Supporting Information

Hybrid Dual Aromatase-Steroid Sulfatase Inhibitors with Exquisite Picomolar Inhibitory Activity

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1) General Methods for Synthesis.

Unless otherwise stated, HPLC grade solvents were used and commercial reagents and starting materials were used without further purification. Sulfamoyl chloride was prepared by an adaptation of the method of Appel and Berger[1] and was stored at 4 ^{o}C under positive N_2 pressure as a solution in toluene, as described by Woo *et al.*[2] Thin-layer chromatography (TLC) was performed on precoated plates (Merck TLC aluminum sheets silica gel 60 F254, Art. No. 5554). Product(s) and starting materials(s) were detected by either viewing under UV light or treatment with an ethanolic solution of phosphomolybdic acid followed by heating. Flash column chromatography was performed on glass columns packed with silica gel (Sorbsil/Matrex C60) or on FlashMaster II run Argonaut pre-packed columns or on ISCO CombiFlash Rf Automated Flash Chromatography System run RediSep Rf disposable flash columns. Nuclear magnetic resonance spectra were recorded on either a Jeol Delta 270 MHz or Varian Mercury VX 400 MHz spectrometer. ^{1}H NMR

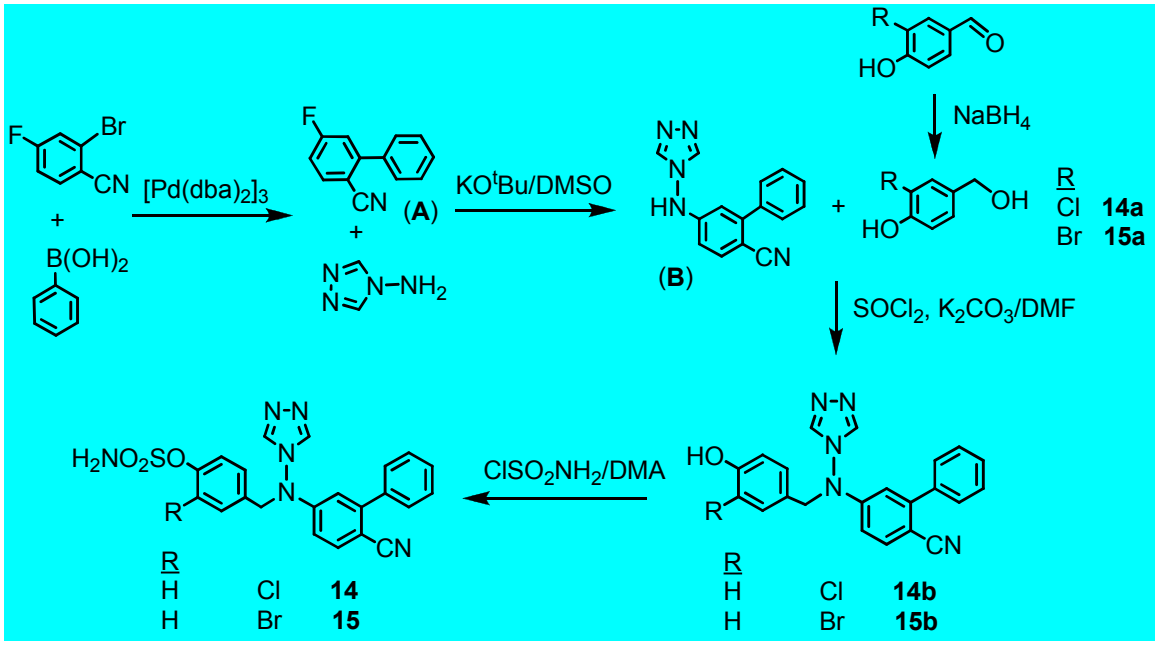
spectra were recorded at 270 MHz or 400 MHz with shifts reported in parts per million (ppm, δ) relative to residual chloroform (δ_H = 7.26 ppm) or residual DMSO (δ_H = 2.50 ppm). Coupling constants, *J*, are reported in hertz. ^{13}C NMR spectra were recorded at 100.6 MHz with the central peak of chloroform (δ_C = 77.16 ppm) or DMSO (δ_C = 39.52 ppm) as internal standard. The following abbreviations are used to describe resonances in ^1H NMR spectra: br, broad; s, singlet; d, doublet; dd, double doublet; q, quartet; m, multiplet; t, triplet. HPLC analyses were performed on a Waters Millenium 32 instrument equipped with a Waters 996 PDA detector. For chromatograhic conditions, refer to the experimental data of individual compound. All biologically tested compounds attained a purity level of 95% or above by HPLC. LC-MS analysis was performed on a Waters 2790 Alliance linked up with a ZQ MicroMass spectrometer and a Waters 996 PDA detector. Atmospheric pressure chemical ionisation (APCI) or electrospray (ES) high resolution mass spectra were recorded on a Bruker micrOTOF Focus. Elemental analyses were performed by the Microanalysis Service, University of Bath. Melting points (mp) were determined using a Stanford Research Systems Optimelt MPA100 automated melting point system and are uncorrected.

