Triarylethylene-Indolin-2, 3-dione Molecular Conjugates: Design, Synthesis, Docking Studies and Anti-Proliferative Evaluation

Sumit Kumar[a], Gabriella Palma[b], ShanenPerumal,[b]MandeepKaur[b], Ashona Singh-Pillay[c], Raghu Raj[d], ParveshSingh[c] and Vipan Kumar*[a]

[a]*Department of Chemistry, Guru Nanak DevUniversity, Amritsar-143005, India*
[b]*School of Molecular and Cell Biology, University of the Witswatersrand, Private Bag 3, Wits-2050, Johannesburg, South Africa.*
[c] *School of Chemistry and Physics, University of KwaZulu Natal, P/Bag X54001, Westvile, Durban 4000, South Africa.*
[d]*Department of Chemistry, DAV College, Amritsar-143001, India*
*Corresponding Author. +91 183 2258802-09*3320 (V.K.); Fax: +91 183 2258819-20, Email address: vipan_org@yahoo.com (V. Kumar)

Experimental Section

General Information

Melting points were determined in capillaries and are uncorrected. ^{1}H and ^{13}C NMR were recorded on JEOL 400, Bruker 500 and 125, 100 MHz spectrometer respectively using $CDCl_3$ as solvent. Chemical shifts were reported in parts per million (ppm) using tetramethylsilane (TMS) as an internal standard and coupling constants *J* indicated in hertz. Spliting patterns were indicated as s: singlet, d: doublet, t: triplet, m: multipletdd: double doublet, ddd: doublet of a doublet of a doublet, and br: broad peak. Mass spectrometric analysis was carried out on BrukermicrOTOF QII equipment using ESI as the source. Column chromatography was performed on a silica gel (60–120 mesh) using an ethyl acetate: hexane mixture as eluent.

General Procedure for synthesis of (Z)-(4-chloro-1-(4-methoxyphenyl)but-1-ene-1,2-diyl)dibenzene4 and (Z)-2-(4-(4-chloro-1,2-diphenylbut-1-en-1-yl)phenoxy)ethan-1-ol 5:A stirred solution of zinc (3.8 mmol) and $TiCl_4$ (2.5 mmol) in dry THF under nitrogen atmosphere was refluxed for 2 h hours and brought to room temperature with sequential addition of a mixture of compound **1**(1mmol) with**2** (1mmol) or **3** (1 mmol) in dry THF. The reaction mixture was heated to reflux for 2 h and the progress was monitored using TLC. On completion, the reaction mixture was cooled to room temperature, charged with toluene and quenched with 37% HCl. The organic layer was separated, washed twice with toluene and dried over anhydrous Na_2SO_4. Evaporation of the solvent under reduced pressure gave crude oil which was purified *via* column chromatography using ethyl acetate: hexane (40:60)

mixture to yield (Z)-(4-chloro-1-(4-methoxyphenyl)but-1-ene-1,2-diyl)dibenzene**4**or (Z)-2-(4-(4-chloro-1,2-diphenylbut-1-en-1-yl)phenoxy)ethan-1-ol **5**respectively.

General Procedure for the synthesis of (Z)-(4-azido-1-(4-methoxyphenyl)but-1-ene-1,2-diyl)dibenzene 6 and (Z)-2-(4-(4-azido-1,2-diphenylbut-1-en-1-yl)phenoxy)ethan-1-ol 7: To the stirred solution of (Z)-(4-chloro-1-(4-methoxyphenyl)but-1-ene-1,2-diyl)dibenzene**4**or (Z)-2-(4-(4-chloro-1,2-diphenylbut-1-en-1-yl)phenoxy)ethan-1-ol **5**indry DMF was added sodium azide(1.2 mmol). The reaction mixture was allowed to stir at 60 ^{0}Cfor 4 h. The progress of reaction mixture was monitored *via* TLC. After usual work up with ethyl acetate and water, the organic layers were combined, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to yield white colour solid, **6** or **7** respectively.

General Procedure for the synthesis of*O*-methylated Ospemifene-isatin/isatin-spiroketal conjugates 12 and 13:To the stirred solution of (Z)-(4-azido-1-(4-methoxyphenyl)but-1-ene-1,2-diyl)dibenzene**6**and*N*-alkylazido-isatin**10**(1 mmol) or *N*-alkylazidospiro-isatin**11**(1 mmol)in ethanol: water (85:15) mixture, was added $CuSO_4.5H_2O$ (0.055 mmol) and sodium ascorbate (0.143 mmol). The reaction mixture was allowed to stir at room temperature for 6-8 h, with progress monitored *via* TLC. On completion, the reaction mixture was extracted with ethyl acetate and water and the combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under vacuum to afford the desired conjugate which was purified *via* column chromatography using ethyl acetate: hexane (60:40) mixture.

(Z)-1-((1-(4-(4-methoxyphenyl)-3,4-diphenylbut-3-en-1-yl)-1H-1,2,3-triazol-4-yl)methyl)indoline-2,3-dione (12a): Yield 83%; light white solid; m.p. 101-102 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.01 (t, *J*=7.2 Hz, 2H, -CH_2-), 3.70 (s, 3H, -OCH_3), 4.29 (t, *J*=7.3 Hz, 2H, -CH_2-N-), 4.90 (s, 2H, -CH_2-N-), 6.55 (d, *J*=8.8 Hz, 2H,Ar-H), 6.70 (d, *J*=8.8 Hz, 2H, Ar-H), 6.79-6.82 (m, 2H, Ar-H), 6.90-6.92 (m, 2H, Ar-H), 7.08-7.20 (m, 8H, Ar-H), 7.32-7.36 (m, 3H, 2Ar-H+triazole-H);^{13}C NMR (125 MHz, $CDCl_3$): δ 35.2, 48.9, 55.0, 65.9, 101.9, 111.0, 112.6, 113.9, 118.7, 125.6, 126.8, 127.1, 127.3, 128.4, 128.5, 128.9, 129.5, 130.0, 130.3, 131.7, 133.9, 134.4, 139.1, 140.4, 142.2, 142.6, 157.3, 182.7; HRMS Calcd for $C_{34}H_{28}N_4O_3$ $[M]^+$ 540.2161 found 540.2150.

(Z)-5-bromo-1-((1-(4-(4-methoxyphenyl)-3,4-diphenylbut-3-en-1-yl)-1H-1,2,3-triazol-4-yl)methyl)indoline-2,3-dione (12b): Yield 79%; light white solid; m.p. 111-112 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.01 (t, *J*=7.1 Hz, 2H, -CH_2-), 3.70 (s, 3H, -OCH_3), 4.28 (t, *J*=6.9 Hz, 2H, -CH_2-N-), 4.89 (s, 2H, -CH_2-N-), 6.55 (d, *J*=8.7 Hz, 2H, Ar-H), 6.70 (d, *J*=8.8 Hz, 2H,

Ar-H), 6.79-6.82 (m, 2H, Ar-H), 6.90-6.93 (m, 2H, Ar-H), 7.12-7.22 (m, 7H, Ar-H), 7.34-7.39 (m, 3H, 2Ar-H+triazole-H);^{13}C NMR (100 MHz, $CDCl_3$): δ 35.1, 49.2, 54.0, 65.9, 102.9, 112.2, 113.3, 113.9, 118.9, 125.2, 126.9, 127.3, 127.6, 128.4, 128.7, 128.9, 129.8, 130.1, 130.3, 131.4, 133.9, 134.4, 139.4, 140.5, 142.2, 142.5, 157.3, 183.1; HRMS Calcd for $C_{34}H_{27}BrN_4O_3$ [M+2] 620.1246 found 620.1265.

(Z)-5,7-dibromo-1-((1-(4-(4-methoxyphenyl)-3,4-diphenylbut-3-en-1-yl)-1H-1,2,3-triazol-4-yl)methyl)indoline-2,3-dione (12c): Yield 81%; light white solid; m.p. 108-109 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.00 (t, *J*=7.2 Hz, 2H, -CH_2-), 3.69 (s, 3H, -OCH_3), 4.29 (t, *J*=7.3 Hz, 2H, -CH_2-N-), 4.90 (s, 2H, -CH_2-N-), 6.55 (d, *J*=8.8 Hz, 2H, Ar-H), 6.72 (d, *J*=8.8 Hz, 2H,Ar-H), 6.80-6.83 (m, 2H, Ar-H), 6.90-6.92 (m, 2H, Ar-H), 7.13-7.21 (m, 7H, Ar-H), 7.34-7.39 (m, 2H, Ar-H+triazole-H);^{13}C NMR (100 MHz, $CDCl_3$): δ 35.1, 48.7, 55.1, 65.7, 101.2, 111.3, 112.5, 113.6, 118.5, 125.4, 126.6, 127.1, 127.3, 128.,5 128.6, 128.9, 129.7, 130.1, 130.3, 131.3, 133.9, 134.4, 139.3, 140.4, 142.3, 142.9, 157.1, 183.3; HRMS Calcd for $C_{34}H_{26}Br_2N_4O_3$ [M+2]700.0351 found 700.0371.

(Z)-5-chloro-1-((1-(4-(4-methoxyphenyl)-3,4-diphenylbut-3-en-1-yl)-1H-1,2,3-triazol-4-yl)methyl)spiro[indoline-3,2'-[1,3]dioxolan]-2-one (13a): Yield 78%; light white solid; m.p. 97-98 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.00 (t, *J*=7.1 Hz, 2H, -CH_2-), 3.69 (s, 3H, -OCH_3), 4.24-4.28 (m, 2H, -N-CH_2-), 4.33 (t, *J*=6.1 Hz, 2H, -CH_2-O-), 4.58 (t, J = 5.7 Hz 2H, -CH_2-O-), 4.89 (s, 2H, -CH_2-N-), 6.55 (d, *J*=8.6 Hz, 2H, Ar-H), 6.71 (d, *J*=8.7 Hz, 2H, Ar-H), 6.77-6.81 (m, 2H, Ar-H), 7.08-7.13 (m, 3H, Ar-H), 7.16-7.22 (m, 7H,Ar-H), 7.33-7.34 (m, 2H, triazole-H+ArH);^{13}C NMR (100 MHz, $CDCl_3$): δ 35.2, 36.4, 48.9, 55.0, 66.0, 101.8, 111.2, 112.9, 113.8, 122.6, 125.3, 125.5, 126.2, 126.8, 127.1, 127.4, 128.4, 128.9, 129.6, 129.9, 130.4, 131.6, 133.8, 134.3, 140.5, 141.8, 142.6, 157.9, 172.6; HRMS Calcd for $C_{36}H_{31}ClN_4O_4$ $[M]^+$ 618.2034 found 618.2041.

