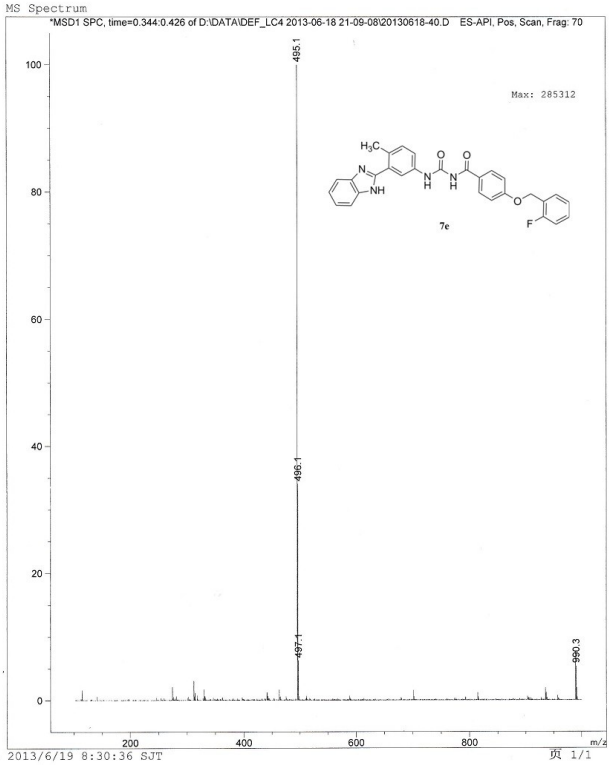


MS spectrum of compound **7e**

打印窗口 80: MS Spectrum
数据文件: : D:\DATA\DEF_LC4 2013-06-18 21-09-08\20130618-40.D
样品名称 : TS05

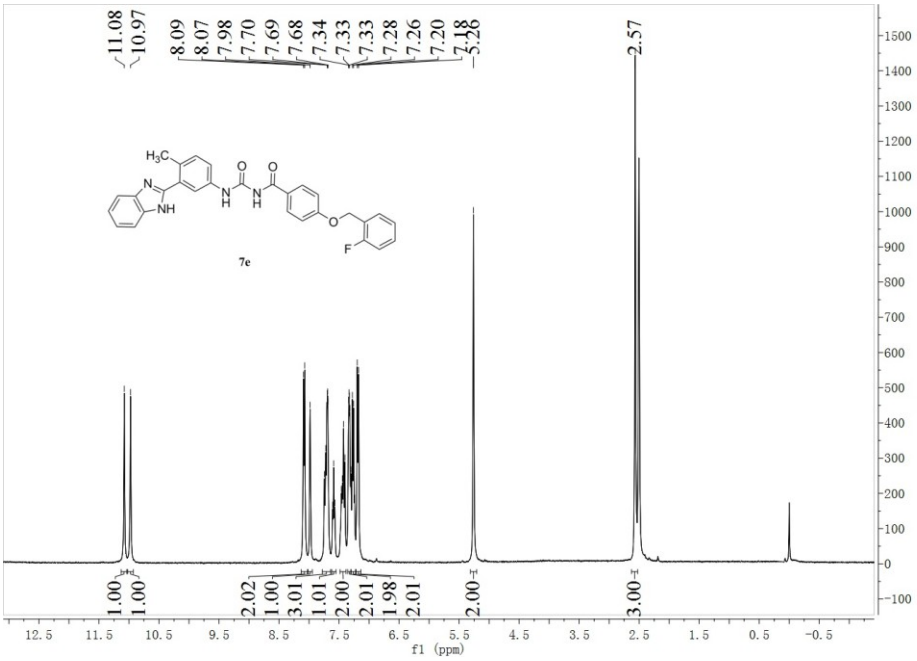
操作者 : SJT 序列行 : 50
仪器 : 仪器 1 位置 : 样品瓶 50
进样日期 : 2013/6/19 1:35:54 进样次数 : 1
进样量 : 5.000 µl
采集方法 : D:\DATA\DEF_LC4 2013-06-18 21-09-08\ESI-B-LAMPOFF-2MIN.M
最后修改 : 2013/3/21 20:23:37 : JH
分析方法 : D:\METHODS\ESI-B-LAMPOFF-2MIN.M
最后修改 : 2013/6/15 12:29:11 : SJT
(调用后修改)
方法信息 : ESI-B-LAMPOFF-2MIN

