Supporting Information

Circumventing Anti-Androgen Resistance by Molecular Design

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Experimental Procedures

General Considerations

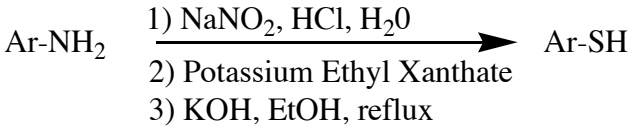
All compounds were purchased from Acros Organics (Morris Plains, NJ) and Aldrich Chemical Co. (Milwaukee, WI) unless otherwise specified. THF was distilled from sodium and methylene chloride was distilled from calcium hydride. Silica gel (60Å) was purchased from Silicycle (Quebec, Canada) and TLC plates (60Å, 250 μM) were purchased from Merck (Whitehouse Station, NJ). NMR spectra were recorded on a Bruker 400 MHz spectrometer at the University of Delaware. Mass spectrometry was performed at the University of Delaware Mass Spectrometry Laboratory. All of the designed oligonucleotides for mutagenesis were purchased from Integrated DNA Technologies (Coralville, IA). CV-1 and COS-7 cells were purchased from ATCC (American Type Tissue Collection, Manassas, VA). LNCaP cells were graciously donated by Professor Sikes (University of Delaware, Newark, DE). Dihydrotestosterone (5α Androstan-17β-OL-3-One), [1,2,4,5,6,7-3H(N)] ([^{3}H]DHT) was purchased from Perkin Elmer (Wellesley, MA). All compounds were made and tested in their racemic form. The purity of final compounds was determined using a C-18 reverse phase, 5 micron column (Higgins Analytical, Inc. Mountain View, CA) with detection at 254 nM on a Shimadzu HPLC (5% Acetonitrile/Water to 50% Acetonitrile/Water, 30 min, 50% Acetonitrile/Water, 20 min.).

Modeling: A computational site model representing the AR(W741L) binding pocket was constructed in FLO/Qxp (Thistlesoft) based on the published X-ray coordinates of the bicalutamide-AR(W741L) complex (J. Dalton et al. PNAS (2005) 102: 6201-6206.). The site model included all residues within 12 Å of the bound bicalutamide and all residues directly adjacent to the bound ligand were free to move during simulations. The AR(wt), AR(W741C) and AR(T877A) site models were generated by changing the appropriate residue and using a conformational search to place the side chain in its lowest energy conformation within the neighboring residues. Designed analogs, control analogs and bicalutamide were subjected to a comprehensive 1000 iteration Monte Carlo docking simulation in each of the four site models that searched random conformations and

orientations of the ligand. The lowest energy conformation of each was further subjected 10 cycles of molecular dynamics/simulated annealing (DynDock). A model of the antagonist conformation was constructed by deleting residues 893-903 representing Helix-12 appropriate site models. Designed analogs, control analogs and bicalutamide were also subjected to a comprehensive 1000 iteration Monte Carlo docking simulation in each of the four site models without Helix-12 that searched random conformations and orientations of the ligand. The lowest energy conformation of each was further subjected to 10 cycles of molecular dynamics/simulated annealing (DynDock).

3-aminobiphenyl: 3-aminobiphenyl was synthesized by the hydrogenation of 3-nitrobiphenyl in the presence of 10 % palladium on carbon as previously described (Thompson, C.M., et al, J. Med. Chem., 45, 11, 2002, 2260-2276).

General procedure for synthesis of thiols (**1b-1e**):



2-benzylthiophenol (1b): This thiol was made following the general procedure of J.I.G. Cadogan, Susan H. Hutchinson and Hamish McNab, J. Chem. Soc. Perkin Trans, 1, 1988, 2875-2880. Briefly, to a solution of 1 equiv. of 2-benzylaniline (500 mg, 2.7 mmol) in water (7.3 mL) at 0 °C was added concentrated hydrochloric acid (0.4 mL). A cold solution of 1 equiv. of sodium nitrite (188 mg, 2.7 mmol) in water (1.5 mL) was added slowly and stirred for 15 minutes. The cold diazonium solution was added slowly to a solution of 1.3 equiv. of potassium ethyl xanthate (525 mg, 3.3 mmol) in water (0.65 mL) at 45 °C. The reaction mixture was stirred for an additional 30 minutes at 45 °C and then cooled to room temperature. The reaction mixture was extracted with diethyl ether (3 x 50 mL). The combined organic extracts were washed with 10 % NaOH solution (100 mL), water (3 x 50 mL), brine (50 mL), dried over $MgSO_4$, filtered and evaporated under reduced pressure. The resulting crude aryl xanthate product was dissolved in ethanol (8 mL) and heated to reflux. Potassium hydroxide pellets (654 mg, 11.6 mmol) were added and refluxing continued overnight. The solution was cooled to room temperature and the ethanol was evaporated under reduced pressure. The residue was dissolved in water and

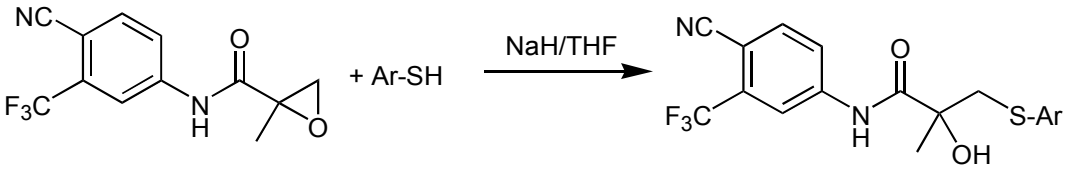
washed with diethyl ether (100 mL). The aqueous layer was acidified with 2 N HCl and extracted with diethyl ether (3 x 50 mL). The organic extracts were washed with water (50 mL), brine (50 mL), dried over $MgSO_4$, filtered and evaporated under reduced pressure to afford 488 mg (89%) of crude product (overall, 3 steps). The unrefined thiol was used directly in the next step without further purification.

3-benzylbenzenethiol (1c): Compound 1c was made following the general procedure for **1b,** using 3-benzylaniline (255 mg (93%)), which was used directly in the next step without further purification.

3-phenylbenzenethiol (1d): Compound 1d was made following the general procedure for **1b,** using 3-aminobiphenyl (171 mg (48%)), which was used directly in the next step without further purification.

2-phenylbenzenethiol (1e): Compound 1e was made following the general procedure for **1b,** using 2-aminobiphenyl (441 mg (74%)), which was used directly in the next step without further purification.

General procedure for coupling of thiols (2a-2i):



***N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methyl-3-(naphthalen-1-ylthio)propanamide (2a):** Compound 2a was made following the general procedure used by B. Chen, et al (J. Org. Chem., 2003, 68, 10181-10182) . To a solution of 1.31 equiv. of sodium hydride (60% dispersed in mineral oil, 9.7 mg, 0.242 mmole) in THF (0.1 mL) at 0 °C was added a solution of 1.26 equiv. of 1-naphthalenethiol (37 mg, 0.23 mmol) dissolved in THF (0.2 mL) and stirred for 5 minutes. A solution of 1 equiv. of N-(4-Cyano-3-trifluorophenyl)methacrylamide epoxide (J. Org. Chem, 68, 2003, 10181-

10182) (50 mg, 0.185 mmol) in THF (0.7 ml) was added to the reaction mixture. The reaction was allowed to warm to room temperature and stir overnight. The solvent was evaporated. The residue was diluted with water and extracted with ethyl acetate (3 x 50 mL). The organic extracts were washed with water (50 mL), brine (50 mL), dried over $MgSO_4$, filtered and evaporated under reduced pressure. The resulting crude product was purified by flash column chromatography on silica gel (50% EtOAc/Hexane) to yield 31 mg (39%) of desired product as a colorless oil. HRMS(ESI) calculated for $[C_{22}H_{17}F_3N_2O_2S + Na]$ 453.0860, found 453.0855. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.78 (bs, NH), 8.39 (d, *J*=8.4 Hz, 1H), 7.73 (dd, *J* =0.80 Hz, 7.2 Hz, 1H), 7.71 (d, *J* =0.80 Hz, 1H), 7.70-7.58 (m, 3H), 7.55-7.40 (m, 3H), 7.29 (d, *J* =8.0 Hz, 1H), 3.93 (d, *J* =14.4 Hz, 1H), 3.71 (s, OH), 3.16 (d, *J* =14.0 Hz, 1H), 1.51 (s, 3H). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 172.8, 140.8, 135.4, 133.9, 133.4, 131.8, 130.0, 129.0, 128.8, 127.0, 126.4, 125.5, 124.6, 123.4, 121.2, 120.7, 116.8, 115.5, 104.1, 75.2, 45.5, 26.2.

3-(2-benzylphenylthio)-*N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide (2b): Compound 2b was made following the general procedure for **2a**, using 2-benzylthiophenol **(1b)** (56 mg (47%)), as a colorless oil. HRMS(ESI) calculated for $[C_{25}H_{21}F_3N_2O_2S + Na]$ 493.1173, found 493.1192. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.72 (bs, NH), 7.85 (d, *J*=1.6 Hz, 1H), 7.73 (d, *J* =8.4 Hz, 1H), 7.68 (dd, *J* =2.0 Hz, 8.0 Hz, 1H), 7.45 (d, *J*=6.0 Hz, 1H) 7.30-7.10 (m, 8H), 4.13 (s, 2H), 3.73 (d, *J*=14.4 Hz, 1H), 3.14 (s, OH), 3.06 (d, *J*=14.0 Hz, 1H), 1.43 (s, 3H). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 173.0, 141.4, 141.1, 139.9, 135.6, 132.8, 131.6, 130.9, 128.9, 128.5, 127.6, 127.3, 126.3, 123.4, 121.7, 120.7, 117.3, 115.4, 104.5, 75.1, 44.2, 40.0, 26.0.

3-(3-benzylphenylthio)-*N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide (2c): Compound 2c was made following the general procedure for **2a**, using 3-benzylthiophenol **(1c)** (15 mg (16%)), as a colorless oil. HRMS(ESI) calculated for $[C_{25}H_{21}F_3N_2O_2S + Na]$ 493.1173, found 493.1195. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.99 (bs, NH), 7.90 (s, 1H), 7.67 (s, 2H), 7.30-7.07 (m, 8H), 6.95 (d, *J*=7.6 Hz, 1H), 3.82 (d, *J*=4.4 Hz, 2H), 3.78 (d, *J*=14.4 Hz, 1H), 3.76 (s, OH), 3.12 (d, *J*=14.4 Hz, 1H), 1.53 (s, 3H). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 173.1, 142.3, 141.2, 140.2, 135.6,

133.6, 133.4, 131.3, 129.2, 128.8, 128.5, 128.2, 126.3, 123.5, 121.7, 120.7, 117.2, 115.5, 104.4, 75.3, 44.5, 41.5, 26.1.

3-(2-phenylphenylthio)-*N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide (2d): Compound 2d was made following the general procedure for **2a**, using 3-phenylbenzenethiol **(1d)** (41 mg (49%)), as a colorless oil. HRMS(ESI) calculated for [$C_{24}H_{19}F_3N_2O_2S$ + Na] 479.1017, found 479.1040. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.97 (bs, NH), 7.83, (dd, *J*=1.6 Hz, 1H), 7.62-7.57 (m, 3H), 7.4-7.25 (m, 8H), 3.89 (d, *J*=14.4 Hz, 1H), 3.72 (bs, OH), 3.13 (d, *J*=14.4 Hz, 1H), 1.54 (s, 3H). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 173.1, 142.0, 141.1, 139.6, 135.5, 133.5, 130.0, 129.9, 129.5, 128.8, 127.8, 126.8, 126.3, 123.4, 121.5, 120.6, 117.0, 115.5, 104.4, 75.1, 44.8, 26.3.

3-(biphenyl-2-ylthio)-*N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide (2e): Compound 2e was made following the general procedure for **2a**, using 2-phenylbenzenethiol **(1e)** (72 mg (76%)), as a colorless oil. HRMS(ESI) calculated for [$C_{24}H_{19}F_3N_2O_2S$ + Na] 479.1017, found 479.1012. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.77 (bs, NH), 7.84 (d, *J*=2.0 Hz, 1H), 7.76 (d, *J*=8.4 Hz, 1H), 7.68 (dd, *J*=8.4 Hz, 2.0 Hz, 1H), 7.60-7.58 (m, 1H), 7.43-7.34 (m, 5H), 7.23-7.22 (m, 3H), 3.55 (d, *J*=14.0 Hz, 1H), 3.22 (s, OH), 2.85 (d, *J*=14.0 Hz, 1H), 1.37 (s, 3H). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 172.9, 144.5, 141.1, 140.2, 135.6, 134.0, 133.6, 131.4, 129.6, 128.1, 128.1, 128.0, 127.7, 123.4, 121.6, 120.7, 117.2, 115.5, 104.4, 75.0, 45.0, 25.9.

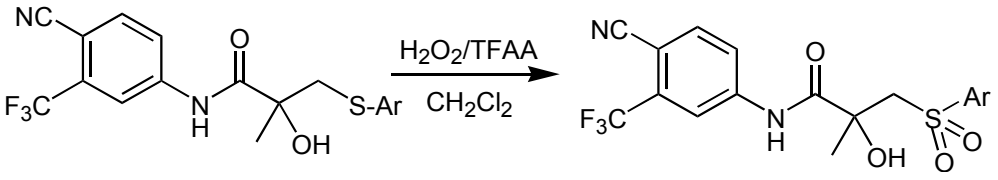
***N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methyl-3-(naphthalen-2-ylthio)propanamide (2f):** Compound 2f was made following the general procedure for **2a,** using 2-naphthalenethiol (153 mg (67%)), as a colorless oil. HRMS(ESI) calculated for [$C_{22}H_{17}F_3N_2O_2S$ + Na] 453.0860, found 453.0872. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.92 (bs, NH), 7.80 (d, *J*=1.2 Hz, 1H), 7.66-7.63 (m, 3H), 7.50-7.35 (m, 6H), 4.00 (d, *J*=14.0 Hz, 1H), 3.89 (s, OH), 3.49 (d, *J*=14.4 Hz, 1H), 1.56 (s, 3H). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 173.1, 140.9, 135.2, 133.1, 132.2, 130.4, 129.6, 128.8, 128.4, 127.5, 126.9, 126.7, 126.5, 123.3, 121.0, 120.5, 116.5, 115.5, 104.0, 74.8, 44.4, 26.3.

3-(3-methoxyphenylthio)-*N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide (2g): Compound 2g was made following the general procedure for **2a**, using 3-methoxybenzenethiol (55 mg (41%)), as a colorless oil. HRMS(ESI) calculated for [$C_{19}H_{17}F_3N_2O_3S$ + Na] 433.0810, found 433.0814. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.04 (bs, NH), 7.94 (s, 1H), 7.73 (d, *J*=1.6 Hz, 2H), 7.09 (t, *J*=8.0 Hz, 1H), 6.96 (dt, *J*=8.4 Hz, 0.80 Hz, 1H), 6.90 (t, *J*=2.0 Hz, 1H), 6.62 (ddd, *J*=8.0 Hz, 2.4 Hz, 0.80 Hz, 1H), 3.79 (d, *J*=14.4 Hz, 1H), 3.77 (s, 3H), 3.72 (s, OH), 3.42 (d, *J*=14.4 Hz, 1H), 1.55 (s, 3H). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 173.2, 159.7, 141.2, 135.5, 134.6, 129.9, 123.4, 122.8, 121.6, 120.7, 117.1, 116.4, 115.5, 112.7, 104.1, 75.2, 55.1, 44.4, 26.0.

3-(3,5-dimethylphenylthio)-*N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide (2h): Compound 2h was made following the general procedure for **2a**, using 3,5-dimethylthiophenol (420 mg (86%)), as a colorless oil. HRMS(ESI) calculated for [$C_{20}H_{19}F_3N_2O_2S$ + Na] 431.1017, found 431.1016. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.07 (bs, NH), 7.94 (s, 1H), 7.72 (s, 2H), 6.99 (s, 2H), 6.69 (s, 1H), 3.88 (s, OH), 3.81 (d, *J*=14.4 Hz, 1H), 3.08 (d, *J*=14.0 Hz, 1H), 2.13 (s, 6H), 1.55 (s, 3H). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 173.4, 141.4, 138.7, 135.4, 132.4, 129.2, 128.7, 123.3, 121.5, 120.6, 117.0, 115.5, 103.6, 75.0, 44.4, 25.8, 20.7.

3-(4-*tert*-butylphenylthio)-*N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide (2i): Compound 2i was made following the general procedure for **2a**, using 4-*tert*-Butylbenzenthiol (200 mg (64%)), as a colorless oil. HRMS(ESI) calculated for [$C_{22}H_{23}F_3N_2O_2S$ + Na] 459.1330, found 459.1325. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.08 (bs, NH), 7.97 (dd, *J*=2.0 Hz, 1H), 7.79 (dd, *J*=8.8 Hz, 2.0 Hz, 1H), 7.73 (d, *J*=8.4 Hz, 1H), 7.33 (d, *J*=8.4 Hz, 2H), 7.21 (d, *J*=8.4 Hz, 2H), 3.77 (d, *J*=14.0 Hz, 1H), 3.12 (d, *J*=14.0 Hz, 1H), 1.54 (s, 3H), 1.20 (s, 9H). ($CDCl_3$, 400 MHz): δ 173.3, 150.9, 141.4, 135.6, 131.1, 129.8, 126.1, 123.4, 121.6, 120.6, 117.0, 115.4, 104.2, 75.2, 45.0, 34.3, 31.0, 26.1.