(Z)-5-fluoro-1-((1-(4-(4-methoxyphenyl)-3,4-diphenylbut-3-en-1-yl)-1H-1,2,3-triazol-4-yl)methyl)spiro[indoline-3,2'-[1,3]dioxolan]-2-one (13b): Yield 85%; light white solid; m.p. 99-101 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.00 (t, *J*=7.1 Hz, 2H, -CH_2-), 3.69 (s, 3H, -OCH_3), 4.24-4.28 (m, 2H, -N-CH_2-), 4.33 (t, *J*=6.3 Hz, 2H, -CH_2-O-), 4.59 (t, J = 5.8 Hz 2H, -CH_2-O-), 4.89 (s, 2H, -CH_2-N-), 6.55 (d, *J*=8.7 Hz, 2H,Ar-H), 6.71 (d, *J*=8.7 Hz, 2H, Ar-H), 6.78-6.81 (m, 2H, Ar-H), 7.06-7.16 (m, 8H, Ar-H), 7.19-7.22 (m, 2H,Ar-H), 7.31-7.33 (m, 2H, triazole-H+ArH);^{13}C NMR (100 MHz, $CDCl_3$): δ 35.2, 36.4, 48.9, 55.0, 65.9, 101.9, 111.0, 112.8, 113.8, 118.1, 122.6, 126.8, 127.1, 127.4, 128.3, 128.4, 128.8, 129.6, 130.0,

130.4, 131.6, 133.8, 134.3, 139.1, 140.5, 142.0, 142.5, 157.9, 172.8; HRMS Calcd for $C_{36}H_{31}FN_4O_4$ $[M]^+$ 602.2329 found 602.2339.

(Z)-5-bromo-1-((1-(4-(4-methoxyphenyl)-3,4-diphenylbut-3-en-1-yl)-1H-1,2,3-triazol-4-yl)methyl)spiro[indoline-3,2'-[1,3]dioxolan]-2-one (13c):Yield 76%; light white solid; m.p. 106-107 °C;^{1}H NMR (500 MHz, $CDCl_3$): δ 3.01 (t, *J*=7.1 Hz, 2H, -CH_2-), 3.70 (s, 3H, -OCH_3), 4.24-4.27 (m, 2H, -N-CH_2-), 4.32 (t, *J*=6.1 Hz, 2H, -CH_2-O-), 4.57 (t, J = 5.7 Hz, 2H, -CH_2-O-),4.90 (s, 2H, -CH_2-N-), 6.55 (d, *J*=8.6 Hz, 2H,Ar-H), 6.72 (d, *J*=8.8 Hz, 2H, Ar-H), 6.77-6.81 (m, 2H, Ar-H), 7.09-7.14 (m, 3H, Ar-H), 7.16-7.24 (m, 7H,Ar-H), 7.33-7.34 (m, 2H, triazole-H+ArH);^{13}C NMR (100 MHz, $CDCl_3$): δ 35.4, 36.5, 48.8, 55.2, 66.1, 101.3, 111.4, 112.6, 113.8, 122.5, 125.6, 125.8, 126.3, 126.8, 127.2, 127.5, 128.5, 128.9, 129.6, 129.9, 130.3, 131.7, 133.7, 134.6, 140.7, 141.8, 142.6, 157.4, 172.6; HRMS Calcd for $C_{36}H_{31}BrN_4O_4$ [M+2] 664.1508 found 664.1493.

General Procedure for the synthesis ofOspemifene-isatin conjugates 14 and 15: To the stirred solution of(Z)-2-(4-(4-azido-1,2-diphenylbut-1-en-1-yl)phenoxy)ethan-1-ol **7** (1 mmol)and *N*-alkylazido-isatin**10**(1 mmol) or *N*-alkylazidospiro-isatin**11**(1 mmol) in ethanol: water (85:15) mixture, was added $CuSO_4.5H_2O$ (0.055 mmol) and sodium ascorbate (0.143 mmol). The reaction mixture was allowed to stir at room temperature for 6-8 h, with progress monitored *via* TLC. On completion, the reaction mixture was extracted with ethyl acetate and water and the combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under vacuum to afford the desired conjugate which was purified *via* column chromatography using ethyl acetate: hexane (60:40) mixture.

(Z)-1-((1-(4-(4-(2-hydroxyethoxy)phenyl)-3,4-diphenylbut-3-en-1-yl)-1H-1,2,3-triazol-4-yl)methyl)indoline-2,3-dione (14a): Yield 82%; light orange solid; m.p. 92-93 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.97 (t, *J*=7.0 Hz, 2H, -CH_2-), 3.86 (t, *J*=3.6 Hz, 2H, -O-CH_2-), 3.93 (t, *J*=4.0 Hz, 2H, -CH_2-O-), 4.25 (t, *J*=7.1 Hz, 2H, -CH_2-N-), 4.96 (s, 2H, -CH_2-N-), 6.53 (d, *J*=8.7 Hz, 2H,Ar-H), 6.67 (d, *J*=8.7 Hz, 2H,Ar-H), 6.84-6.86 (m, 2H, Ar-H), 7.05-7.17 (m, 8H, 7Ar-H+-CH_2-OH Exchangeable with D_2O), 7.21-7.22 (m, 2H,Ar-H), 7.29-7.33 (m, 2H, Ar-H), 7.54-7.58 (m, 2H, triazole-H+ArH);^{13}C NMR (100 MHz, $CDCl_3$): δ 35.4, 36.5, 49.2, 61.4, 68.9, 113.5, 113.6, 117.0, 118.7, 123.1, 126.9, 127.0, 127.3, 128.0, 128.5, 128.8, 128.9, 129.6, 131.7, 133.9, 134.7, 140.6, 140.9, 141.2, 142.6, 142.7, 148.9, 157.1, 182.0; HRMS Calcd for $C_{35}H_{30}N_4O_4$ $[M]^+$ 570.2267 found 570.2260.

(Z)-5-chloro-1-((1-(4-(4-(2-hydroxyethoxy)phenyl)-3,4-diphenylbut-3-en-1-yl)-1H-1,2,3-triazol-4-yl)methyl)indoline-2,3-dione (14b): Yield 78%; orange solid; m.p. 102-103 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.97 (t, *J*=7.2 Hz, 2H, -CH_2-), 3.86 (t, *J*=4.0 Hz, 2H, -O-CH_2-), 3.94 (t, *J*=4.7 Hz, 2H, -CH_2-O-), 4.26 (t, *J*=7.4 Hz, 2H, -CH_2-N-), 4.94 (s, 2H, -CH_2-N-), 6.54 (d, *J*=8.8 Hz, 2H,Ar-H), 6.67 (d, *J*=8.8 Hz, 2H, Ar-H), 6.86-6.88 (m, 2H, Ar-H), 7.05-7.07 (m, 3H, Ar-H), 7.10-7.18 (m, 4H,Ar-H), 7.23-7.24 (m, 1H, Ar-H), 7.28 (s, 1H, Ar-H), 7.32-7.34 (m, 1H, Ar-H), 7.51-7.54 (m, 2H, triazole-H+Ar-H);^{13}C NMR (100 MHz, $CDCl_3$): δ 35.4, 36.5, 49.2, 61.4, 68.9, 113.1, 113.6, 123.1, 125.2, 125.5, 125.6, 127.0, 127.3, 128.5, 128.8, 129.6, 130.0, 130.5, 131.7, 133.9, 134.7, 138.0, 140.6, 141.2, 142.6, 142.7, 148.5, 157.0, 182.1; HRMS Calcd for $C_{35}H_{29}ClN_4O_4$ $[M]^+$ 604.1877 found 604.1881.

(Z)-5-fluoro-1-((1-(4-(4-(2-hydroxyethoxy)phenyl)-3,4-diphenylbut-3-en-1-yl)-1H-1,2,3-triazol-4-yl)methyl)indoline-2,3-dione (14c): Yield 81%; light orange solid; m.p. 98-99 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.98 (t, *J*=7.1 Hz, 2H, -CH_2-), 3.86-3.88 (m, 2H, -O-CH_2-), 3.94 (t, *J*=4.7 Hz, 2H, -CH_2-O-), 4.26 (t, *J*=7.4 Hz, 2H, -CH_2-N-), 4.94 (s, 2H, -CH_2-N-), 6.54 (d, *J*=8.8 Hz, 2H, Ar-H), 6.67 (d, *J*=8.8 Hz, 2H,Ar-H), 6.86-6.88 (m, 2H, Ar-H), 7.05-7.08 (m,2H, Ar-H), 7.10-7.18 (m, 4H,Ar-H), 7.23-7.24 (m, 1H, Ar-H), 7.26-7.30 (m, 4H, triazole-H+3Ar-H), 7.33-7.36 (m, 1H, Ar-H);^{13}C NMR (100 MHz, $CDCl_3$): δ 35.4, 36.5, 49.2, 61.4, 68.9, 112.2, 112.4, 113.0, 113.1, 113.6, 123.1, 124.9, 125.1, 127.0, 127.3, 128.5, 128.8, 129.6, 131.7, 132.2, 133.9, 134.7, 134.8, 140.6, 141.3, 142.6, 142.7, 157.0, 182.4; HRMS Calcd for $C_{35}H_{29}FN_4O_4$ $[M]^+$ 588.2173 found 588.2180.

(Z)-5-bromo-1-((1-(4-(4-(2-hydroxyethoxy)phenyl)-3,4-diphenylbut-3-en-1-yl)-1H-1,2,3-triazol-4-yl)methyl)indoline-2,3-dione (14d): Yield 77%; light orange solid; m.p. 91-92 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.97 (t, *J*=7.2 Hz, 2H, -CH_2-), 3.86 (t, *J*=4.2 Hz, 2H, -O-CH_2-), 3.95 (t, *J*=4.8 Hz, 2H, -CH_2-O-), 4.26 (t, *J*=7.4 Hz, 2H, -CH_2-N-),4.94 (s, 2H, -CH_2-N-), 6.55 (d, *J*=8.6 Hz, 2H, Ar-H), 6.67 (d, *J*=8.8 Hz, 2H, Ar-H), 6.86-6.88 (m, 2H, Ar-H), 7.05-7.07 (m, 3H, Ar-H), 7.10-7.18 (m, 4H,Ar-H), 7.23-7.24 (m, 1H, Ar-H), 7.28 (s, 1H, Ar-H), 7.32-7.34 (m, 1H, Ar-H), 7.51-7.54 (m, 2H, triazole-H+Ar-H);^{13}C NMR (100 MHz, $CDCl_3$): δ 35.4, 36.5, 49.2, 61.4, 68.9, 113.1, 113.8, 123.1, 125.4, 125.8, 127.0, 127.6, 127.8, 128.5, 128.9, 129.6, 130.0, 130.5, 131.7, 133.9, 134.7, 138.0, 140.6, 141.2, 142.5, 142.9, 148.5, 157.3, 182.5; HRMS Calcd for $C_{35}H_{29}BrN_4O_4$[M+2] 650.1352 found 650.1343.