References

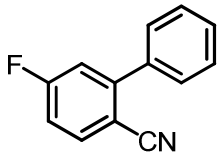
1) Appel, R.; Berger, G. Uber das Hydrazidosulfamid (On hydrazidosulfamide.) Chem. Ber. ***1958,*** *91, 1339-1341.*

2) Woo, L. W. L.; Lightowler, M.; Purohit, A.; Reed, M. J.; Potter, B. V. L. Heteroatom-substituted analogues of the active-site directed inhibitor estra-1,3,5(10)-trien-17-one-3-sulphamate inhibit estrone sulphatase by a different mechanism. J. Steroid Biochem. Mol. Biol. ***1996****, 57, 79-88.*

2) Syntheses of compounds ***14*** *and* ***15.***

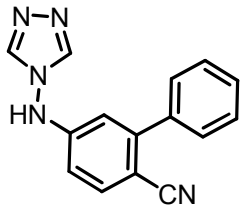


5-Fluoro-biphenyl-2-carbonitrile (A).



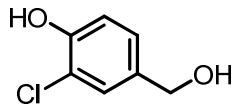
2-Bromo-4-fluorobenzonitrile (5.60 g, 28.0 mmol), phenyl boronic acid (4.27 g, 35.0 mmol) and $Pd_2(dba)_3$ (0.10 g, 0.11 mmol) were added to dimethoxyethane (25 mL) and 2M Na_2CO_3 (40 mL) under N_2-atmosphere. The suspension was heated to reflux for 4 h (TLC-control) before it was cooled to room temperature and filtered (celite). The product was extracted with EtOAc (3 x 50 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by flash chromatography [SiO_2, $CHCl_3$/hexane (1:10) followed by recrystallization from hexane to give **A** (4.29 g, 78%) as fine colorless crystals. Mp. 79-80 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.14 (dt, *J* = 8.6, 2.7 Hz, 1H), 7.22 (dd, *J* = 9.4, 2.7 Hz, 1H), 7.46-7.57 (m, 5H), 7.77 (dd, *J* = 8.6, 5.5 Hz, 1H); LRMS (ES+): *m/z* 197.9 (100%, $[M+H]^+$); HRMS (ES+) calcd for $C_{13}H_9FN$ $[M+H]^+$: 198.0714, found 198.0706; Anal. Calcd for $C_{13}H_8FN$: C, 79.17; H, 4.09; N, 7.10. Found C, 79.3; H, 4.08; N, 7.04. HPLC: Sunfire C_{18} reverse phase column, 4.6 x 150 mm, 3.5µm pore size, isocratic 90% acetonitrile and 10% water at 1.0 mL/min, t_R = 1.90 min, purity >99%.

5-(4*H*-1,2,4-Triazol-4-ylamino)biphenyl-2-carbonitrile (B)



