Supporting Information

Novel Chromene-Derived Selective Estrogen Receptor Modulators Useful for Alleviating Hot Flush and Vaginal Dryness

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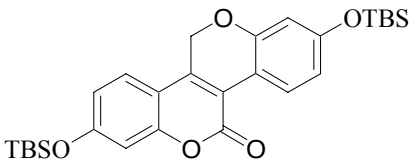
General Information. Optical Rotations were measured on a Perkin Elmer model 341 polarimeter. ^{1}H and ^{13}C spectra were measured on either a Bruker 300 or 400 MHz or 500 MHz instrument. In the case of ^{13}C spectra, these measurements were taken with full proton decoupling. Data for proton spectra were reported as follows: chemical shifts are reported in ppm, utilizing the residual solvent as an internal standard, integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, nd = narrow doublet), coupling constants (Hz). Analytical high performance liquid chromatography (HPLC) coupled with MS and uv diodray detectors, was performed on an agilent 1100 series instrument at 280 nM (UV detector) and mass ranging from 300-1000 (MS detector). (a) Phenonmenex, luna 5μ, phrnyl-hexyl 150x4.60 mm, Solvent A: H_2O (0.1% TFA), Solvent B: CH_3CN (0.1% TFA), gradient 20-90% of solvent A to B, flow rate: 1ml/min, total run time: 15 min. (b) phenonmenex, luna 5μ 150x4.60 mm, Solvent A: H_2O (0.1% TFA), Solvent B: CH_3CN (0.1% TFA), gradient 20-90% of solvent A to B, flow rate: 1ml/min, total run time: 15 min. (c) YMC diol 120 100 x 4.6 mm (achiral, normal phase) column, Solvent system 50% IPA in hexanes, Isocratic solvent sytem, flow rate: 1 min / mL, run time: 20 min. and (d) a Diacel ChiralPak AD 250 x 4.6 mm (chiral) column using an isocratic solvent mixture 50:50 IPA/Hexanes, at a flow rate of 1 mL/min.

For thin layer chromatography (TLC) analysis throughout this work, Analtech Uniplate precoated plates were used in conjunction with a variety of developing reagents including phosphomolybdic acid (PMA) and para-anisaldehyde (PAA) in addition to UV light. Purification of materials was carried out using an ISCO chromatography system with pre-packed silica gel columns. High-resolution mass spectrometry (HRMS) was performed by M-Scan, Inc. and elemental analyses by QTI technologies. All reagents and solvents were used as received from commercial source.

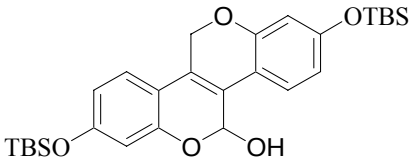
Synthesis of 2, 8-Bis- (*tert*-butyl-dimethyl-silyloxy)-11*H*-chromeno[4,3-c]chromen-5-one (**3b)**



3b

A slurry of 2, 8-dihydroxy-11*H*-chromeno[4,3-c]chromen-5-one (0.322 g, 1.1412 mmol, 1 eq), in dichloromethane (10 mL) was treated with triethylamine (0.8 mL, 5.70 mmol, 5 eq), followed by the addition of t-butyldimethylsilyl chloride (0.585 g, 3.88 mmol, 3.4 eq). The reaction mixture was stirred at room temperature under nitrogen for 18 hours. (The slurry was observed to become a clear solution after about 30 minutes of stirring.) The reaction mixture was diluted with hexane (~35 mL) and washed once with brine. The aqueous washing was re-extracted with hexane. The combined organic extracts were dried (anhydrous sodium sulphate), filtered and evaporated in vacuum to yield a yellow solid residue. The solid residue was recrystallized from hexane to yield the title compound as a light yellow crystalline solid. Mp 150-151°C; MS (Cl) m/z 533 $(M+Na)^{+1}$, ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.43 (1H, d, J = 8.6 Hz), 7.33 (1H, d, J = 8.3 Hz), 6.84 (1H, s), 6.83 (1H, d, J =9.1 Hz), 6.57 (1H, d, d, J = 2.4, 8.7 Hz), 6.47 (1H, d, J = 2.22 Hz), 5.26 (2H, s), 1.00 (9H, s), 0.99 (9H, s), 0.26 (3H, s), 0.23 (6H, s), IR (KBr): 2957, 2927,2883, 2855, 1713, 1618, 1567, 1498, 1287 cm^{-1}. Anal. Calc. for $C_{28}H_{38}O_5Si_2$: C, 65.84; H, 7.50. Found: C, 65.81; H, 7.56.

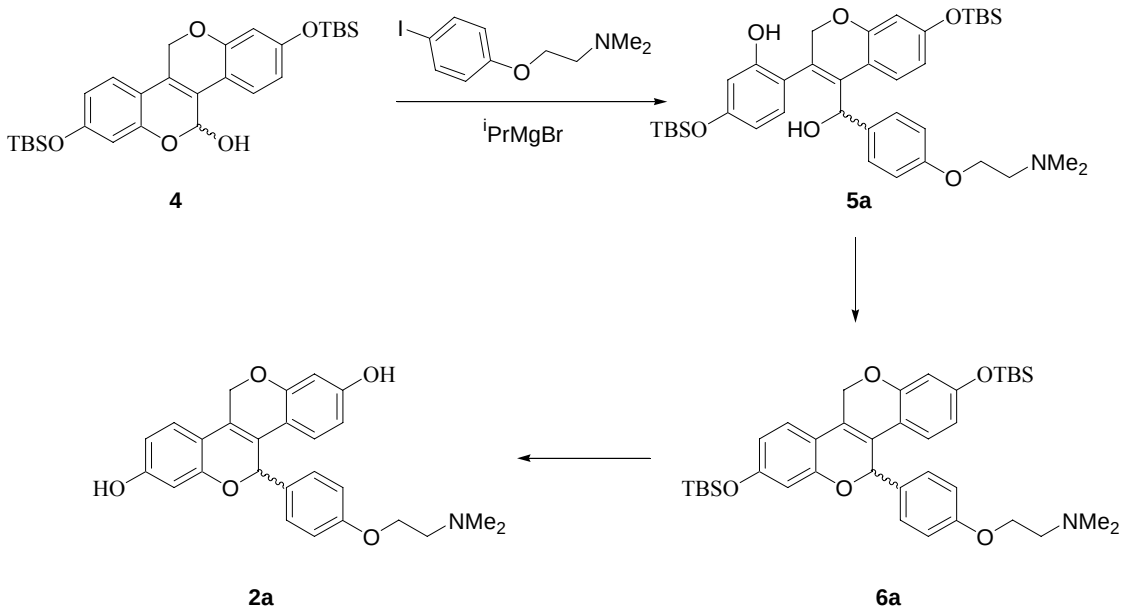
Synthesis of 8-Bis-(*tert*-Butyl-dimethyl-silanlyoxy)-5,11-dihydro-chromeno [4,3-c]-chromen-5-ol (**4)**



4

A solution of 2, 8-bis-(*tert*-butyl-dimethyl-silyloxy)-11*H*-chromeno[4,3-c]chromen-5-one (5.016 g, 9.82 mmol, 1eq) in toluene (525 mL) was cooled to -78°C in a 1L 3-neck round bottom flask equipped with a mechanical stirrer, a nitrogen inlet and a dropping funnel. To the reaction mixture was slowly added a toluene solution of diisobutylaluminum hydride (19 mL of 1.5 M, 28.48 mmol, 2.9 eq), with the temperature of the reaction mixture maintained at less than -70^0 C. The reaction was stirred for 5 hours, quenched with addition of methanol (25 mL) followed by 10% citric acid solution (~140 mL). The resulting solution was diluted with dichloromethane (525 mL), the solution washed with a saturated solution of Rochelle salt (250 mL), then washed with brine, dried on anhydrous sodium sulphate, filtered and evaporated to yield the crude compound as a yellow solid. The solid was recrystallized from a dichloromethane:hexane mixture (1:1) to yield the title product as an ivory, crystalline solid. mp 150-151^o C (hexane). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.43 (1H, d, J = 9.1 Hz), 7.33 (1H, d, J = 8.3 Hz), 6.84 (1H, s), 6.83 (1H, d, J = 9.1 Hz), 6.57 (1H, d, d, J = 2.4, 8.7 Hz), 6.47 (1H, d, J = 2.22 Hz), 5.26 (2H, s), 1.00 (9H, s), 0.99 (9H, s), 0.26 (3H, s), 0.23 (6H, s); IR (KBr): 2957, 2927, 2883, 2855, 1713, 1618, 1567, 1498, 1287 cm^{-1} ; MS (CI) m/z 311(MH^+), 333 (M^+Na). Anal. Calc. For: $C_{28}H_{40}O_5Si_20.5CH_2Cl_2$: C, 61.65; H, 7.44. Found: C61.66; H, 7.44.

Synthesis of 5-[4-(2-Dimethylamino-ethoxy)-phenyl]-5,11-dihydro-chromeno[4,3-c]chromene-2,8-diol (**2a**)



In a single neck, 50 mL round bottom flask was dissolved and stirred 4-[2-(N, N-dimethyl)-ethoxy]-iodobenzene (0.727 g, 2.5 mmol, 3 eq), in tetrahydrofuran (10 mL) under argon, and the mixture cooled to –22°C. After 5 minutes of stirring, an ether solution of isopropylmagnesium bromide (1.244 mL of 2.13 M, 2.65 mmol, 3 eq) was added via syringe. The reaction mixture was then stirred for 2 hours at about -22°C. A tetrahydrofuran solution of 2, 8-bis-(*tert*-Butyl-dimethyl-silanlyoxy)-5,11-dihydro-chromeno [4,3-c]-chromen-5-ol, **4** (0.512 g, 1 mmol, 1 eq, in 10 mL), was then added, the cooling bath was removed and the reaction mixture was allowed to warm to room temperature overnight. After about 18 hours, the reaction was worked-up with addition of saturated ammonium acetate solution (15 mL) and extraction with ethyl ether (2 x 25 mL). The combined organic extracts were washed with brine and water, dried with anhydrous sodium sulphate, filtered and evaporated to yield a sticky semisolid residue. The **5a** was isolated as a viscous, colorless, semisolid foam via chromatography on silica

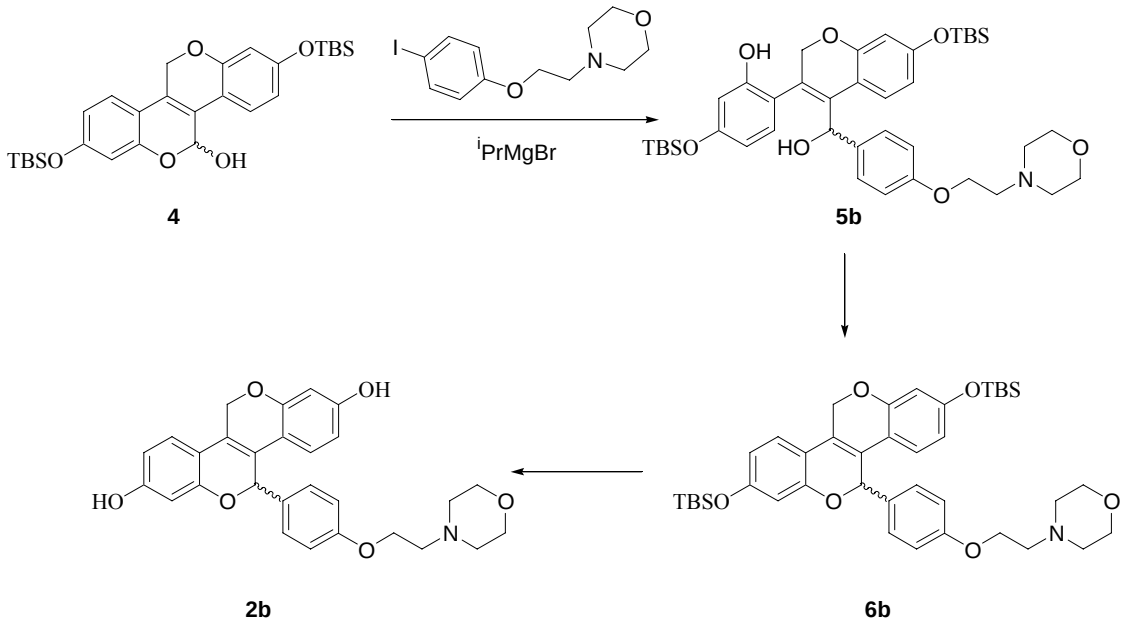
gel eluted with 3% methanol/dichloromethane, (HPLC purity, 88%, t^a_r=4.6) MS (Cl) m/z 678 $(M+H)^+$, 700 $(M+Na)^+$; loop negative 706 (M-H), HRMS, m/z calcd for $C_{38}H_{65}NO_6Si_2$ $(M+H^+)$ 678.3646 found 678.3539. ^{1}H NMR (300 MHz , $CDCl_3$ δ 7.09 (4H, m), 6.94 (2H, d, J = 8.10 Hz), 6.58–6.33 4H, m), 5.50 (1H, brs,), 4.82 (2H, brs), 4.00 (2H, t), 2.78 (2H, m), 2.38 (6H, s), 0.98 (9H, s), 0.94 (9H, s), 0.20 (6H, s), 0.15 (6H, s)