(Z)-5,7-dibromo-1-((1-(4-(4-(2-hydroxyethoxy)phenyl)-3,4-diphenylbut-3-en-1-yl)-1H-1,2,3-triazol-4-yl)methyl)indoline-2,3-dione (14e): Yield 74%; light orange solid; m.p. 106-

107 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.01 (t, *J*=6.7 Hz, 2H, -CH_2-), 3.73-3.79 (m, 2H, -O-CH_2-), 3.87-3.91 (m, 2H, -O-CH_2-), 3.97 (t, J = 3.5 Hz, 2H, -O-CH_2-), 4.25 (t, *J*=6.6 Hz, 2H, -CH_2-N-), 5.46 (s, 2H, -CH_2-N-), 6.57 (d, *J*=8.1 Hz, 2H, Ar-H), 6.73 (d, *J*=8.0 Hz, 2H,Ar-H), 6.84 (d, *J*=7.3 Hz, 2H, Ar-H), 7.06-7.09 (m, 2H, Ar-H), 7.14-7.21 (m, 3H, Ar-H), 7.26-7.31 (m, 4H,Ar-H), 7.49 (s, 1H, Ar-H), 7.58 (s, 1H, triazole-H);^{13}C NMR (125 MHz, $CDCl_3$): δ 35.3, 36.5, 48.9, 61.0, 68.9, 101.3, 112.7, 113.0, 113.5, 123.0, 125.1, 126.9, 127.2, 127.8, 128.5, 128.8, 129.5, 129.9, 130.0, 131.6, 133.8, 134.6, 137.9, 140.5, 142.5, 142.8, 148.4, 157.3, 182.4; HRMS Calcd for $C_{35}H_{28}Br_2N_4O_4$ [M+2] 730.0457 found 730.0476.

(Z)-1-((1-(4-(4-(2-hydroxyethoxy)phenyl)-3,4-diphenylbut-3-en-1-yl)-1H-1,2,3-triazol-4-yl)methyl)spiro[indoline-3,2'-[1,3]dioxolan]-2-one (15a): Yield 81%; light white solid; m.p. 102-103 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.97 (t, *J*=7.2 Hz, 2H, -CH_2-), 3.85 (t, *J*=4.2 Hz, 2H, -O-CH_2-), 3.91 (t, *J*=4.5 Hz, 2H, -CH_2-O-), 4.22 (t, *J*=7.2 Hz, 2H, -CH_2-N-), 4.28-4.31 (m, 2H, -O-CH_2-), 4.52-4.55 (m, 2H, -O-CH_2-), 4.85 (s, 2H, -CH_2-N-), 6.55 (d, *J*=8.8 Hz, 2H, Ar-H), 6.67 (d, *J*=8.8 Hz, 2H, Ar-H), 6.82-6.85 (m, 2H, Ar-H), 7.01-7.07 (m, 3H, Ar-H), 7.11-7.19 (m, 6H,Ar-H), 7.22-7.23 (m, 2H, Ar-H), 7.40-7.43 (m, 2H, triazole-H+2ArH);^{13}C NMR (100 MHz, $CDCl_3$): δ 35.1, 36.4, 49.0, 61.4, 66.1, 68.9, 101.2, 111.3, 113.4, 116.5, 122.2, 125.3, 126.4, 127.5, 128.2, 128.6, 128.8, 129.2, 129.6, 131.8, 134.1, 134.5, 134.9, 140.2, 141.9, 142.3, 142.5, 142.6, 157.0, 172.3; HRMS Calcd for $C_{37}H_{34}N_4O_5$ $[M]^+$ 614.2523 found 614.2515.

(Z)-5-chloro-1-((1-(4-(4-(2-hydroxyethoxy)phenyl)-3,4-diphenylbut-3-en-1-yl)-1H-1,2,3-triazol-4-yl)methyl)spiro[indoline-3,2'-[1,3]dioxolan]-2-one (15b): Yield 76%; light white solid; m.p. 98-99 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.97 (t, *J*=7.1 Hz, 2H, -CH_2-), 3.86 (t, *J*=4.0 Hz, 2H, -O-CH_2-), 3.93 (t, *J*=4.4 Hz, 2H, -CH_2-O-), 4.21 (t, *J*=7.2 Hz, 2H, -CH_2-N-), 4.27-4.30 (m, 2H, -O-CH_2-), 4.51-4.55 (m, 2H, -O-CH_2-), 4.87 (s, 2H, -CH_2-N-), 6.55 (d, *J*=8.7 Hz, 2H, Ar-H), 6.68 (d, *J*=8.8 Hz, 2H, Ar-H), 6.82-6.85 (m, 2H, Ar-H), 7.02-7.08 (m, 3H, Ar-H), 7.11-7.19 (m, 5H, Ar-H), 7.22-7.24 (m, 2H, Ar-H), 7.40-7.43 (m, 2H, triazole-H+ArH);^{13}C NMR (100 MHz, $CDCl_3$): δ 35.2, 36.3, 49.2, 61.4, 66.3, 68.9, 101.3, 111.5, 113.6, 118.3, 122.3, 125.5, 126.8, 127.7, 128.1, 128.5, 128.6, 128.9, 129.4, 131.5, 134.1, 134.6, 134.9, 140.3, 141.9, 142.3, 142.5, 142.6, 157.1, 172.5; HRMS Calcd for $C_{37}H_{33}ClN_4O_5$ $[M]^+$ 648.2149 found 648.2163.

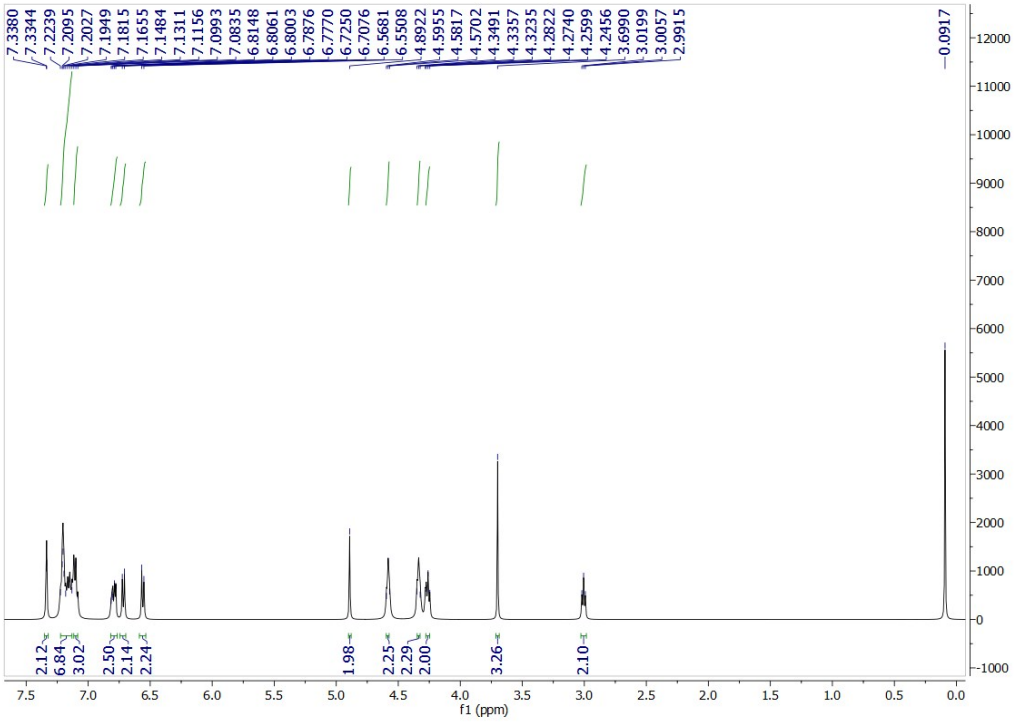
(Z)-5-fluoro-1-((1-(4-(4-(2-hydroxyethoxy)phenyl)-3,4-diphenylbut-3-en-1-yl)-1H-1,2,3-triazol-4-yl)methyl)spiro[indoline-3,2'-[1,3]dioxolan]-2-one (15c): Yield 79%; light white

solid; m.p. 103-104 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.97 (t, *J*=7.1 Hz, 2H, -CH_2-), 3.86 (t, *J*=4.0 Hz, 2H, -O-CH_2-), 3.93 (t, *J*=4.4 Hz, 2H, -CH_2-O-), 4.22 (t, *J*=7.1 Hz, 2H, -CH_2-N-), 4.28-4.31 (m, 2H, -O-CH_2-), 4.54-4.57 (m, 2H, -O-CH_2-), 4.86 (s, 2H, -O-CH_2-N-), 6.53 (d, *J*=8.8 Hz, 2H, Ar-H), 6.68 (d, *J*=8.8 Hz, 2H, Ar-H), 6.82-6.84 (m, 2H, Ar-H), 6.97-7.02 (m, 2H, Ar-H), 7.05-7.13 (m, 6H, 5Ar-H +-CH_2-OH Exchangeable with D_2O), 7.13-7.19 (m, 3H, Ar-H), 7.22-7.23 (m, 2H, triazole-H+ArH);^{13}C NMR (100 MHz, $CDCl_3$): δ 35.3, 36.4, 49.0, 61.4, 68.9, 102.0, 111.1, 112.7, 113.0, 113.5, 118.0, 118.2, 122.7, 125.5, 126.9, 127.2, 128.6, 128.9, 129.6, 131.8, 134.1, 134.9, 139.2, 140.5, 142.0, 142.5, 142.6, 156.9, 172.9; HRMS Calcd for $C_{37}H_{33}FN_4O_5$ $[M]^+$ 632.2435 found 632.2445.

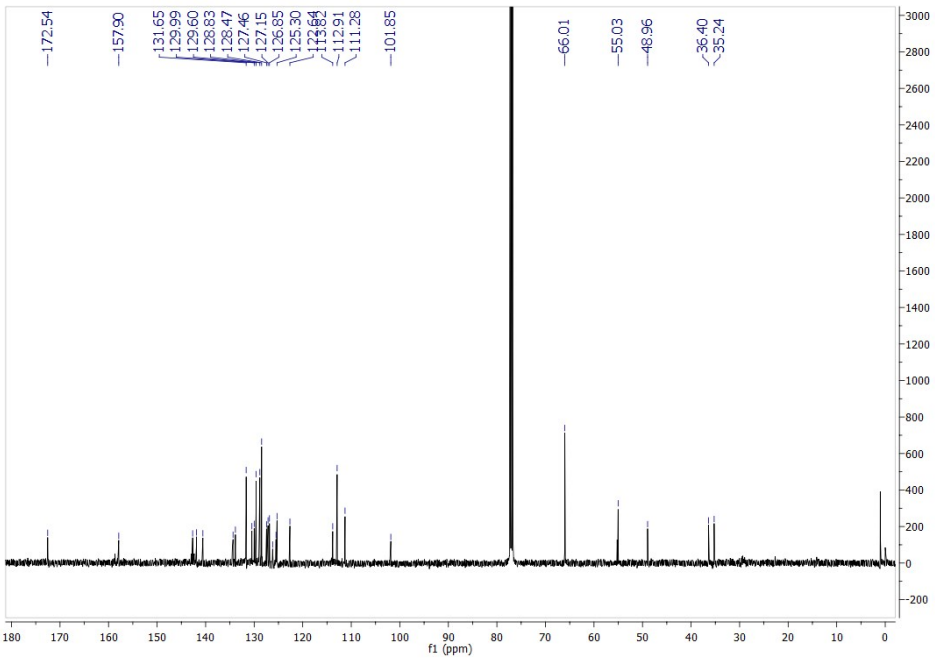
(Z)-5-bromo-1-((1-(4-(4-(2-hydroxyethoxy)phenyl)-3,4-diphenylbut-3-en-1-yl)-1H-1,2,3-triazol-4-yl)methyl)spiro[indoline-3,2'-[1,3]dioxolan]-2-one (15d):

Yield 83%; light white solid; m.p. 104-105 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.97 (t, *J*=7.1 Hz, 2H, -CH_2-), 3.86 (t, *J*=4.0 Hz, 2H, -O-CH_2-), 3.93 (t, *J*=4.6 Hz, 2H, -CH_2-O-), 4.22 (t, *J*=7.1 Hz, 2H, -CH_2-N-), 4.28-4.31 (m, 2H, -O-CH_2-), 4.52-4.56 (m, 2H, -O-CH_2-), 4.85 (s, 2H, -CH_2-N-), 6.54 (d, *J*=8.8 Hz, 2H, Ar-H), 6.68 (d, *J*=8.8 Hz, 2H, Ar-H), 6.82-6.85 (m, 2H, Ar-H), 7.01-7.07 (m, 3H, Ar-H), 7.11-7.19 (m, 5H,Ar-H), 7.22-7.23 (m, 2H, Ar-H), 7.40-7.43 (m, 2H, triazole-H+ArH);^{13}C NMR (100 MHz, $CDCl_3$): δ 35.2, 36.4, 49.0, 61.4, 66.1, 68.9, 101.8, 111.8, 113.6, 116.2, 122.7, 125.8, 126.9, 127.2, 128.1, 128.5, 128.6, 128.9, 129.6, 131.8, 134.1, 134.6, 134.9, 140.5, 141.9, 142.3, 142.5, 142.6, 157.0, 172.5; HRMS Calcd for $C_{37}H_{33}BrN_4O_5$ [M+2] 694.1614 found 694.1601.