To a solution of 4-amino-4*H*-1,2,4-triazole (3.29 g, 39.14 mmol) in DMSO (40 mL) was added KO^{t}Bu (4.39 g, 39.14 mmol). The mixture was stirred for 0.5 h at room temperature before **A** (3.86 g, 19.57 mmol) was added and stirring was continued for 1 h. The mixture was poured into crushed ice and neutralised with 2M $KHSO_4$-solution. The white precipitate was filtered off, washed with water and recrystallized from 2-propanol to give **B** (3.62 g, 71%) as light yellow crystals. Mp. 181-182 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.51 (d, J = 2.5 Hz, 1H), 6.60 (dd, J = 8.5, 2.5 Hz, 1H), 7.42-7.58 (m, 5H), 7.81 (d, J = 8.5 Hz, 1H), 8.89 (s, 2H), 10.34 (s, 1H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 101.3, 111.4, 112.6, 119.0, 128.4, 128.8, 128.9, 135.7, 137.8, 144.1, 146.4, 150.9; LRMS (ES+): *m/z* 262.0 (100%, $[M+H]^+$); HRMS (ES+) calcd for $C_{15}H_{11}N_5$ $[M+H]^+$: 262.1087, found 262.1080. HPLC: Symmetry C_{18} reverse phase column, 4.6 x 75 mm, 3.5µm pore size, isocratic 90% methanol and 10% water at 1.0 mL/min, t_R = 1.25 min, purity >99%.

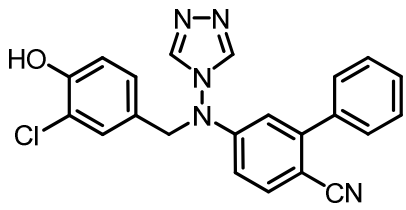
2-Chloro-4-hydroxymethyl-phenol (14a)



To a solution of 3-chloro-4-hydroxybenzaldehyde (4.697 g, 30.0 mmol) and trimethoxyborate (0.5 mL) in THF (20 mL) and EtOH (20 mL) was added $NaBH_4$ (2.0 g, 52.87 mmol) in small portions over a period of 8 h. The mixture was stirred overnight and EtOAc (100 mL) and water (50 mL) were added. The organic layer was separated and washed with 2M $KHSO_4$ (30 mL) and brine

(30 mL), dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by flash chromatography [SiO_2, $CHCl_3$/acetone (3:1)] followed by recrystallization from EtOAc/hexane to give **14a** (3.82 g, 80%) as fine colorless needles: Mp. 126-127 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.36 (d, J = 6.5 Hz, 2H), 5.11 (t, J = 6.5 Hz, 1H), 6.90 (d, J = 8.2 Hz, 1H), 7.06 (dd, J = 8.2, 2.0 Hz, 1H), 7.25 (d, J = 2.0 Hz, 1H), 9.99 (s, 1H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 62.1, 116.3, 119.2, 126.4, 128.1, 134.5, 151.7; LRMS (ES-): m/z 156.9 (100%, $[M-H]^-$).

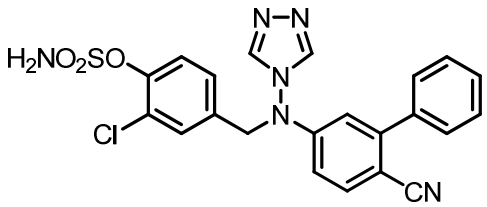
5-((3-Chloro-4-hydroxybenzyl)(4*H*-1,2,4-triazol-4-yl)amino)biphenyl-2-carbonitrile (14b)



A solution of 2-chloro-4-(hydroxymethyl)phenol (**14a**) (0.317 g, 2.0 mmol) in $SOCl_2$ (5 mL) was stirred at room temperature for 0.5 h. The excess $SOCl_2$ was removed under reduced pressure and the residue was dissolved in DMF (5 mL). Then **B** (0.523 g, 2.0 mmol) and K_2CO_3 (1.38 g, 10 mmol) were added and the mixture was stirred intensively overnight. EtOAc (60 mL) and 2M $KHSO_4$ (30 mL) were added, the organic layer was separated, washed with water (10 mL) and brine (20 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by flash chromatography ([SiO_2], acetone/chloroform 1 : 2) to give **14b** (0.490 g, 61%) as a white solid: Mp. 226-228 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.00 (s, 2H), 6.70 (dd, J = 8.6, 2.7 Hz. 1H), 6.80 (d, J = 2.4 Hz, 1H), 6.87 (d, J = 8.6 Hz, 1H), 7.02 (dd, J = 8.6, 2.4 Hz, 1H), 7.46-7.56 (m, 5H), 7.29 (d, J = 2.7 Hz, 1H), 7.85 (d, J = 8.6 Hz, 1H), 8.78 (s, 2H), 10.28 (s, 1H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 56.1, 101.9, 112.7, 114.1, 116.6, 118.7, 119.6, 126.1, 128.5, 128.6, 128.8, 128.9, 130.4, 135.5, 137.8, 143.4, 146.3, 151.4, 152.9; LRMS (ES+): m/z 402.3 (100%, $[M+H]^+$);

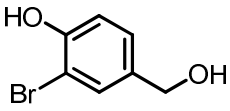
HRMS (ES+) calcd for $C_{22}H_{17}ClN_5O$ $[M+H]^+$: 402.1116, found 402.1105; HPLC: Sunfire C_{18} reverse phase column, 4.6 x 150 mm, 3.5μm pore size, isocratic 90% acetonitrile and 10% water at 0.8 mL/min, t_R = 1.42 min, purity >99%.