General procedure for oxidation to sulfones (PLM1-PLM4, PLM6-7, C1-C3):



***N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methyl-3-(naphthalen-1-ylsulfonyl)propanamide (PLM1):** PLM1 was made following the general procedure used by B. Chen, et al (J. Org. Chem., 2003, 68, 10181-10182). To a solution of 1 equiv. of **2a** (31 mg, 0.072 mmol) in methylene chloride (0.2 mL) cooled to -78 °C was added dropwise 8 equiv. of 30 % hydrogen peroxide solution (16.7 μL, 0.58 mmol), followed by the slow addition of 6 equiv. of trifluoroacetic anhydride (62 μL, 0.43 mmol). The reaction was allowed to warm to room temperature and stir overnight. The reaction was diluted with methylene chloride, cold water and brine were added, and the reaction was stirred for 20 minutes. The organic layer was separated, dried over $MgSO_4$, filtered and evaporated under reduced pressure. The crude product was purified by flash column chromatography (50% EtOAc/Hexane) to yield 25 mg (76%) of desired product as a white solid. The purity determined by HPLC was found to be 96.9%. HRMS(ESI) calculated for [$C_{22}H_{17}F_3N_2O_4S$ + Na] 485.0759, found 485.0771. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.14 (bs, NH), 8.65 (d, *J* =8.8 Hz, 1H), 8.21 (dd, *J* =1.2 Hz, 7.2 Hz, 1H), 8.09 (d, *J* =8.4 Hz, 1H), 7.99 (d, *J* =8.0 Hz, 1H), 7.89 (d, *J* =1.6 Hz, 1H), 7.81-7.66 (m, 4H), 7.41(t, *J* =8.0 Hz, 1H), 5.29 (s, OH), 4.26 (d, *J* =14.4 Hz, 1H), 3.64 (d, *J* =14.4 Hz, 1H), 1.59 (s, 3H). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 171.4, 141.1, 136.2, 135.6, 134.1, 134.0, 133.7, 130.3, 129.5, 129.4, 128.5, 127.5, 124.0, 123.4, 121.8, 120.7, 117.3, 115.4, 104.7, 74.5, 60.7, 27.7.

3-(2-benzylphenylsulfonyl)-*N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide (PLM2): PLM2 was made from the oxidation of **2b** following the general procedure for **PLM1** to give 24 mg (40%) of desired product as a colorless oil. The purity determined by HPLC was found to be 97.9%. HRMS(ESI) calculated for [$C_{25}H_{21}F_3N_2O_4S$ + Na] 525.1072, found 525.1062. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.02 (bs, NH), 7.94 (d, *J*=1.2 Hz, 1H), 7.91 (d, *J*=1.6 Hz, 1H), 7.76 (d, *J*=8.4 Hz, 1H), 7.48

(dt, *J*=7.6 Hz, 1.2 Hz, 1H), 7.28-7.19 (m, 8H), 5.15 (s, OH), 4.48 (s, 2H), 3.79 (d, *J*=14.4 Hz, 1H), 3.18 (d, *J*=14.4 Hz, 1H), 1.48 (s, 3H). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 171.4, 141.5, 141.0, 138.8, 136.9, 135.7, 134.5, 133.1, 130.0, 129.4, 128.8, 127.0, 126.8, 123.4, 121.8, 120.7, 117.3, 115.4, 104.8, 74.3, 60.7, 38.2, 27.7.

3-(3-benzylphenylsulfonyl)-*N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide (PLM3): PLM3 was made from the oxidation of **2c** following the general procedure for **PLM1** to give 11 mg (66%) of desired product as a colorless oil. The purity determined by HPLC was found to be 94.9%. HRMS(ESI) calculated for [$C_{25}H_{21}F_3N_2O_4S$ + Na] 525.1072, found 525.1055. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.16 (bs, NH), 7.98 (s, 1H), 7.79-7.70 (m, 4H), 7.45-7.22 (m, 5H), 7.13 (d, *J*=8.0 Hz, 2H), 5.21 (s, OH), 3.98 (d, *J*=14.4 Hz, 1H), 3.97 (s, 2H), 3.48 (d, *J*=14.0 Hz, 1H), 1.61 (s, 3H). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 171.5, 143.3, 141.1, 139.1, 139.0, 135.7, 135.1, 129.5, 128.8, 128.8, 128.0, 126.7, 125.6, 123.4, 121.9, 120.7, 117.3, 115.3, 104.8, 74.4, 61.3, 41.4, 27.7.

3-(2-phenylphenylsulfonyl)-*N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide (PLM4): PLM4 was made from the oxidation of **2d** following the general procedure for **PLM1** to give 36 mg (82%) of desired product as a colorless oil. The purity determined by HPLC was found to be >99.5%. HRMS(ESI) calculated for [$C_{24}H_{19}F_3N_2O_4S$ + Na] 511.0915, found 511.0907. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.08 (bs, NH), 8.03 (t, *J*=1.6 Hz, 1H), 7.87-7.82 (m, 3H), 7.66-7.57 (m, 3H), 7.46-7.40 (m, 5H), 5.28 (bs, OH), 4.08 (d, *J*=14.8 Hz, 1H), 3.51 (d, *J*=14.8 Hz, 1H), 1.60 (s, 3H). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 171.4, 142.5, 140.9, 139.3, 138.1, 135.6, 132.8, 130.0, 129.0, 128.6, 126.7, 126.4, 126.1, 123.3, 121.6, 120.6, 117.1, 115.3, 104.5, 74.2, 61.3, 27.9.

3-(biphenyl-2-ylsulfonyl)-*N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide (PLM6): PLM6 was made from the oxidation of **2e** following the general procedure for **PLM1** to give 63 mg (70%) of desired product as a colorless oil. The purity determined by HPLC was found to be 98.4%. HRMS(ESI) calculated for [$C_{24}H_{19}F_3N_2O_4S$ + Na] 511.0915, found 511.0897. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.96

(s, NH), 8.00 (dd, *J*=8.0 Hz, 1.2 Hz, 1H), 7.84 (d, *J*=2.0 Hz, 1H), 7.77 (d, *J*=8.4 Hz, 1H), 7.63-7.48 (m, 7H), 7.71 (dd, *J*=7.6 Hz, 1.2 Hz, 1H), 7.29 (dd, *J*=8.0 Hz, 1.2 Hz, 1H), 5.21 (s, OH), 3.58 (d, *J*=14.8 Hz, 1H), 3.02 (d, *J*=14.8 Hz, 1H), 1.26 (s, 3H). ^{13}C NMR (Acetone-d_6, 400 MHz): δ 172.2, 143.8, 143.0, 139.9, 139.9, 136.9, 134.2, 133.8, 131.2, 129.8, 129.1, 128.7, 128.5, 125.1, 123.7, 122.4, 118.5, 116.5, 104.4, 74.5, 62.6, 27.8.

***N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methyl-3-(naphthalen-2-ylsulfonyl)propanamide (PLM7):** PLM7 was made from the oxidation of **2f** following the general procedure for **PLM1** to give 48 mg (71%) of desired product as a white solid. The purity determined by HPLC was found to be 97.8%. HRMS(ESI) calculated for [$C_{22}H_{17}F_3N_2O_4S$ + Na] 485.0759, found 485.0740. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.0 (bs, NH), 8.35 (s, 1H), 7.96 (d, *J*=8.4 Hz, 1H), 7.86 (d, *J*=8.4 Hz, 1H), 7.81 (dd, *J*=8.8 Hz, 2.0 Hz, 1H), 7.68-7.64 (m, 3H), 7.56-7.46 (m, 3H), 5.34 (s, OH), 4.14 (d, *J*=14.4 Hz, 1H), 3.50 (d, *J*=14.8 Hz, 1H), 1.56 (s, 3H). ^{13}C NMR (DMSO-d_6, 400 MHz): δ 173.6, 143.1, 137.5, 136.0, 134.7, 131.5, 129.6, 129.4, 129.1, 128.9, 127.7, 127.4, 124.9, 123.3, 122.5, 122.9, 117.2, 115.8, 101.3, 72.9, 63.4, 27.2

3-(3-methoxyphenylsulfonyl)-*N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide (C1): C1 was made from the oxidation of **2g** following the general procedure for **PLM1** to give 19 mg (61%) of desired product as a colorless oil. The purity determined by HPLC was found to be 95.7%. HRMS(ESI) calculated for [$C_{19}H_{17}F_3N_2O_5S$ + Na] 465.0708, found 465.0694. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.19 (s, NH), 8.01 (d, (*J*=1.6 Hz, 1H), 7.81-7.76 (m, 2H), 7.45 (dt, *J*=8.0 Hz, 1.2 Hz, 1.2 Hz, 1H), 7.40 (t, *J*=8.0 Hz, 1H), 7.34 (t, *J*=2.0 Hz, 1H), 7.12 (ddd, *J*=8.0 Hz, 2.4 Hz, 0.80 Hz, 1H), 5.19 (s, OH), 4.03 (d, *J*=14.4 Hz, 1H), 3.77 (s, 3H), 3.50 (d, *J*=14.4 Hz, 1H), 1.61 (s, 3H). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 171.5, 160.0, 141.1, 139.9, 135.6, 130.6, 123.4, 121.8, 120.6, 120.4, 119.8, 117.3, 115.4, 112.6, 104.7, 74.3, 61.4, 55.6, 27.7.

3-(3,5-dimethylphenylsulfonyl)-*N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide (C2): C2 was made from the oxidation of **2h** following the general procedure for **PLM1** to give 357 mg (79%) of desired product as a white solid. The

purity determined by HPLC was found to be 97.7%. HRMS(ESI) calculated for [$C_{20}H_{19}F_3N_2O_4S$ + Na] 463.0915, found 463.0922. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.26 (bs, NH), 8.01 (s, 1H), 7.80-7.74 (m, 2H), 7.46 (s, 2H), 7.21 (s, 1H), 5.27 (bs, OH), 4.02 (d, *J*=14.4 Hz, 1H), 3.48 (d, *J*=14.4 Hz, 1H), 2.26 (s, 6H), 1.59 (s, 3H). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 171.6, 141.2, 139.6, 138.4, 136.1, 135.5, 125.2, 123.3, 121.8, 120.6, 117.2, 115.4, 104.5, 74.2, 61.3, 27.7, 20.9.

3-(4-*tert*-butylphenylsulfonyl)-*N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide (C3): C3 was made from the oxidation of **2i** following the general procedure for **PLM1** to give 165 mg (76%) of desired product as a white solid. The purity determined by HPLC was found to be 98.2%. HRMS(ESI) calculated for [$C_{22}H_{23}F_3N_2O_4S$ + Na] 491.1228, found 491.1209. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.25 (bs, NH), 8.04 (dd, *J*=1.6 Hz, 1H), 7.86-7.75 (m, 4H), 7.53 (dt, *J*=20.8 Hz, 2.0 Hz, 2H), 5.26 (bs, OH), 4.00 (d, *J*=14.4 Hz, 1H), 3.50 (d, *J*=14.4 Hz, 1H), 1.60 (s, 3H), 1.29 (s, 9H). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 171.7, 158.8, 141.3, 135.8, 135.7, 127.7, 126.4, 123.4, 121.9, 120.6, 117.3, 115.4, 104.6, 74.4, 61.5, 35.3, 30.8, 27.7.

Bicalutamide: This compound was made following the procedure of B. Chen et al (J. Org. Chem., 2003, 68, 10181-10182). ^{1}H and ^{13}C NMR are consistent with the literature.

Plasmid Constructs: Mammalian expression vectors pSG5-hAR(W741L), pSG5-hAR(W741C) and pSG5-hAR(T877A) were created from parent plasmid pSG5-hAR(wt) (kindly provided by Professor Shutsung Liao, University of Chicago) using the QuickChange mutagenesis kit (Stratagene, LaJolla, CA) following manufacturers protocol. The following primers were used:
W741L f: CT GTC ATT CAG TAC TCC **TTG** ATG GGG CTC ATG GTG TTT G
W741L r: C AAA CAC CAT GAG CCC CAT **CAA** GGA GTA CTG AAT GAC AG
W741C f: CT GTC ATT CAG TAC TCC **TGT** ATG GGG CTC ATG GTG TTT G
W741C r: C AAA CAC CAT GAG CCC CAT **ACA** GGA GTA CTG AAT GAC AG
T877A f: AGA GAG CTG CAT CAG TTC **GCT** TTT GAC CTG CTA ATC AAG
T877A r: CTT GAT TAG CAG GTC AAA **AGC** GAA CTG ATG CAG CTC TCT

The constructs were sequenced over the entire coding region of AR to confirm the presence of only the desired mutation.

Transcription Assays: Twenty-four hours prior to transfection, CV-1 cells were seeded at a density of 45,000 cells per well in 24-well cell culture plates and grown in phenol red free Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% cosmic calf serum (CCS) (HyClone, Logan, UT). Transfections were performed using Lipofectamine (Invitrogen, Carlsbad, CA) following manufacturer's protocol with 0.14 μg of ARE-luciferase reporter, 0.03 μg Renilla-Luc as the internal standard and 0.08 μg of AR(wt) or AR(mutant) per well. Five hours after transfection, media was added containing the appropriate concentrations of ligands. The cells were allowed to incubate for 38 hours before harvesting by passive lysis buffer. Cell extracts were immediately assayed using the Dual Luciferase Assay (Promega #E1960, Madison, WI) with a Perkin-Elmer Microbeta Luminometer. All experiments were run in triplicate. Activity is reported in relative light unit (RLU), determined as the ratio of inducible firefly luciferase luminescence divided by the luminescence of the renilla luciferase control normalized to 10 nM DHT in AR(wt). Dose-response data was analyzed by nonlinear regression analysis using GraphPad Prism.

Competitive Binding Assays: Twenty-four hours prior to transfection, COS-7 cells were seeded at a density of 70,000 cells per well in 24-well cell culture plates and grown in phenol red free Dulbecco's Modified Eagle Medium (DMEM) supplement with 10% cosmic calf serum (CCS). Transfections were performed using Lipofectamine (Invitrogen) following manufacturer's protocol with 0.5 μg of AR(wt) or AR(mutant) per well. The cells were allowed to grow for 30 hours and then labeled for 2 hours at 37 °C with [^{3}H]DHT and the appropriate concentration of ligands. Cells were washed with PBS and harvested in 2% SDS, 10% glycerol, and 10 mM Tris, pH 6.8, and diluted into scintillation cocktail (ScintiSafe Econo 2 Cocktail) before counting with a Perkin-Elmer Microbeta Luminometer. Competitive binding data was analyzed by nonlinear regression analysis using GraphPad Prism.

Cell Growth Assays: LNCaP cells were maintained in T-medium supplemented with 5% Fetal Bovine Serum (FBS) and 100 I.U./mL of penicillin and 100 μg/mL of streptomycin. LNCaP cells were seeded at a density of 2,000 cells per well in 96-well cell culture plates and grown in phenol red free RPMI supplemented with 5% Dextran Coated Charcoal FBS (DCC-FBS) and 100 I.U./mL of penicillin and 100 μg/mL of streptomycin. Twenty-four hours after plating, cells were treated with various concentrations of ligand with or without R1881 (DuPont). Every other day, half of the media was removed and replaced with fresh media containing the appropriate concentration of ligands. At different time points, all of the media was removed and the plates were frozen at -78 °C. At the end of the treatment, relative fluorescence was measured using the CyQUANT cell proliferation assay kit (Invitrogen) with a 96-well fluorescent plate reader (Perkin Elmer Fusion Plate Reader) following manufacturer's protocol. Statistical analysis was performed using one-way ANOVA. The criterion for statistical significance was $P < 0.05$ with 95% confidence.