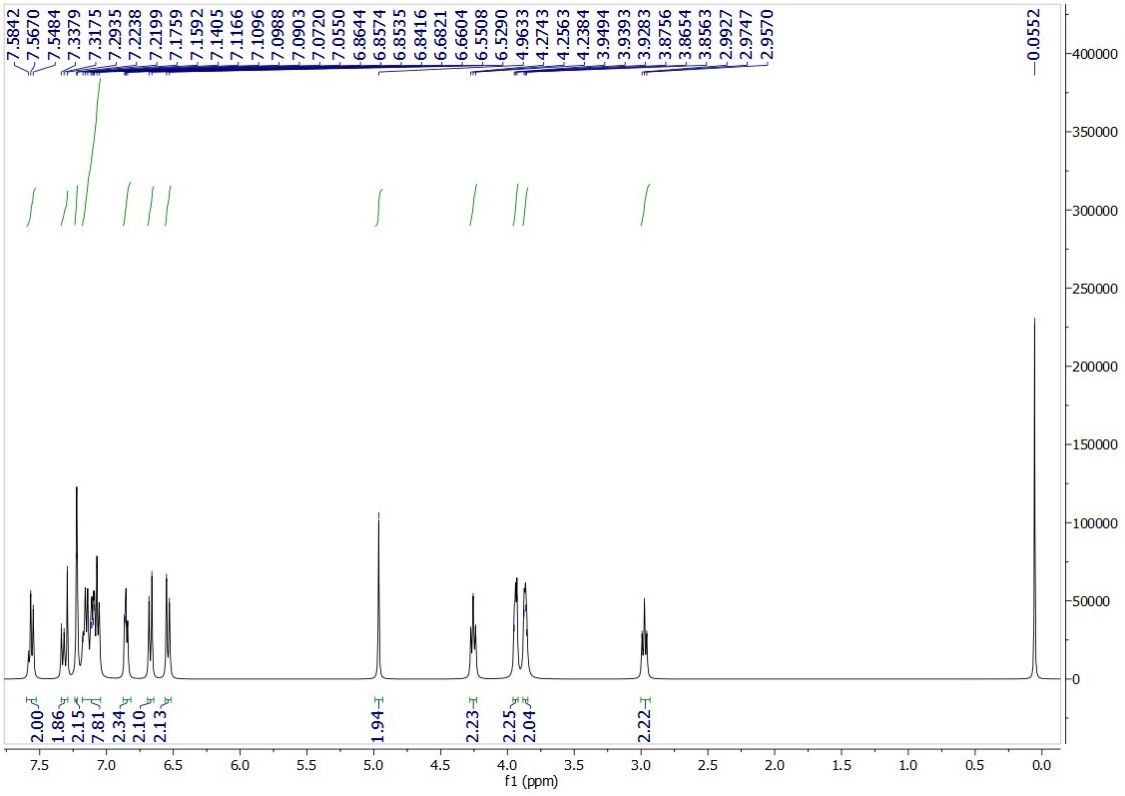
^{1}H-NMR of (Z)-5-chloro-1-((1-(4-(4-methoxyphenyl)-3,4-diphenylbut-3-en-1-yl)-1H-1,2,3-triazol-4-yl)methyl)spiro[indoline-3,2'-[1,3]dioxolan]-2-one (13a)



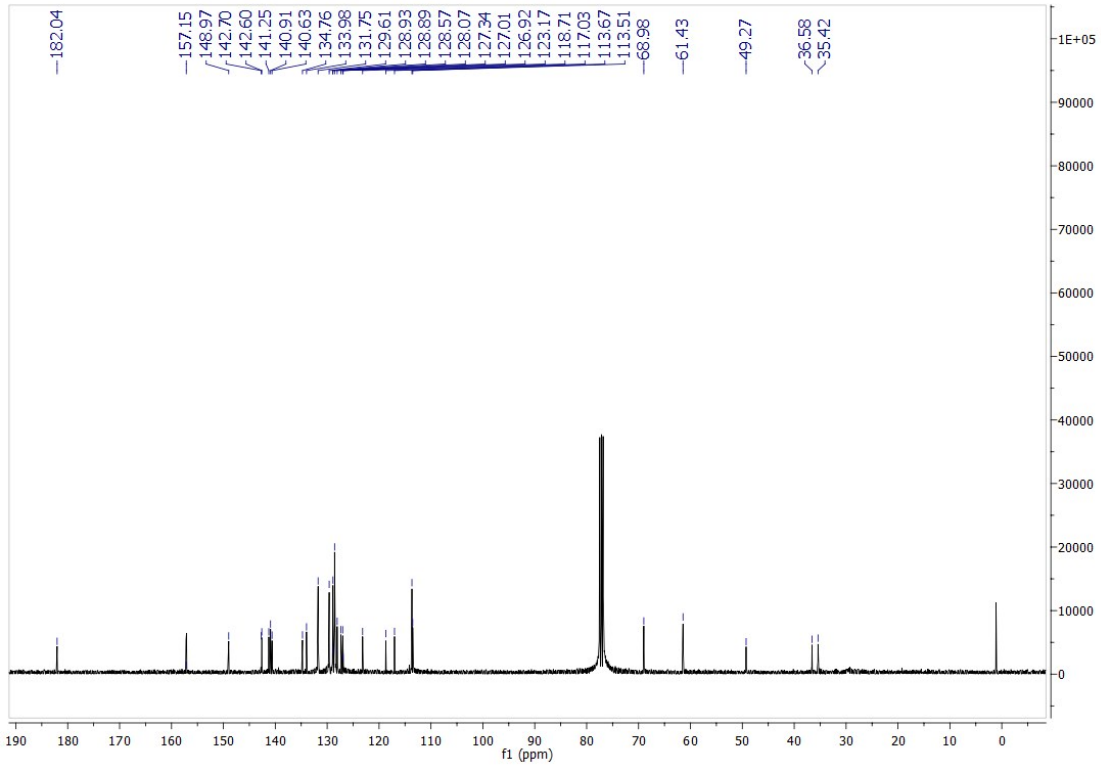
^{13}C-NMR of (Z)-5-chloro-1-((1-(4-(4-methoxyphenyl)-3,4-diphenylbut-3-en-1-yl)-1H-1,2,3-triazol-4-yl)methyl)spiro[indoline-3,2'-[1,3]dioxolan]-2-one (13a)



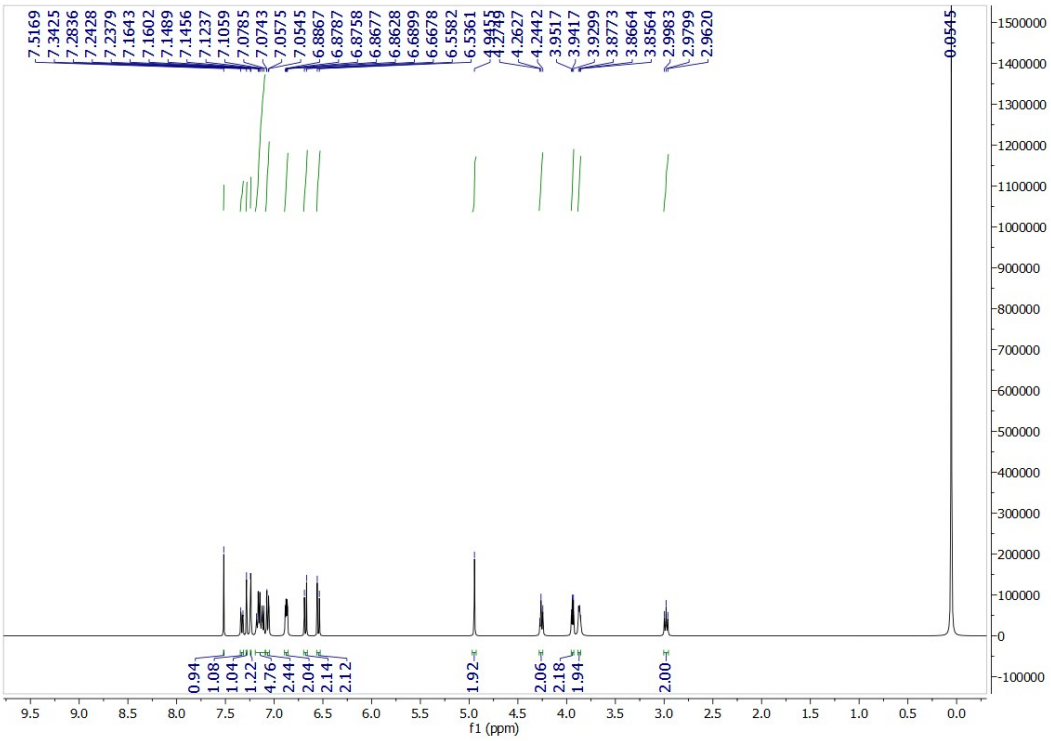
^{1}H NMR of(Z)-1-((1-(4-(4-(2-hydroxyethoxy)phenyl)-3,4-diphenylbut-3-en-1-yl)-1H-1,2,3-triazol-4-yl)methyl)indoline-2,3-dione (14a)