2-Chloro-4-(((6-cyanobiphenyl-3-yl)(4*H*-1,2,4-triazol-4-yl)amino)methyl)phenyl sulfamate (14)



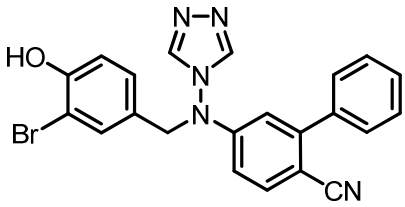
To a solution of sulfamoyl chloride (0.399 g, 3.45 mmol) in DMA (1 mL) was added a solution of **14b** (0.20 g, 0.498 mmol) in DMA (5 mL) at 0 °C. The clear solution was stirred for 2 h at 0 °C and then for 4 h at room temperature. EtOAc (50 mL) and water (20 mL) were added, the organic layer was separated, washed with water (2 x 30 mL) and brine (30 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by flash chromatography [SiO_2, $CHCl_3$/acetone (2:1)] to give **14** (0.158 g, 66%) as a white solid. Mp. 201-204 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.16 (s, 2H), 6.66 (dd, J = 8.6, 2.4 Hz, 1H), 6.77 (d, J = 2.4 Hz, 1H), 7.38 (dd, J = 8.6, 2.3 Hz, 1H), 7.44 (d, J = 8.6 Hz, 1H), 7.46-7.53 (m, 5H), 7.59 (d, J = 2.3 Hz, 1H), 7.83 (d, J = 8.6 Hz, 1H), 8.29 (s, 2H), 8.89 (s, 2H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 56.2, 102.2, 112.6, 114.0, 118.7, 123.7, 126.5, 128.1, 128.6, 128.8, 129.0, 130.3, 134.9, 135.6, 137.7, 143.4, 145.8, 146.3, 151.2; LRMS (ES+): *m/z* 481.2 (100%, $[M+H]^+$); HRMS (ES+) calcd for $C_{22}H_{18}ClN_6O_3S$ $[M+H]^+$: 481.0844, found 481.0830; HPLC: Sunfire C_{18} reverse phase column, 4.6 x 150 mm, 3.5μm pore size, isocratic 90% acetonitrile and 10% water at 1.0 mL/min, t_R = 1.32 min, purity 99.5%.

2-Bromo-4-hydroxymethyl-phenol (15a)



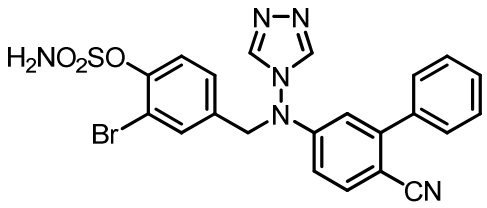
Prepared in the same manner as compound **5i** in Woo *et al.* J. Med. Chem. **2007**, *50*, 3540-3560.

5-[(3-Bromo-4-hydroxy-benzyl)-[1,2,4]triazol-4-yl-amino]-biphenyl-2-carbonitrile (15b).