Biological Data

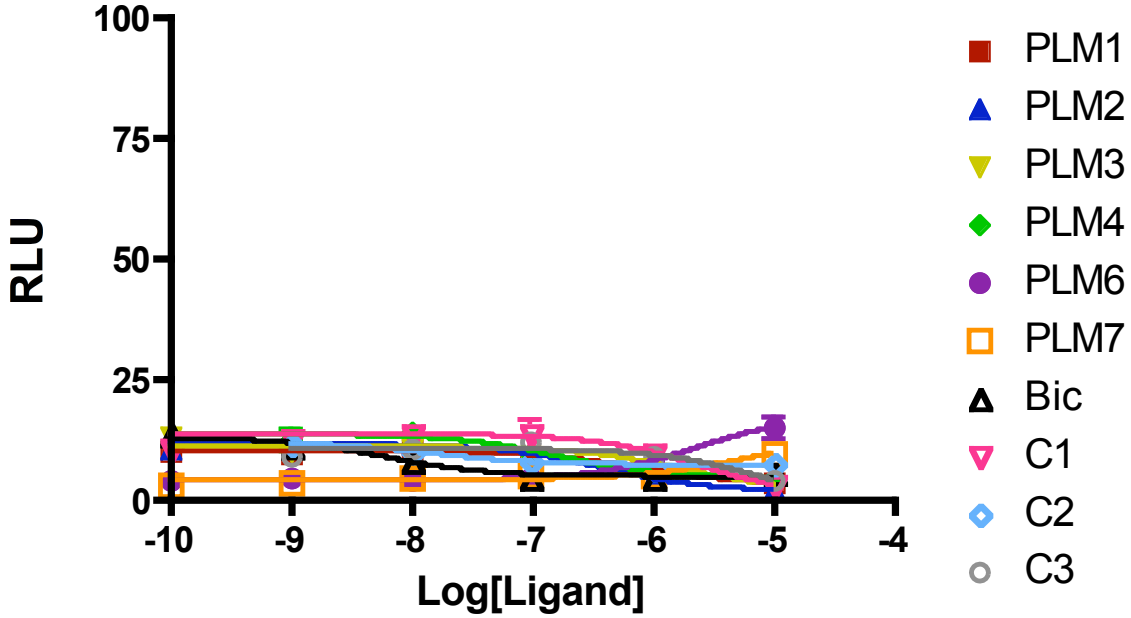


Figure 1: Dose response curve of PLM1-4, PLM6-7, C1-C3 and Bicalutamide in AR(wt).

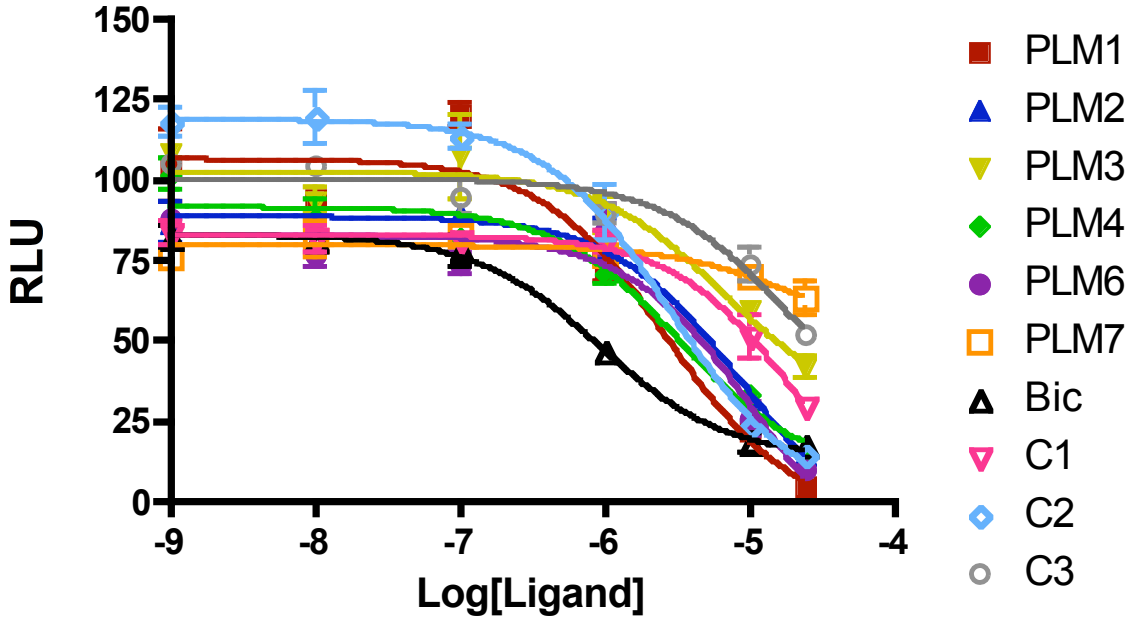


Figure 2: Dose response curve of PLM1-4, PLM6-7, C1-C3 and Bicalutamide in competition with 3 nM DHT in AR(wt).

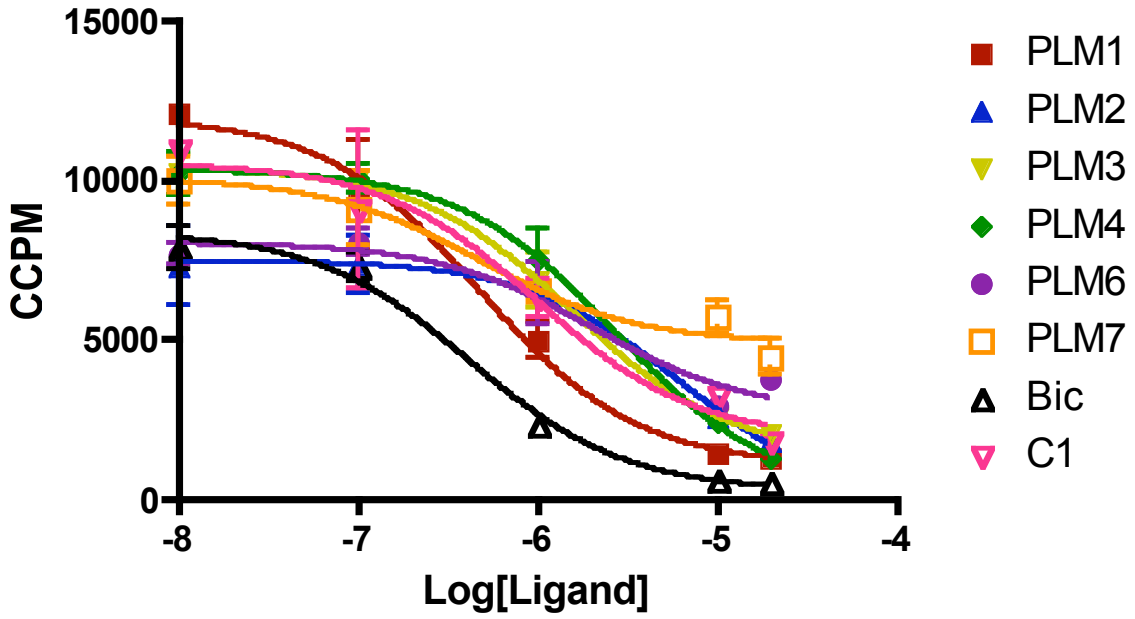


Figure 3: Cellular radio-ligand displacement assay of PLM1-4, PLM6-7, C1 and Bicalutamide using 8 nM [^{3}H]DHT in AR(wt).

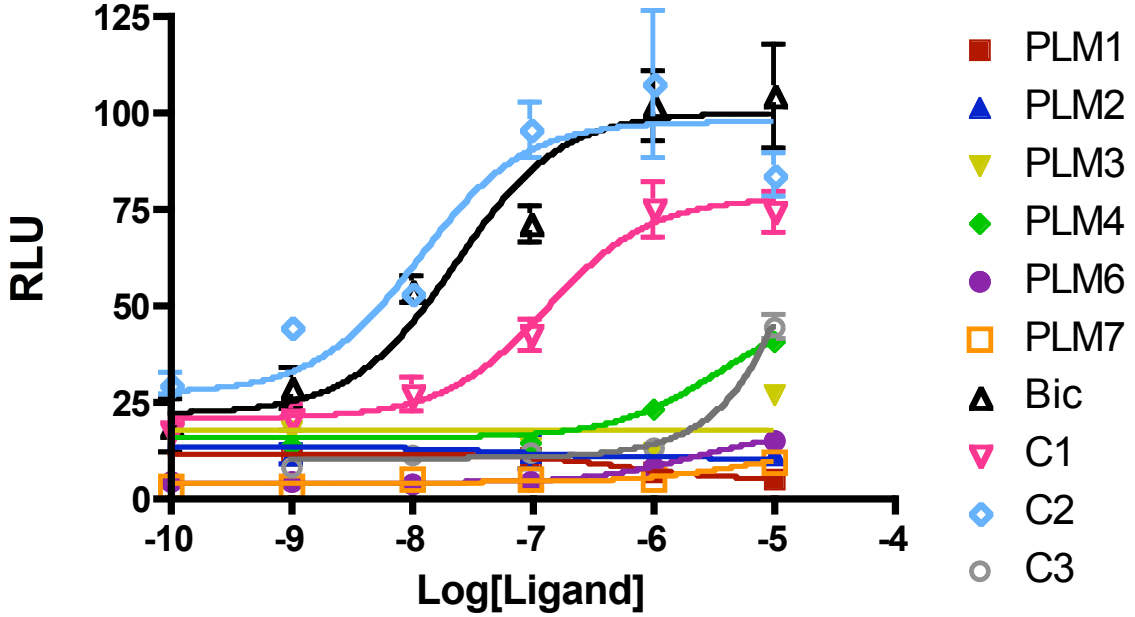


Figure 4: Dose response curve of PLM1-4, PLM6-7, C1-C3 and Bicalutamide in AR(W741L).

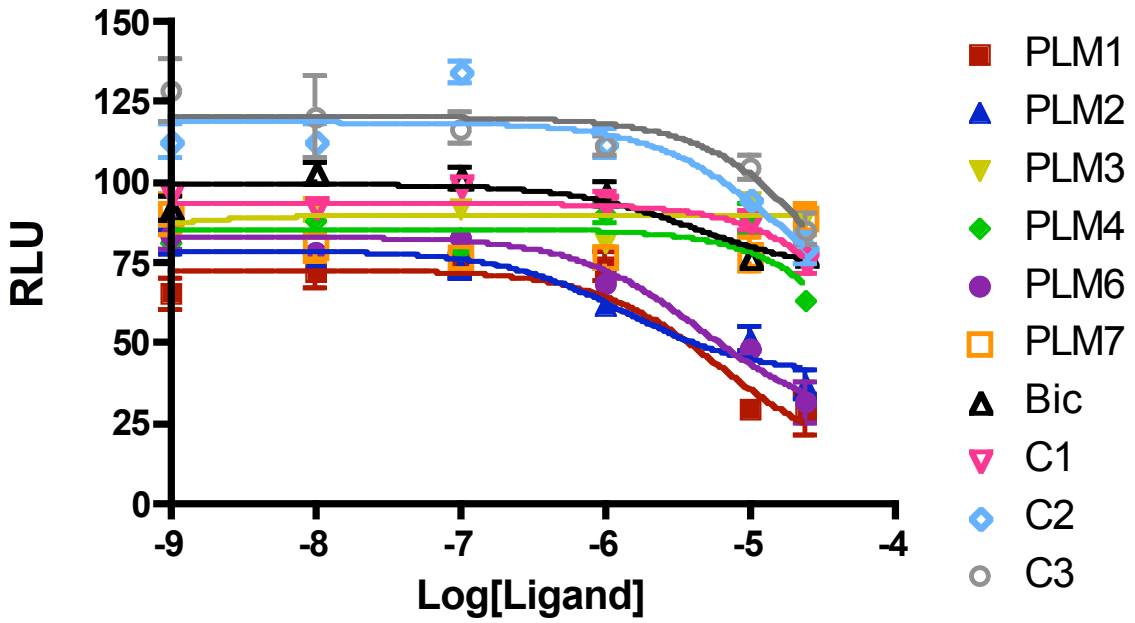


Figure 5: Dose response curve of PLM1-4, PLM6-7, C1-C3 and Bicalutamide in competition with 250 nM DHT in AR(W741L).

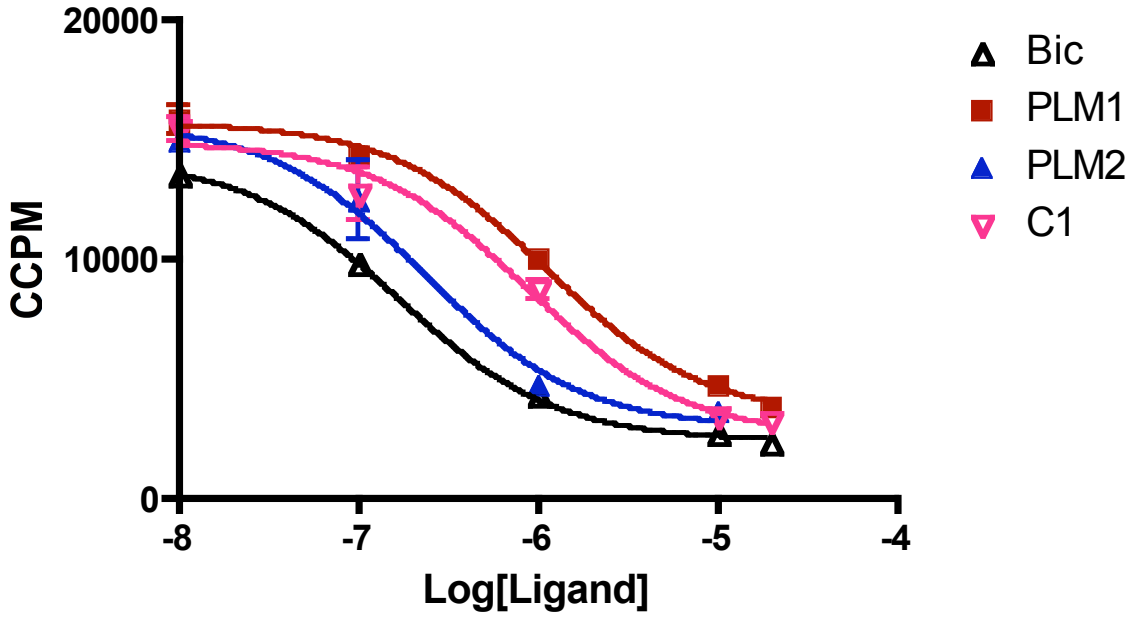


Figure 6: Cellular radio-ligand displacement assay of PLM1, PLM2, C1 and Bicalutamide using 100 nM [^{3}H] DHT in AR(W741L).

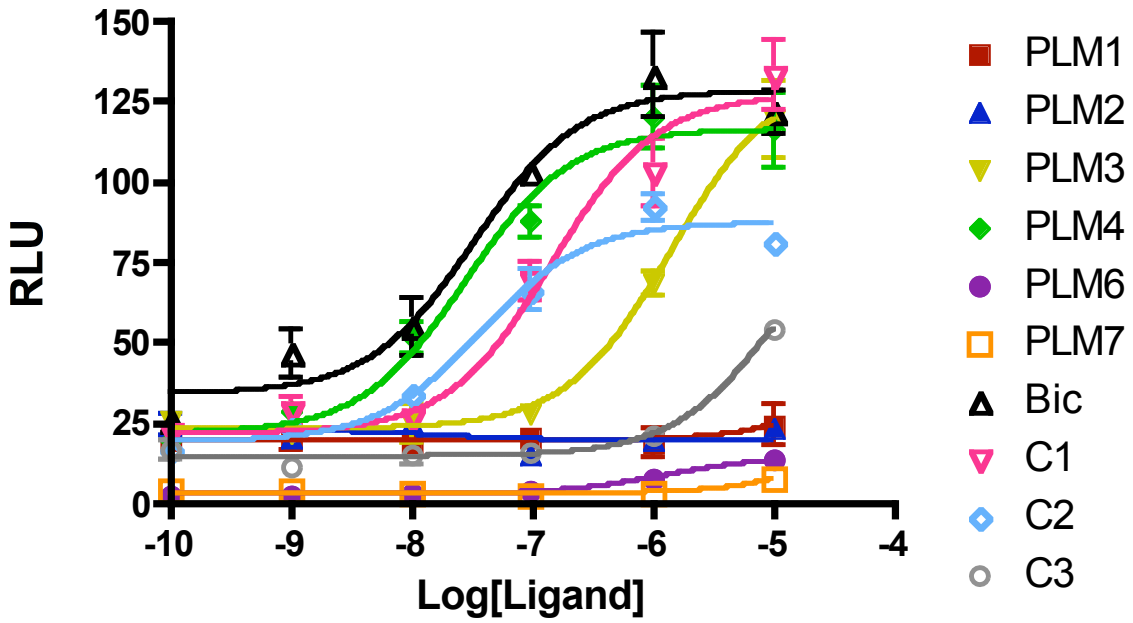


Figure 7: Dose response curve of PLM1-4, PLM6-7, C1-C3 and Bicalutamide in AR(W741C).