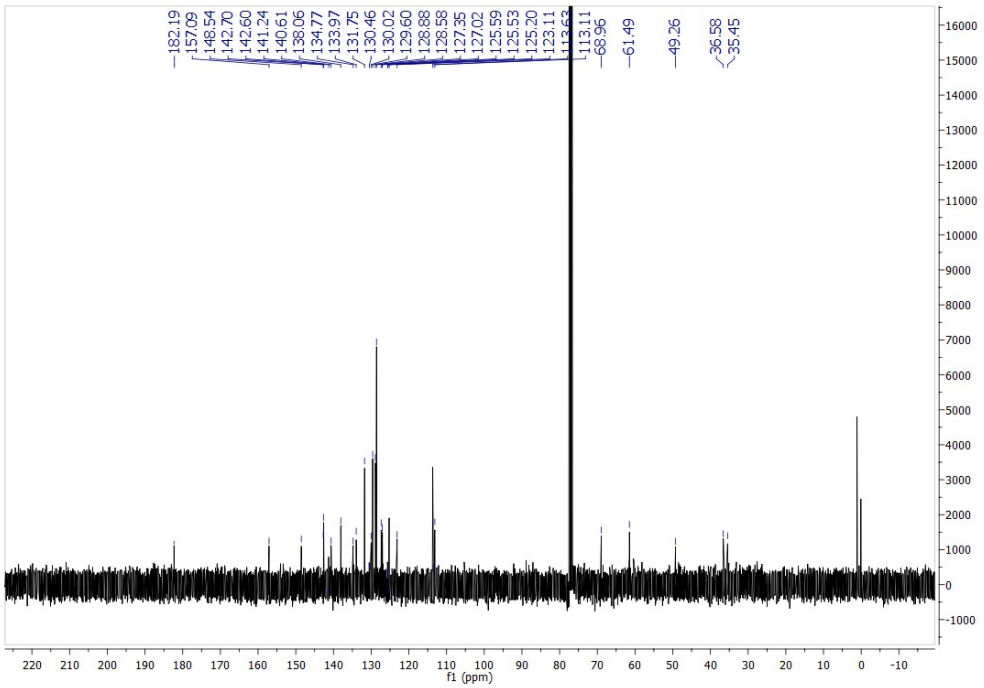
^{13}C NMR of(Z)-1-((1-(4-(4-(2-hydroxyethoxy)phenyl)-3,4-diphenylbut-3-en-1-yl)-1H-1,2,3-triazol-4-yl)methyl)indoline-2,3-dione (14a)



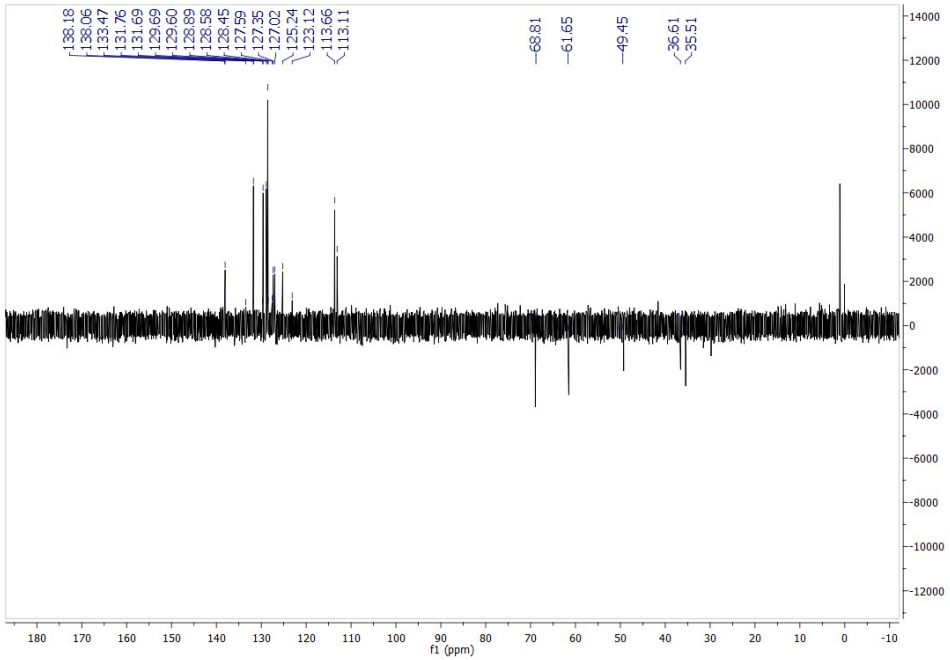
^{1}H NMR of (Z)-5-chloro-1-((1-(4-(4-(2-hydroxyethoxy)phenyl)-3,4-diphenylbut-3-en-1-yl)-1H-1,2,3-triazol-4-yl)methyl)indoline-2,3-dione (14b)



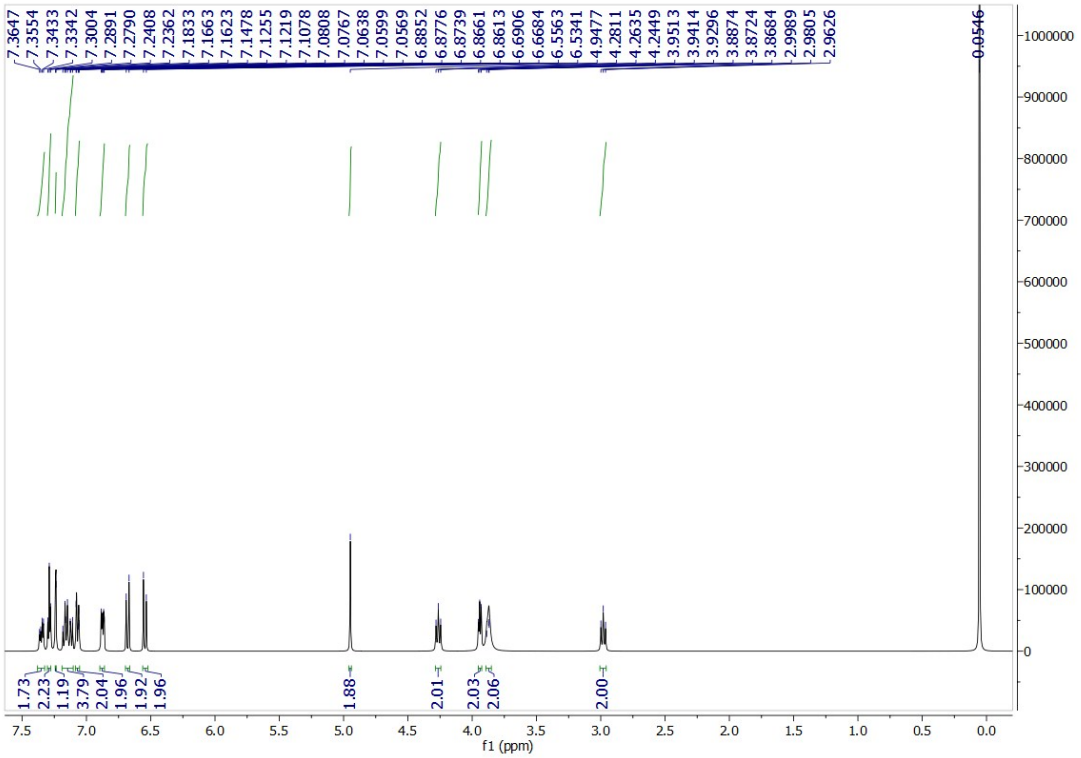
^{13}C NMR of (Z)-5-chloro-1-((1-(4-(4-(2-hydroxyethoxy)phenyl)-3,4-diphenylbut-3-en-1-yl)-1H-1,2,3-triazol-4-yl)methyl)indoline-2,3-dione (14b)



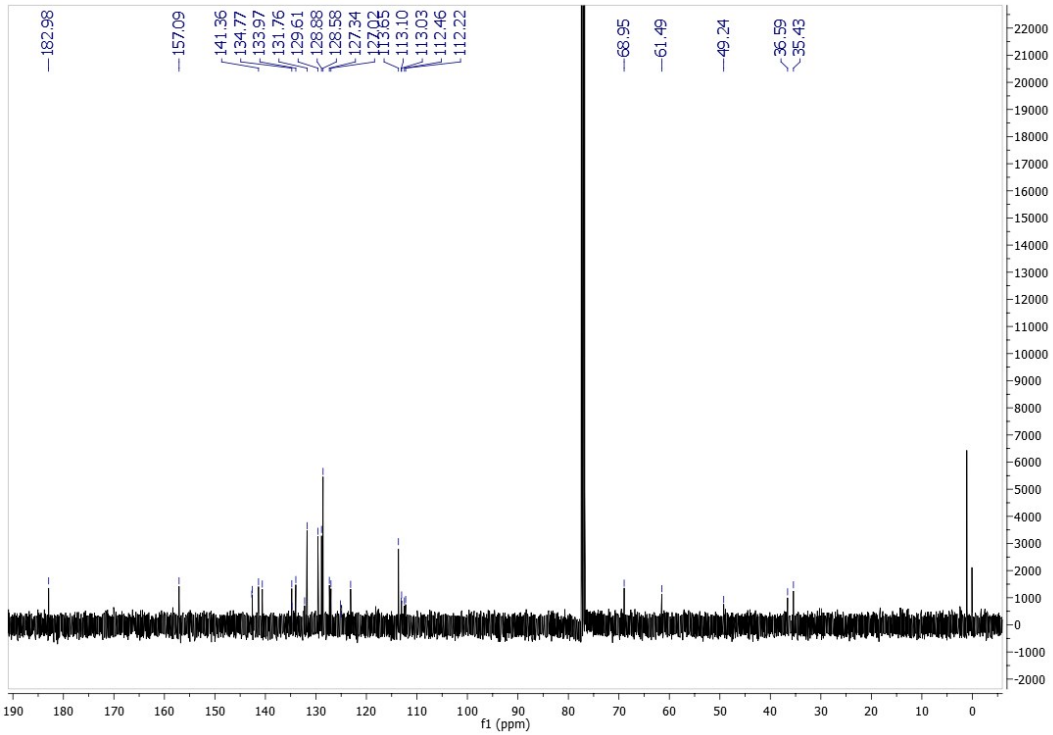
^{13}C DEPT NMR of (Z)-5-chloro-1-((1-(4-(4-(2-hydroxyethoxy)phenyl)-3,4-diphenylbut-3-en-1-yl)-1H-1,2,3-triazol-4-yl)methyl)indoline-2,3-dione (14b)