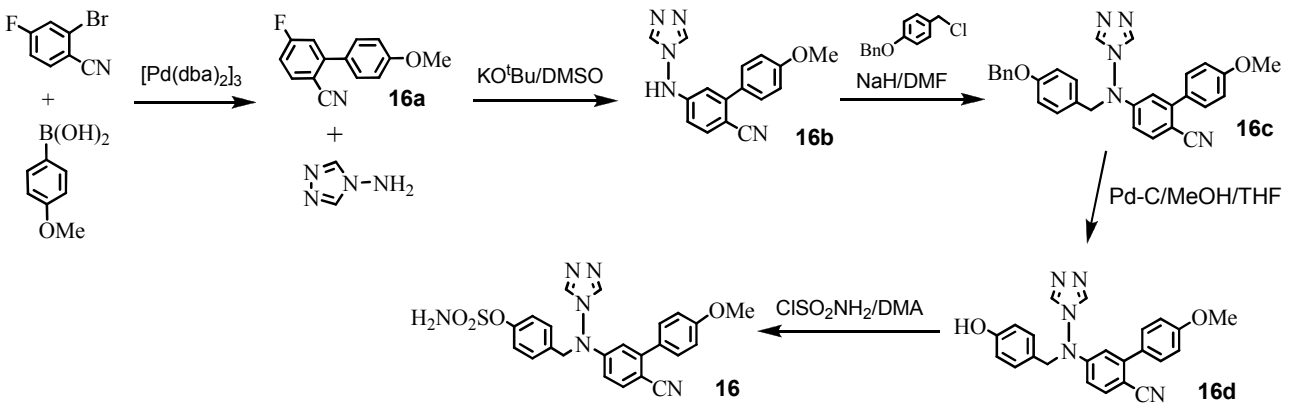
A solution of 2-bromo-4-(hydroxymethyl)phenol (**15a**) (0.305 g, 1.5 mmol) in $SOCl_2$ (5 mL) was stirred at room temperature for 0.5 h. The excess $SOCl_2$ was removed under reduced pressure and the residue was dissolved in DMF (5 mL). Then **B** (0.392 g, 1.5 mmol) and K_2CO_3 (1.38 g, 10 mmol) were added and the mixture was stirred intensively overnight. EtOAc (60 mL) and 2M $KHSO_4$ (30 mL) were added, the organic layer was separated, washed with water (10 mL) and brine (20 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by flash chromatography ([SiO_2], acetone/chloroform 1 : 2) to give **15b** (0.288 g, 43%) as a white solid: Mp. 218-220 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.00 (s, 2H), 6.70 (dd, J = 8.6, 2.4 Hz, 1H), 6.82 (d, J = 2.4 Hz, 1H), 6.86 (d, J = 8.2 Hz, 1H), 7.07 (dd, J = 8.2, 2.0 Hz, 1H), 7.44 (d, J = 2.0 Hz,1H), 7.48-7.58 (m, 5H), 7.86 (d, J = 8.2 Hz, 1H), 8.79 (s, 2H), 10.38 (s, 1H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 56.0, 101.9, 109.2, 112.7, 114.2, 116.3, 118.8, 126.5, 128.6, 128.8, 129.0, 129.3, 133.4, 135.6, 137.8, 143.5, 146.3, 151.4, 154.0; LRMS (ES+): *m/z* 446.2 (100%, $[M+H]^+$); HRMS (ES+) calcd for $C_{22}H_{17}BrN_5O$ $[M+H]^+$: 446.0624, found 446.0617. HPLC: Sunfire C_{18} reverse phase column, 4.6 x 150 mm, 3.5µm pore size, isocratic 90% acetonitrile and 10% water at 1.0 mL/min, t_R = 1.37 min, purity >99%.

2-Bromo-4-(((6-cyanobiphenyl-3-yl)(4*H*-1,2,4-triazol-4-yl)amino)methyl)phenyl sulfamate (15).