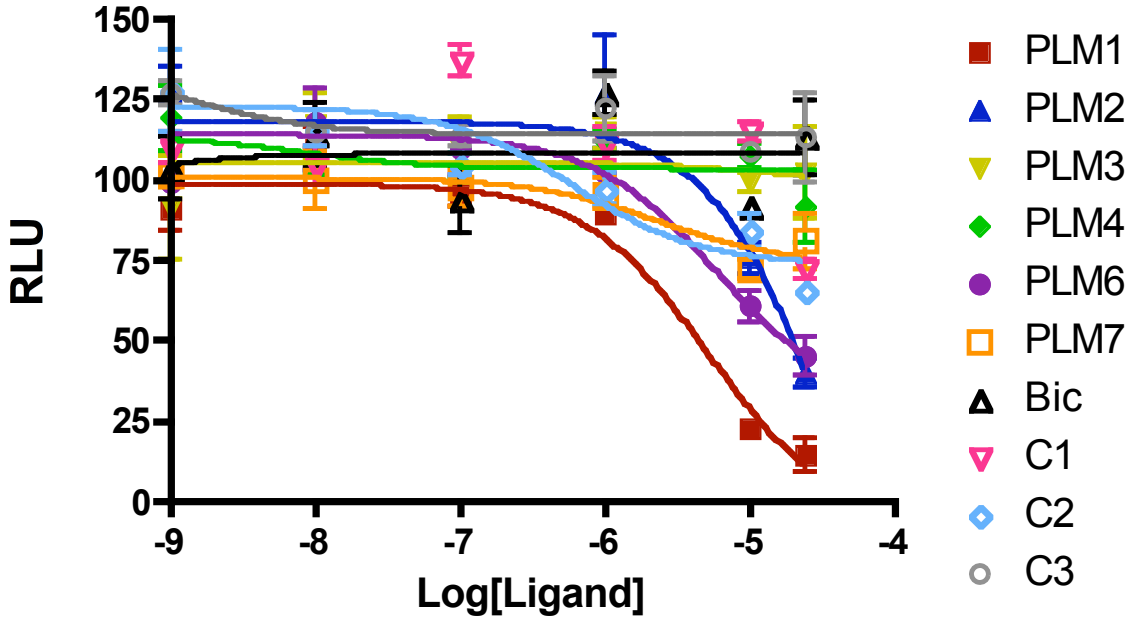


Figure 8: Dose response curve of PLM1-4, PLM6-7, C1-C3 and Bicalutamide in competition with 200 nM DHT in AR(W741C).

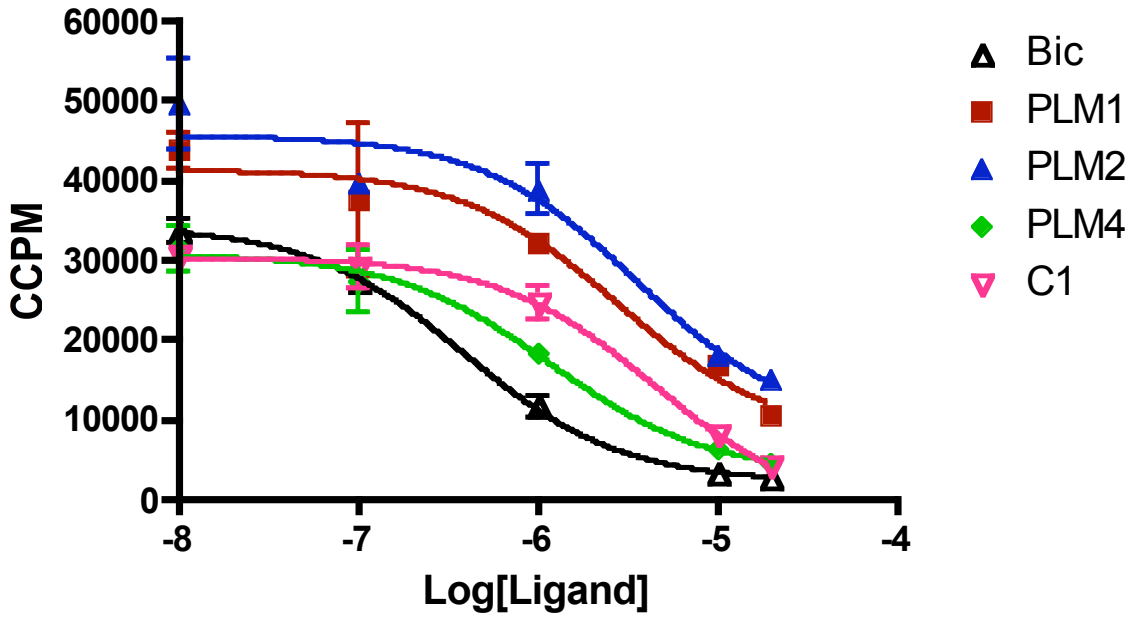


Figure 9: Cellular radio-ligand displacement assay of PLM1, PLM2, PLM4, C1 and Bicalutamide using 75 nM [^{3}H]DHT in AR(W741C).

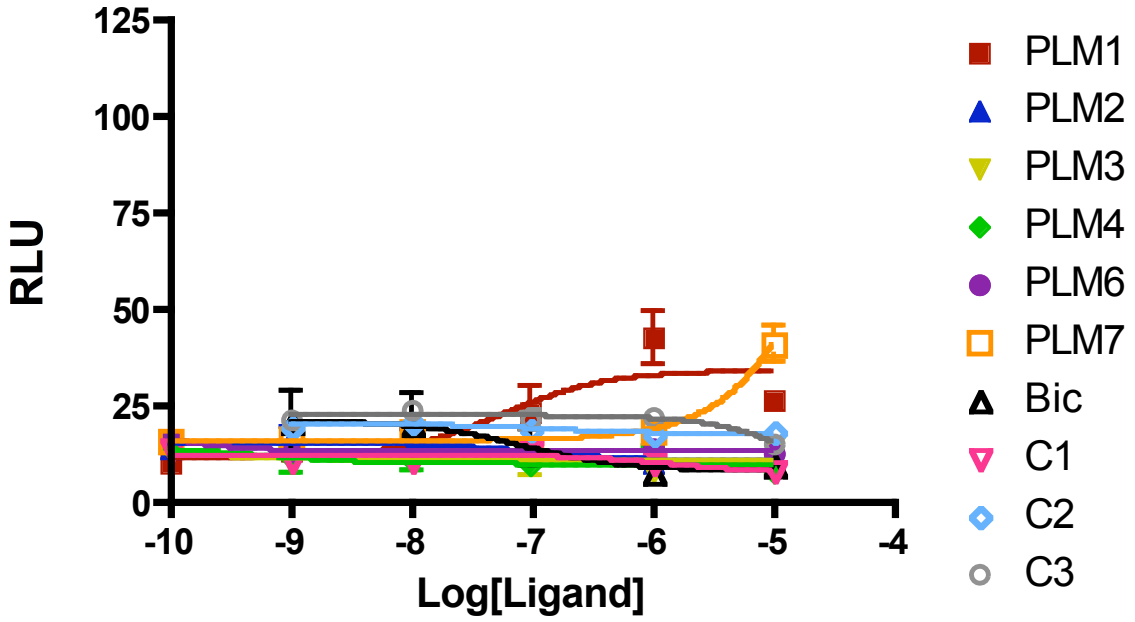


Figure 10: Dose response curve of PLM1-4, PLM6-7, C1-C3 and Bicalutamide in AR(T877A).

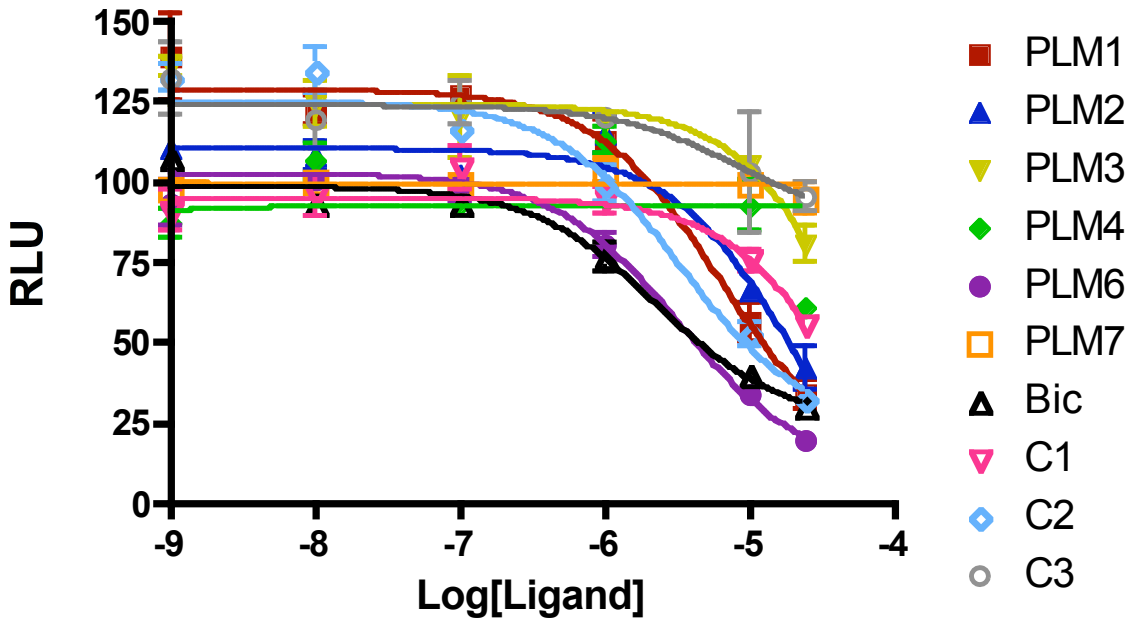


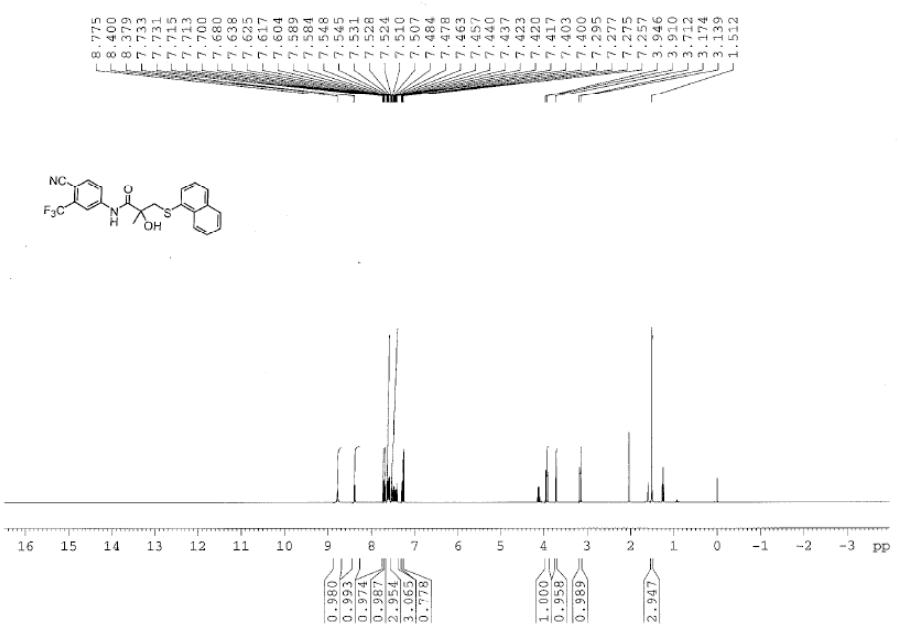
Figure 11: Dose response curve of PLM1-4, PLM6-7, C1-C3 and Bicalutamide in competition with 10 nM DHT in AR(T877A).

Table 1: Potentcies, efficacies, and competitive binding data for PLM1-4, PLM6-7, C1-3 and Bicalutamide in AR(wt), AR(W741L), AR(W741C) and AR(T877A).

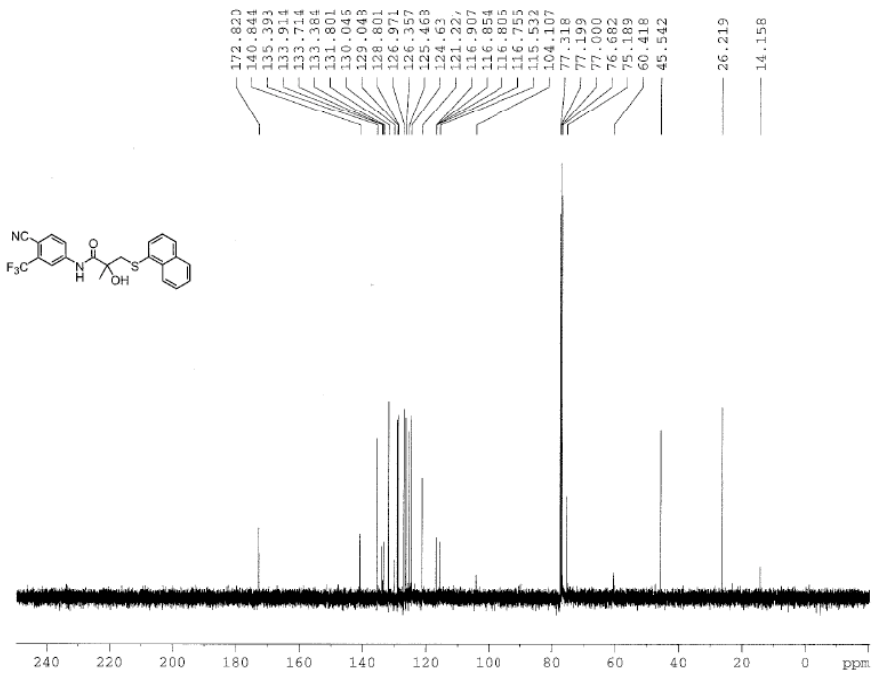
	AR(wt)			AR(W741L)			AR(W741C)			AR(T877A)	
	IC50 μM	EC50 nM	K_i μM	IC50 μM	EC50 nM	K_i μM	IC50 μM	EC50 nM	K_i μM	IC50 μM	EC50 nM
Bic	1.0 ±0.08	--	0.4	--	52.0 ±14.6 82%	0.2 ±0	--	107 ±23 74%	0.7 ±0.5	1.9 ±0.4	--
PLM1	3.8 ±0.7	--	0.5	9.7 ±1.9	--	2.1 ±2.2	3.3 ±1.1	--	5.9 ±6.2	6.0 ±0.4	--
PLM2	12.5 ±2.2	--	6.3	23.0 ±6.2	--	2.9 ±1.1	11.6 ±1.2	--	2.8 ±1.6	16.5 ±4.6	--
PLM3	12.9 ±3.6	--	1.6	--	--	--	--	630 ±67 65%	nd	--	--
PLM4	7.4 ±2.5	--	2.7	--	--	--	--	33.0 ±9.8 66%	2.7 ±2.9	--	--
PLM6	21.4 ±9.9	--	2.1	7.9 ±1.9	--	nd	7.4 ±0.9	--	nd	5.4 ±1.7	--
PLM7	--	--	--	--	--	--	--	--	--	--	--
C1	17.2 ±1.9	--	1.0	--	155 ±45 70%	5.2 ±8.6	--	162 ±9.2 83%	9.1 ±9.1	--	--
C2	3.8 ±0.53	--	--	--	8.7 ±1.7 94%	nd	--	29.6 ±5.2 83%	nd	3.6 ±0.29	--
C3	22.0 ±6.7	--	--	--	3551 ±1050 38%	nd	--	4379 ±1387 43%	nd	--	--

IC50 and EC50 values are reported as the average of three independent experiments run in triplicate ± sem. The EC50 percentage is the maximum inducible activity found compared to 10 nM DHT in AR(wt) expressed as the average of three independent experiments run in triplicate. K_i values for AR(W741L) and AR(W741C) are reported as the average of two independent experiments run in duplicate ± deviation. K_i values for AR(wt) are expressed as the average of a single experiment run in duplicate. 'nd' indicates values were not determined and '--' indicates no effect.