To a stirred solution of **5a** (1.0633 g, 1.48 mmol, 1eq), in tetrahydrofuran (50 mL) under argon at room temperature were added powdered molecular sieve (4 Å, 0.250 g) and triphenyl phosphine (0.7829 g, 2.99 mmol, 2 eq) followed by diethyl diazodicarboxylate (0.52 g = 0.466 mL, 2.96 mmol). The reaction mixture was let run overnight (about 18 hours). The reaction mixture was evaporated to dryness, triturated with ether and the resulting colorless solid of triphenyl phosphine oxide removed by filtration. The filtrate was evaporated to dryness to yield a residue which was purified by column chromatography on silica gel using 3% methanol in dichloromethane as an eluent to yield the **6a** as a viscous semisolid. (HPLC purity, 98%, t^a_r=6.8, t^b_r=5.2), MS (Cl) m/z 660 $(M+H)^+$, HRMS, m/z calcd for $C_{38}H_{54}NO_5Si_2$ 660.3541 found 660.3494. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.31 (2H, d, J = 8.69 Hz), 6.87 (1H, d, J = 8.32 Hz), 6.81 (2H, d, J = 8.68 Hz), 6.70 (1H, d, J = 8.42 Hz), 6.41 – 6.27 (4H, m), 6.14 (1H, s), 5.30 (1H, d, J = 13.83 Hz), 4.91 (1H, d, d, J = 1.50, 13.88 Hz), 3.99 (2H, t, J = 5.79 Hz), 2.68 (2H, t, J = 5.79 Hz), 2.29 (6H, s), 0.95 (9H, s), 0.93 (9H, s), 0.18 (6H, s), 0.16 (6H, s).

To a stirred solution of **6a** (0.19 g, 0.287 mmol, 1 eq), in tetrahydrofuran (15 mL) under nitrogen was added *tetra*-n-butyl ammonium fluoride (1M in tetrahydrofuran, 1.36 mL, 1.36 mmol, 5 eq) and the mixture was stirred for 3 hours. The reaction mixture was diluted with ethyl acetate (30 mL) and then washed with saturated aqueous ammonium chloride solution (35 mL). The precipitated inorganic salts were removed by filtration and washed with ethyl acetate. The combined organic phases were washed with saturated aqueous sodium bicarbonate solution (50 mL), dried (anhydrous sodium sulphate), filtered and evaporated to dryness to yield the crude product. The crude product was purified by column chromatography on silica gel using a 1:1 mixture of hexane and 10% ammoniated methanol containing 10% ammonium hydroxide to yield the purified **2a** as a brownish, foamy solid. (HPLC purity, 95%, t^a_r=4.2, t^b_r=2.8, Table 4) (MS (Cl) m/z 432 $(M+H)^+$; loop negative 430 (M-H), HRMS, m/z calcd for $C_{26}H_{25}NO$ (M+) 431.1733

found 431.1699. ^{1}H NMR (300 MHz, d-6 acetone) δ 7.37 (2H, d, J = 8.63 Hz), 7.03 (1H, d, J = 8.36 Hz), 6.84 (3H, d, J = 8.49 HZ), 6.43 – 6.27 (5H, m), 5.38 (1H, d, J = 14.11 Hz), 5.06 (1H, d, d, J = 1.39, 14.11 Hz), 4.02 (2H, t, J = 5.88 Hz), 2.63 (2H, t, J = 5.85 Hz), 2.23 (6H, brs), Anal. Calc. for $C_{26}H_{25}O_5N$ 0.4 MeOH: C, 71.37; H, 6.03; N, 3.15 Found: C, 71.36; H, 6.06; N, 3.19

Synthesis of 5-[4-(2-Morpholin-4-yl-ethoxy)-phenyl]-5,11-dihydro-chromeno[4,3-c]chromene-2,8-diol (**2b**)



In a single neck, 50 mL round bottom flask was dissolved and stirred 4-[2-(4-Iodo-phenoxy)-ethyl]-morpholine (0.7 g, 2.1 mmol), in tetrahydrofuran (10 mL) under argon, and the mixture cooled to –18°C. After 5 minutes of stirring, an ether solution of isopropylmagnesium bromide (2.2 mL of 1 M, 2.2 mmol) was added via syringe. The reaction mixture was then stirred for 4 hours at about -18°C. A tetrahydrofuran solution of 2, 8-bis-(*tert*-Butyl-dimethyl-silanlyoxy)-5,11-dihydro-chromeno [4,3-c]-chromen-5-ol, **4** (0.48 g, 0.93 mmol, in 10 mL), was then added, the cooling bath was removed and the reaction mixture was allowed to warm to room temperature overnight. After about 11 hours, the reaction was worked-up with addition of saturated ammonium acetate solution

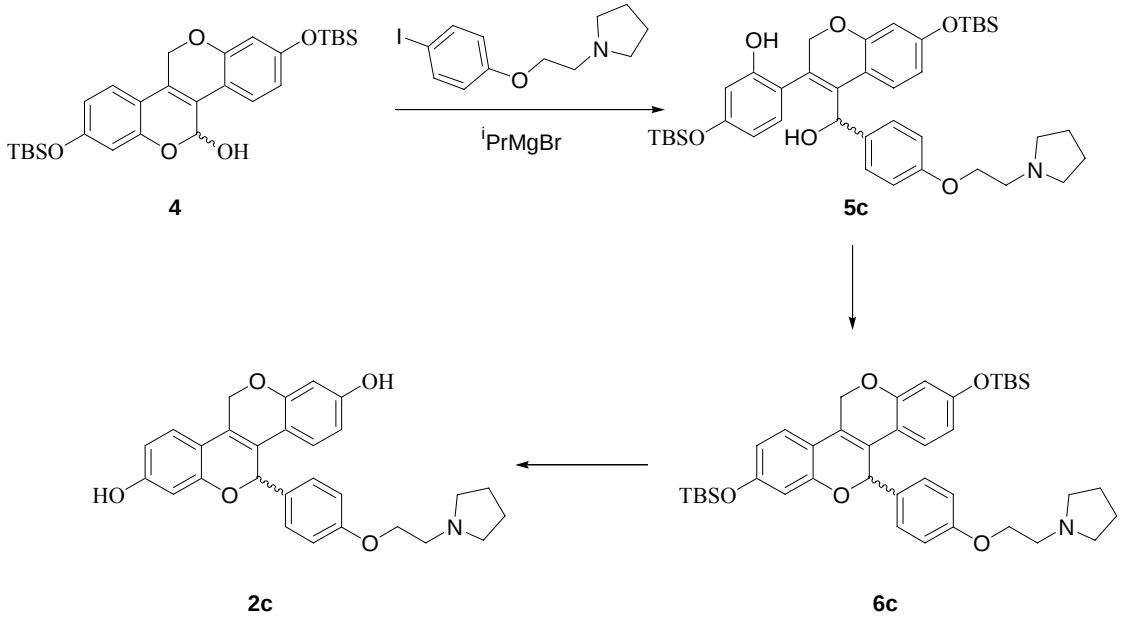
(40 mL) and extraction with ethyl ether (2 x 50 mL). The combined organic extracts were washed with brine and water, dried with anhydrous sodium sulphate, filtered and evaporated to yield a sticky semisolid residue. The **5b** was isolated as a viscous, colorless, semisolid foam via chromatography on silica gel eluted with 3% methanol/dichloromethane, (HPLC purity, 96%, t^a_r = 6.1, t^b_r = 4.66, Table 4), MS (Cl) m/z 720 $(M+H)^+$, 742 $(M+Na)^+$; loop negative 718 (M-H), HRMS, m/z calcd for $C_{40}H_{58}NO_7Si_2$ (M+H+) 720.3752 found 720.3788. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.06–7.02 (4H, m), 6.77 (2H, d, J = 7.98 Hz), 6.43 – 6.18 (4H, m), 5.67 (1H, brs,), 4.81 (2H, brs), 4.05 (2H, t), 3.72 (4H, m), 2.77 (2H, t), 2.56 (4H, m), 0.96 (9H, s), 0.93 (9H, s), 0.19 (6H, s), 0.15 (6H,s).

To a stirred solution of **5b** (0.89 g, 1.23 mmol, 1eq), in tetrahydrofuran (50 mL) under argon at room temperature were added powdered molecular sieve (4 Å, 0.250 g) and triphenyl phosphine (0.73 g, 2.99 mmol) followed by diethyl diazodicarboxylate (0.52 g = 0.466 mL, 2.96 mmol). The reaction mixture was let run overnight (about 18 hours). The reaction mixture was evaporated to dryness, triturated with ether and the resulting colorless solid of triphenyl phosphine oxide removed by filtration. The filtrate was evaporated to dryness to yield a residue which was purified by column chromatography on silica gel using 2% methanol in dichloromethane as an eluent to yield the **6b** as a viscous semisolid (HPLC purity, 95%, t^a_r=6.1, t^b_r=3.6, Table 4) MS (Cl) m/z 702 $(M+H)^+$, HRMS, m/z calcd for $C_{40}H_{56}NO_6Si_2$ 702.3646 found 702.3641. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.31 (2H, d, J = 8.65 Hz), 6.88 (1H, d, J = 8.33 Hz), 6.79 (2H, d, J = 8.74 Hz), 6.70 (1H, d, J = 8.43 Hz), 6.41 – 6.27 (4H, m), 6.15 (1H, brs), 5.30 (1H, d, J = 13.77 Hz), 5.10 (1H, d, d, J = 1.52, 13.77 Hz), 4.04 (2H, t) 3.74 – 3.69 (4H, m), 2.75 (2H, t), 2.55-2.52 (4H, m), 0.95 (9H, s), 0.93 (9H, s), 0.18 (6H, s), 0.16 (6H, s).

To a stirred solution of **6b** (0.21 g, 0.299 mmol), in tetrahydrofuran (15 mL) under nitrogen was added *tetra*-n-butyl ammonium fluoride (1M in tetrahydrofuran, 1.36 mL, 1.36 mmol) and the mixture was stirred for 3 hours. The reaction mixture was diluted with ethyl acetate (80 mL) and then washed with saturated aqueous ammonium chloride solution (65 mL). The precipitated inorganic salts were removed by filtration and washed with ethyl acetate. The combined organic phases were washed with saturated aqueous sodium bicarbonate solution (50 mL), dried (anhydrous sodium sulphate), filtered and

evaporated to dryness to yield the crude product. The crude product was purified by column chromatography on silica gel using a 1:1 mixture of hexane and 10% ammoniated methanol containing 10% ammonium hydroxide to yield the purified **2b** as a brownish, foamy solid (HPLC purity, 97%, t^a_r= 4, t^b_r = 2.1, Table 4), MS (Cl) m/z 474 $(M+H)^+$; loop negative 472 (M-H) ^{1}H HRMS, m/z calcd for $C_{28}H_{28}NO_6$(M+H+) 474.1917 found 474.1914. NMR (300 MHz, d-6 acetone) δ 8.58 (2H, br hump), 7.37 (2H, d, J = 8.68 Hz), 7.04 (1H, d, J = 8.73 Hz), 6.84 (3H, d, J = 8.73 Hz), 6.42 (1H, d, d, J = 2.37, 8.34 Hz), 6.38 (2H, d, 2.37 Hz), 6.33 (1H, d, d, J = 2.41, 8.33 Hz), 6.27 (2H, d, J = 2.33 Hz), 6.27 (1H, s), 5.38 (1H, d, J = 14.11 Hz), 5.06 (1H, d, d, J = 1.56, 14.13 Hz), 4.06 (2H, t, J = 5.81 Hz), 3.57 (4H, t, J = 4.01 Hz), 2.92 (4H, brs), 2.69 (2H, t, J = 3.45). Anal. Calc. for $C_{28}H_{27}O_6N$ 0.6 MeOH: C, 69.71; H, 6.01; N, 2.84 Found: C, 69.81; H, 6.05; N, 2.86.