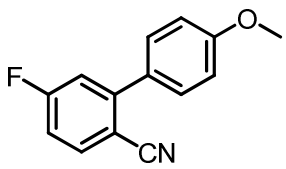


To a solution of sulfamoyl chloride (0.399 g, 3.45 mmol) in DMA (1 mL) was added a solution of **15b** (0.103 g, 0.231 mmol) in DMA (5 mL) at 0 °C. The clear solution was stirred for 2 h at 0 °C and then for 4 h at room temperature. EtOAc (50 mL) and water (20 mL) were added, the organic layer was separated, washed with water (2 x 30 mL) and brine (30 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by flash chromatography [SiO_2, $CHCl_3$/acetone (1:1)] to give **15** (0.073 g, 60%) as a white solid. Mp. > 200 °C (dec.); ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.18 (s, 2H), 6.67 (dd, J = 8.6, 2.4 Hz, 1H), 6.79 (d, J = 2.4 Hz, 1H), 7.40-7.56 (m, 7H), 7.74 (d, J = 1.6 Hz, 1H), 7.87 (d, J = 8.6 Hz, 1H), 8.31 (s, 2H), 8.91 (s, 2H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 56.1, 102.1, 112.6, 114.0, 115.8, 118.7, 123.2, 128.5, 128.8, 129.0, 133.4, 135.0, 135.6, 137.7, 143.4, 146.3, 147.1, 151.2; LRMS (ES+): *m/z* 527.2 (100%, $[C_{22}H_{18}{}^{81}BrN_6O_3S]^+$), 525.2 (90%, $[C_{22}H_{18}{}^{79}BrN_6O_3S]^+$); HRMS (ES+) calcd for $C_{22}H_{18}{}^{79}BrN_6O_3S$ $[M+H]^+$: 525.0339, found 525.0320. HPLC: Sunfire C_{18} reverse phase column, 4.6 x 150 mm, 3.5μm pore size, isocratic 90% acetonitrile and 10% water at 0.8 mL/min, t_R = 1.35 min, purity >99%.

3) Synthesis of compound ***16***

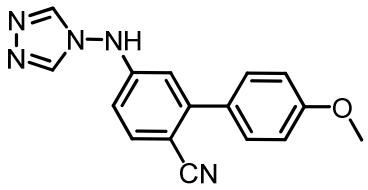


5-Fluoro-4'-methoxybiphenyl-2-carbonitrile (16a)



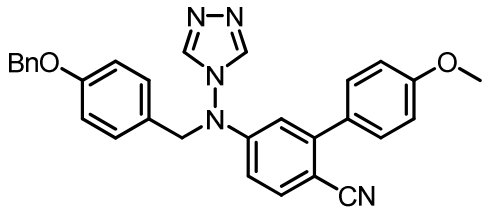
A mixture of 2-bromo-4-fluorobenzonitrile (5.0 g, 25.0 mmol), 4-methoxyphenylboronic acid (4.56 g, 30 mmol), dimethoxyethane (25 mL) and 2M Na_2CO_3 (40 mL) was heated to reflux before $Pd_2(dba)_3$ (0.10 g) was added and heating was continued for 5 h. After cooling to room temperature $CHCl_3$ (50 mL) was added to dissolve the product, which crystallised from the organic layer. The mixture was filtered through celite, the organic layer was separated, dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by flash chromatography [SiO_2, $CHCl_3$] followed by recrystallization from EtOH to give **16a** as a white solid (4.03 g, 71%). Mp. 145-147 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.87 (s, 3H), 7.02 (*para* AA'BB', 2H), 7.07-7.13 (m, 1H), 7.19 (dd, *J* = 9.4, 2.7 Hz, 1H), 7.51 (*para* AA'BB', 2H), 7.75 (dd, *J* = 8.6, 5.5 Hz, 1H); LRMS (ES+): *m/z* 228.2 (100%, $[M+H]^+$); HRMS (ES+) calcd for $C_{14}H_{11}FNO$ $[M+H]^+$: 228.0819, found 228.0816; Anal. Calcd for $C_{14}H_{10}FNO$: C, 74.00; H, 4.44; N, 6.16. Found C, 73.7; H, 4.40; N, 6.09.

5-(4*H*-1,2,4-Triazol-4-ylamino)-4'-methoxybiphenyl-2-carbonitrile (16b).



To a solution of 4*H*-1,2,4-triazol-4-amine (3.441 g, 40.928 mmol) in DMSO (40 mL) was added KO^{t}Bu (4.593 g, 40.928 mmol). The mixture was stirred for 0.5 h at room temperature before **16a** (4.65 g, 20.468 mmol) was added and stirring was continued for 1 h. The mixture was poured into crushed ice and neutralized with 2M $KHSO_4$ solution. The precipitate was filtered off and recrystallised from acetone/Et_2O to give **16b** (5.07 g, 85%) as a light yellow crystalline solid. Mp. 176-177 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.81 (s, 3H), 6.47 (d, J = 2.4 Hz, 1H), 6.54 (dd, J = 8.6, 2.4 Hz, 1H), 7.07 (*para* AA'BB', 2H), 7.43 (*para* AA'BB', 2H), 7.77 (J = 8.6 Hz, 1H), 8.86 (s, 2H), 10.28 (s, 1H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 55.3, 101.2, 111.0, 112.3, 114.2, 119.1, 129.7, 130.0, 135.6, 144.1, 146.1, 150.8, 159.8; LRMS (ES+): *m/z* 292.3 (100%, $[M+H]^+$); HRMS (ES+) calcd for $C_{16}H_{14}N_5O$ $[M+H]^+$: 292.1193, found 292.1180; Anal. Calcd for $C_{16}H_{13}N_5O$: C, 65.97; H, 4.50; N, 24.04. Found C, 65.9; H, 4.56; N, 23.8. HPLC: Sunfire C_{18} reverse phase column, 4.6 x 150 mm, 3.5µm pore size, isocratic 90% acetonitrile and 10% water at 0.8 mL/min, t_R = 1.41 min, purity >99%.