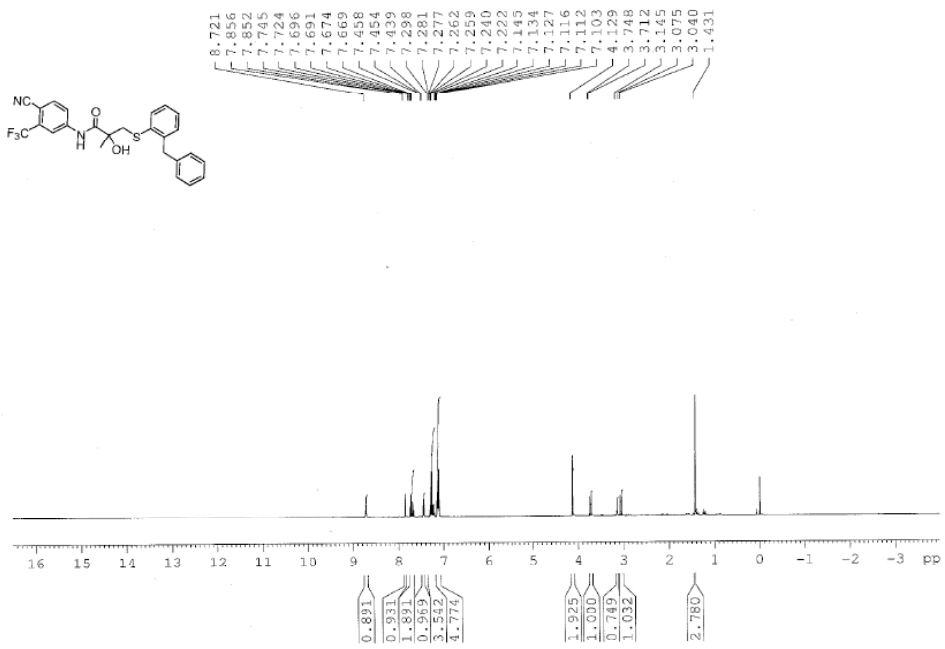
^{1}H NMR Spectrum of *N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methyl-3-(naphthalen-1-ylthio)propanamide (2a)



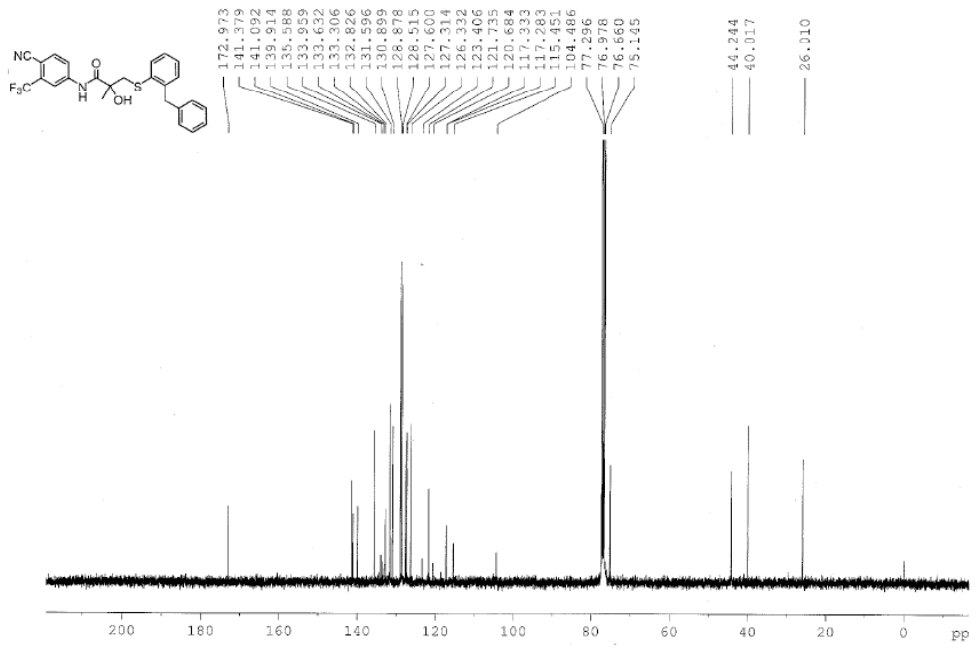
^{13}C NMR Spectrum of *N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methyl-3-(naphthalen-1-ylthio)propanamide (2a)



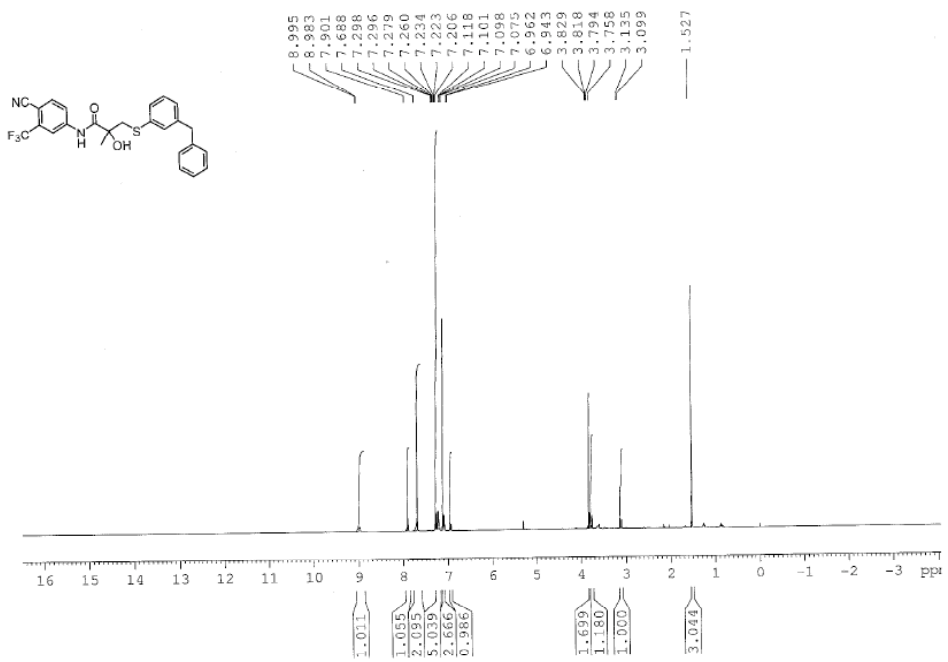
^{1}H NMR Spectrum of 3-(2-benzylphenylthio)-*N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide (2b)



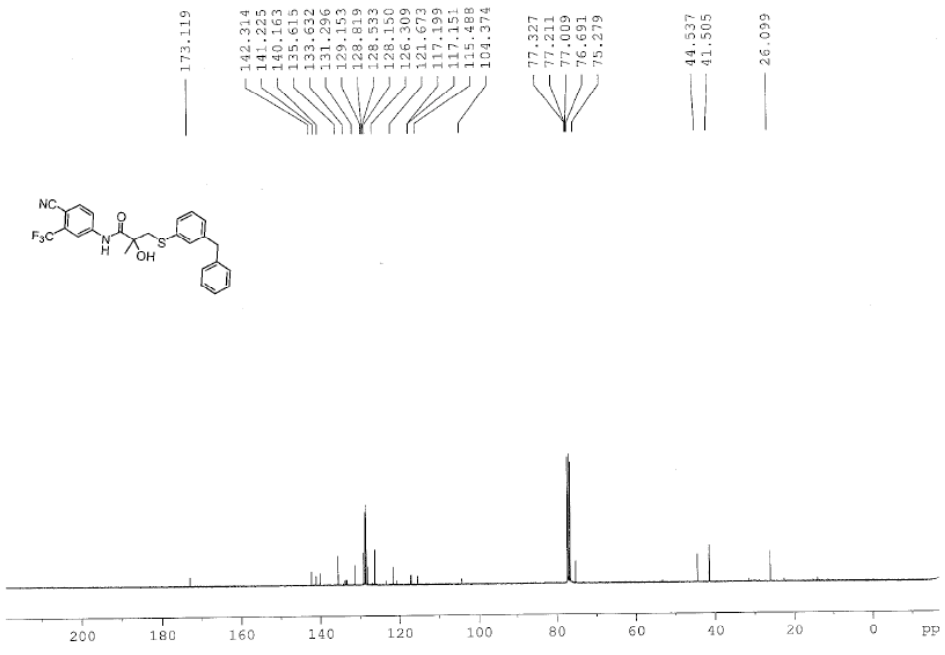
^{13}C NMR Spectrum of 3-(2-benzylphenylthio)-*N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide (2b)



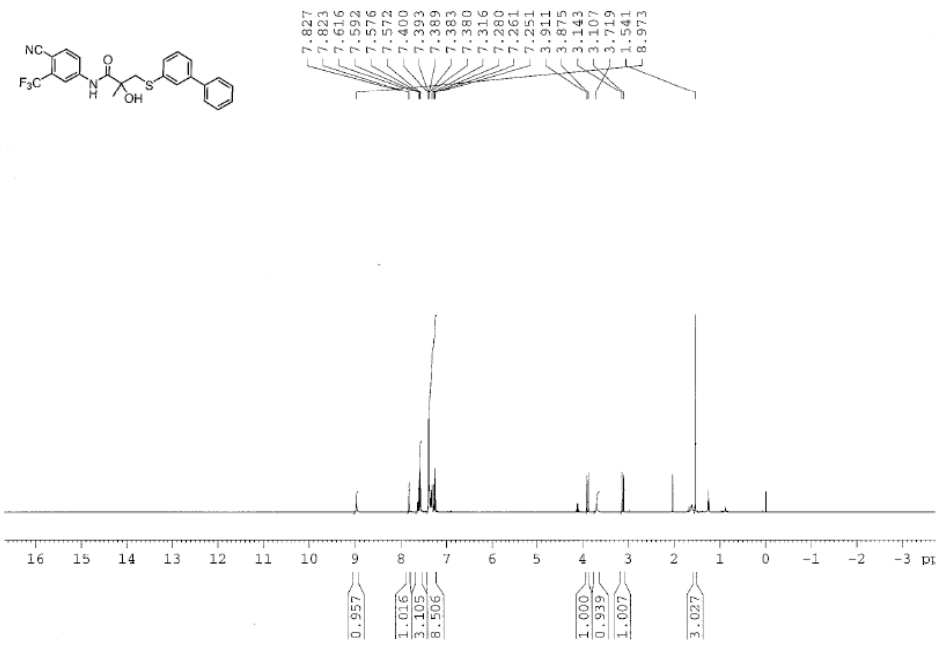
^{1}H NMR Spectrum of 3-(3-benzylphenylthio)-*N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide (2c)



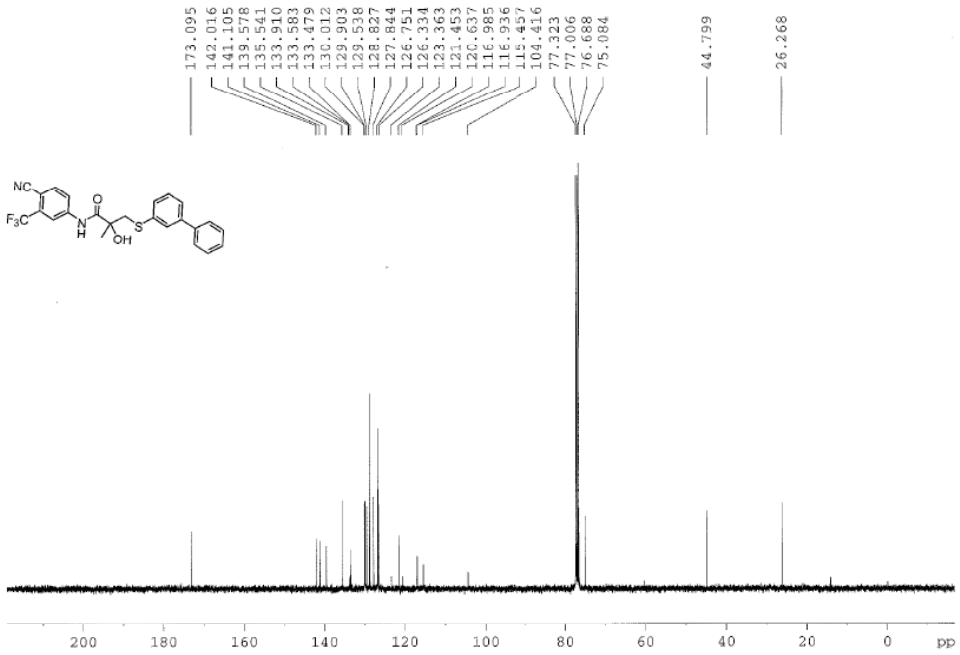
^{13}C NMR Spectrum of 3-(3-benzylphenylthio)-*N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide (2c)



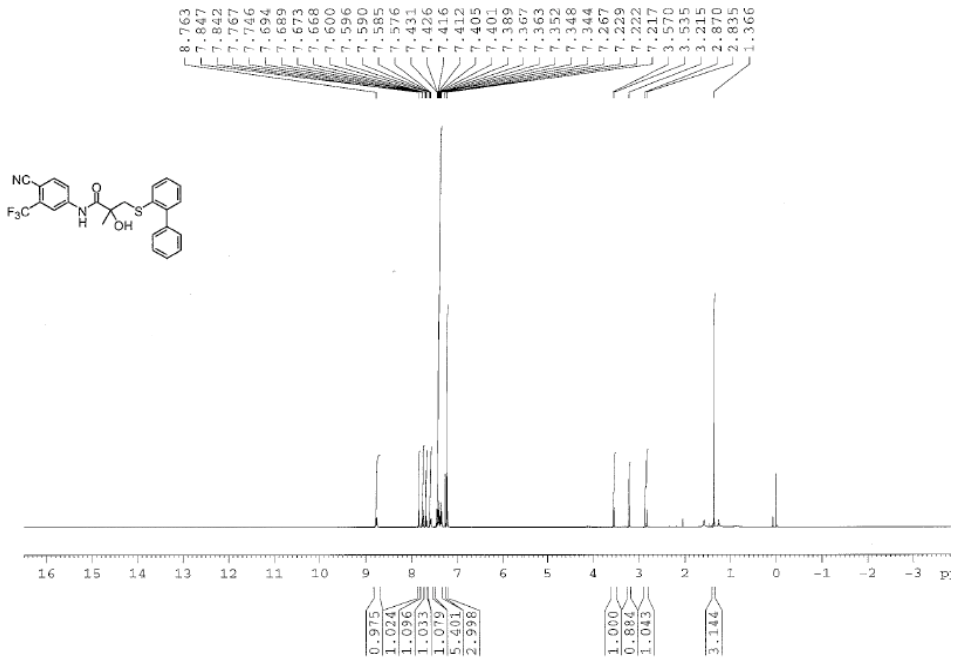
[1]H NMR Spectrum of 3-(2-phenylphenylthio)-*N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide (2d)



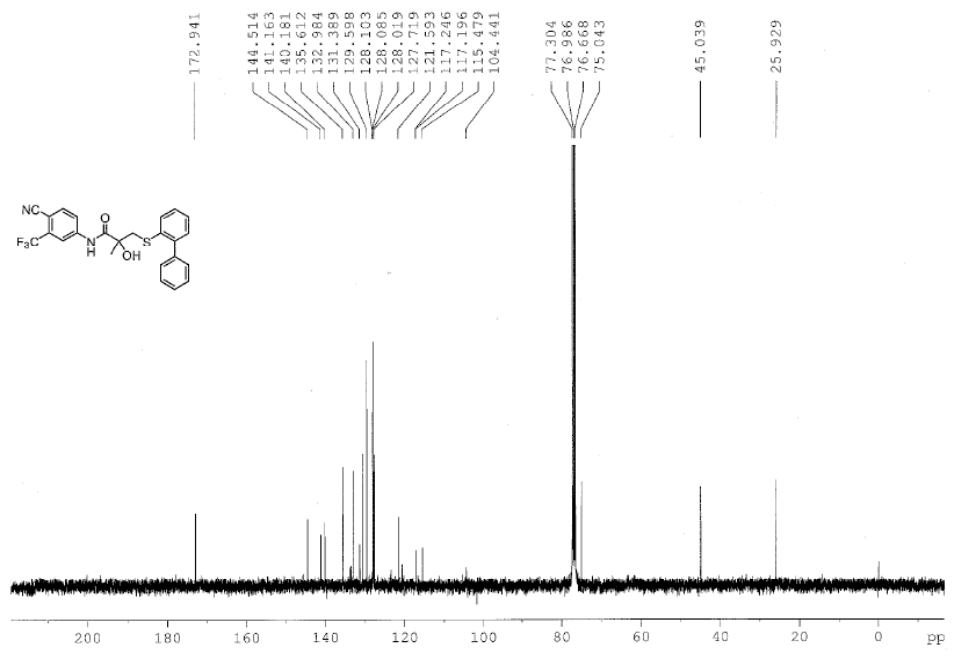
[13]C NMR Spectrum of 3-(2-phenylphenylthio)-*N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide (2d)



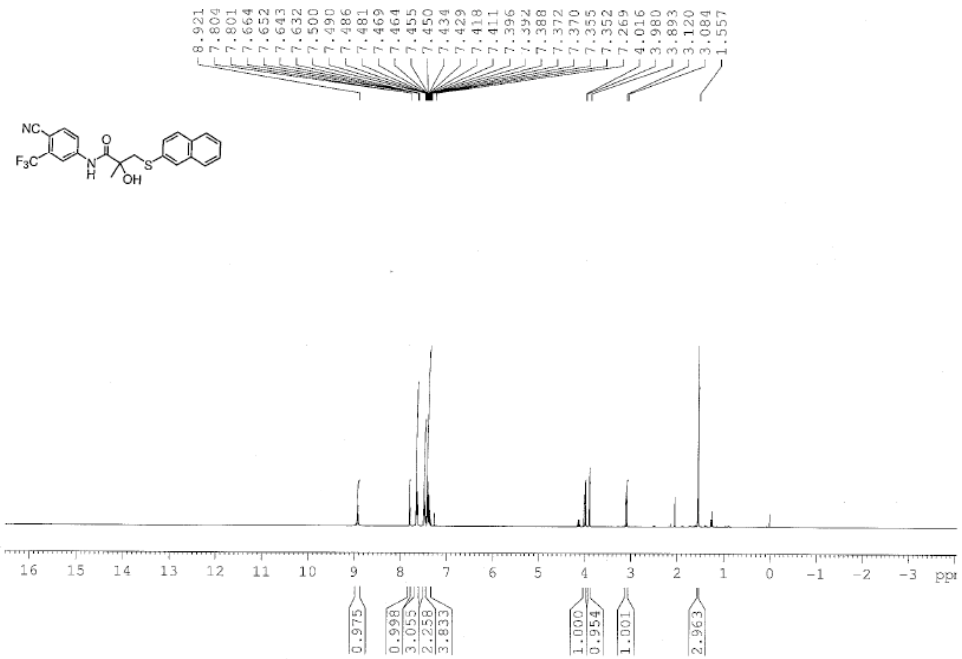
^{1}H NMR Spectrum of 3-(3-methoxyphenylthio)-*N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide (2e)