MS Spectrum

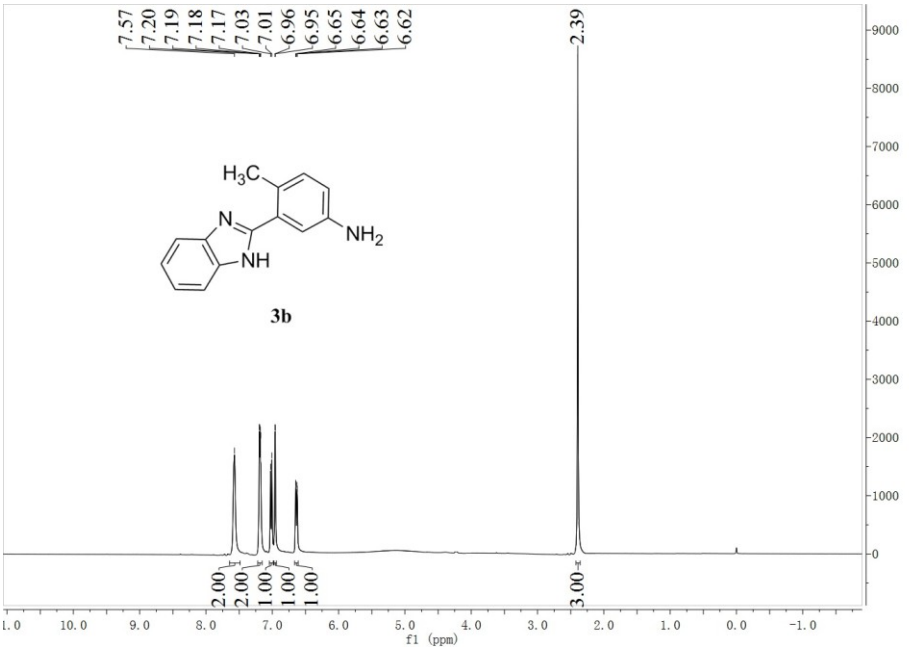


仪器 1 2013/6/19 8:30:36 SJT 页 1/1

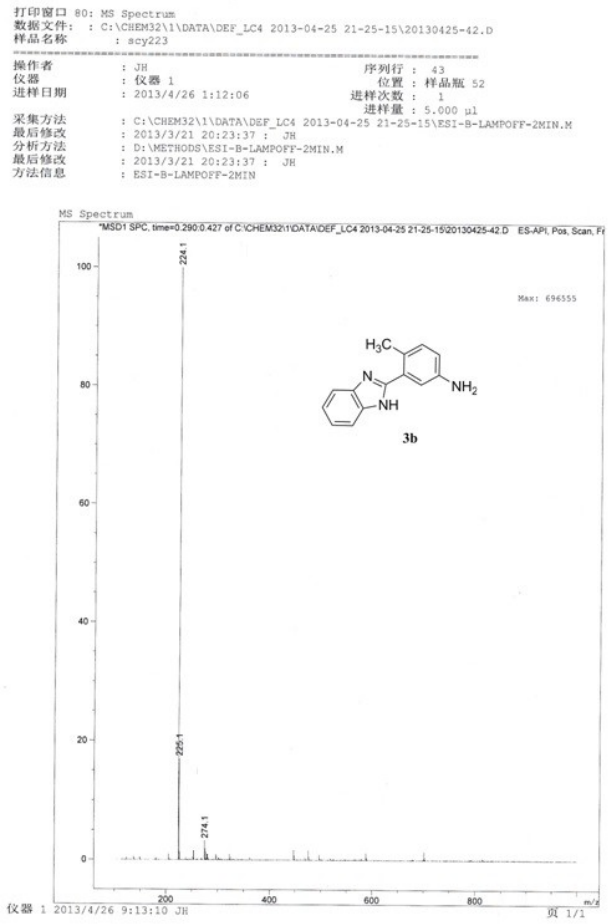
^{1}H-NMR spectrum of compound **7e**



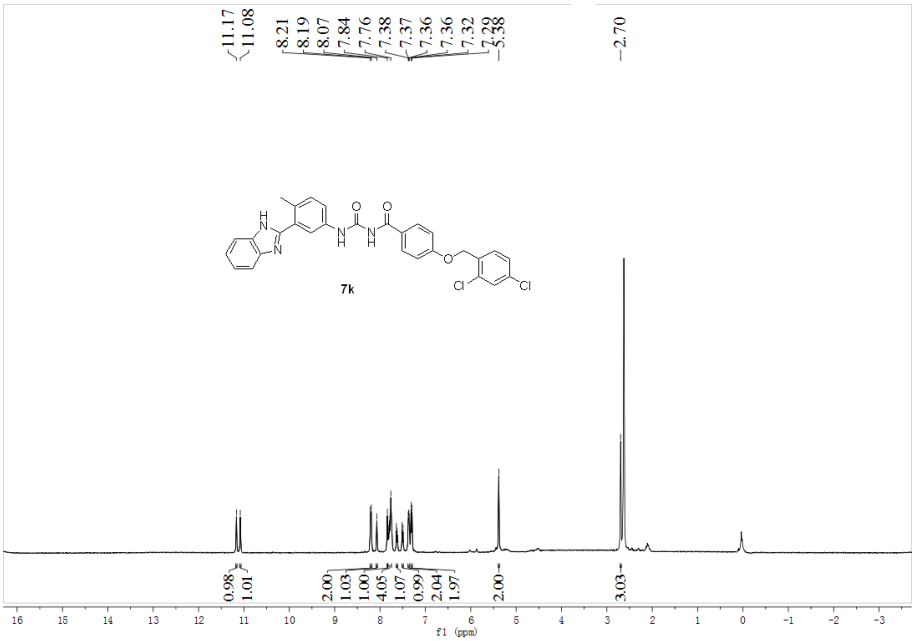
^{1}H-NMR spectrum of intermediate **3b**