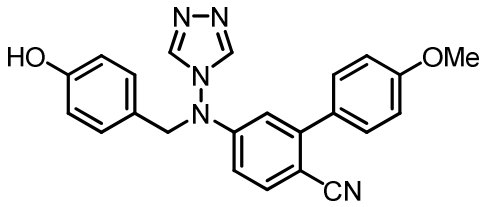
5-((4-(Benzyloxy)benzyl)(4*H*-1,2,4-triazol-4-yl)amino)-4'-methoxybiphenyl-2-carbonitrile (16c)



To a solution of **16b** (0.874 g, 3.0 mmol) in DMF (20 mL) was added NaH (0.120 g, 3.0 mmol, 60% in mineral oil) at room temperature. The mixture was stirred for 0.5 h before benzyloxybenzyl chloride (0.698 g, 3.0 mmol) was added and stirring was continued for 18 h. The reaction mixture

was diluted with EtOAc (50 mL), washed with water (3 x 30 mL) and brine (30 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was recrystallised from 2-propanol to give **16c** (1.112 g, 76%) as colorless needles. Mp. 219-221 ˚C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.81 (s, 3H), 5.04 (s, 2H), 5.06 (s, 2H), 6.69 (dd, J = 8.6, 2.4 Hz, 1H), 6.74 (d, J = 2.4 Hz, 1H), 6.95 (*para* AA'BB', 2H), 7.07 (*para* AA'BB', 2H), 7.23 (*para* AA'BB', 2H), 7.30-7.44 (m, 5H), 7.47 (*para* AA'BB', 2H), 7.82 (d, J = 8.6 Hz, 1H), 8.77 (s, 2H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 55.3, 56.5, 69.2, 101.7, 112.3, 113.8, 114.2, 114.8, 119.0, 126.6, 127.8, 127.9, 128.4, 129.9, 130.0, 130.1, 135.5, 136.9, 143.4, 146.0, 151.5, 158.2, 159.9; LRMS (ES+): *m/z* 488.2 (100%, $[M+H]^+$); HRMS (ES+) calcd for $C_{30}H_{26}N_5O_2$ $[M+H]^+$: 488.2087, found 488.2063; Anal. Calcd for $C_{30}H_{25}N_5O_2$: C, 73.90; H, 5.17; N, 14.36. Found C, 73.7; H, 5.10; N, 14.3. HPLC: Sunfire C_{18} reverse phase column, 4.6 x 150 mm, 3.5µm pore size, isocratic 90% acetonitrile and 10% water at 1.0 mL/min, t_R = 1.63 min, purity 99.5%.

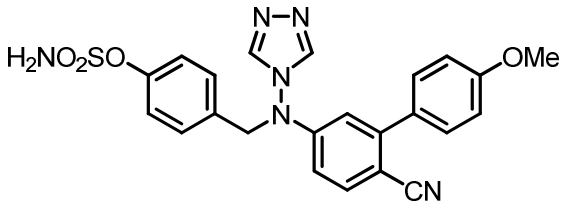
5-((4-Hydroxybenzyl)(4*H*-1,2,4-triazol-4-yl)amino)-4'-methoxybiphenyl-2-carbonitrile (16d)