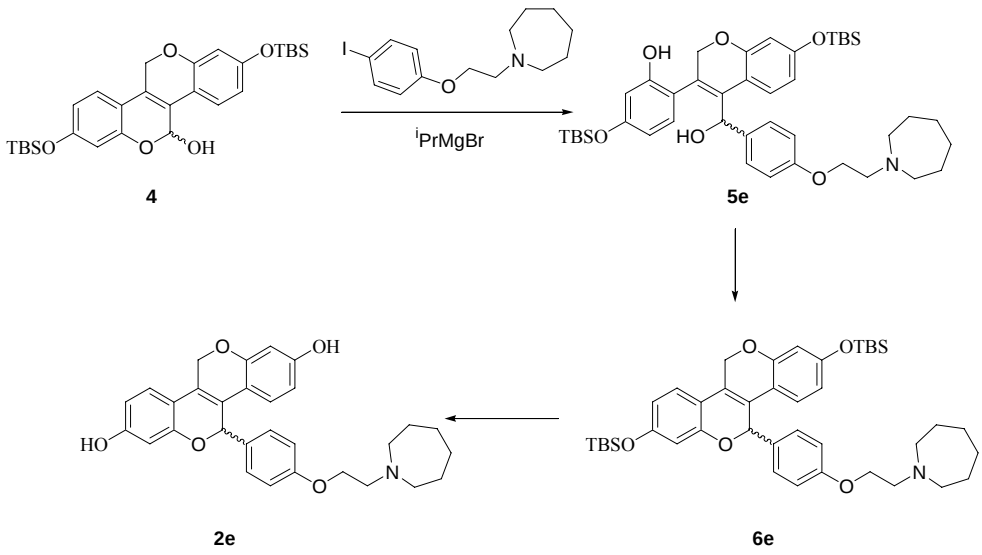
Synthesis of 5-[4-(2-Pyrrolidin-1-yl-ethoxy)-phenyl]-5,11-dihydro-chromeno[4,3-c]chromene-2,8-diol (**2c**)



In a single neck, 50 mL round bottom flask was dissolved and stirred 1-[2-(4-Iodo-phenoxy)-ethyl]-pyrrolidine (0.81 g, 2.55 mmol), in tetrahydrofuran (12 mL) under argon, and the mixture cooled to –18°C. After 5 minutes of stirring, an ether solution of isopropylmagnesium bromide (2.55 mL of 1 M, 2.55 mmol) was added via syringe. The reaction mixture was then stirred for 7 hours at about -18°C. A tetrahydrofuran solution of 2, 8-bis-(*tert*-Butyl-dimethyl-silanlyoxy)-5,11-dihydro-chromeno [4,3-c]-chromen-5-ol, **4** (0.51 g, 1 mmol, in 10 mL of THF), was then added, the cooling bath was removed and the reaction mixture was allowed to warm to room temperature overnight. After about 11 hours, the reaction was worked-up with addition of saturated ammonium acetate solution (80 mL) and extraction with ethyl ether (2 x 150 mL). The combined organic extracts were washed with brine and water, dried with anhydrous sodium sulphate, filtered and evaporated to yield a sticky semisolid residue. The **5c** was isolated as a viscous, colorless, semisolid foam via chromatography on silica gel eluted with 3% methanol/dichloromethane and was carried over further purification. To a stirred solution of **5c** (0.81 g, 1.23 mmol, 1eq), in tetrahydrofuran (50 mL) under argon at room temperature were added powdered molecular sieve (4Å, 0.250 g) and triphenyl phosphine (0.73 g, 2.99 mmol) followed by diethyl diazodicarboxylate (0.52 g = 0.466 mL, 2.96 mmol). The reaction mixture was let run overnight (about 18 hours). The reaction mixture was evaporated to dryness, triturated with ether and the resulting colorless solid of triphenyl phosphine oxide removed by filtration. The filtrate was evaporated to dryness to yield a residue which was purified by column chromatography on silica gel using 2% methanol in dichloromethane as an eluent to yield the **6c** as a viscous semisolid. (HPLC purity, 93%, t^a_r=5.9, t^b_r=3.6, Table 4) MS (Cl) m/z 686 $(M+H)^+$ HRMS, m/z calcd for $C_{40}H_{56}NO_5Si_2$ $(M+H^+)$ 686.3697 found 686.3689 ^{1}H NMR (300 MHz, $CDCl_3$): δ 731 (2H, d, J = 8.59 Hz), 6.87 (1H, d, J = 8.32 Hz), 6.80 (2H, d, J = 8.70 Hz), 6.70 (1H, d, J = 8.41 Hz), 6.15 (1H, s), 5.30 (1H, d, J = 13.88 Hz), 5.10 (1H, d, J = 14.04 Hz), 4.05 (2H, t, J = 5.88 Hz), 2.87 (2H, t, J = 5.98 Hz), 2.61 (4H, brs), 0.95 (9H, s), 0.93 (9H, s), 0.18 (6H, s), 0.16 (6H, s).

To a stirred solution of **6c** (0.21 g, 0.299 mmol), in tetrahydrofuran (15 mL) under nitrogen was added *tetra*-n-butyl ammonium fluoride (1M in tetrahydrofuran, 1.36 mL, 1.36 mmol) and the mixture was stirred for 3 hours. The reaction mixture was diluted with ethyl acetate (80 mL) and then washed with saturated aqueous ammonium chloride solution (65 mL). The precipitated inorganic salts were removed by filtration and washed with ethyl acetate. The combined organic phases were washed with saturated aqueous sodium bicarbonate solution (50 mL), dried (anhydrous sodium sulphate), filtered and evaporated to dryness to yield the crude product. The crude product was purified by column chromatography on silica gel using a 1:1 mixture of hexane and 10% ammoniated methanol containing 10% ammonium hydroxide to yield the purified **2c** as a brownish, foamy solid (HPLC purity, 96%, t^a_r=4.1, t^b_r=2.7, Table 4) MS (Cl) m/z 458 $(M+H)^+$; loop negative 456 (M-H) HRMS, m/z calcd for $C_{28}H_{28}NO_5$ $(M+H)^+$ 458.1967 found 458,1959. ^{1}H NMR (300 MHz, d_6 acetone) δ 7.36 (2H, d, J = 8.63 Hz), 7.01 (1H, d, J = 8.34 Hz), 6.84 – 6.79 (3H, m), 6.44 – 6.26 (5H, m), 5.36 (1H, d, J = 14.14 Hz), 5.05 (1H, d, d, J = 1.22, 14.13 Hz), 4.82 (2H, br hump), 4.03 (2H, t, J = 5.85 Hz), 2.81 (2H, t, J = 5.83), 2.54 (4H, m) 1.71-1.68 (4H, m).

Synthesis of 5-[4-(2-Azepan-1-yl-ethoxy)-phenyl]-5,11-dihydro-chromeno[4,3-c]chromene-2,8-diol (**2e**)

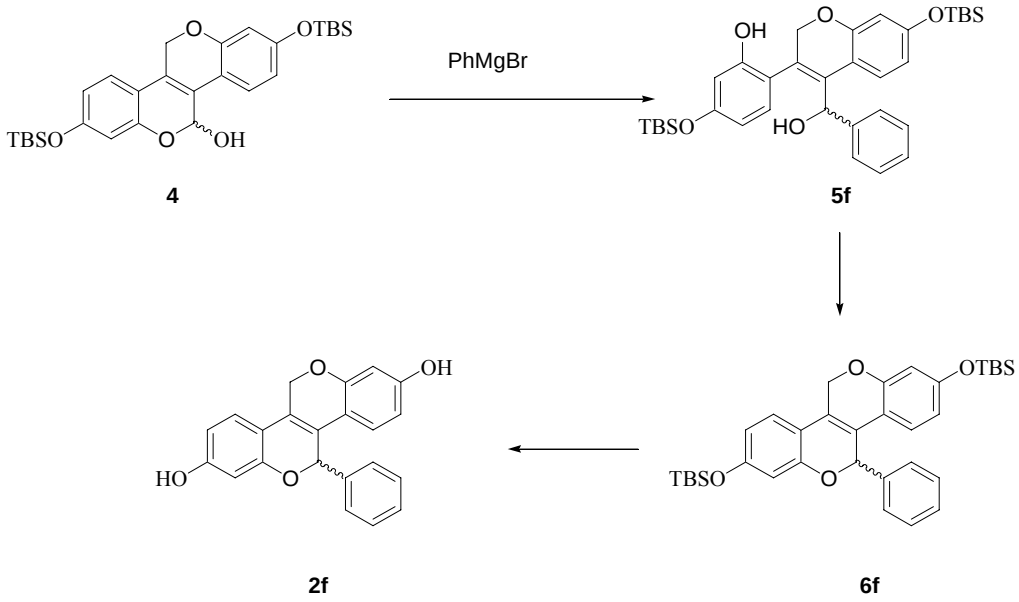


In a single neck, 50 mL round bottom flask was dissolved and stirred 1-[2-(4-Iodo-phenoxy)-ethyl]-azepane (0.76 g, 2.22 mmol), in tetrahydrofuran (12 mL) under argon, and the mixture cooled to –18°C. After 5 minutes of stirring, an ether solution of isopropylmagnesium bromide (2.55 mL of 1 M, 2.55 mmol) was added via syringe. The reaction mixture was then stirred for 7 hours at about -18°C. A tetrahydrofuran solution of 2, 8-bis-(*tert*-Butyl-dimethyl-silanlyoxy)-5,11-dihydro-chromeno [4,3-c]-chromen-5-ol, **4** (0.51 g, 1 mmol, in 10 mL of THF), was then added, the cooling bath was removed and the reaction mixture was allowed to warm to room temperature overnight. After about 11 hours, the reaction was worked-up with addition of saturated ammonium acetate solution (80 mL) and extraction with ethyl ether (2 x 150 mL). The combined organic extracts were washed with brine and water, dried with anhydrous sodium sulphate, filtered and evaporated to yield a sticky semisolid residue. The **5c** was isolated as a viscous, colorless, semisolid foam via chromatography on silica gel eluted with 3% methanol/dichloromethane and was carried over further purification. To a stirred solution of **5e** (0.81 g, 1.23 mmol, 1eq), in tetrahydrofuran (50 mL) under argon at room temperature were added powdered molecular sieve (4 Å, 0.250 g) and triphenyl phosphine (0.73 g, 2.99 mmol) followed by diethyl diazodicarboxylate (0.52 g = 0.466 mL, 2.96 mmol). The reaction mixture was let run overnight (about 18 hours). The reaction mixture was evaporated to dryness, triturated with ether and the resulting colorless solid of triphenyl phosphine oxide removed by filtration. The filtrate was evaporated to dryness to yield a residue which was purified by column chromatography on silica gel using 2% methanol in dichloromethane as an eluent to yield the **6e** as a viscous semisolid (HPLC purity, 94%, t^a_r=7.1, t^b_r=4.7, Table 4) MS (Cl) m/z 714 $(M+H)^+$, HRMS, m/z calcd for $C_{42}H_{60}NO_5Si_2$ 714.4010 found 714.3998, ^{1}H NMR (300 MHz, $CDCl_3$): δ 731 (2H, d, J = 8.72 Hz), 6.87 (1H, d, J = 8.32 Hz), 6.79 (2H, d, J = 8.70 Hz), 6.70 (1H, d, J = 8.44 Hz), 6.14 (1H, s), 5.30 (1H, d, J = 13.88 Hz), 5.10 (1H, d, d, J = 1.55, 13.88 Hz 4.01 (2H, t, J = 6.20 Hz), 2.91 (2H, t, J = 6.20 Hz), 2.81-2.73 (4H, m), 1.70–1.60 (8H, m), 0.95 (9H, s), 0.93 (9H, s), 0.18 (6H, s), 0.16 (6H, s)