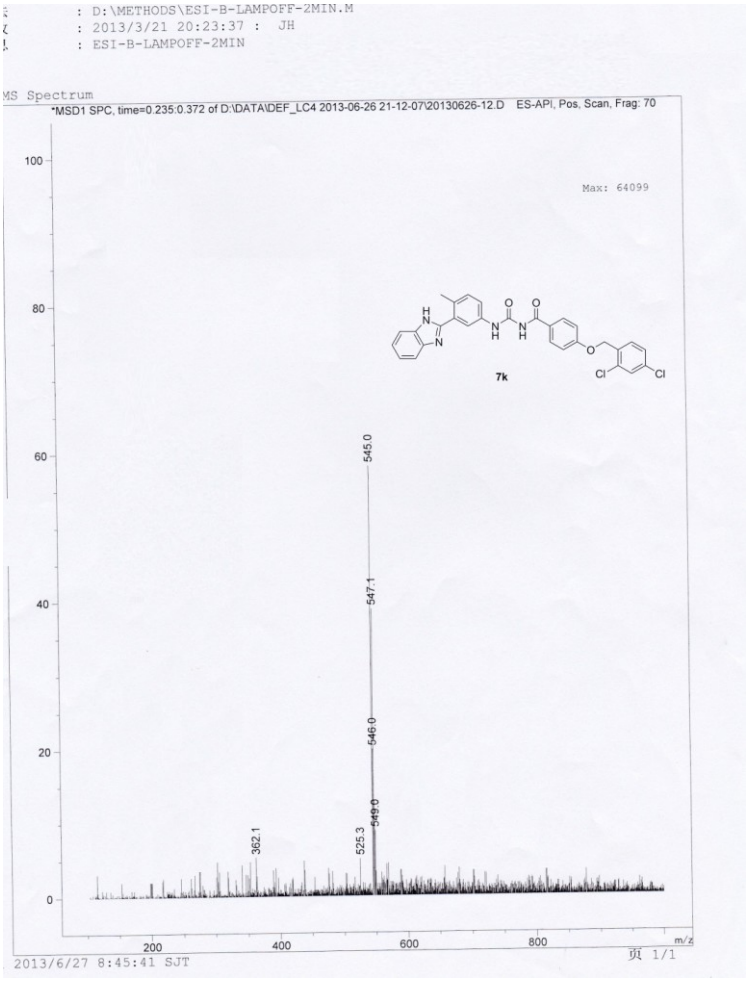
MS spectrum of intermediate **3b**



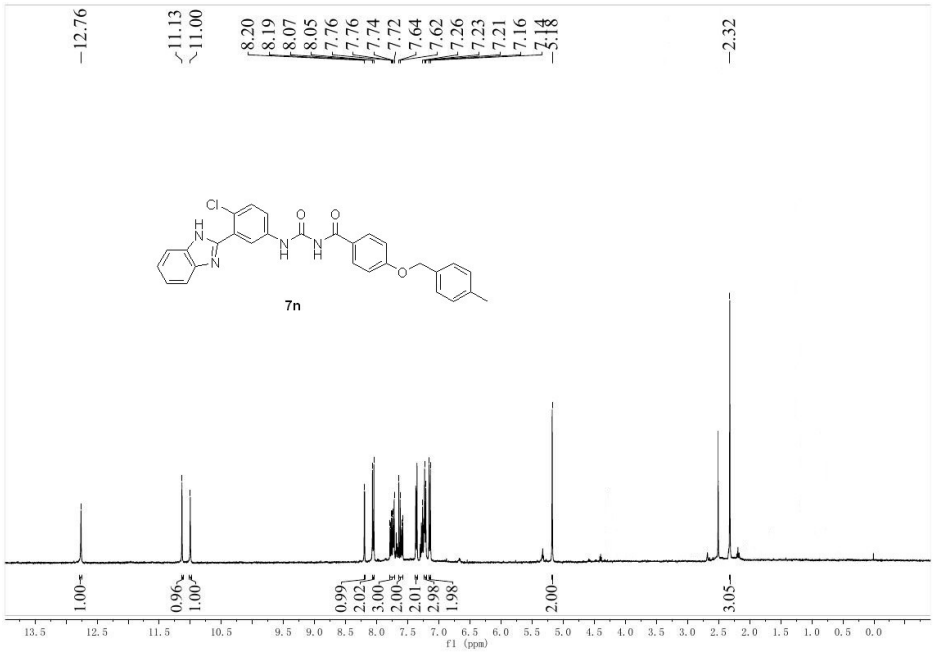
^{1}H-NMR spectrum of compound **7k**



MS spectrum of compound **7k**



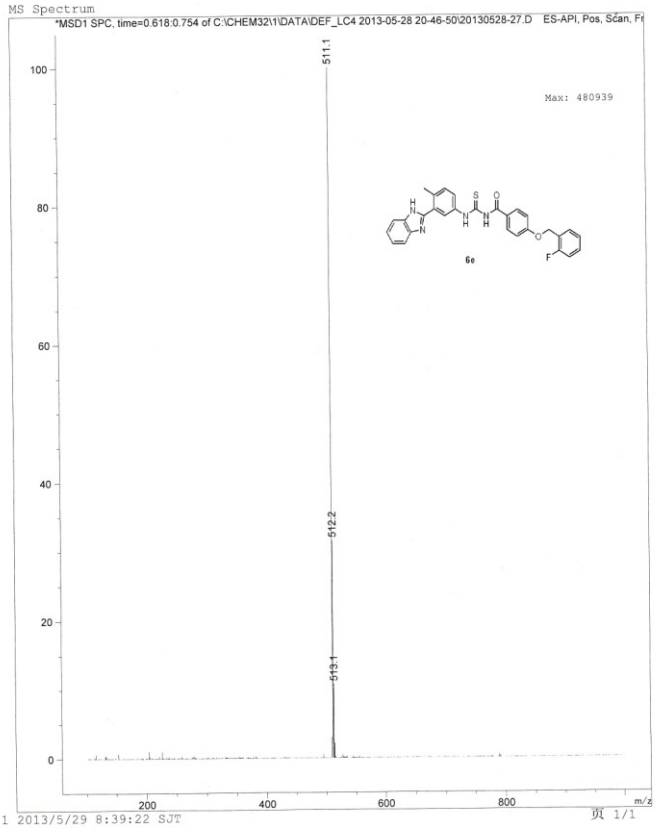