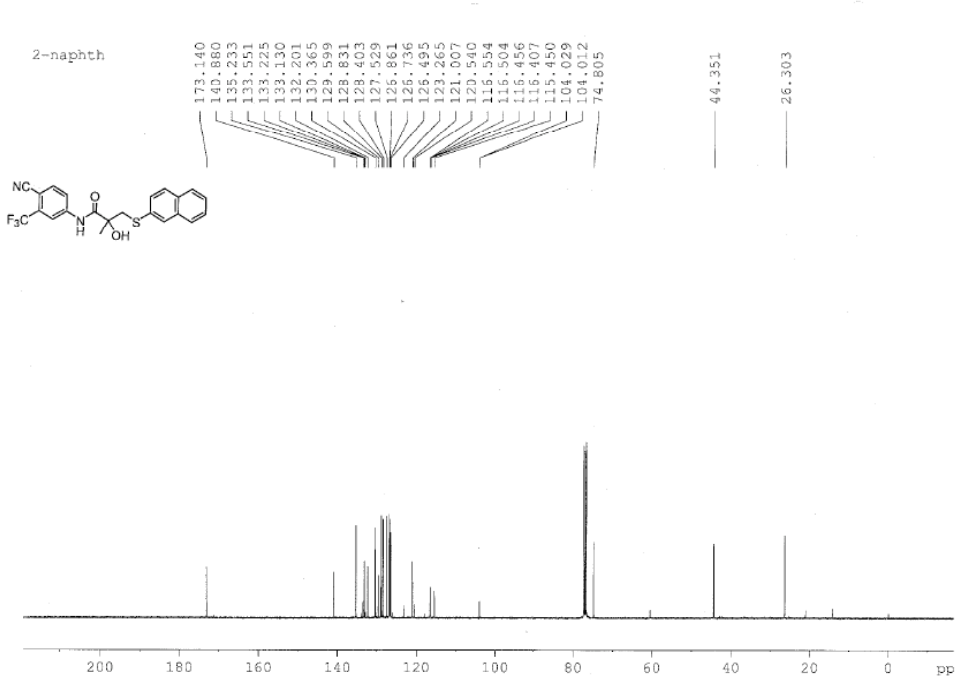
^{13}C NMR Spectrum of 3-(3-methoxyphenylthio)-*N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide (2e)



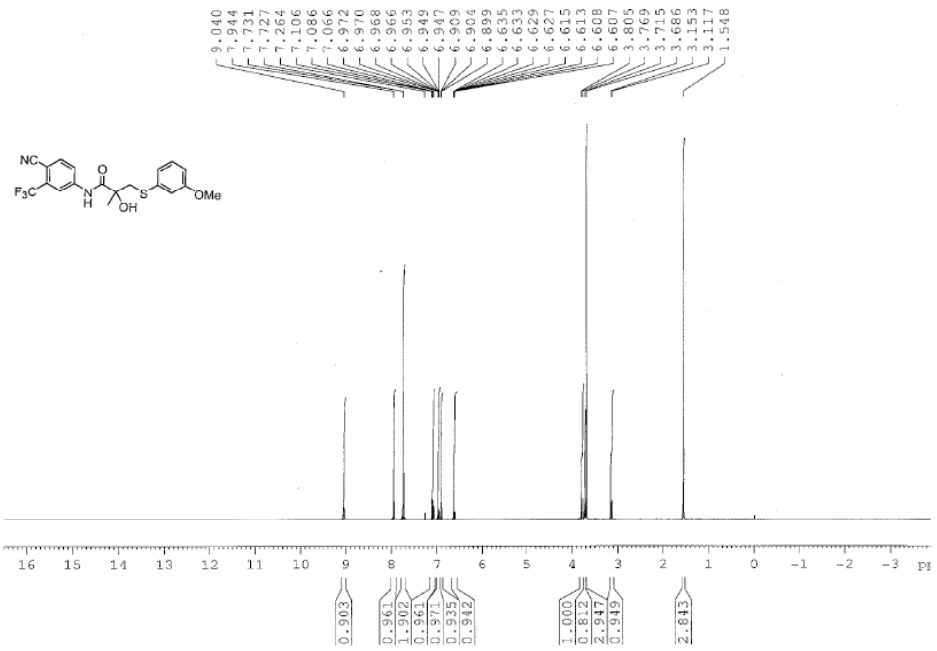
^{1}H NMR Spectrum of *N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methyl-3-(naphthalen-2-ylthio)propanamide (2f)



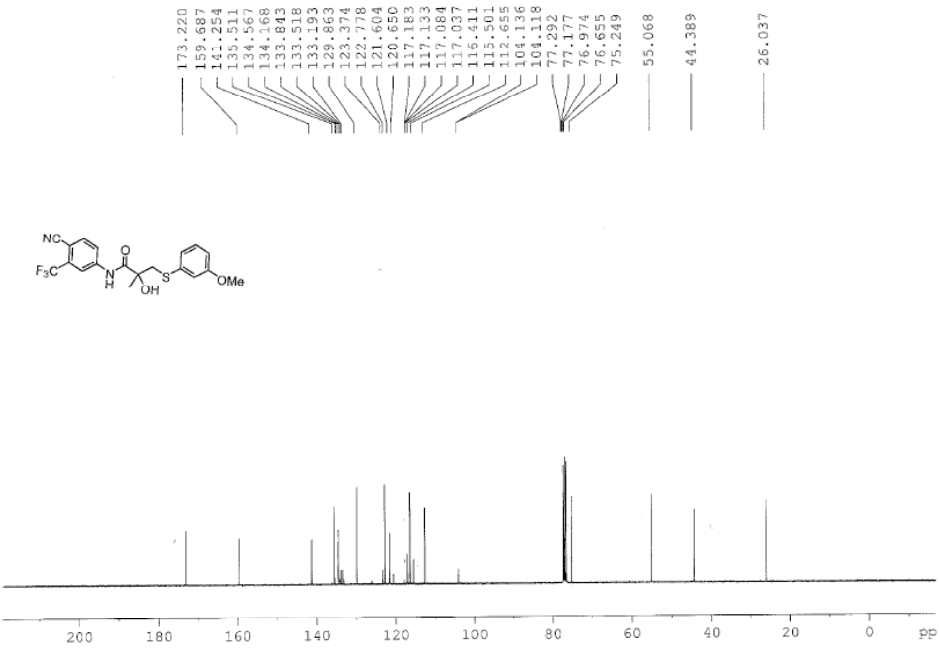
^{13}C NMR Spectrum of *N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methyl-3-(naphthalen-2-ylthio)propanamide (2f)



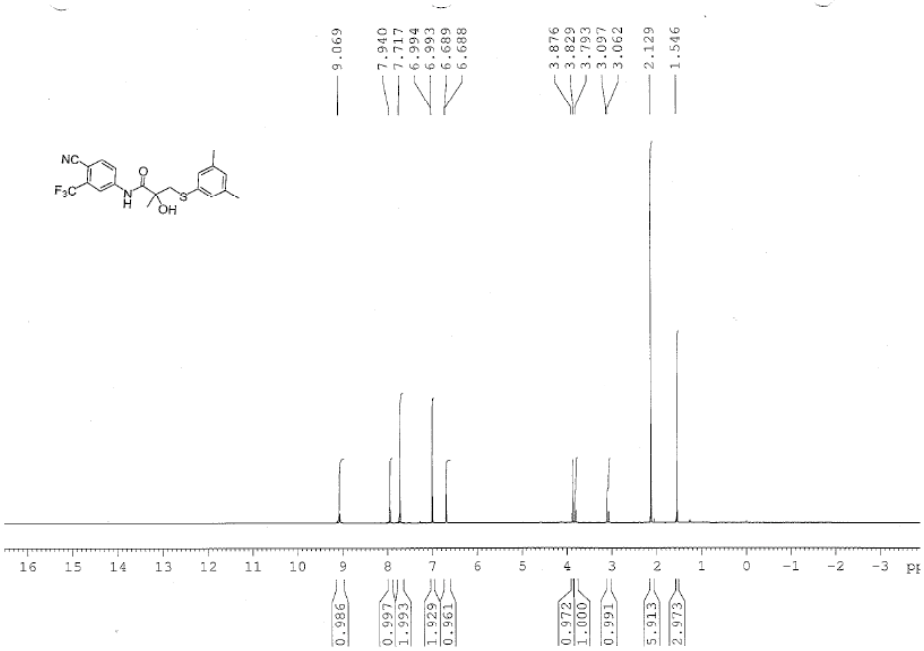
^{1}H NMR Spectrum of 3-(biphenyl-2-ylthio)-*N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide (2g)



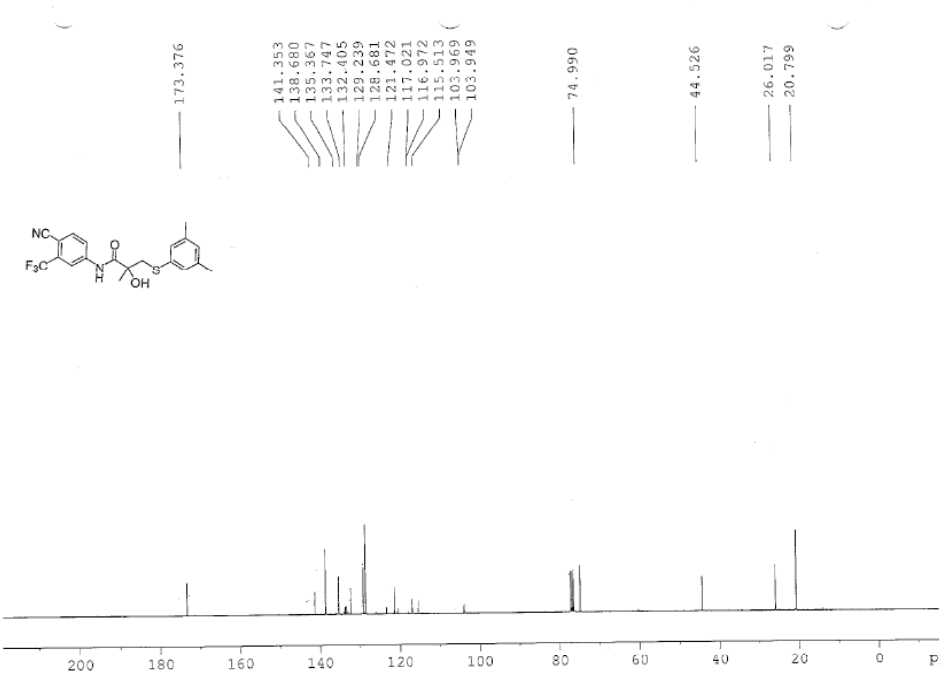
^{13}C NMR Spectrum of 3-(biphenyl-2-ylthio)-*N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide (2g)



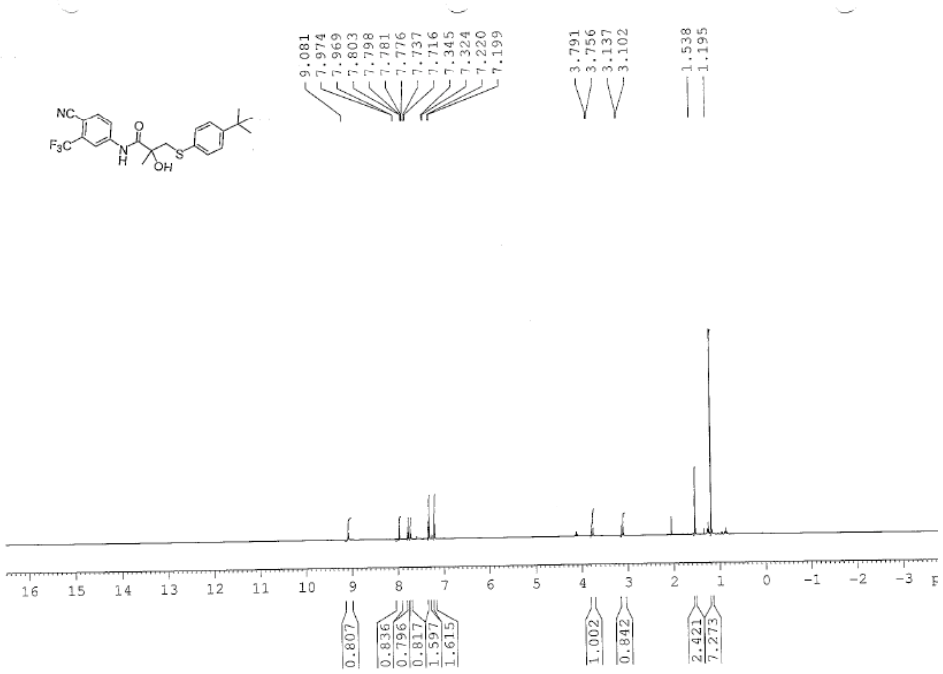
^{1}H NMR Spectrum of 3-(3,5-dimethylphenylthio)-*N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide (2h)



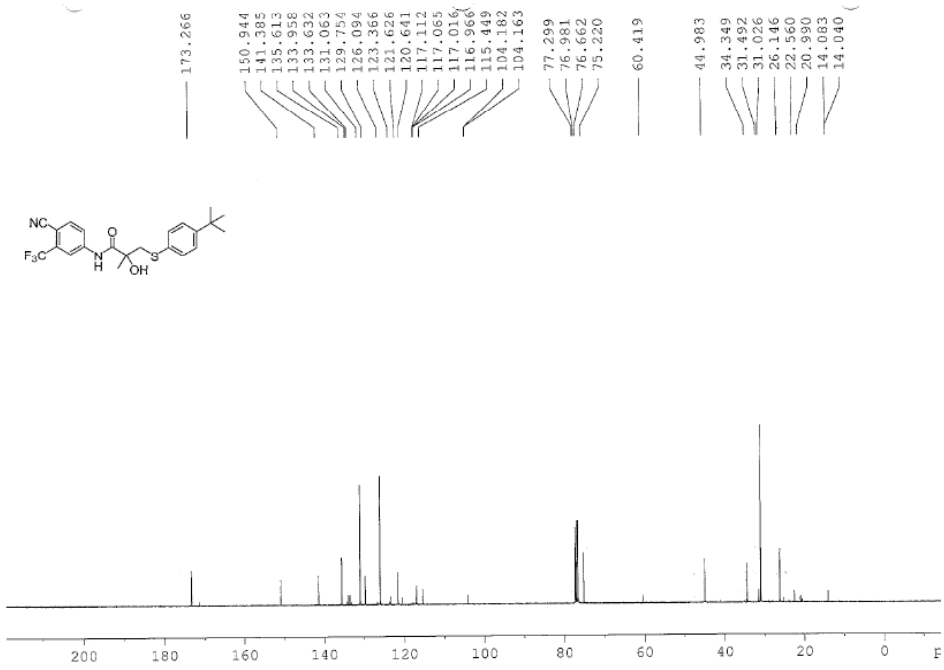
^{13}C NMR Spectrum of 3-(3,5-dimethylphenylthio)-*N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide (2h)



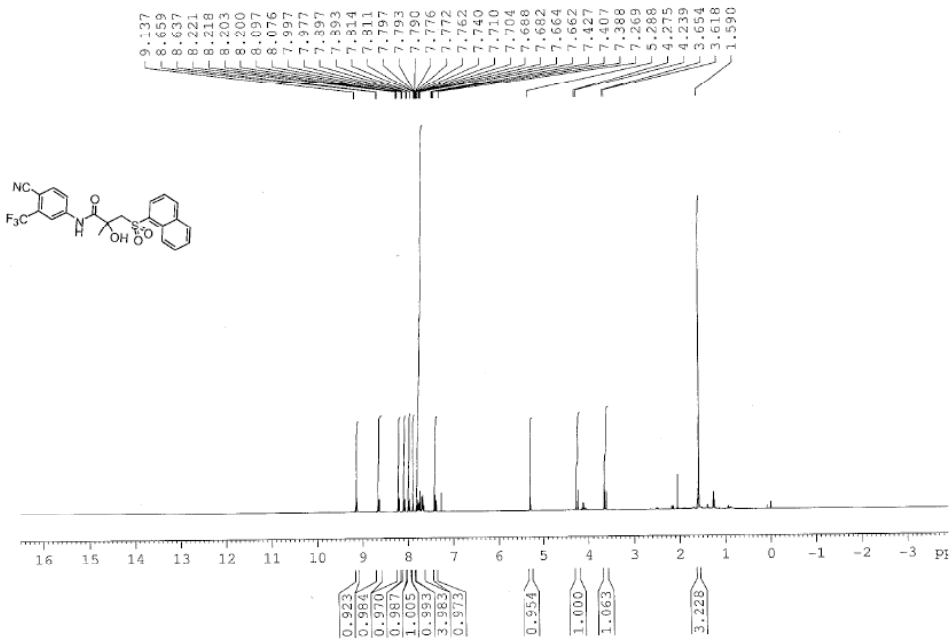
^{1}H NMR Spectrum of 3-(4-*tert*-butylphenylthio)-*N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide (2i)