To a stirred solution of **6e** (0.31 g, 0.41 mmol), in tetrahydrofuran (15 mL) under nitrogen was added *tetra*-n butyl ammonium fluoride (1M in tetrahydrofuran, 0.82 mL, 0.82 mmol) and the mixture was stirred for 3 hours. The reaction mixture was diluted

with ethyl acetate (90 mL) and then washed with saturated aqueous ammonium chloride solution (85 mL). The precipitated inorganic salts were removed by filtration and washed with ethyl acetate. The combined organic phases were washed with saturated aqueous sodium bicarbonate solution (50 mL), dried (anhydrous sodium sulphate), filtered and evaporated to dryness to yield the crude product. The crude product was purified by column chromatography on silica gel using a 1:1 mixture of hexane and 10% ammoniated methanol containing 10% ammonium hydroxide to yield the purified **2e** as a brownish, foamy solid. (HPLC purity, 96%, t^a_r=4.3, t^b_r=2.8, Table 4) MS (Cl) m/z 486 $(M+H)^+$; loop negative 484 (M-H), HRMS, m/z calcd for $C_{30}H_{32}NO_5$ 486.2280 found. ^{1}H NMR (300 MHz, d_6 acetone) δ 7.32 (2H, d, J = 8.70 Hz), 7.03 (1H, d, J = 8.37 Hz), 6.84 (3H, d, J = 8.60 Hz), 6.43 - 6.26 (5H, m), 5.37 (1H, d, J = 14.14 Hz), 5.06 (1H, d, d, J = 1.67, 14.14 Hz), 4.00 (2H, t, J = 6.14 Hz), 2.85 (2H, t, J = 6.11 Hz), 1.56 (8H, m).

Synthesis of 5-Phenyl-5,11-dihydro-chromeno[4,3-c]chromene-2,8-diol (**2f**)



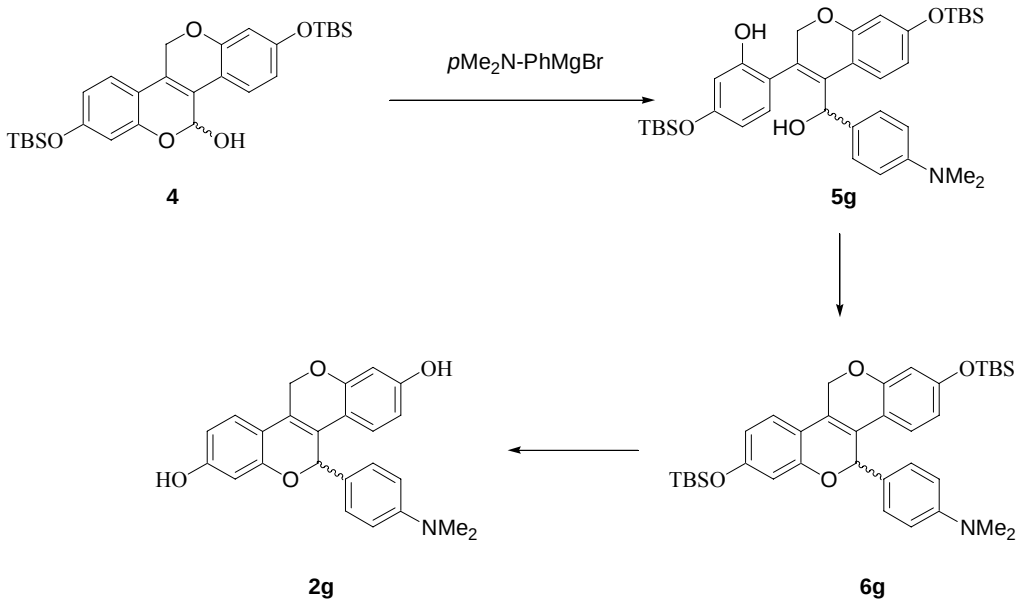
In a single neck, 50 mL round bottom flask was charged with PhMgBr (4.4 ml, 0.5 M in THF, 2.2 mmol) and cooloed to 0 °C. A tetrahydrofuran solution of 2, 8-bis-(*tert*-Butyl-dimethyl-silanlyoxy)-5,11-dihydro-chromeno [4,3-c]-chromen-5-ol, **4** (0.51 g, 1 mmol, in 10 mL of THF), was then added, the cooling bath was removed and the reaction mixture

was allowed to warm to room temperature overnight. After about 14 hours, the reaction was worked-up with addition of saturated ammonium acetate solution (80 mL) and extraction with ethyl ether (2 x 150 mL). The combined organic extracts were washed with brine and water, dried with anhydrous sodium sulphate, filtered and evaporated to yield a sticky semisolid residue. The **5f** was isolated as a viscous, colorless, semisolid foam via chromatography on silica gel eluted with 3% methanol/dichloromethane and was carried over to next reaction. To a stirred solution of **5f** (0.81 g, 1.23 mmol, 1eq), in tetrahydrofuran (50 mL) under argon at room temperature were added powdered molecular sieve (4 Å, 0.250 g) and triphenyl phosphine (0.73 g, 2.99 mmol) followed by diethyl diazodicarboxylate (0.52 g = 0.466 mL, 2.96 mmol). The reaction mixture was let run overnight (about 18 hours). The reaction mixture was evaporated to dryness, triturated with ether and the resulting colorless solid of triphenyl phosphine oxide removed by filtration. The filtrate was evaporated to dryness to yield a residue which was purified by column chromatography on silica gel using 2% methanol in dichloromethane as an eluent to yield the **6f** as a viscous semisolid MS (Cl) m/z 573 $(M+H)^+$, $(M+Na)^+$ ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.41 (2H, m), 7.28 (2H, m), 6.87 (1H, d, J = 8.30 Hz), 6.54 (1H, d, J = 8.40 Hz), 6.41 (1H, d, J = 2.30 Hz), 6.40 (1H, d, d, J = 2.34, 7.94 Hz), 6.21 (s, 1H, s), 5.31 (1H, d, J = 13.90 Hz), 5.10 (1H, d, d, J = 1.44, 13.90 Hz), 0.96 (9H, s), 0.93 (9H, s), 0.19 (6H, s), 0.16 (6H, s).

To a stirred solution of **6f** (0.31 g, 0.41 mmol), in tetrahydrofuran (15 mL) under nitrogen was added *tetra*-n-butyl ammonium fluoride (1M in tetrahydrofuran, 0.82 mL, 0.82 mmol) and the mixture was stirred for 3 hours. The reaction mixture was diluted with ethyl acetate (90 mL) and then washed with saturated aqueous ammonium chloride solution (85 mL). The precipitated inorganic salts were removed by filtration and washed with ethyl acetate. The combined organic phases were washed with saturated aqueous sodium bicarbonate solution (50 mL), dried (anhydrous sodium sulphate), filtered and evaporated to dryness to yield the crude product. The crude product was purified by column chromatography on silica gel using a 1:2 mixture of hexane and acetate to yield the purified **2f** as a yellow brownish, foamy solid. MS (Cl) m/z 345 $(M+H)^+$; loop negative 343 (M-H,)

^{1}H NMR (500 MHz, acetone-d_6): δ 8.49 (1H, brs), 8.47 (1H, s), 7.46 (2H, d, d, J = 1.76, 8.10 Hz), 7.31 – 7.26 (3H, m), 7.04 (1H, d, J = 8.38 Hz), 6.87 (1H, d, J = 8.38 Hz), 6.47 (1H, d, d, J = 2.43, 8.38 Hz), 6.38 (1H, d, d, J = 2.43, 8.38 Hz), 6.33 (1H, brs), 6.29 (1H, d, J = 2.43), 5.38 (1H, d, J = 14.08), 5.06 (1H, d, d, J = 1.67, 14.08 Hz). Anal. Calc. for $C_{22}H_{16}O_4$ 0.5 EtOAc: C, 72.21; H, 5.59; Found: C, 72.31; H, 5.70

Synthesis of 5-(4-Dimethylamino-phenyl)-5,11-dihydro-chromeno[4,3-c]chromene-2,8-diol (**2g**):



In a single neck, 50 mL round bottom flask was charged with 4-(N,N-dimethyl) aniline magnesium bromide (6 ml, 0.5 M in THF, 3 mmol) and cooloed to 0 °C. A tetrahydrofuran solution of 2, 8-bis-(*tert*-Butyl-dimethyl-silanlyoxy)-5,11-dihydro-chromeno [4,3-c]-chromen-5-ol, **4** (0.51 g, 1 mmol, in 10 mL of THF), was then added, the cooling bath was removed and the reaction mixture was allowed to warm to room temperature overnight. After about 14 hours, the reaction was worked-up with addition of saturated ammonium acetate solution (80 mL) and extraction with ethyl ether (2 x 150 mL). The combined organic extracts were washed with brine and water, dried with

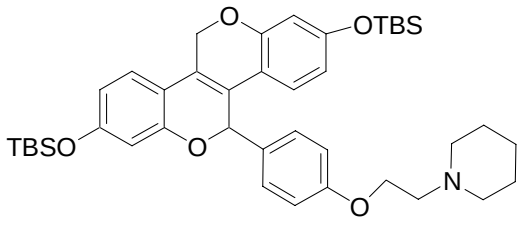
anhydrous sodium sulphate, filtered and evaporated to yield a sticky semisolid residue. The **5g** was isolated as a viscous, colorless, semisolid foam via chromatography on silica gel eluted with 3% methanol/dichloromethane and was carried over to next reaction. To a stirred solution of **5g** (0.77 g, 1.21 mmol, 1eq), in tetrahydrofuran (50 mL) under argon at room temperature were added powdered molecular sieve (4 Å, 0.250 g) and triphenyl phosphine (0.73 g, 2.99 mmol) followed by diethyl diazodicarboxylate (0.52 g = 0.466 mL, 2.96 mmol). The reaction mixture was let run overnight (about 18 hours). The reaction mixture was evaporated to dryness, triturated with ether and the resulting colorless solid of triphenyl phosphine oxide removed by filtration. The filtrate was evaporated to dryness to yield a residue which was purified by column chromatography on silica gel using 2% methanol in dichloromethane as an eluent to yield the **6g** as a viscous semisolid

MS (Cl) m/z 573 $(M+H)^+$, $(M+Na)^+$ ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.41 (2H, m), 7.28 (2H, m), 6.87 (1H, d, J = 8.30 Hz), 6.54 (1H, d, J = 8.40 Hz), 6.41 (1H, d, J = 2.30 Hz), 6.40 (1H, d, d, J = 2.34, 7.94 Hz), 6.21 (s, 1H, s), 5.31 (1H, d, J = 13.90 Hz), 5.10 (1H, d, d, J = 1.44, 13.90 Hz), 2.89 (6H, s), 0.96 (9H, s), 0.93 (9H, s), 0.19 (6H, s), 0.16 (6H, s).