^{1}H-NMR spectrum of compound **7n**



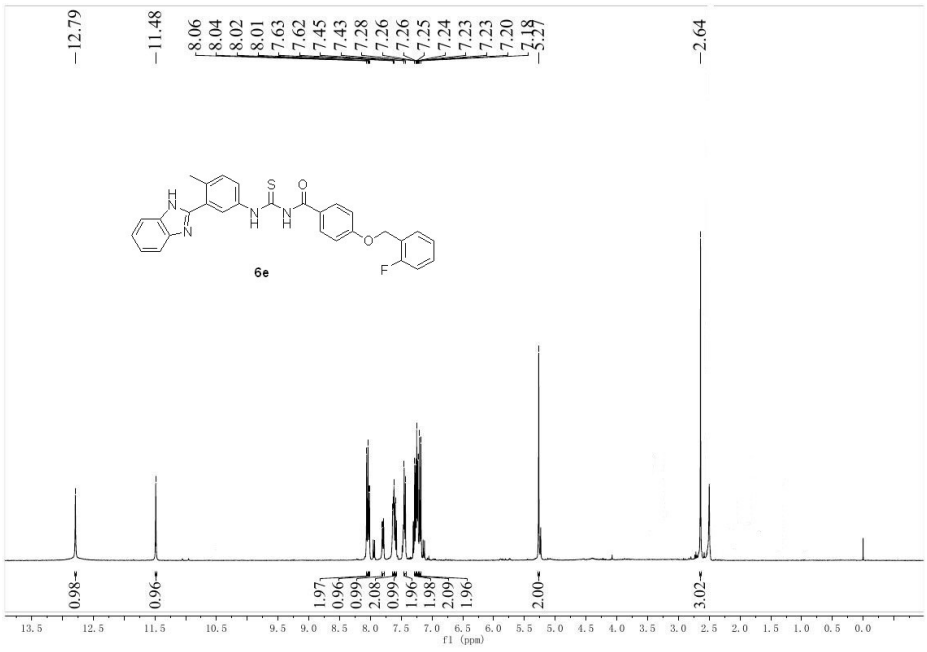
MS spectrum of compound **6e**

打印窗口 80: MS Spectrum
数据文件: : C:\CHEM32\1\DATA\DEF_LC4 2013-05-28 20-46-50\20130528-27.D
样品名称 : CY1305