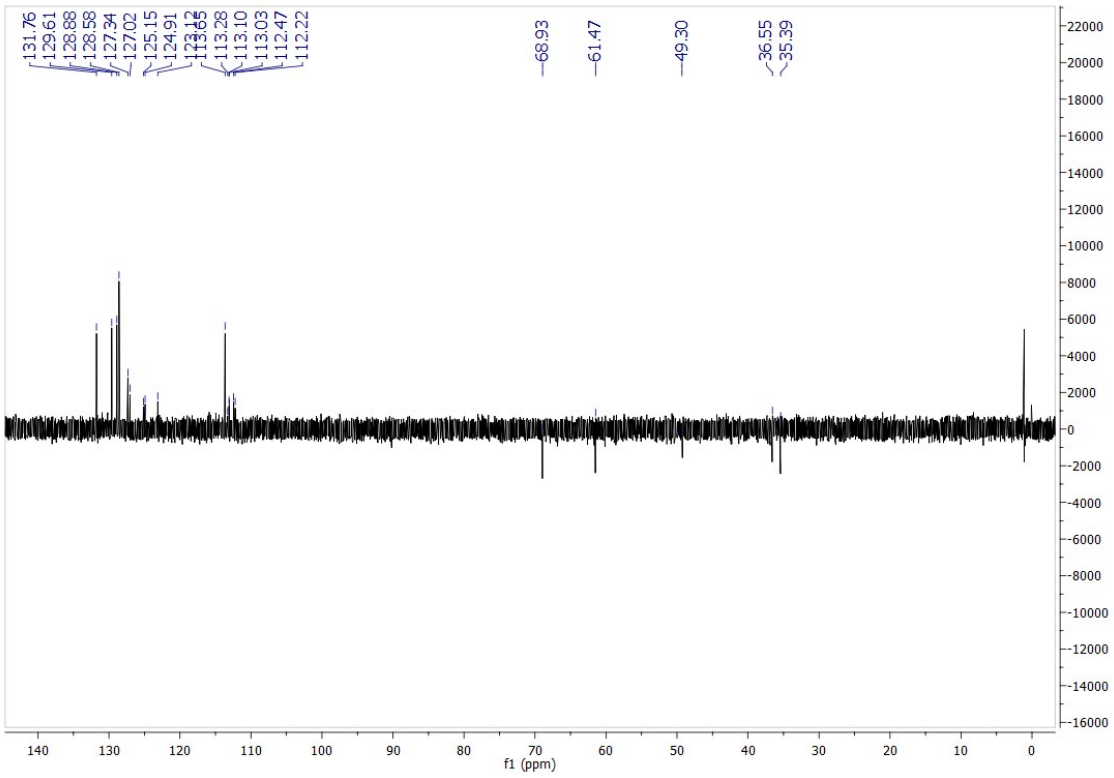
^{1}H NMR (400 MHz) of(Z)-5-fluoro-1-((1-(4-(4-(2-hydroxyethoxy)phenyl)-3,4-diphenylbut-3-en-1-yl)-1H-1,2,3-triazol-4-yl)methyl)indoline-2,3-dione (14c)



^{13}C NMR (100 MHz) of(Z)-5-fluoro-1-((1-(4-(4-(2-hydroxyethoxy)phenyl)-3,4-diphenylbut-3-en-1-yl)-1H-1,2,3-triazol-4-yl)methyl)indoline-2,3-dione (14c)

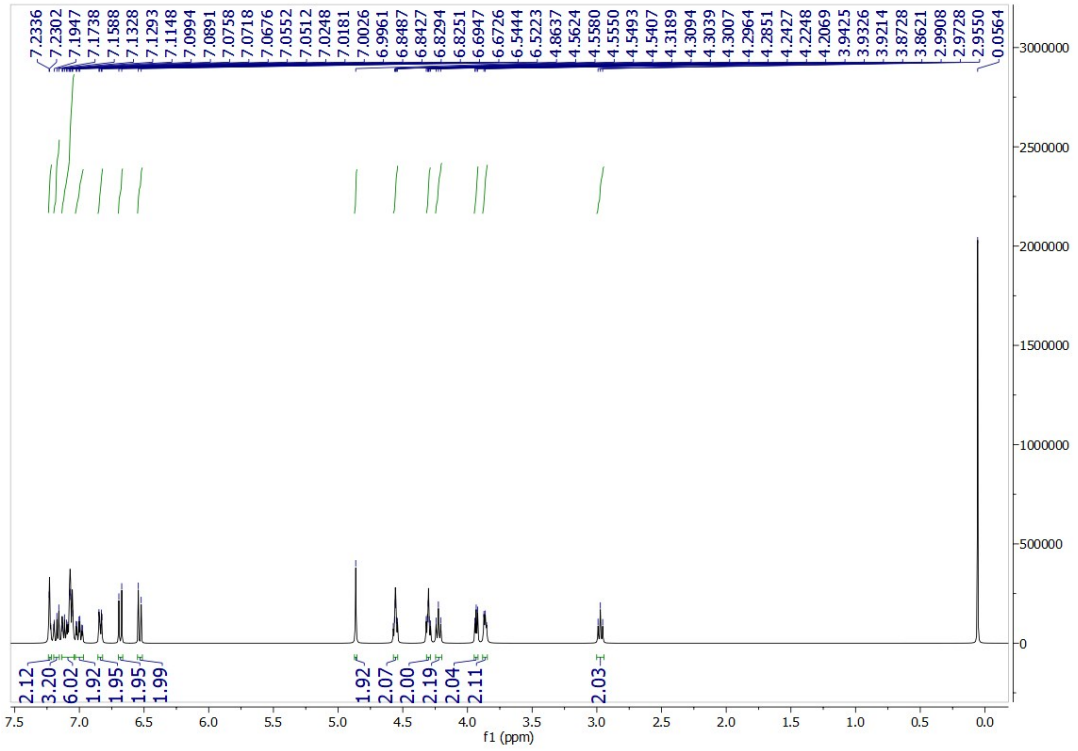


^{13}C DEPT NMR of (100 MHz) (Z)-5-fluoro-1-((1-(4-(4-(2-hydroxyethoxy)phenyl)-3,4-diphenylbut-3-en-1-yl)-1H-1,2,3-triazol-4-yl)methyl)indoline-2,3-dione (14c)

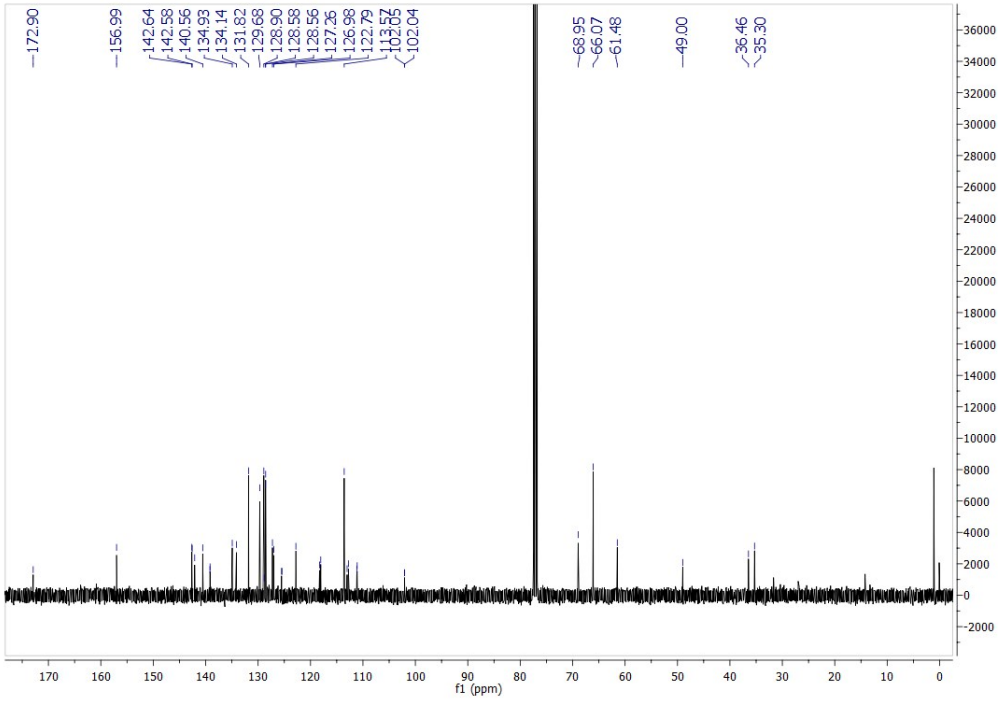


^{1}H NMR of (Z)-1-((1-(4-(4-(2-hydroxyethoxy)phenyl)-3,4-diphenylbut-3-en-1-yl)-1H-1,2,3-triazol-4-yl)methyl)spiro[indoline-3,2'-[1,3]dioxolan]-2-one (15a)

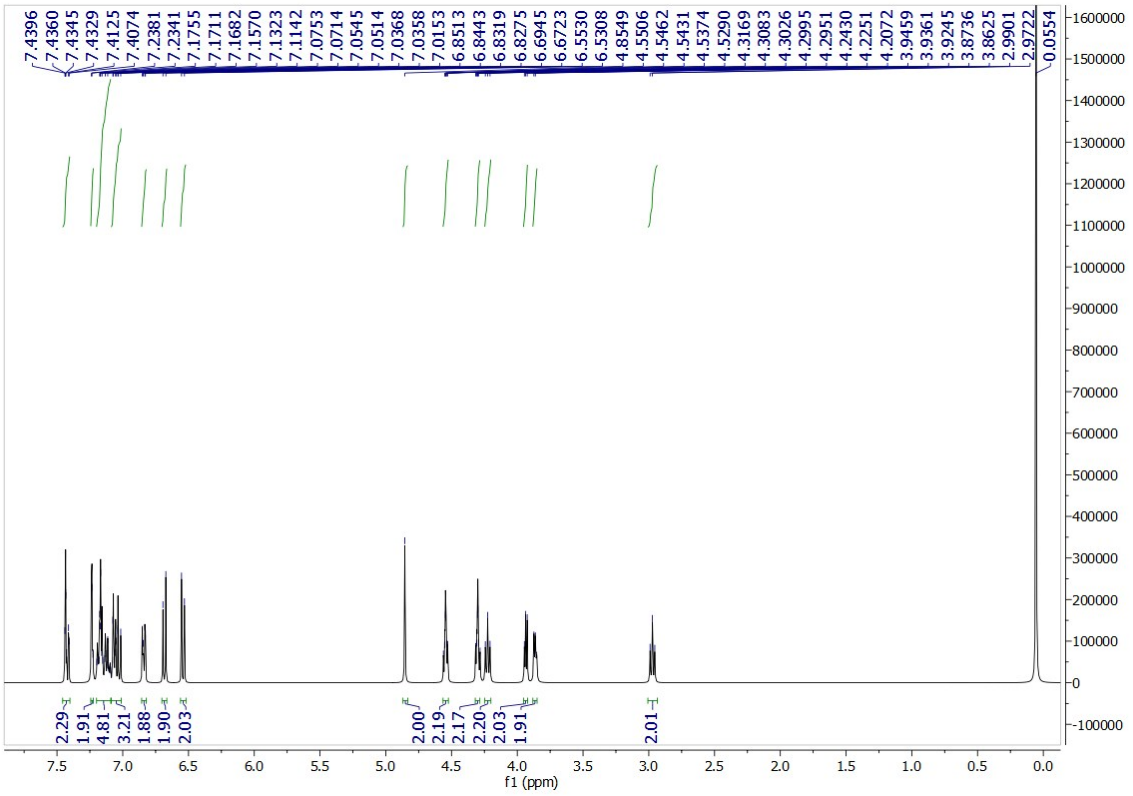
^{1}H NMR of(Z)-5-fluoro-1-((1-(4-(4-(2-hydroxyethoxy)phenyl)-3,4-diphenylbut-3-en-1-yl)-1H-1,2,3-triazol-4-yl)methyl)spiro[indoline-3,2'-[1,3]dioxolan]-2-one (15c)