To a stirred solution of **6g** (0.31 g, 0.41 mmol), in tetrahydrofuran (15 mL) under nitrogen was added *tetra*-n-butyl ammonium fluoride (1M in tetrahydrofuran, 0.82 mL, 0.82 mmol) and the mixture was stirred for 3 hours. The reaction mixture was diluted with ethyl acetate (90 mL) and then washed with saturated aqueous ammonium chloride solution (85 mL). The precipitated inorganic salts were removed by filtration and washed with ethyl acetate. The combined organic phases were washed with saturated aqueous sodium bicarbonate solution (50 mL), dried (anhydrous sodium sulphate), filtered and evaporated to dryness to yield the crude product. The crude product was purified by column chromatography on silica gel using a 1:2 mixture of hexane and ethyl acetate to yield the purified **2g** as a yellow brownish, foamy solid. MS (Cl) m/z 388 $(M+H)^+$; loop negative 386 (M-H) ^{1}H NMR (300 MHz, d_4 methanol) δ 7.21 (2H, d, J = 8.79 Hz), 6.92 (1H, d, J = 8.36 Hz), 6.71 (1H, d, J = 8.41 HZ), 6.64 (2H, d, J = 8.83 Hz), 6.33 (1H, d, d, J = 2.42, 7.70 Hz), 6.30 (1H, d, J = 2.39 Hz), 6.23 (1H, d, d, J = 2.43, 8.36 Hz), 6.12 (1H, d, J = 2.41 Hz), 6.08 (1H, s), 5.26 (1H, d, J = 13.95 Hz), 5.03 (1H, d, d, J = 1.62, 13.95

Hz), 2.86 ((6H, s). Anal. Calc. for $C_{24}H_{21}O_4N$ 0.5 EtOAc: C, 72.37; H, 5.84; N, 3.25 Found: C, 72.36; H, 5.96; N, 3.29

Synthesis of 1-(2-{4-[2,8-Bis-(tert-butyl-dimethyl-silanyloxy)-5,11-dihydro-chromeno[4,3-c]chromen-5-yl]-phenoxy}-ethyl)-piperidine **(6d):**

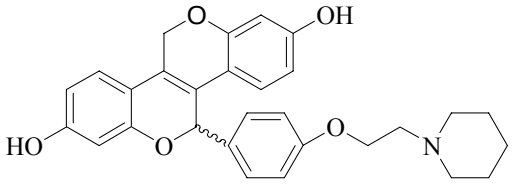


6d

1-[2-(4-Iodo-phenoxy)-ethyl]-piperidine(1.656, 5 mmol) was dissolved in THF and cooled to –78°C. To the reaction mixture, was then added n-butyl lithium (2M solution in pentane, 2.5mL, 10 mmol), slowly over 5 min. The resulting solution was stirred for 1h at –78°C. 2,8-Bis-(tert-butyl-dimethyl-silyloxy)-5,11-dihydro-chromeno[4,3-c]chromen-5-ol1g1.953 mmol) was dissolved in THF (20 mL) and then added to the reaction mixture containing 1-[2-(4-Iodo-phenoxy)-ethyl]-piperidine and n-butyl lithium, slowly over 10 min. The reaction mixture was stirred for an additional hour. The reaction mixture was quenched with MeOH (1mL) and then treated with a saturated solution of ammonium chloride (30 mL) and then diluted with diethyl ether (150 mL). The organic layer was separated and washed with brine (100 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and the solvent evaporated to yield a crude oil.

The crude oil was diluted with toluene (150 mL) and HCl (37%, 6.0 mL) and stirred for 30 min at room temperature. The solution was diluted with EtOAc (300 mL), the organic layer washed twice with water (100 ml) and then with a saturated solution of $NaHCO_3$ (150 ml). The organic layer was separated and dried over anhydrous Na_2SO_4, filtered, and evaporated to yield **6d** as a foamy material. (HPLC purity, 98%, t^a_r=7.1, t^b_r=4.7, Table 4), MS(CI) m/z 700 ($M+H^+$), 723 ($M+Na^+$), HRMS, m/z calcd for $C_{41}H_{58}NO_5Si_2$, ($M+H^+$)700.3854 found 700.3829, ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.30 (2H, d, J = 8.7 Hz), 6.87 (1H, d, J = 8.30 Hz), 6.79 (2H, d, J = 1.91, 6.82 Hz), 6.70 (1H, d, J = 8.42 Hz), 6.39 (2H, m), 6.29 (2H, m), 6.14 (1H, s), 5.30 (1H, d, J =13.90 Hz), 5.10 (1H, d, d, J = 1.654, 13.90 Hz), 4.04 (2H, t, J = 5.97 Hz), 2.48 (2H, t, J = 6.0 Hz), 2.48 (4H, m), 1.58 (4H, m), 1.43 (2H, m), 0.95 (9H, s), 0.93 (9H, s), 0.18 (6H, s), 0.16 (6H, s).

Synthesis of 5-[4-(2-Piperidin-1-yl-ethoxy)-phenyl]-5,11-dihydro-chromeno[4,3-c]chromene-2,8-diol (**2d**):

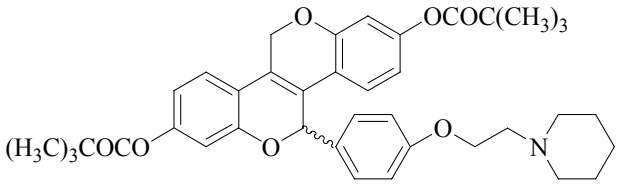


2d

To a stirred solution of **6d** (0.19 g, 0.2714 mmol, 1 eq), in tetrahydrofuran (15 mL) under nitrogen was added *tetra*-n-butyl ammonium fluoride (1M in tetrahydrofuran, 1.36 mL, 1.36 mmol, 5 eq) and the mixture was stirred for 3 hours. The reaction mixture was diluted with ethyl acetate (30 mL) and then washed with saturated aqueous ammonium chloride solution (35 mL). The precipitated inorganic salts were removed by filtration and washed with ethyl acetate. The combined organic phases were washed with saturated aqueous sodium bicarbonate solution (50 mL), dried (anhydrous sodium sulphate), filtered and evaporated to dryness to yield the crude product. The crude product was purified by column chromatography on silica gel using a 1:1 mixture of hexane and 10% ammoniated methanol containing 10% ammonium hydroxide to yield

the purified **2d** as a brownish, foamy solid. (HPLC purity, 96%, t^a_r=3.9, t^b_r=2.4, Table 4) MS (Cl) m/z 472 (M+H)+, 470 (M-H, loop negative), HRMS, m/z calcd for $C_{29}H_{30}NO_5$ 472.2124 found 472.2124, ^{1}H NMR (300 MHz, d-6 acetone): δ 8.46 (2H, br hump), 7.24 (2H, d, d, J = 1.93, 6.6 Hz, 6.91 (1H, d, J = 8.40 Hz), 6.71 (3H, d, J = 6.6 Hz), 6.29 (1H, d, d, J = 2.43, 8.34 Hz), 6.25 (1H, d, J = 2.40 Hz), 6.20 (1H, d, d, J = 2.43, 8.32 Hz), 6.13 (2H, d, J = 2.36 Hz), 5.25 (1H, d, J = 14.15 Hz), 4.93 (1H, d, d, J = 1.66, 14.13 Hz), 3.89 (2H, t, J = 6.02 Hz), 2.51 (2H, t, J = 6.02 Hz), 2.30 (4H, m), 1.37 (4H, m), 1.26 (2H, m).

Synthesis of 2,2-Dimethyl-propionic acid 8-(2,2-dimethyl-propionyloxy)-11-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-5,11-dihydro-chromeno[4,3-c]chromen-2-yl ester [**1**-(±)]:

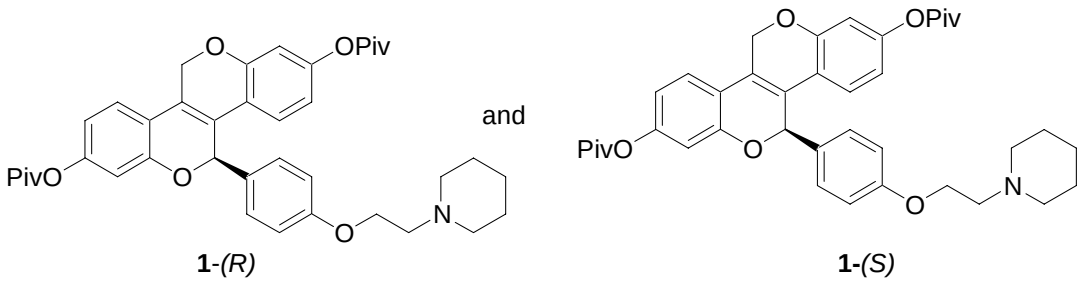


1-(±)

To an ice-cooled and stirred slurry of **2d** (0.200g, 0.424 mmol), in dichloromethane (10 mL) under nitrogen, was added triethylamine (0.2mL, 1.43 mmol, 3.5 eq). After about 10 minutes the reaction mixture was observed to become clear. To the reaction mixture was then slowly added (over a period of about 5 minutes) 2,2-dimethylpropionyl chloride (i.e., pivaloyl chloride, 0.157 mL, 1.3 mmol, 3.18 eq.). The cooling bath was then removed and the reaction mixture was allowed to warm to room temperature overnight. To the reaction mixture was then added saturated $NaHCO_3$ solution (20 mL) and the resulting solution was stirred at room temperature for 1 hour. The organic layer was separated and the aqueous layer re-extracted with dichloromethane (2x20 mL). The combined organic extracts were washed with brine, dried (anhydrous sodium sulphate), filtered and evaporated in vacuo. The residue was purified by chromatography on silica

gel using 2% methanol/ dichloromethane as an eluent to yield **1**-(±) as an ivory, crystalline solid. (HPLC purity, >98%, t^a_r=8.1, t^b_r=4.6, Table 4),
MS (Cl) m/z 640 $(M+H)^+$, HRMS, m/z calcd for $C_{39}H_{46}NO_7(M+H^+)$ 640.3274 found ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.30 (2H, d, J = 8.7 Hz), 7.01 (1H, d, J = 8.4 Hz), 6.83 – 6.78 (3H, m), 6.64 (1H, d,d, J = 2.3, 8.5 Hz), 6.63 (1H, d, J = 2.3 Hz), 6.54 – 6.49 (2H, m), 6.21 (1H, s), 5.37 (1H, d, J = 14 Hz), 5.16 (1H, d, J = 14 Hz), 4.05 (2H, t, J = 6.0 Hz), 2.74 (1H, t, J = 6.0 Hz), 2.49 (4H, brs), 1.59 (4H, m), 1.37 (2H, m), 1.32 (9H, s), 1.30 (9H, s), ^{13}C NMR (300 MHz, $CDCl_3$) δ 176.713, 176.53, 159.357, 154.346, 152.531, 151.960, 151.539, 129.907, 129.181, 123.524, 122.197, 121.683, 120.317, 118,142, 117.589, 114.767, 114.554, 114.367, 110.695, 109.814, 77.013, 74.879, 65.894, 64.253, 57.798, 54.938, 39.069, 39.052, 27.055, 25.916, 24.170 IR (KBr): 2972, 2934, 2872, 1754, 1611, 1585, 1510, 1498, 1220, 1175, 1157, 1127, 1109, 1026 cm^{-1} Anal. Calc. $C_{39}H_{45}NO_7/0.6H_2O$: C, 73.22; H, 7.09; N, 2.19. Found: C, 72.25; H, 7.06; N, 2.08

Chiral sepration of 2,2-Dimethyl-propionic acid 8-(2,2-dimethyl-propionyloxy)-11-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-5,11-dihydro-chromeno[4,3-c]chromen-2-yl ester [**1**-(±)]



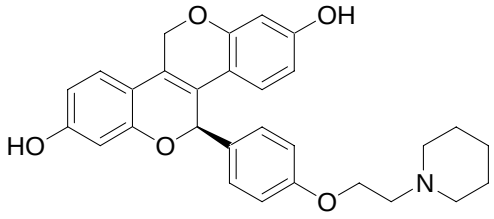
1-*(R)* **1**-*(S)*

The racemic compound (2,2-dimethyl-propionic acid 8-(2,2-dimethyl-propionyloxy)-5[4-(2-piperidin-1-yl-ethoxy)-phenyl]-5,11-dihydro-chromeno[4,3-c]chromen-2-yl ester) (2.5g) was loaded onto a ChiralPak AD chiral HPLC column (5 cm I.D. x 50 cm L) and eluted with 20%MeOH in IPA at the 90 mL/min flow rate. The two peaks were removed under vacuum to yield: **1**-*(S)**, first peak to elute, as white solid (HPLC purity, >99%, t^a_r=8.1, optical purity 99.1%, t^c_r =14.5, Table 4) mp 202-206, [α]= -70 (c = 0.52g/100 mL, $CHCl_3$) CHN analysis Calc'd for $C_{39}H_{45}NO_7$: C, 73.22; H, 7.09; N, 2.19 found: C,