操作者 : SJT
仪器 : 仪器 1
进样日期 : 2013/5/28 23:04:00
序列行 : 26
位置 : 样品瓶 37
进样次数 : 1
进样量 : 5.000 µl
采集方法 : C:\CHEM32\1\DATA\DEF_LC4 2013-05-28 20-46-50\ESI-B-LAMPOFF-2MIN.M
最后修改 : 2013/3/21 20:23:37 : JH
分析方法 : D:\METHODS\ESI-B-LAMPOFF-2MIN.M
最后修改 : 2013/3/21 20:23:37 : JH
方法信息 : ESI-B-LAMPOFF-2MIN



^{1}H-NMR spectrum of compound **6e**

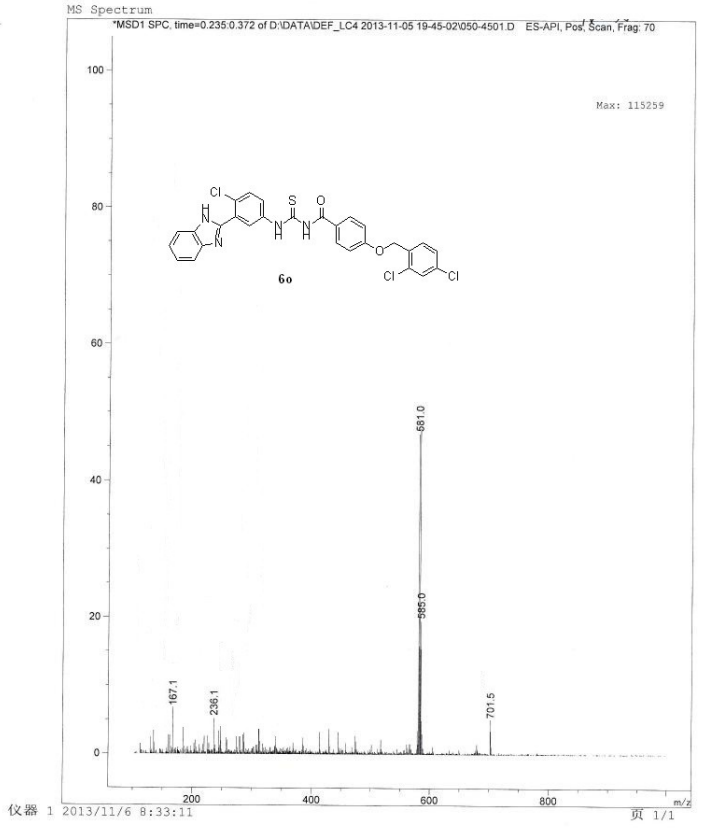


MS spectrum of compound **6o**

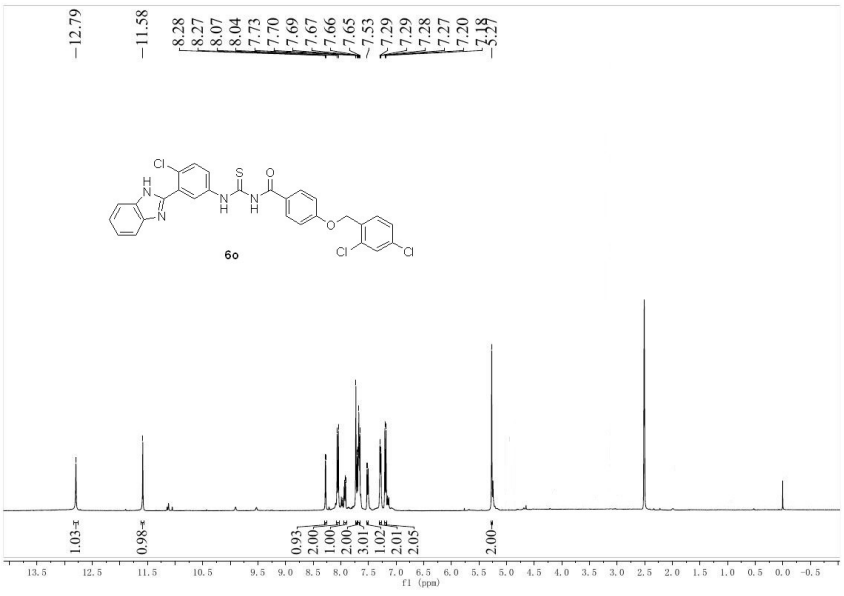
打印窗口 80: MS Spectrum
数据文件: : D:\DATA\DEF_LC4 2013-11-05 19-45-02\050-4501.D
样品名称 : CY1323

操作者 :
仪器 : 仪器 1
进样日期 : 2013/11/5 23:47:33
序列行 : 45
位置 : 样品瓶 50
进样次数 : 1
进样量 : 5.000 µl