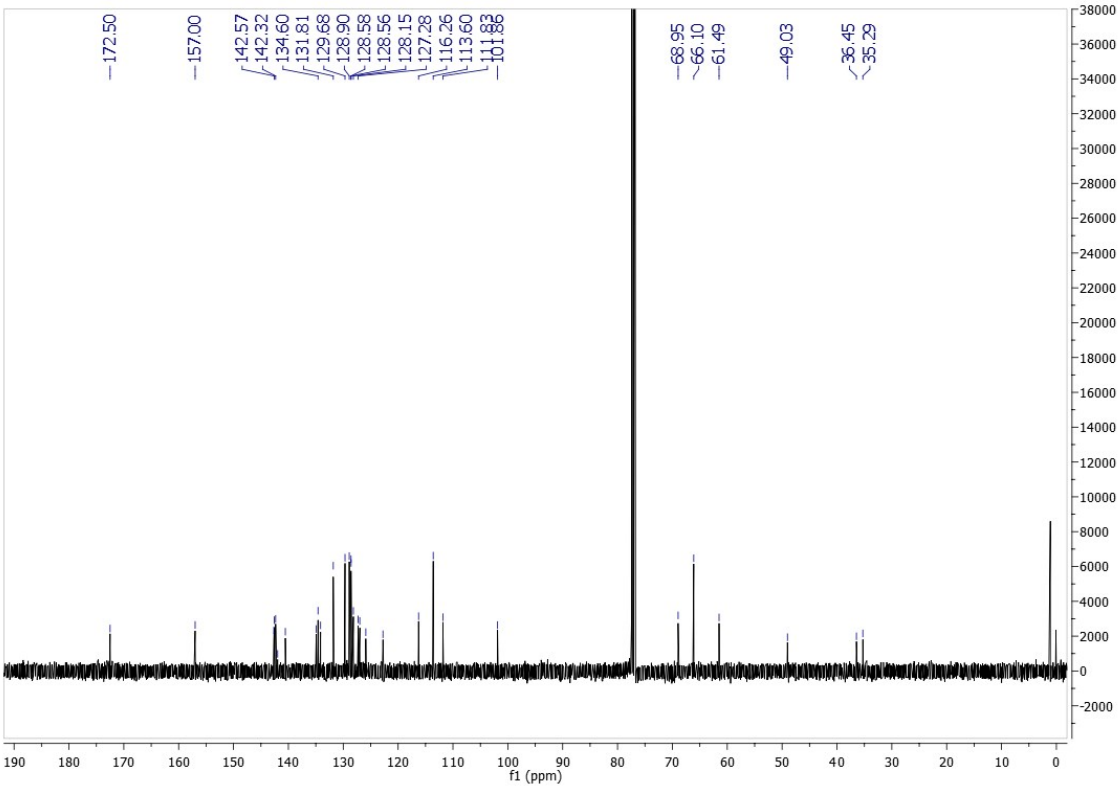
^{13}C NMR of(Z)-5-fluoro-1-((1-(4-(4-(2-hydroxyethoxy)phenyl)-3,4-diphenylbut-3-en-1-yl)-1H-1,2,3-triazol-4-yl)methyl)spiro[indoline-3,2'-[1,3]dioxolan]-2-one (15c)



^{1}H NMR of(Z)-5-bromo-1-((1-(4-(4-(2-hydroxyethoxy)phenyl)-3,4-diphenylbut-3-en-1-yl)-1H-1,2,3-triazol-4-yl)methyl)spiro[indoline-3,2'-[1,3]dioxolan]-2-one (15d)



^{13}C NMR of(Z)-5-bromo-1-((1-(4-(4-(2-hydroxyethoxy)phenyl)-3,4-diphenylbut-3-en-1-yl)-1H-1,2,3-triazol-4-yl)methyl)spiro[indoline-3,2'-[1,3]dioxolan]-2-one (15d)



Materials and Methods:

The cytotoxicity of conjugates *viz.***12a-c, 13a-c, 14a-e, and 15a-d** were tested on two different breast cancer cell lines, namely; estrogen receptor positive (ER+) MCF-7 cells and triple negative MDA-MB-231 (ER-) using MTT (3-(4,5-Dimethylthiazol-z-yl)-2,5-diphenyltetrazolium bromide) assay.[1]

Cell Culturing:

MCF-7 cells were cultured in complete Dulbecco's media Eagle's medium (DMEM) media with 5% FBS and 1% penicillin-streptomycin and MDA-MB-231 cells were cultured in 3:1 DMEM and Ham's F12 with 10% FBS and 1% penicillin-streptomycin, both cell-lines were incubated at 37°C and 5% carbon dioxide.

Biological testing

Cells were seeded in 96-well plates at a density of 5000 cells per well in triplicate in media as described above. After 24 hours, the test compounds (dissolved in DMSO), were diluted in DMEM and added to each well. Cells were treated with a range of different concentrations of drug (1, 5, 10, 20, 50, 100µM) and incubated for 24-hours. This was followed by 24-hour incubation. A further incubation of 2 hours was performed with the addition of sterile 5 mg/mL, MTT (Sigma-Aldrich) dissolved in PBS to each well containing cells and test compound. A solubilisation solution (10% SDS, 10mM HCl) of equal volume to the wells was then added to each well, and incubated with cells for 16 hours at 37 °C. The optical density of each well was determined at a wavelength of 570 nm using a microtiter plate reader (TecanSunrise^{TM}Microplate Reader, Magellan software).

Statistical analysis

The statistical analyses were performed using Excel® and IC_{50}values were estimated using Graphpad Prism5 software (Hearne Scientific Software). The experiments were performed in duplicate and the statistical significance was calculated using the student's t-test. A p-value of less than 0.05 was used to estimate the significance of the observations. A Z-factor was calculated for each 96-well plate and assays having Z-factors above 0.5 were included in the statistical analysis.[2]

Molecular Docking Analysis

The x-ray crystal structure of the alpha-ER (PDB ID: 3ERT) was obtained from the RCSB Protein Data Bank (http://www.rcsb.org/pdb/home/home.do). Using the UCSF Chimera package the ligand and receptor complex was prepared for docking. [3] Chem3D Ultra was used to construct a three-dimensional (3D) structure of the test ligands. The 3D structures of the ligands were further optimized using the force field, MMFF94s in the Avogadro package (V 1.2.0). AutoDock Tools (V 1.5.6) was used to establish grid box parameters. All non-polar hydrogens were merged, Gasteiger partial atomic charges added, and rotatable bonds assigned. The grid box of the receptor had a grid spacing of 0.375 Å, the size of the box and grid points are given in **Table S1**.AutodockVina was used to perform the docking of ligands into the receptors, in triplicate.[4]The docking systems were validated by docking the native ligand of the protein crystal structure into the respective binding site. The Protein-Ligand Interaction Profiler (PLIP) was used to analyse the non-polar interactions between the protein and ligand in complex.[5]

References:

1. S. Sagar, L. Esau, B.Moosa, N. M.Khashab, V. B.Bajic, M. Kaur.*Anti-Cancer Agents in Med. Chem.*2014, **14**, 170.
2. J. H. Zhang, T. D. Chung, K. R. Oldenburg. *J. Biomol. Screen*1999, **4**, 67.
3. E. F. Pettersen,T. D. Goddard,C. C. Huang,G. S.Couch,D. M.Greenblatt,E. C.Meng,T. E. Ferrin. *J. Comput. Chem.*2004, **25**, 1605.
4. O. Trott, A. J. Olson, *J. Comput. Chem.* 2010, **31**, 455.
5. S. Salentin, S. Schreiber, V. J.Haupt,M. F. Adasme,M. Schroeder.*Nucl. Acids Res.* 2015, **43**, 443.

Figure S1: Representative graph of the percentage cell death of MCF7 and MDA-MB-231 cells at selected concentrations of test compounds **14a, 14b, 14c** and **14e**. 40 μM Plumbagin was used as a positive control. Data are mean ± standard deviation S.D. (n=3), where *p ≤ 0.05, **p ≤ 0.01 and ***p ≤ 0.001 significant difference

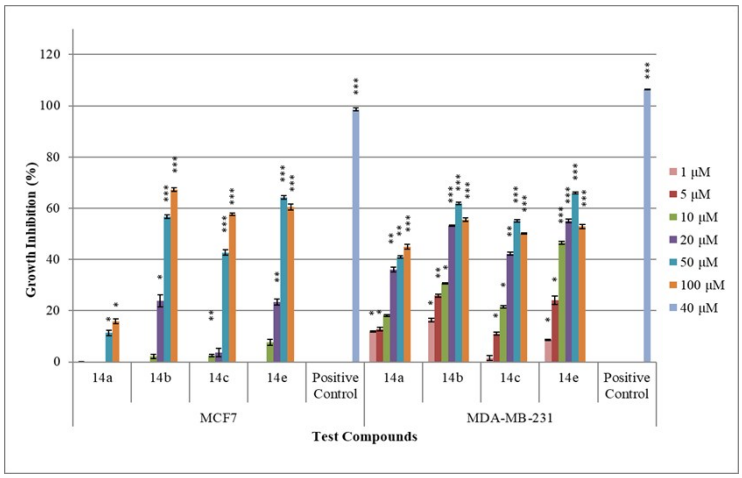


Figure S2: Representative graph of the percentage cell death of MCF7 and MDA-MB-231 cells at selected concentrations of test compounds **14d, 15a, 15b** and **15c**. 40 µM Plumbagin was used as a positive control. Data are mean ± standard deviation S.D. (n=3), where *p ≤ 0.05, **p ≤ 0.01 and ***p ≤ 0.001 significant difference to untreated control

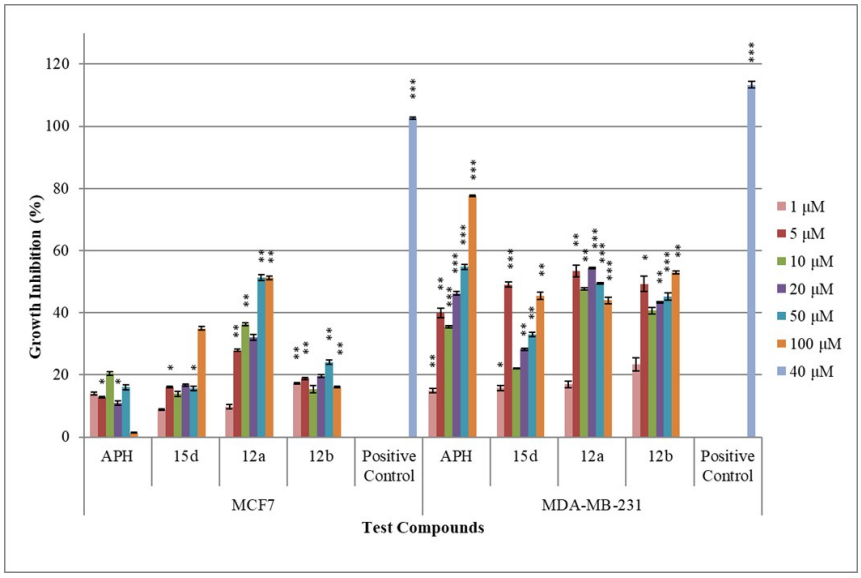


Figure S3: Representative graph of the percentage cell death of MCF7 and MDA-MB-231 cells at selected concentrations of test compounds APH, **15d, 12a** and **12b**. 40 µM Plumbagin was used as a positive control. Data are mean ± standard deviation S.D. (n=3), where *p ≤ 0.05, **p ≤ 0.01 and ***p ≤ 0.001 significant difference to untreated control