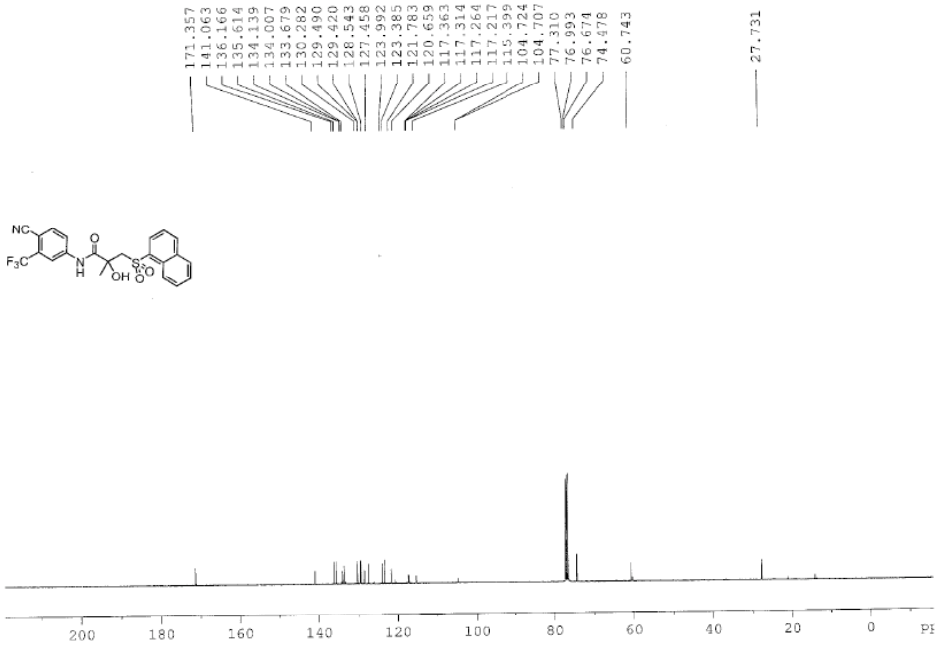
^{13}C NMR Spectrum of 3-(4-*tert*-butylphenylthio)-*N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide (2i)



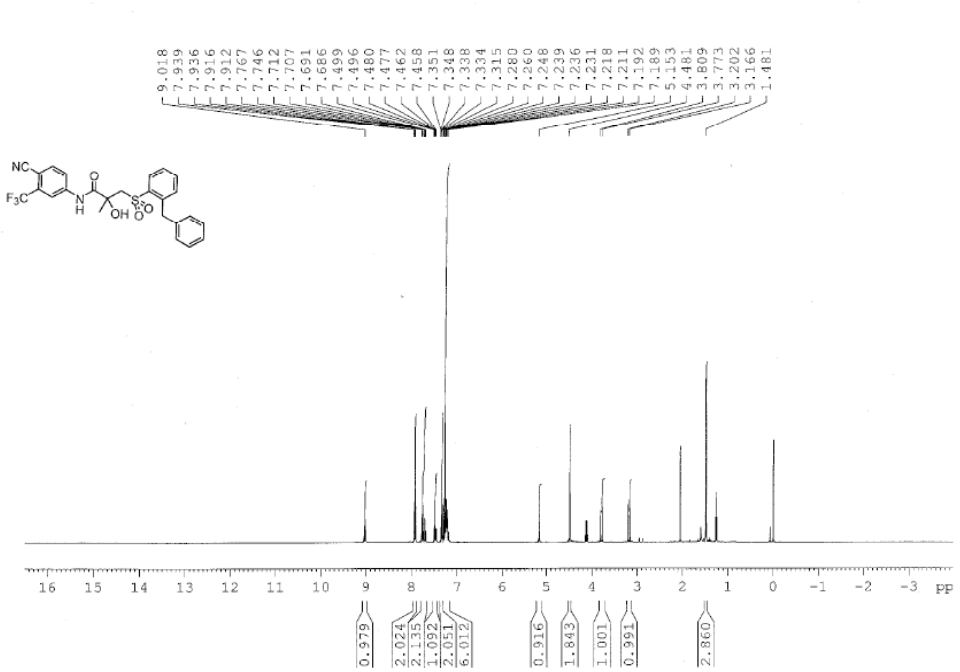
^{1}H NMR Spectrum of *N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methyl-3-(naphthalen-1-ylsulfonyl)propanamide (PLM1)



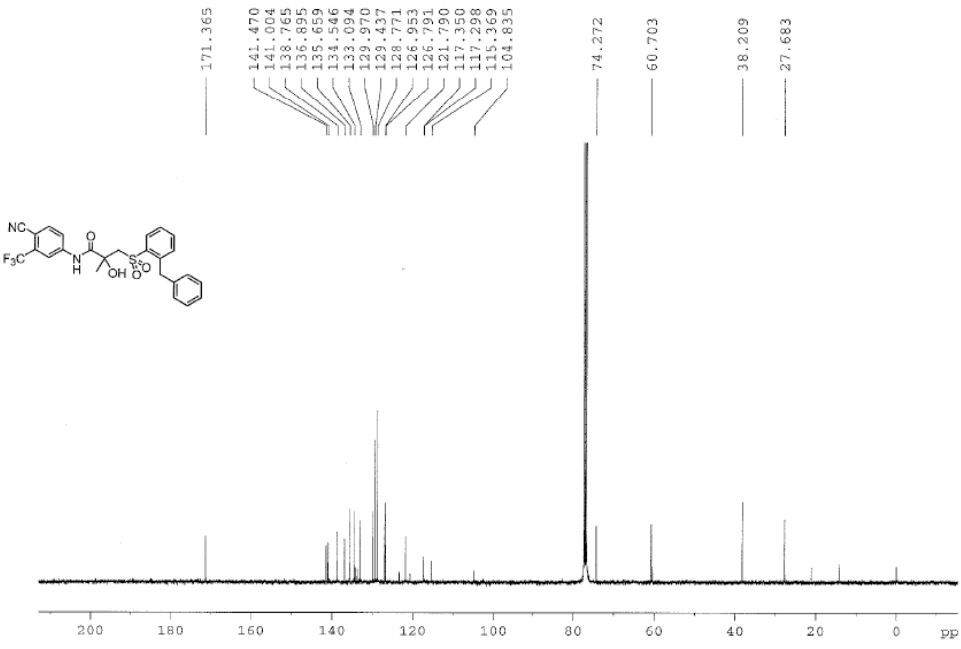
^{13}C NMR Spectrum of *N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methyl-3-(naphthalen-1-ylsulfonyl)propanamide (PLM1)



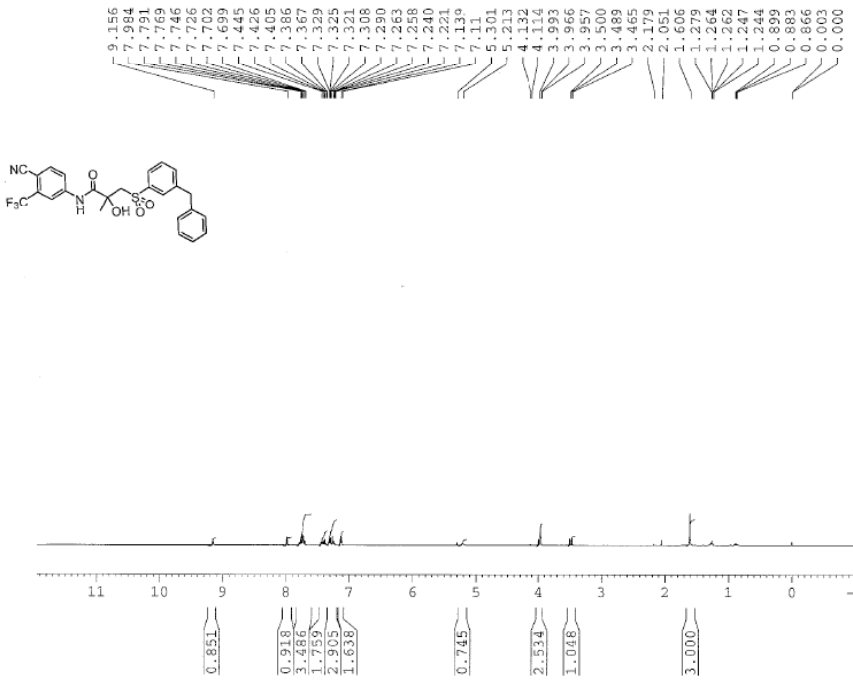
^{1}H NMR Spectrum of 3-(2-benzylphenylsulfonyl)-*N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide (PLM2)



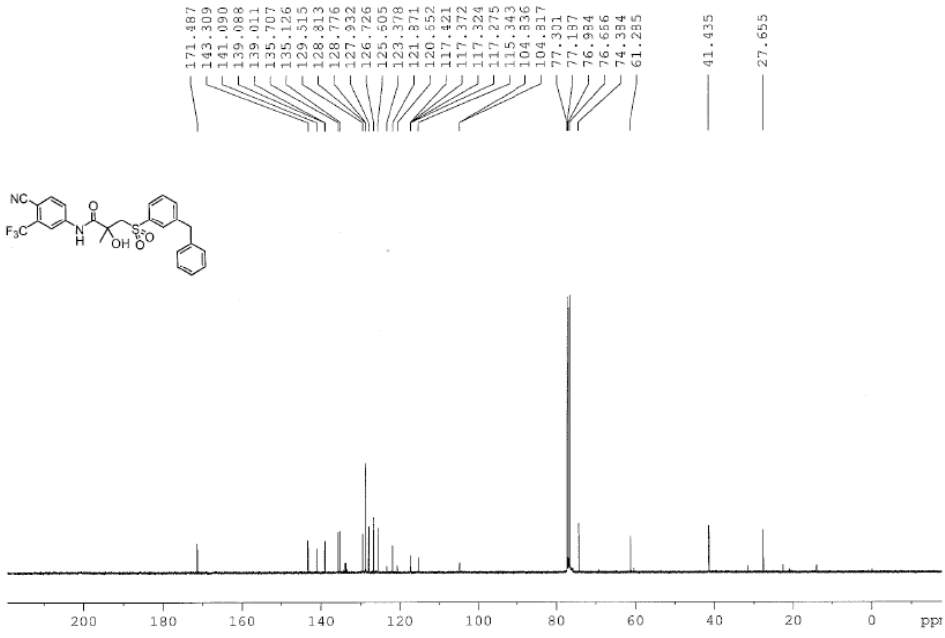
^{13}C NMR Spectrum of 3-(2-benzylphenylsulfonyl)-*N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide (PLM2)



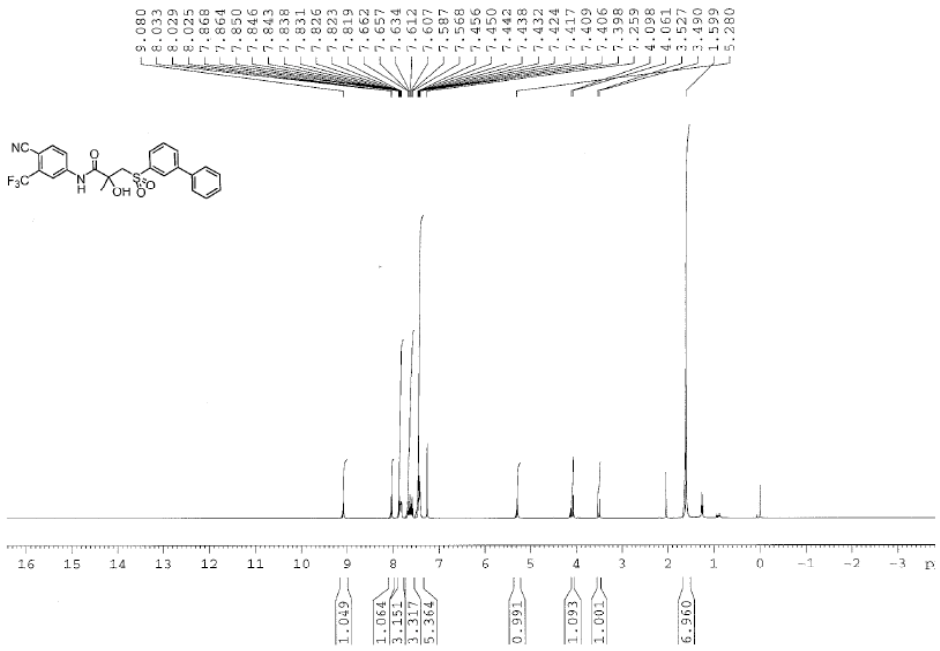
^{1}H NMR Spectrum of 3-(3-benzylphenylsulfonyl)-*N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide (PLM3)



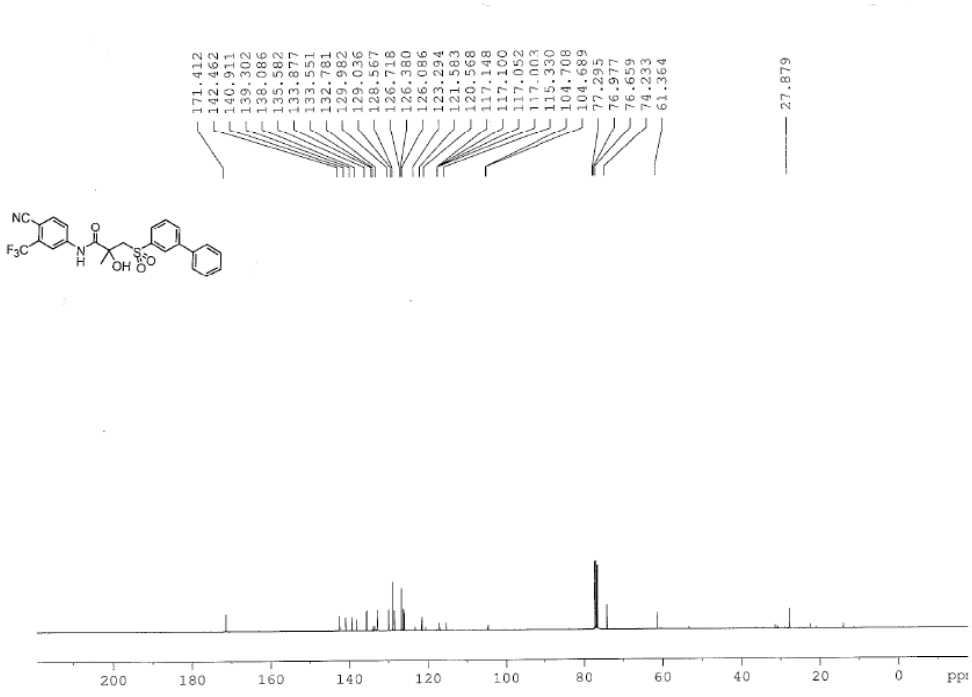
^{13}C NMR Spectrum of 3-(3-benzylphenylsulfonyl)-*N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide (PLM3)



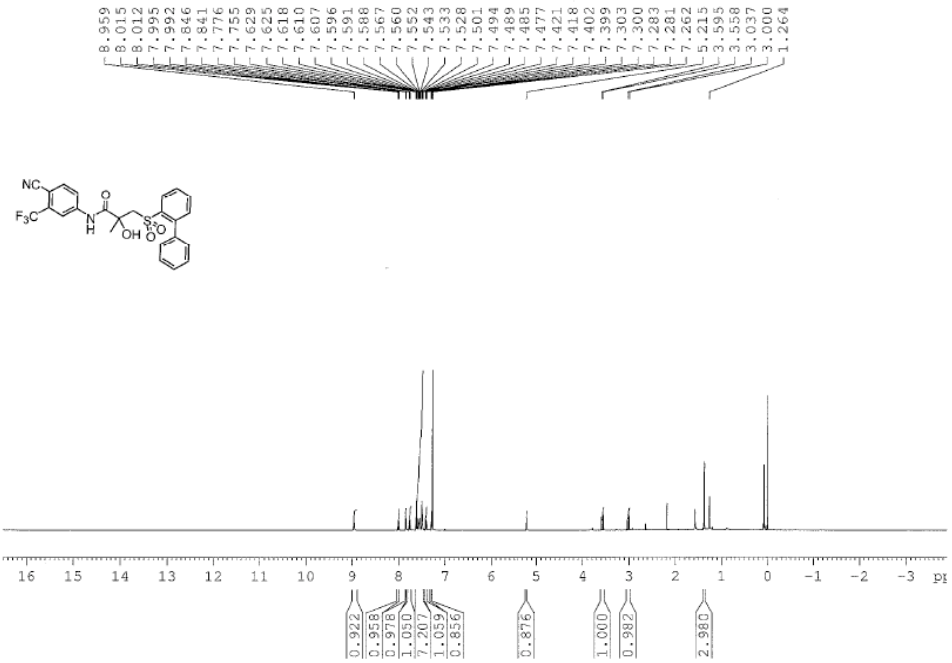
^{1}H NMR Spectrum of 3-(2-phenylphenylsulfonyl)-*N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide (PLM4)