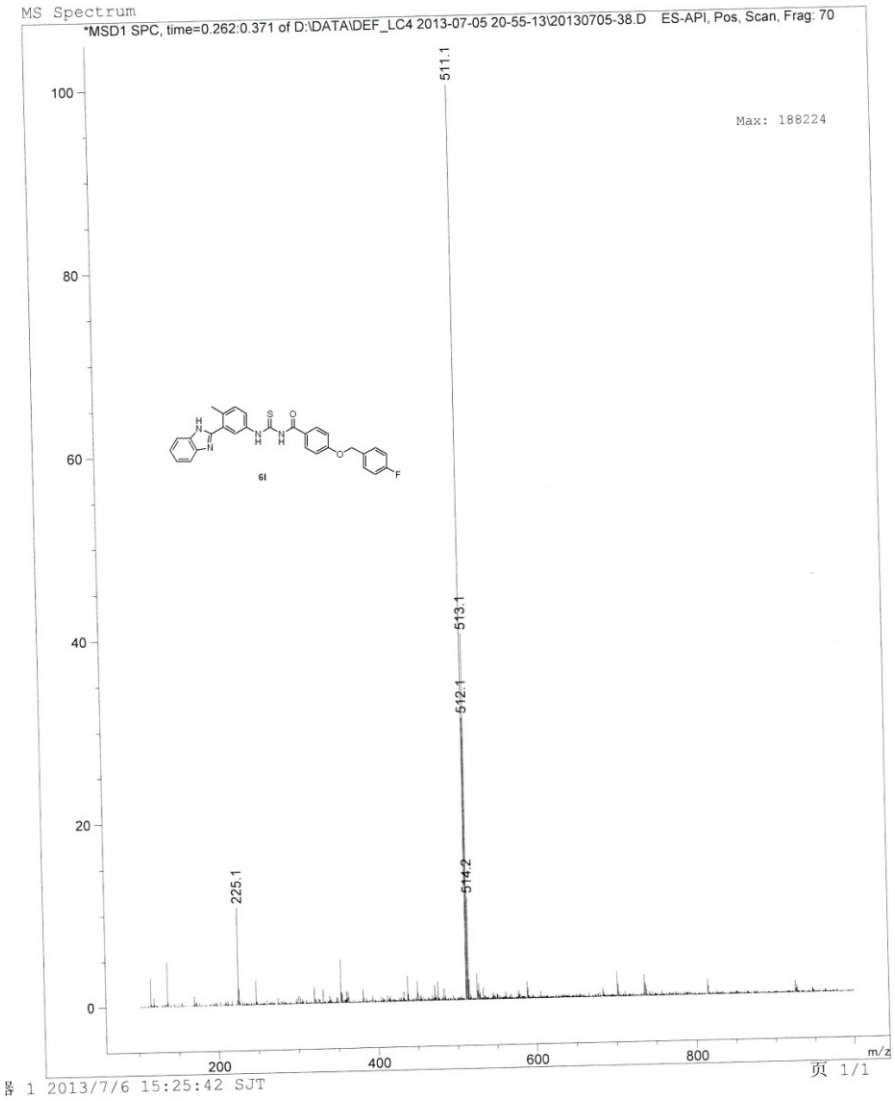
采集方法 : D:\DATA\DEF_LC4 2013-11-05 19-45-02\ESI-B-LAMPOFF-2MIN.M
最后修改 : 2013/3/21 20:23:37 : JH
分析方法 : D:\DATA\DEF_LC4 2013-11-02 10-55-22\ESI-B-LAMPOFF-2MIN.M
最后修改 : 2013/3/21 20:23:37 : JH
方法信息 : ESI-B-LAMPOFF-2MIN