72.79; H, 7.13; N, 2.14 and **1**-*(R)** as second peak to elute (HPLC purity, >99%, t^a_r=8.1, optical purity 99.1%, t^c_r =14.5, Table 4) mp 203-206, [α]= +71 (c = 0.32g/100 mL, $CHCl_3$) CHN analysis Calc'd for $C_{39}H_{45}NO_7$: C, 73.22; H, 7.09; N, 2.19 found: C, 73.20; H, 7.12; N, 2.15

- NMR spectral data were similar to 1-(±).
-

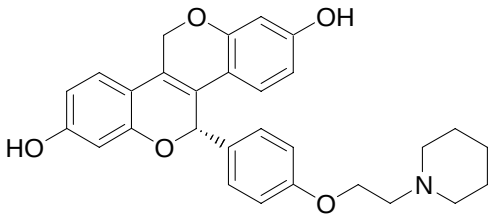
Synthesis of 5-*(R)*-[4-(2-Piperidin-1-yl-ethoxy)-phenyl]-5,11-dihydro-chromeno[4,3-c]chromene-2,8-diol [**2d**-*(R)*]:



2d-*(R)*

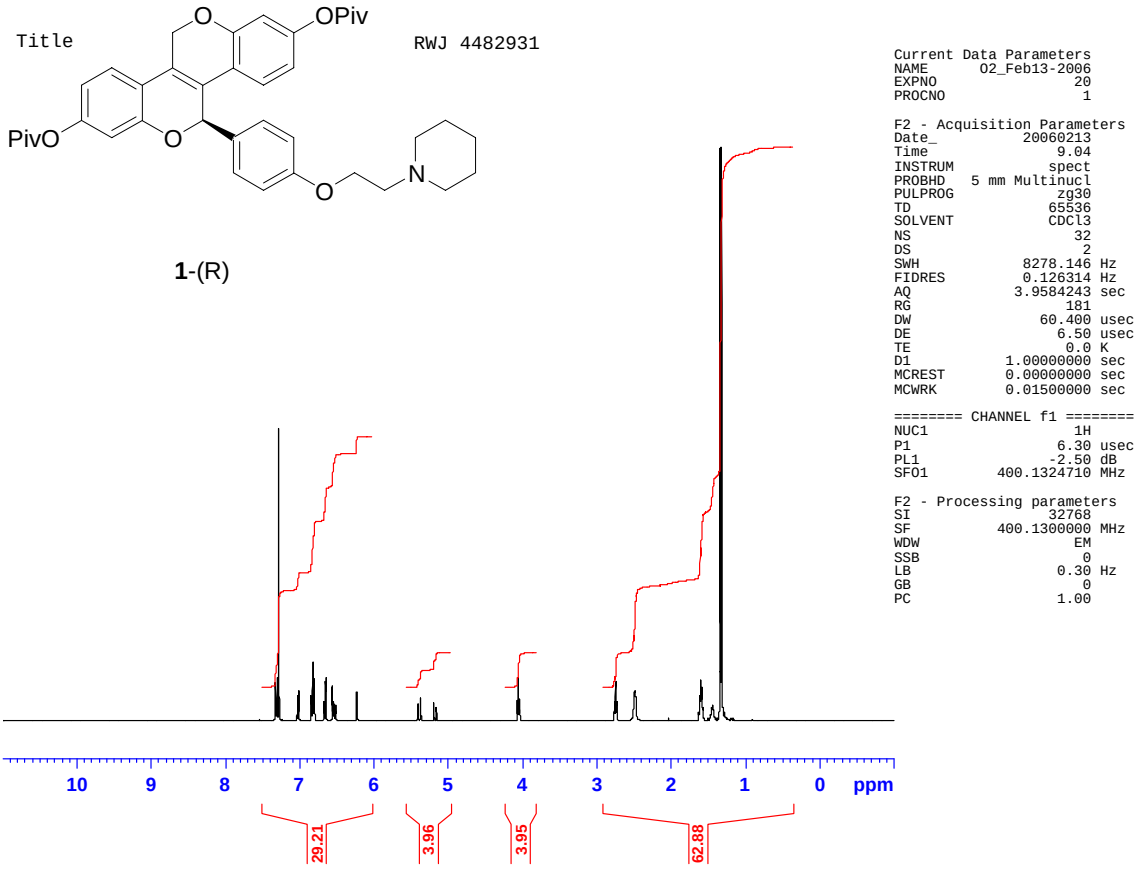
In a 50 mL high pressure vessel was charged with **1**-*(R)* (1.1g, 1.7 mmol), 30ml of MeOH and 10 ml 0f Et_2NH and was sealed. The reaction mixture was heated to 100 ^oC, for 3 hours. After about 3 hours, the reaction was cooled to room temperature and evaporated to yield a sticky semisolid residue. The crude product was purified by chromatography on silica gel eluted with 6-7% methanol/dichloromethane to yield **2d**-*(R)* as dark yelow to brownish solid. (HPLC purity, 95.1%, t^d_r=3.1, optical purity 99.1%, t^c_r =6, Table 4) mp 203-206, [α]= +51 (c =o.16, acetone) HRMS, m/z calcd for $C_{29}H_{30}NO_5$ 472.2124 found 472.2124, Anal. Calc. for $C_{29}H_{29}O_5N$ 2.1 Et_2NH: C, 69.72; H, 8.49; N, 6.09 Found: C, 69.69; H, 8.51; N, 6.11

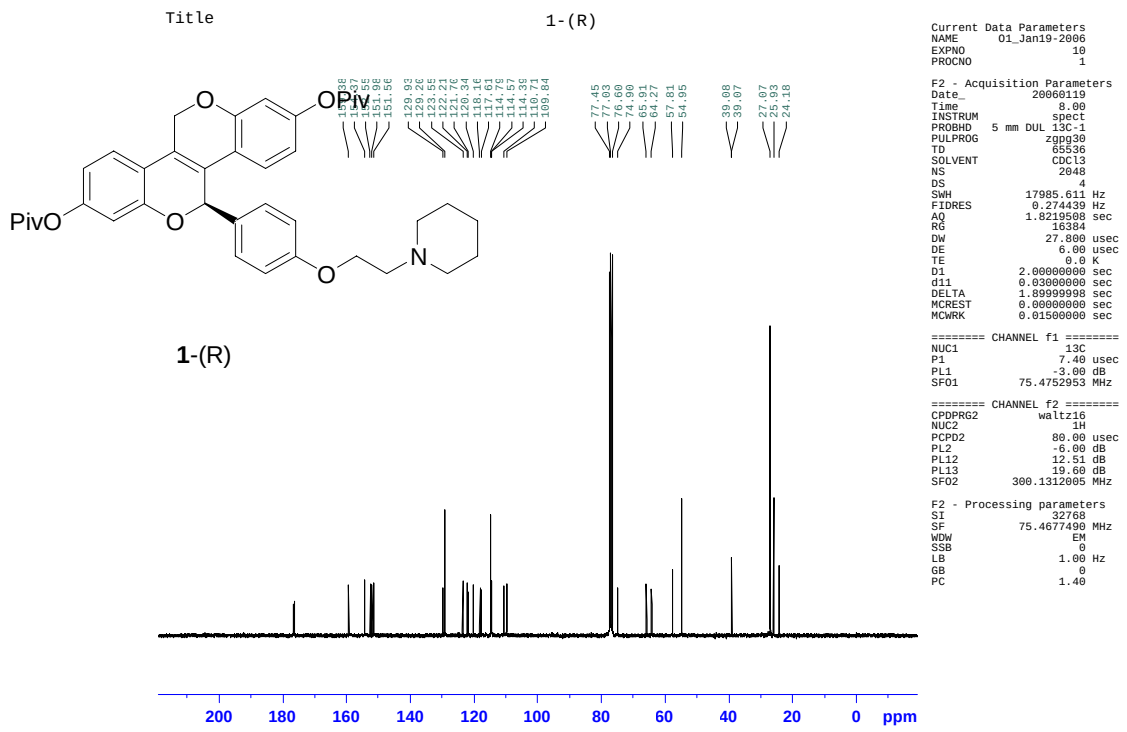
Synthesis of 5-*(R)*-[4-(2-Piperidin-1-yl-ethoxy)-phenyl]-5,11-dihydro-chromeno[4,3-c]chromene-2,8-diol [**2d**-*(S)*]:



2d-*(R)*

Similar procedure as **2d**-*(S)* yielded as dark yelow to brownish solid. (HPLC purity, 94.4%, t^d_r=3.1, optical purity 96.1%, t^c_r = 6, Table 4) mp 203-206, [α]= -47 (c =o.17, acetone) HRMS, m/z calcd for $C_{29}H_{30}NO_5$ 472.2124 found: 472.2128



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F2 - Processing parameters
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Table 4: Purity based on HPLC and HPLC for data for target compounds

Compound	% Purity (Based on HPLC)	Retention time based on major peak*				Analytical Data (HRMS or CHN analysis)
		t^a_r (HPLC condition **a**)	t^b_r(HPLC condition **b**)	t^c_r (HPLC condition **c**)	t^d_r (HPLC condition **d**)	
3b	97%	12.8	--	16.4		Anal. Calc. for $C_{28}H_{38}O_5Si_2$: C, 65.84; H, 7.50. Found: C, 65.81; H, 7.56.
4	>97%	10.4		17.5		Anal. Calc. For: $C_{28}H_{40}O_5Si_2 0.5CH_2Cl_2$: C, 61.65; H, 7.44. Found: C61.66; H, 7.44.
2a	95%	4.2	2.8	-	-	Anal. Calc. for $C_{26}H_{25}O_5N$-0.4 MeOH: C, 71.37; H, 6.03; N, 3.15 Found: C, 71.36; H, 6.06; N, 3.19
2b	97%	4.01	2.1	-	-	Anal. Calc. for $C_{28}H_{27}O_6N$ 0.6 MeOH: C, 69.71; H, 6.01; N, 2.84 Found: C, 69.81; H, 6.05; N, 2.86
2c	96%	4.1	2.7			HRMS, m/z calcd for $C_{28}H_{28}NO_5$ $(M+H)^+$ 458.1967 found 458,1959
2d	96%	3.9	2.4			HRMS, m/z calcd for $C_{29}H_{30}NO_5$ 472.2124 found 472.2124
2e	96%	4.3	2.8			HRMS, m/z calcd for $C_{42}H_{60}NO_5Si_2$ 714.4010 found 714.3998
2f	>95%			13.3		Anal. Calc. for $C_{22}H_{16}O_4$ 0.5 EtOAc: C, 72.21; H, 5.59; Found: C, 72.31; H, 5.70
2g	>97%			16.3		Anal. Calc. for $C_{24}H_{21}O_4N$ 0.5 EtOAc: C, 72.37; H, 5.84; N, 3.25 Found: C, 72.36; H, 5.96; N, 3.29
1-*(S)*	>98% ee: >99	8.1			14.4	Anal. Calc. for $C_{29}H_{29}O_5N$ 2.1 Et_2NH: C, 69.72; H, 8.49; N, 6.09 Found: C, 69.69; H, 8.51; N, 6.11
1-*(R)*	>98% ee: >99	8.1			21.7	CHN analysis Calc'd for $C_{39}H_{45}NO_7$: C, 73.22; H, 7.09; N, 2.19 found: C, 73.20; H, 7.12; N, 2.15

(*) [a] Phenonmenex, luna 5µ, phrnyl-hexyl 150x4.60 mm, Solvent A: H_2O (0.1% TFA), Solvent B: CH_3CN (0.1% TFA), gradient 20-90% of solvent A to B, flow rate: 1ml/min, total run time: 15 min. [b] phenonmenex, luna 5µ150x4.60 mm, Solvent A: H_2O (0.1% TFA), Solvent B: CH_3CN (0.1% TFA), gradient 20-90% of solvent A to B, flow rate: 1ml/min, total run time: 15 min.; [c]YMC diol 120 100 x 4.6 mm (achiral, normal phase) column, solvent system 50% IPA in hexanes, Isocratic solvent system, flow rate: 1 min / mL, run time: 20 min.;[d] Diacel ChiralPak AD 250 x 4.6 mm, 10 µ, (chiral) column using an isocratic solvent mixture 50:50 IPA/Hexanes, at a flow rate of 1 mL/min, run time: 50 min.