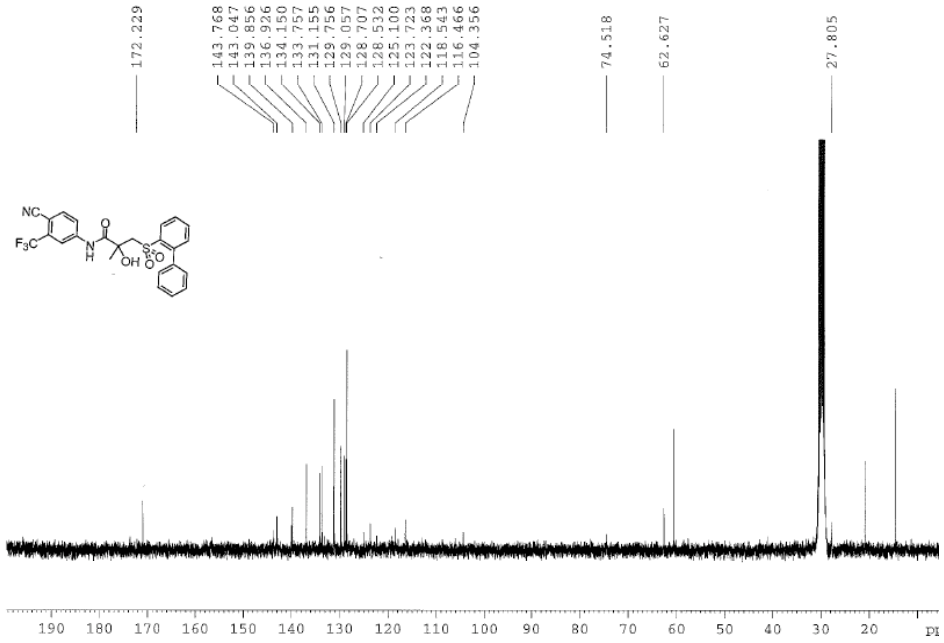
^{13}C NMR Spectrum of 3-(2-phenylphenylsulfonyl)-*N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide (PLM4)



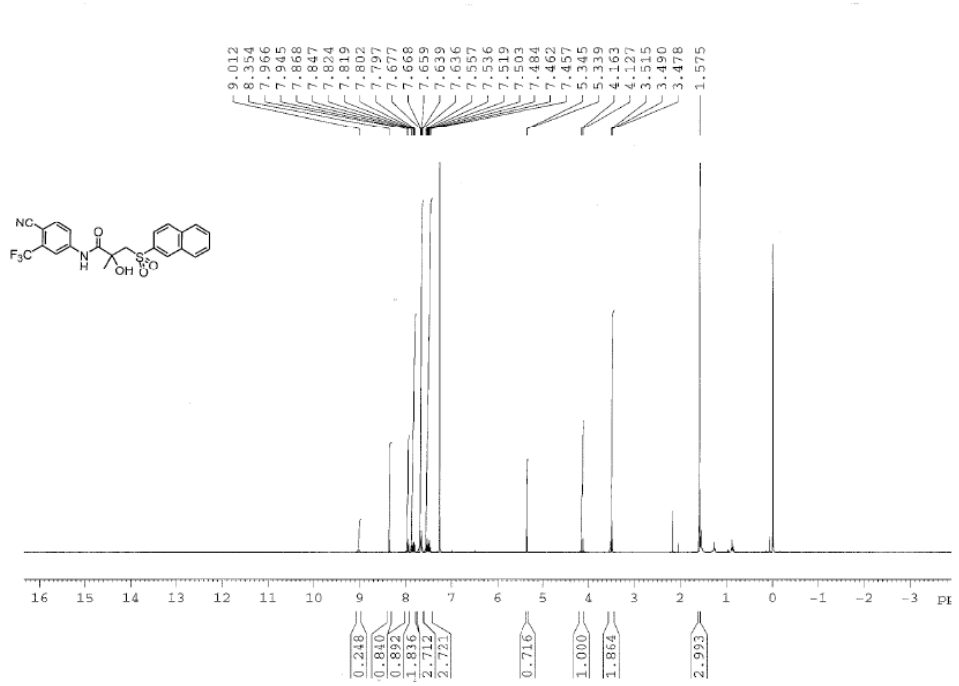
^{1}H NMR Spectrum of 3-(biphenyl-2-ylsulfonyl)-*N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide (PLM6)



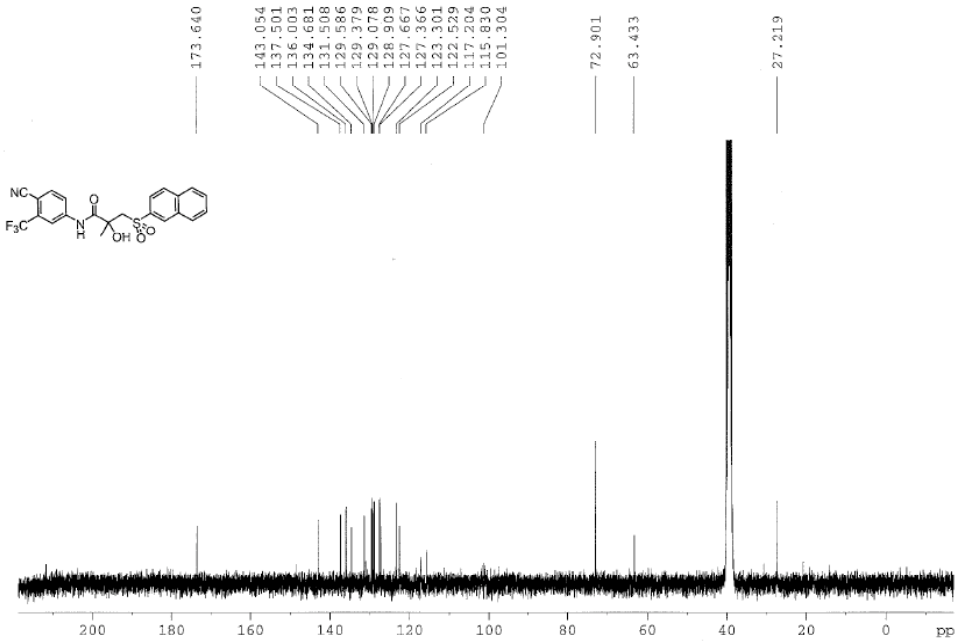
^{13}C NMR Spectrum of 3-(biphenyl-2-ylsulfonyl)-*N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide (PLM6)



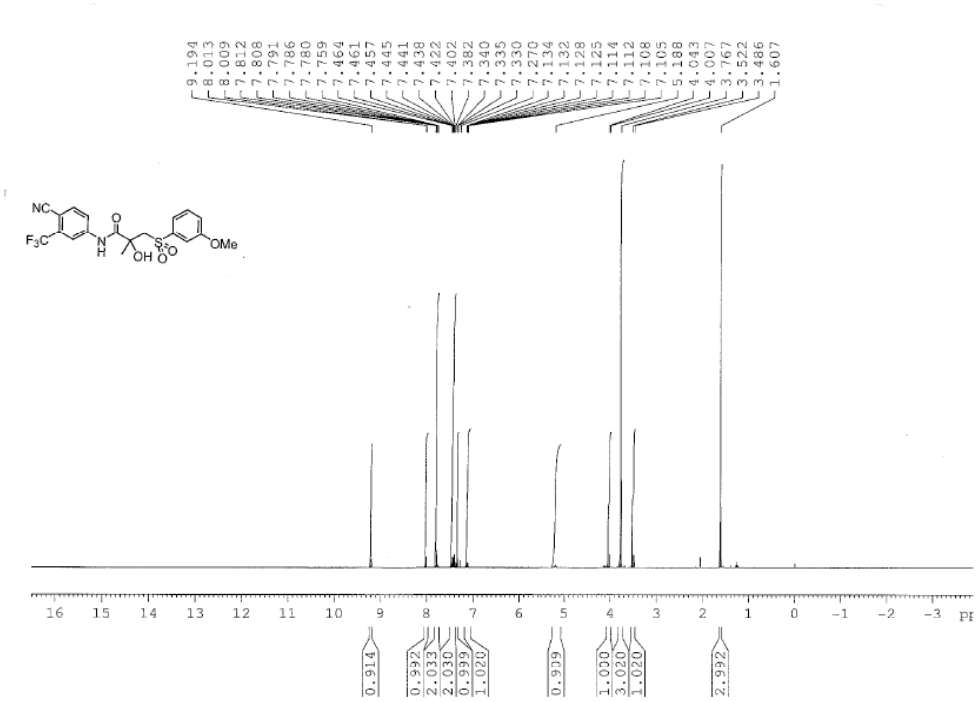
^{1}H NMR Spectrum of *N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methyl-3-(naphthalen-2-ylsulfonyl)propanamide (PLM7)



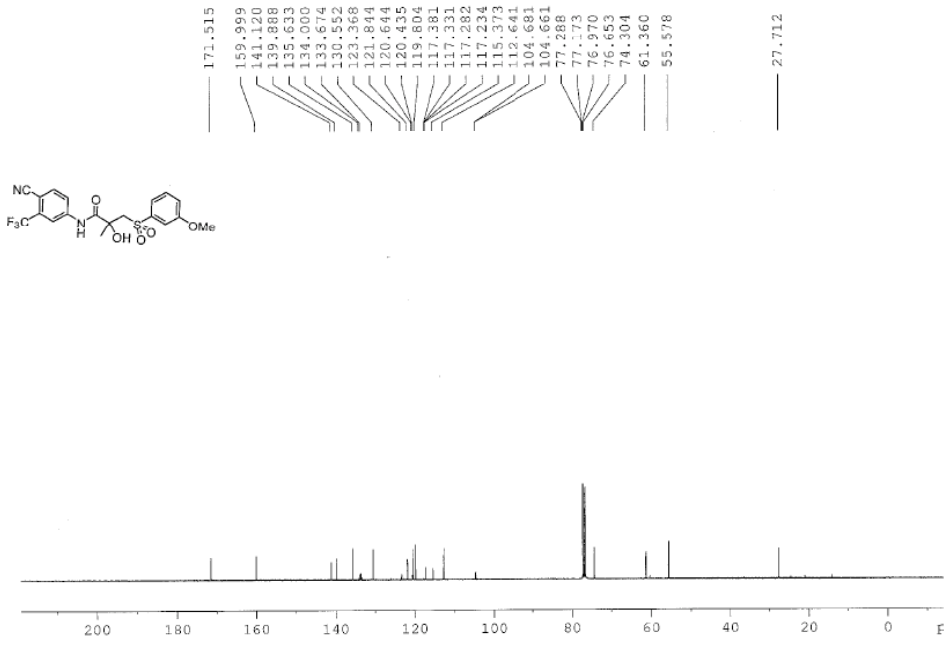
^{13}C NMR Spectrum of *N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methyl-3-(naphthalen-2-ylsulfonyl)propanamide (PLM7)



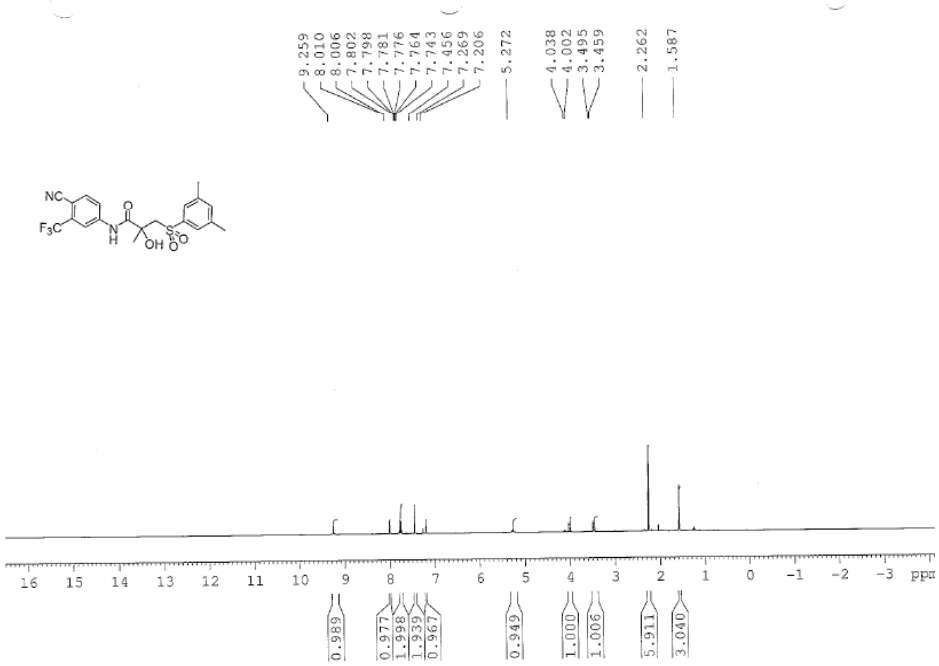
^{1}H NMR Spectrum of 3-(3-methoxyphenylsulfonyl)-*N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide (C1)



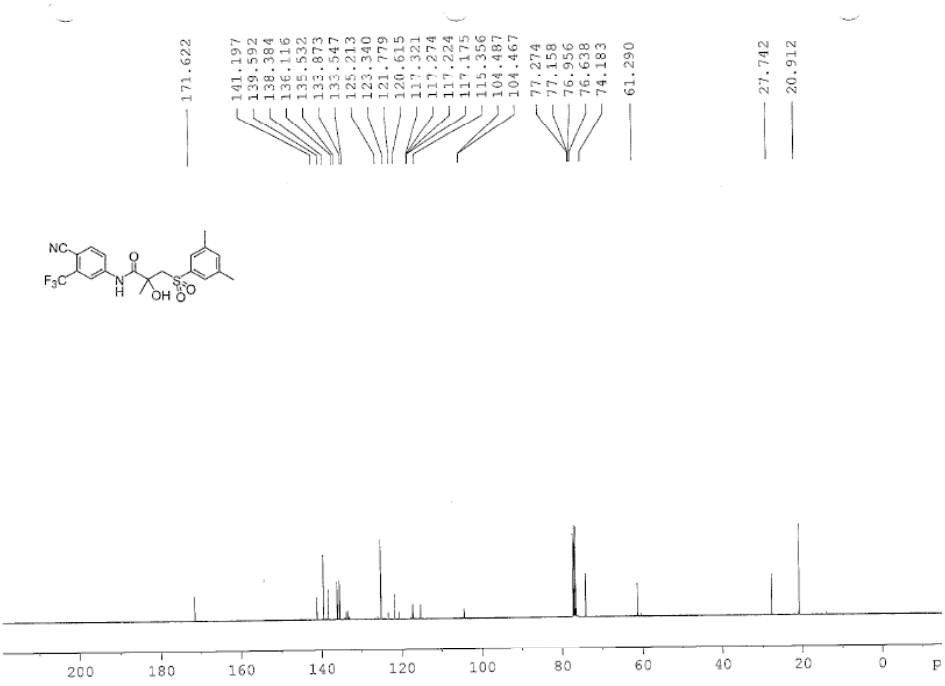
^{13}C NMR Spectrum of 3-(3-methoxyphenylsulfonyl)-*N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide (C1)



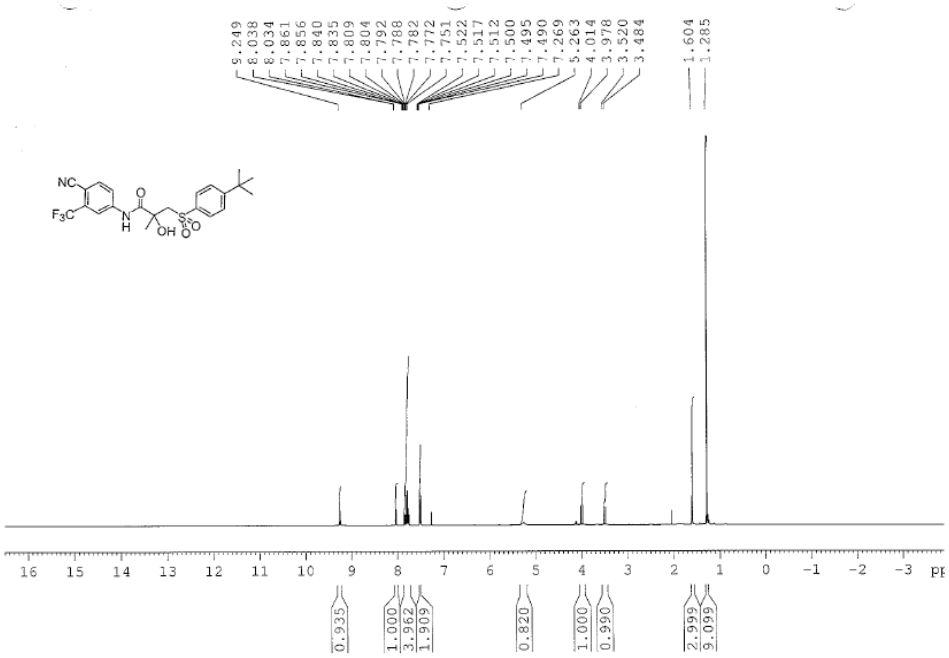
^{1}H NMR Spectrum of 3-(3,5-dimethylphenylsulfonyl)-*N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide (C2)



^{13}C NMR Spectrum of 3-(3,5-dimethylphenylsulfonyl)-*N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide (C2)



^{1}H NMR Spectrum of 3-(4-*tert*-butylphenylsulfonyl)-*N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide (C3)



^{13}C NMR Spectrum of 3-(4-*tert*-butylphenylsulfonyl)-*N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide (C3)