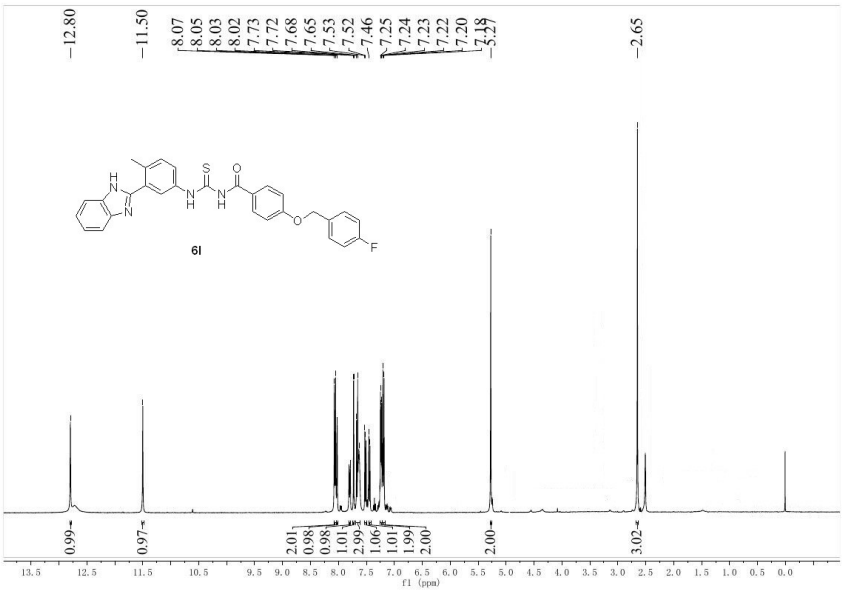
^{1}H-NMR spectrum of compound **6o**



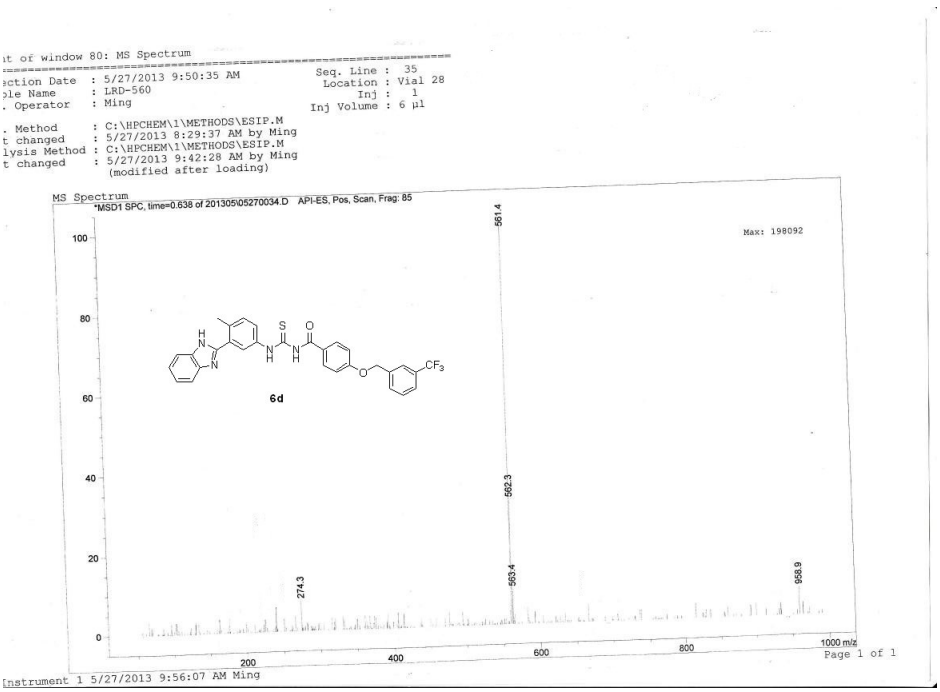
MS spectrum of compound **6l**



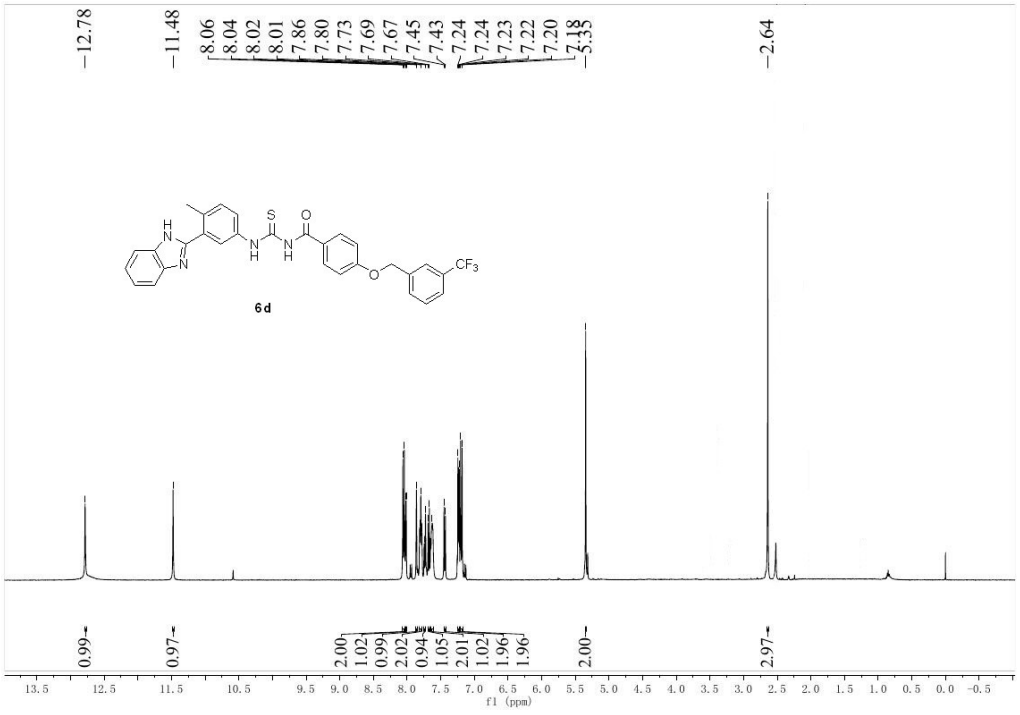
^{1}H-NMR spectrum of compound **6l**



MS spectrum of compound **6d**



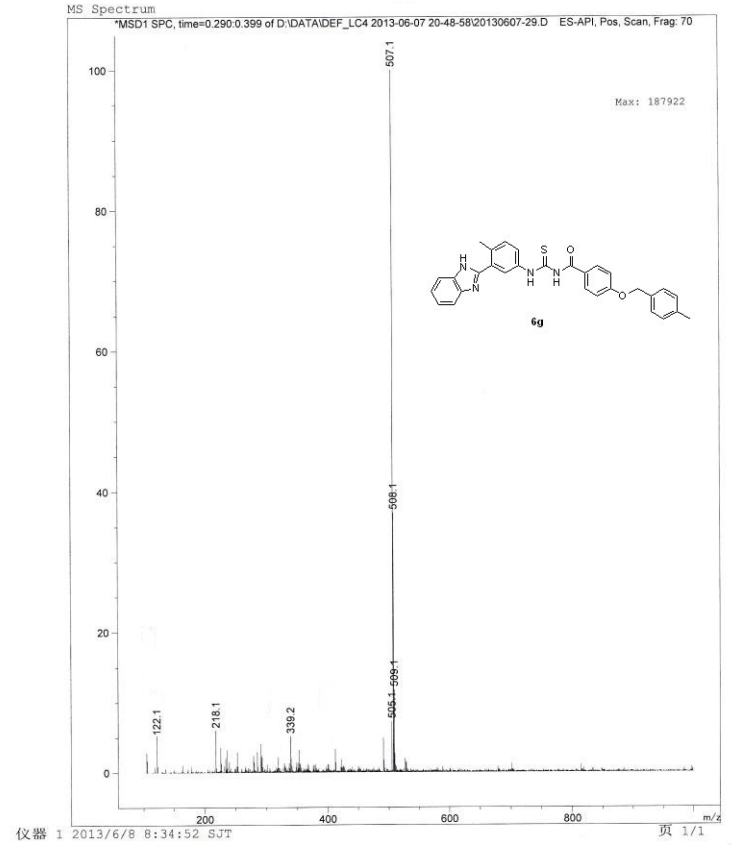
^{1}H-NMR spectrum of compound **6d**



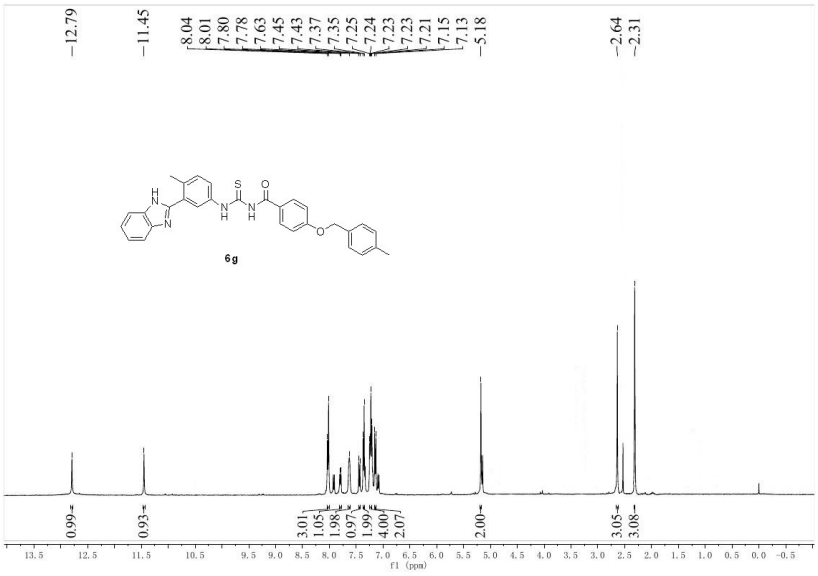
MS spectrum of compound **6g**

打印窗口 80: MS Spectrum
数据文件: : D:\DATA\DEF_LC4 2013-06-07 20-48-58\20130607-29.D
样品名称 : CY1307

操作者	: SJT	序列行	: 31
仪器	: 仪器 1	位置	: 样品瓶 39
进样日期	: 2013/6/7 23:32:57	进样次数	: 1
		进样量	: 5.000 µl
采集方法	: D:\DATA\DEF_LC4 2013-06-07 20-48-58\ESI-B-LAMPOFF-2MIN.M		
最后修改	: 2013/3/21 20:23:37 : JH		
分析方法	: D:\METHODS\ESI-B-LAMPOFF-2MIN.M		
最后修改	: 2013/3/21 20:23:37 : JH		
方法信息	: ESI-B-LAMPOFF-2MIN		



^{1}H-NMR spectrum of compound **6g**



^{1}H-NMR spectrum of compound **6h**