Alkaline Phosphatase Assay in Human Endometrial Ishikawa Cells

This assay was run according to the procedure described by Albert et a., *Cancer Res*, **(9910)**, 50(11), 330-6-10, with minor modification.

Ishikawa cells (from ATCC) were maintained in DMEM/F12 (1:1) phenol red free medium (Gibco) supplemented with 10% calf serum (Hyclone). 24 hours prior to testing, the medium was changed to DMEM/F12 (1:1) phenol red free containing 2% calf serum. Compounds to be tested in the agonist mode were added to the culture media at varying concentrations. Compounds to be treated in the antagonist mode were prepared similarly, and 10 nM 17β-estradiol was also added to the culture media. The cells were then incubated at 37^{o}C for 3 days. On the fourth day, the media was remove, 1 volume of 1X Dilution Buffer (Clontech) was added to the well followed by addition of 1 volume of Assay Buffer (Clontech). The cells were then incubated at room temperature for 5 minutes. 1 volume of freshly prepared Chemiluminescence Buffer (1 volume of chemiluminescent substrate (CSPD) in 19 volume Chemiluminescent Enhancer with final concentration of CSPD at 1.25 mM; Sigma Chemical Co.) was added. The cells were incubated at room temperature for 10 minutes and then quantified on a luminometer. The increase of chemiluminescence over vehicle control was used to calculate the increase in alkaline phosphatase activity.

MCF-7 Cell Proliferation Assay

This assay was run according to the procedure described by Welshons, et al., (*Breast Cancer Res. Treat.,* **1987**, 10(2), 169-75), with minor modification. Briefly, MCF-7 cells (from Dr. C. Jordan, Northwestern University) were maintained in RPMI 1640 phenol red free medium (Gibco) in 10% FBS (Hyclone), supplemented with bovine insulin and non-essential amino acid (Sigma). The cells were initially treated with 4-hydoxyltamoxifen (10^{-8} M) and let stand at 37^{o}C for 24 hours. Following this incubation with tamoxifen, the cells were treated with compounds at various concentrations. Compounds to be tested in the agonist mode were added to the culture media at varying concentrations. Compounds to be treated in the antagonist mode were prepared similarly,

and 10 nM 17β-estradiol was also added to the culture media. The cells were incubated for 24 hours at 37°C. Following this incubation, 0.1 μCi of ^{14}C-thymidine (56mCi/mmol, Amersham) was added to the culture media and the cells were incubated for an additional 24 hours at 37°C. The cells were then washed twice with Hank's buffered salt solution (HBSS) (Gibco) and counted with a scintillation counter. The increase in the ^{14}C-thymidine in the compound treated cells relative to the vehicle control cells were reported as percent increase in cell proliferation.

Estrogen Receptor β Fluorescence Polarization Assay

This assay monitors binding of a fluorescent analog of estrogen (Fluormone ES2, Panvera) to the estrogen receptor. Plates are read in a fluorometer that can be set to polarization mode. A decrease in fluorescence relative to vehicle control is an indication of binding of a compound to the receptor.

It is crucial to avoid introduction of air bubbles into the reaction in each well of the 96 well plate throughout this procedure. (Bubbles on the surface of the reaction disrupt light flow, affecting the polarization reading.) However, it is also crucial to effectively mix the reaction components upon addition to the well.

On ice, a 2X standard mixture of Assay Buffer (Panvera), 10 nM DTT and 40 nM ES2 was prepared. On ice, a 2X reaction mixture of Assay Buffer (Panvera), and 20 nM hER-β (Panvera) and 40 nM ES2 was also prepared.

Dilutions of test compound were prepared in 30% (v/v) dimethyl sulfoxide/50 mM HEPES, pH 7.5. At this point, the dilutions were 40X the final required concentration.

The standard mixture at 50 μL was then added to each well. The reaction mixture at 48 μL was added to all wells. The compound dilution at 2.5 μL was added to the appropriate wells. The reaction mixtures were mixed using a manual pipette, a roll of aluminum foil adhesive cover was placed on the plate and the plate incubated at room temperature for 1 hour.

Each well on the plate was then read in an LjL Analyst with an excitation wavelength of 265 nm and an emission wavelength of 538.

Estrogen Receptor α Flash Plate Assay

This assay monitors binding of radiolabeled estrogen to the estrogen receptor. It is performed on a BioMek 2000 (Beckman). Plates are read in a scintillation counter (Packard TopCount), with decreased counts an indication of binding of a compound to the receptor. The assay was run according to the procedure described by Allan, et al., *Anal. Biochem.* **(1999)**, 275(2), 243-247.

On day one, 100 μL of Estrogen Screening Buffer (ESB, Panvera) containing 5mM dithiothreitol (DTT, Panvera), 0.5 μg mouse anti-estrogen receptor monoclonal antibody (SRA-1010, Stressgen) and 50 ng purified human estrogen receptor α (Panvera) were added to each well of a 96 well FlashPlate Plus plate crosslinked with goat anti-mouse antibodies (NEN Life Sciences). The plate was sealed and incubated at 4°C overnight.

On day two, each well was washed three times with 200 μL PBS, pH 7.2, at room temperature. To each well was then added 98 μL radiolabeled estrogen (0.5 nM, which equals 6 νCi for a 120 Ci/mmol batch, Amersham), diluted in ESB and 5mM dithiothreitol (DTT). To individual wells were then added 2.5 μL test compound diluted in 30% (v/v) dimethyl sulfoxide/50 mM HEPES, pH 7.5. The wells were mixed three times by aspiration, the plate sealed and incubated at room temperature for one hour. The wells were then counted for 1 min in a TopCount scintillation counter (Packard)

Table 1. SAR of chromene-derived bisbenzopyrans[a]

Compounds	ERα Binding RBA, %	ERβ Binding RBA, %	Ishikawa Cells[b] IC_{50} (nM)	MCF-7 Cells[c] IC_{50} (nM)
2a	61(4)	40(4)	180(4)	220(4)
2b	31(3)	90(3)	410(3)	1400(3)
2c	35(3)	47(3)	80(4)	150(4)
2d	340(5)	170(5)	12(4)	110(4)
2e	55(3)	50(3)	13(4)	660(4)
2f	13(3)	110(3)	(24)[d](4)	(94)[d](4)
2g	6.4(3)	42(3)	3800(3)	(71)[d](3)
Raloxifene	64(3)	58(3)	23(3)	222(3)

[a]All compounds were tested more than 3 times in these assays with high reproducibility and *n* values are in parenthesis. .[b]No agonist activity were found at 10 μM concentration except compound **2f**. [c] No agonist activity were found at 10 μM concentration except compound **2f** and **2g**. [d]Agonists, EC_{50} shown in parentheses; showed 13-22% inhibition at 10 μM concentration in antagonist format

Table 2. In vitro profiles of **1**-*(R)* and **1**-*(S)* and its active metabolites **2d**-*(R)* and **2d**-*(S)*[a]

Compound	ERα Binding IC_{50} (nM)	ERβ Binding IC_{50} (nM)	Human Endometrial Ishikawa Cell[b] IC_{50} (nM)	Human Breast MCF-7 Cell[b] IC_{50} (nM)
Ethinyl estradiol	2.0(10)	8.1(10)	NA	NA
Raloxifene	1.8 (5)	8.2(5)	24.6(5)	222(5)
1-*(R)*	>10,000(10)	>10,000(10)	175[c](5)	510[c](5)
2d-*(R)*	51(5)	20.3(5)	43.3(5)	599(5)
1-*(S)*	>1,000(10)	>10,000(10)	99[c](5)	670[c](5)
2d-*(S)*	0.61(5)	1.3(5)	17.1(5)	416(5)

[a]All compounds were tested more than three times in these assays with high reproducibility and *n* values are in parenthesis. [b]No agonist activity were found at 10 μM concentration. [c] **1**-*(R)* and **1**-*(S)* may be getting hydrolyzed to **2d**-*(R)* and **2d**-*(S)* respectivelly[17]

Protocol for immature rat uterotropic study

Introduction

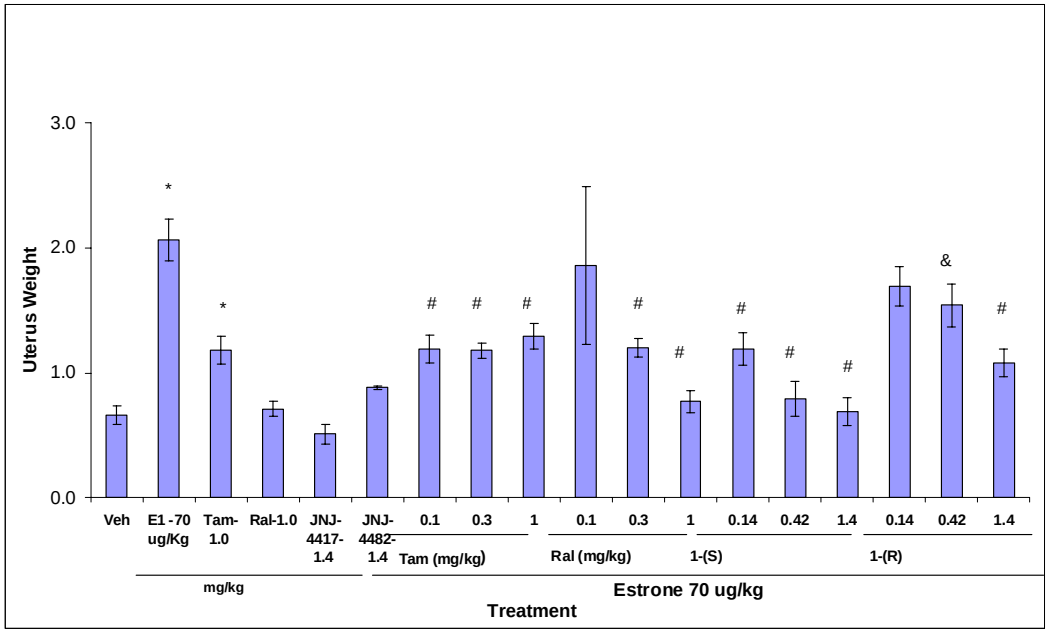
Selective Estrogen Receptor Modulators (SERM) act as estrogen agonist or antagonist on uterus. It is well established that estrogens are known for their uterotropic activities to stimulate uterine growth. The immature rat uterotropic model (Reference 1-3) is used to get a rapid and accurate assessment of the activity of a compound in the uterus. This can be used in either the agonist mode (compound alone) or antagonist mode (compound + estrogen). Because the animals have not matured sexually, there is minimal endogenous estrogen to complicate the evaluation. Immature rats which are unexposed to estrogen, are administered with an estrogen, Estrone for three days. The uteri grow rapidly, and the weight of uterus increases sharply in three days. Co-treatment with estrogen antagonist could block the stimulation, whiles estrogen agonist synergistically enhances the stimulation. The difference between the weight of uterus from vehicle control animals and that from treated animals is a sensitive indicator of estrogen agonist or antagonist activity. This model has been used as a classical measure to evaluate activities of estrogen agonists and antagonists including SERMs. The affects of these compounds in this model have been predictive of the clinical responses in reported women.

Materials and methods

Eighteen Nineteen days old immature rats (45-55 gm) are obtained from Charles River Laboratories (Wilmington, MA). They are housed in groups of three in wire-mesh cages at an ambient temperature of 21 to 23 °C with an automated 12/12 hour light/dark cycle and access to water and a commercial rodent food ad libitum. The rats are treated daily for three consecutive days with Estrone (Sigma, St. Louise, MO), at 70 μg/kg/day (in 0.1 ml of sesame oil, s.c..) alone or along with testing compounds with oral administration by gavaging. There are three animals per group. The rats are euthanized 24 hours after the final dose, and their uteri are were excised, cleaned of surrounding fat and connective tissue, incised slightly to release luminal fluid, blotted on filter paper, and weighed. The

ratio of uterine weight and body weight is expressed as an indicator of uterotropic activity.