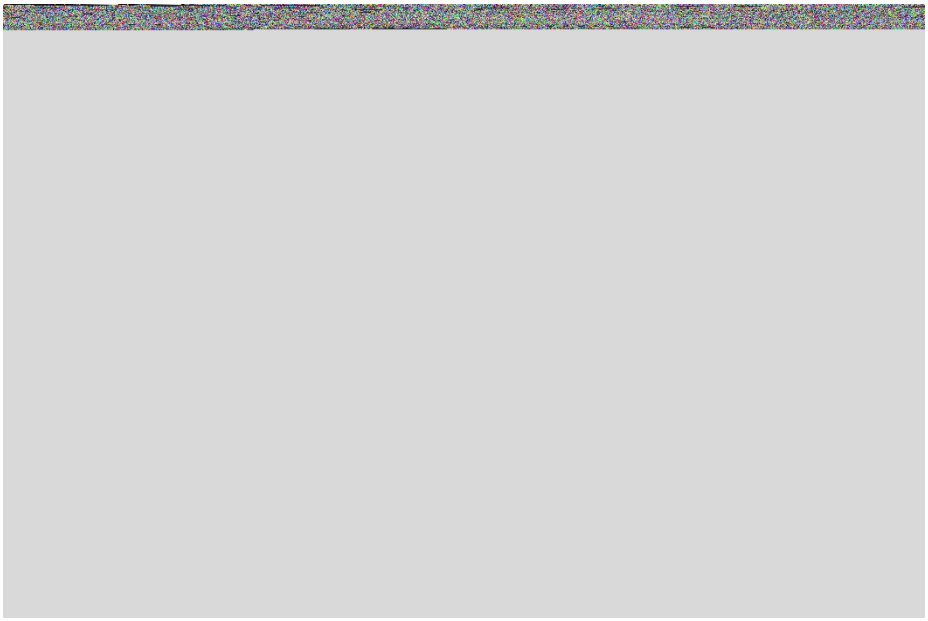


Figure S4: Representative graph of the percentage cell death of MCF7 and MDA-MB-231 cells at selected concentrations of test compounds **12c, 13a, 13b** and **13c**. 40 µM Plumbagin was used as a positive control. Data are mean ± standard deviation S.D. (n=3), where *p ≤ 0.05, **p ≤ 0.01 and ***p ≤ 0.001 significant difference to untreated control.

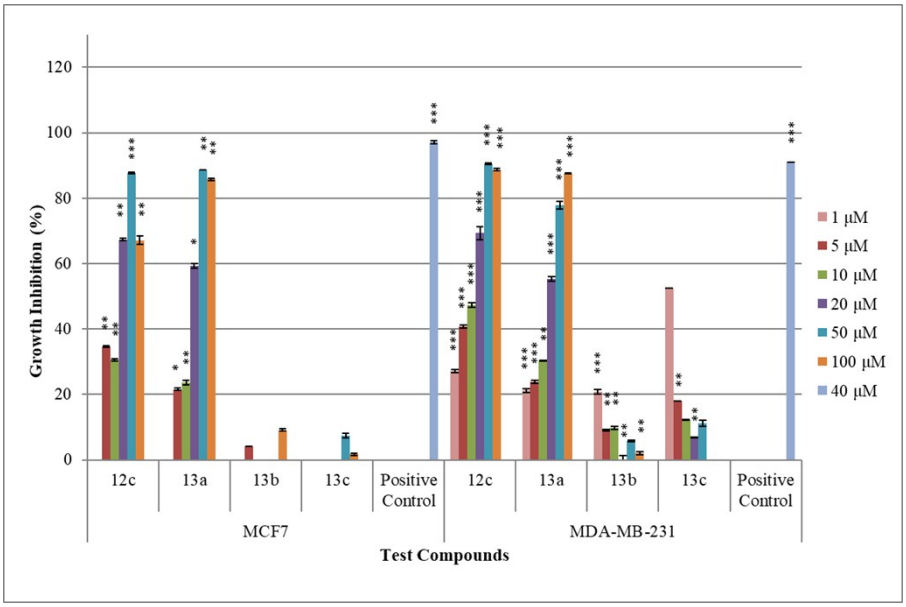
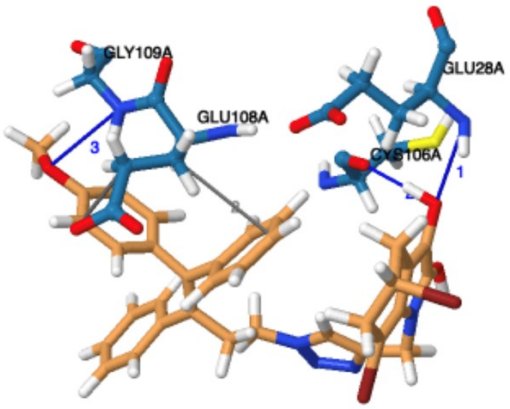
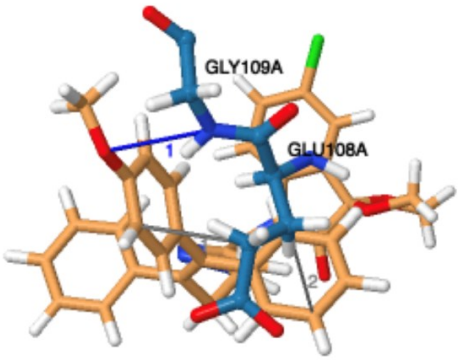


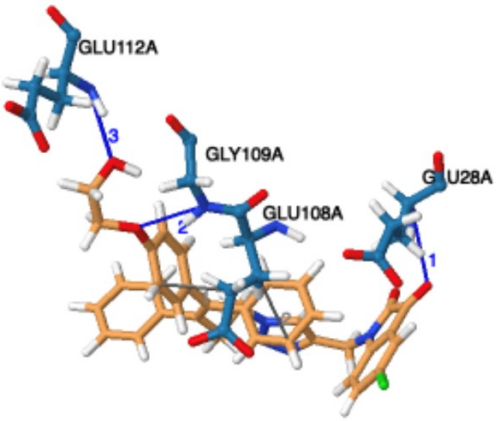
Figure S5: Three-dimensional illustration of docked ligands in the active site of estrogen receptor beta (PDB ID: 3OLS)



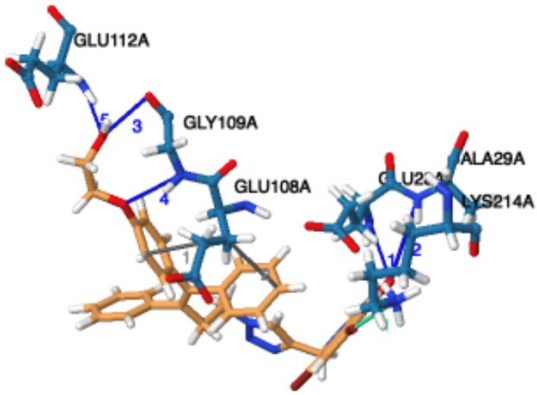
12c



13a

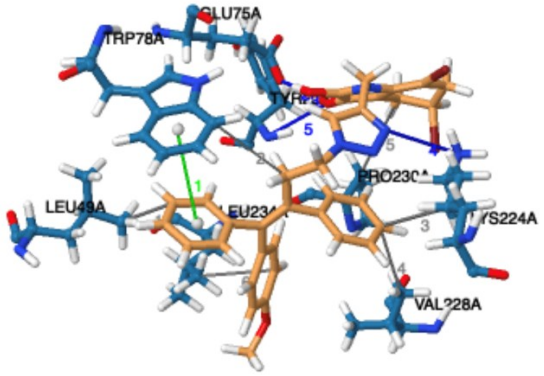


14b

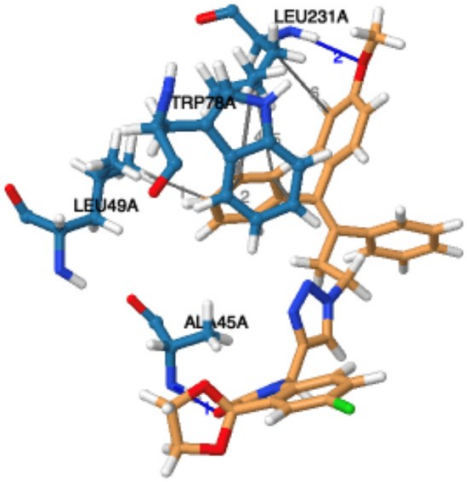


14e

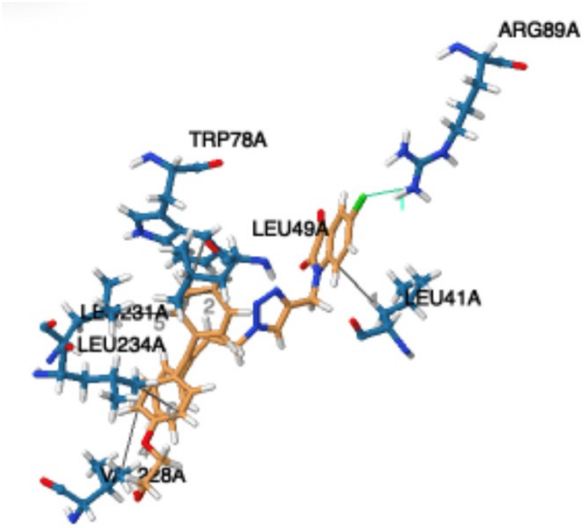
Figure S6: Three-dimensional illustration of docked ligands in the active site of estrogen receptor alpha (PDB ID: 3ERT)



12c



13a



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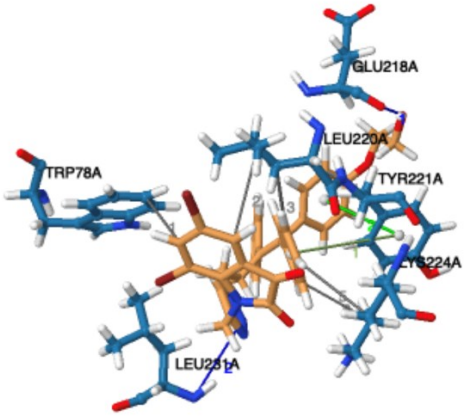

14e

Table S1: Docking of the most active compounds in the estrogen receptor subtypes

Ligand Identifier	Docking score/ (kcal/mol)	RMSD i.b.	RMSD u.b.
3ERT			
12c	-9.0	0.000	0.000
13a	-10.1	1.901	2.954
14b	-10.3	2.586	3.453
14e	-8.5	0.000	0.000
3OLS			
12c	-7.0	1.968	2.639
13a	-7.5	0.000	0.000
14b	-7.2	0.000	0.000
14e	-7.2	0.000	0.000

Table S2: Grid Point Parameters

PDB: 3ERT (ERα)	
Grid center co-ordinates	**Grid size**
X = 30.173 Y = -1.997 Z = 24.207	X = 40 Y = 30 Z = 40
PDB: 3OLS (ERβ)	
Grid center co-ordinates	**Grid size**
X = 25.135 Y = -27.145 Z = -11.468	X = 26 Y = 40 Z = 28