To a solution of **16c** (0.95 g, 1.95 mmol) in THF (20 mL) and methanol (20 mL) was added Pd-C (0.10 g, 5% Pd). The mixture was stirred under H_2-atmosphere for 48 h before the catalyst was filtered off (celite) and the volatiles were removed under reduced pressure. The residue was recrystallised from EtOH give **16d** (0.69 g, 89%) as colorless crystals. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.81 (s, 3H), 4.96 (s, 2H), 6.65-6.71 (m, 3H), 6.74 (d, J = 8.6 Hz, 1H), 7.04-7.10 (m, 4H), 7.46 (*para* AA'BB', 2H), 7.81 (d, J = 8.6 Hz, 1H), 8.71 (s, 2H), 9.49 (s, 1H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 55.3, 56.6, 101.6, 112.3, 113.8, 114.2, 115.4, 119.0, 124.5, 129.9, 130.0, 130.2, 135.5, 143.5, 146.0, 151.5, 157.2, 159.9; LRMS (ES+): *m/z* 398.2 (100%, $[M+H]^+$); HRMS (ES+) calcd

for $C_{23}H_{19}N_5O_2Na$ $[M+Na]^+$: 420.1431, found 420.1410; Anal. Calcd for $C_{23}H_{19}N_5O_2$: C, 69.51; H, 4.82; N, 17.62. Found C, 69.3; H, 4.80; N, 17.65. HPLC: Sunfire C_{18} reverse phase column, 4.6 x 150 mm, 3.5µm pore size, isocratic 90% acetonitrile and 10% water at 1.0 mL/min, t_R = 1.34 min, purity >99%.

4-(((6-Cyano-4'-methoxybiphenyl-3-yl)(4*H*-1,2,4-triazol-4-yl)amino)methyl)phenyl sulfamate (16)



To a solution of sulfamoyl chloride (0.399 g, 3.45 mmol) in DMA (1 mL) was added a solution of **16d** (0.200 g, 0.503 mmol) in DMA (5 mL) at 0 °C. The clear solution was stirred for 2 h at 0 °C and then for 4 h at room temperature. EtOAc (50 mL) and water (20 mL) were added, the organic layer was separated, washed with water (2 x 30 mL) and brine (30 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was recrystallised from EtOAc to give **16** (0.194 g, 81%) as a white solid. Mp. 184-186 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.81 (s, 3H), 6.65 (dd, J = 8.6, 2.3 Hz, 1H), 6.71 (d, J = 2.3 Hz, 1H), 7.07 (*para* AA'BB', 2H), 7.24 (*para* AA'BB', 2H), 7.43 (*para* AA'BB', 2H), 7.45 (*para* AA'BB', 2H), 7.83 (d, J = 8.6 Hz, 1H), 8.03 (s, 2H), 8.85 (s, 2H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 55.3, 56.6, 101.8, 112.2, 113.7, 114.2, 118.9, 122.2, 129.7, 129.9, 130.0, 133.3, 135.5, 143.4, 146.0, 149.8, 151.3, 159.9; LRMS (ES+): *m/z* 477.3 (100%, $[M+H]^+$); HRMS (ES+) calcd for $C_{23}H_{21}N_6O_4S$ $[M+H]^+$: 477.1340, found 477.1328; HPLC: Sunfire C_{18} reverse phase column, 4.6 x 150 mm, 3.5µm pore size, isocratic 90% acetonitrile and 10% water at 1.0 mL/min, t_R = 1.298 min, purity >99%.

4) JEG-3 cells assay

The extent of in vitro inhibition of STS and aromatase activity by compounds was assessed using JEG-3 cells. Cells were seeded into 24-well culture plates and maintained in MEM (Flow Laboratories, Irvine, U.K.) containing supplements and used when 80% confluent. To determine STS activity, cells were incubated for 1 h with [6,7-^{3}H]E1S (5 pmol, 7 x 10^{5} dpm, 60 Ci/mmol; Perkin-Elmer LS, Wellesley, MA) in the presence or absence of (0.001-10000 nmol/L) inhibitor. The product E1 was separated from E1S by toluene partition using [4-^{14}C]E1 to monitor procedural losses, and the radioactivity was measured by scintillation spectrometry. Similarly, for aromatase activity, [1β-^{3}H]androstenedione (2-3 nM, 30 Ci/mmol; Perkin-Elmer Life Sciences, MA) was incubated with JEG-3 cells for 1 h in the presence or absence of inhibitor. The product, E1, was separated using dextran-coated charcoal at 4 °C for 2 h, and remaining radioactivity was measured by scintillation spectrometry. Each IC_{50} represents the mean ± SE of triplicate measurements.