Figure 5. Effect of **1**-*(R)* and **1**-*(S)* on Uterine Weight in Immature Rats



Note: *: significant difference compared to Veh control (p<0.001);
#: significant difference compared to E1-70 ug/kg (p<0.001);
&: significant difference compared to E1-70 ug/kg (p<0.05);
Tam: Tamoxifen; Ral: Raloxifene; E1: Estrone

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Protocol for in vivo ovariectomized rat model

Introduction:

The adult ovariectomized estrogen-deficiency rat model is applied to evaluate the tissue selective effects of Selective Estrogen Receptor Modulators (SERM). The model is useful because the responses in several tissues can be used to evaluate the tissue selective properties of SERM compounds. It provides information on ovariectomy-induced bone loss, and plasma lipids, uterine and vaginal effects, as well as other pathological changes in cardiovascular system, and reproductive system. This model has been used to characterize many estrogen agonist and antagonist activities of SERMs (1). The treatment of testing compounds can be adjusted for 2 weeks or 6 weeks. Bone density measurement on isolated bones is conducted in the 6-weeks animal model. The affects of these compounds in this model have been in line with the clinical responses in reported women (2).

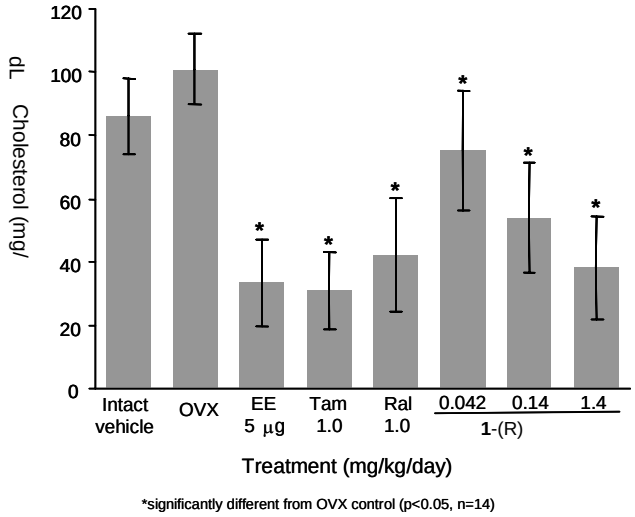
Materials and methods

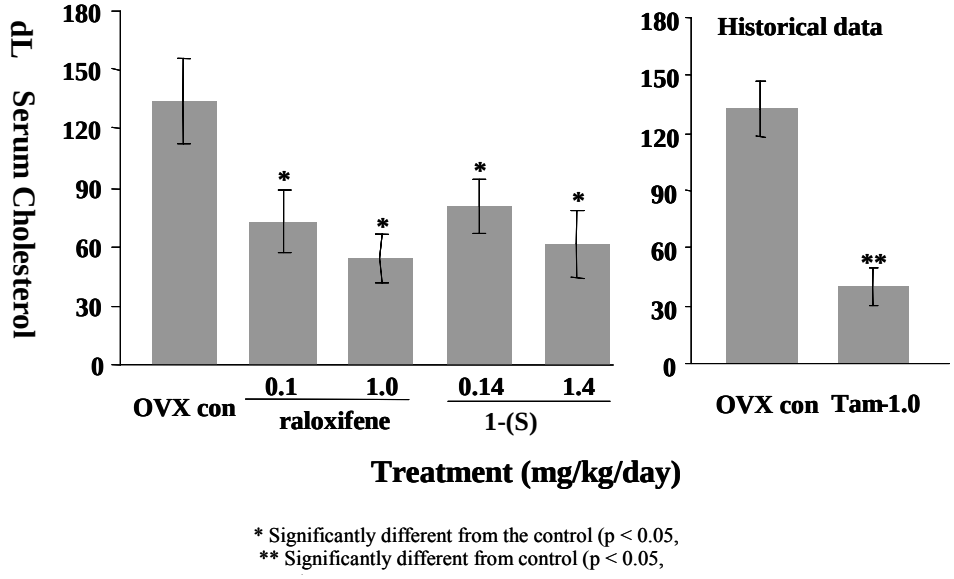
Adult female animals (> 6 months old, Charles River Laboratories, Wilmington, MA) are used. The rats are housed individualy in wire-mesh cages at an ambient temperature of 21 to 23 °C with an automated 12/12 hour light/dark cycle and access to water and a commercial rodent food ad libitum. Each treatment group consisted of 6-14 animals. The animals are ovariectomized under sterile condition and anesthesia. Twenty four hours after the surgery, testing compounds are administered daily by gavaging for 6 weeks. Other reference treatment groups include sham-operated control, and ovariectomized control, ethanylestradiol (EE, 5 mg/kg/day), and raloxifene (1 mg/kg/day). 0.5% Methocel is used as the vehicle for all compounds.

Measurement of serum total cholesterol levels

Blood samples are collected orbitally, after 2 weeks of treatment and or at the end of study. Serum samples are shipped to LarCorp LabCorp (Burlington, NC) and analyzed with a Roche Hitachi 717 Chemistry Analyzer. All reagents are obtained from Roche Diagnostics.

Figure 6: Effect of **1**-*(R)* and **1**-*(S)* on Serum Total Cholesterol Levels in Ovariectomized Rats after Two Weeks of Treatment.





Vaginal cytology

Vaginal smear is taken by flushing the vagina with water using a pipette. The water containing vaginal epithelial cells is put on to a slide followed by examination of

cytology of the epithelial cells under microscope to determine the cycling stage of the animal.

Measurement of uterine weight

Animals are euthanatized at the end of study with CO_2. The uteri are excised, cleaned of surrounding fat and connective tissue, incised slightly to release luminal fluid, blotted on filter paper, and weighed.

Measurement of uterine epithelial thickness

1. Immunohistochemistry

Immunohistochemistry is performed as described in Reference 5. All incubations are performed at room temperature. After microwaving the slides in Target (Dako, Carpenturia, CA), the slides are placed in PBS, then 3% H2O2, rinsed in PBS and then appropriate blocking serum was added for 10 minutes. Subsequently, the primary antibodies (cocktail: pan-cytokeratin (1:25, Sigma, St. Louis, MO) and smooth muscle actin (1:100, Dako) are applied to the slides for 30 minutes. Proper species isotype antibody (Vector Labs, Burlingham, CA) is substituted as the primary antibody for the negative control. After several PBS washes, a biotinylated secondary antibody (Vector Labs) is placed on the slides for 30 minutes. Subsequently, the slides are washed in PBS and then the avidin-biotin complex (ABC, Vector Labs) was applied to the cells for 30 minutes. The presence of the primary antibodies is detected by adding DAB (3'-diaminobenzidine HCl; Biomeda, Foster City, CA) for 2 times 5 minutes. Slides are briefly exposed to Mayer's hematoxylin for 1 minute, dehydrated and coverslipped.

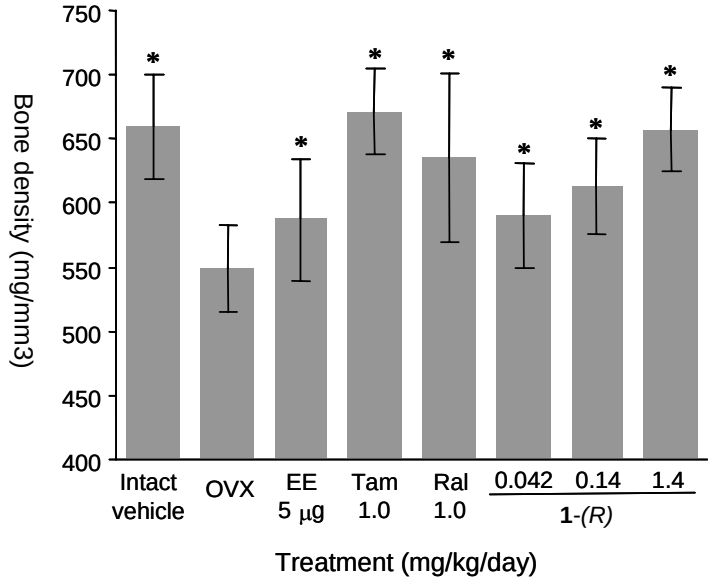
2. Image analysis methods

Image analysis is applied to count the thickness of the epithelium, which is easily identified by the antibody labeling. The image analysis is performed using an Olympus BX50 light microscope, Sony 3CCD digital camera interfaced with a IBM 350PC using Image Pro (v 3.0) analysis software (Phase 3 Imaging Systems, Glen Mills, PA). A special tool is used to draw a line at the base of the epithelial cells of the uterus, while another line is draw along the apical, luminal surface of the epithelial cells. The computer calculates average distance between the two traced lines.

Measurement of bone mineral density

After euthanatization, the left tibia is removed from the animal, and is defleshed and fixed in 10% of Formalin. Ex-Vivo pQCT is conducted at Dr. Jee's laboratory at the Radiobiology Division, University of Utah, Salt Lake City, UT. The trabecular and cortical BMD and BMC measurements are carried out using a XCT□960A peripheral quantitative computerized tomography system (pQCT, Norland Medical Systems, Fort Atkinson, WI) on the proximal tibial metaphysis at 5 mm and 6 mm distal to the knee joint. A voxel size of 0.148 mm is used and a threshold of 0.600cm□1 for cancellous bone is used.

Figure 7: Effect of **1**-(R) on Total Bone Mineral Density of Tibia in Ovariectomized Rats



*significantly different from OVX control (p<0.05,

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Protocol for ovariectomized rat hot flush model

Introduction

Selective estrogen receptor modulators (SERM) have divergent activities depending on the tissue it is acting upon. That is it has agonist properties in some tissues and antagonist properties in another. One of the important activities of SERM compounds is the ability to increase or reduce the incidence and severity of the hot flush, a symptom that frequently occurs in postmenopausal women (Reference 1). This activity is rtested in a rodent model for hot flush.

In the ovariectomized rat hot flush model, morphine-addicted rats undergo morphine withdrawal, after which they experience a “hot flush” that can be measured by their tail skin temperature. Estrogens have been shown to block this hot flush (2-6). This model has been used to characterize several SERMs including raloxifene and bazedoxifene (6).

The affects of these compounds in this model have been predictive of the clinical responses in reported women (2, 7-9).

Materials and methods

Adult female Sprague-Dawley rats (3 months old, Charles River Laboratories, Wilmington, MA) are used. Each treatment group consisted of 8 to 25 animals. They are housed individually in wire-mesh cages at an ambient temperature of 21 to 23 °C with an automated 12/12 hour light/dark cycle and access to water and a commercial rodent food ad libitum. The rats are ovariectomized under anesthesia. Six days after ovariectomy, treatment of the rats is initiated. All compounds are administered either testing compounds or vehicle (sesame oil), ethinyl estradiol (EE), and raloxifene orally by gavage. The rats are injected (s.c.) with a suspension containing 75 mg and 150 mg of morphine (freebase) on day 3 and day 5 of treatment, respectively. On the last day of treatment, the animals are lightly anesthetized with ketamine (80 mg/kg, i.m.). Following the anesthesia, a thermistor (YSI 400 series, YSI Precision Temperature Group, Dayton, OH), connected to a data acquisition system (Acquisition interface Model ACQ-10, Gould 6600 Amplifier, Gould Instrument System Inc. Valley view, OH), is placed on the tail of the animals. Following the measurement of the baseline tail skin temperature for about 20 minutes, naloxone (2.0 mg/kg, s.c. Sigma, St. Louis, MO) is administered to induce the morphine withdrawal. Tail skin temperature is then measured for an additional 60 minutes. Multiple comparisons among the treatment groups at each time point are used for analysis. The values of maximal temperature change (ΔT) are reported. Statistical analysis (t-test) is conducted by Preclinical Biostatistics, JJPRD with an analysis program of Wilcoxon Ranl Sum Test from Statxact (Version 4, Statistical Solutions, Saugus, MA).

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