Supporting information for

Identification of Potent and Selective Diphenylpropanamide RORγ Inhibitors

Jun R. Huh,[‡] Erika E. Englund,[†] Hang Wang,[†] Ruili Huang,[†] Pengxiang Huang,[⊥] Fraydoon Rastinejad,[⊥] James Inglese,[†] Christopher P. Austin,[†] Ronald L. Johnson,[†≠] Wenwei Huang,[†*] Dan R. Littman[‡ #*]

[†]NIH Chemical Genomics Center, National Center for Advancing Translational Sciences, National Institutes of Health, 9800 Medical Center Drive, Bethesda, MD, 20892-3370.

[‡]Molecular Pathogenesis Program, The Kimmel Center for Biology and Medicine of the Skirball Institute, New York University School of Medicine, New York, NY 10016, USA

[#]Howard Hughes Medical Institute

[⊥]Sanford-Burnham Medical Research Institute at Lake Nona, 6400 Sanger Road, Orlando, FL 32827, USA

[≠]Present address: Division of Cancer Biology, National Cancer Institute

*Corresponding Author: WH Tel: 301-217-5740. Email: huangwe@mail.nih.gov.
Or DRL Tel: 212-263-5711. Email: Dan.Littman@med.nyu.edu.

Materials and Methods

Biology

The RORγ assay employed *Drosophila* Schneider cells that stably expressed two vectors: a vector encoding a fusion of the Gal4 DNA binding domain and RORγt transactivation domain under the control of the metallothionine promoter, and a *Photinus* luciferase reporter regulated by the Gal4 binding site enhancer, UAS. Addition of 700 μM copper to the medium induced expression of the Gal4-RORγt fusion, which subsequently led to expression of the luciferase reporter. Small molecule inhibitors of RORγ activity were detected by a decrease in luciferase reporter activity.

To identify nonspecific and nonselective inhibitors, three other engineered *Drosophila* Schneider cell lines were used; these employed the same methodology, but differed only in the Gal4-transactivation domain fusion. The VP16 assay utilized a fusion of the Gal4 DNA binding domain to the transactivation domain of VP16, a herpes virus-encoded protein that functions as a general transcriptional activator. The VP16 assay was used as a counter screen for the RORγ reporter assay in both the primary and confirmatory screens to identify nonspecific inhibitors that suppress general transcription or luciferase activity. The other two assays, termed RORα and DHR3, utilized the Gal4 DNA binding domain fusion to the transactivation domain of RORα or DHR3 (*Drosophila* hormone receptor 3). These assays were used to confirm the selectivity of RORγ inhibitors. The itemized protocol used for all four assays is shown below in Table 1. Screen and follow up assay data were deposited in PubChem (AID 2604).

Table 1. Itemized protocol for RORγ, VP16 and RORα reporter assays used in the qHTS and follow up studies.

Step	Parameter	Value	Description
1	Reagent	4 uL	600 cells/well
2	Compound	23 nL	46 uM – 1.7 nM
3	Time	1 hr	Ambient incubation
4	Reagent	1 uL	0.7 mM copper sulfate
5	Centrifuge	30 sec	1000 RPM
6	Time	20 hr	Ambient incubation
7	Reagent	1.5 uL	*Photinus* luciferase detection mix
8	Time	10 min	Ambient incubation
9	Detector	Luminescence	ViewLux

Notes. Cells (600/well) were dispensed into white solid 1536-well plates (Greiner) using a solenoid-based dispenser. Following transfer of 23 nL compound or DMSO vehicle by a pin tool, the plates were incubated 1 h at ambient temperature, and 1 µL/well copper sulfate (700 µM final concentration) was added. The plates were centrifuged 15 s at 1000 RPM and incubated 20 h at ambient temperature. After addition of 1.5 µL *Photinus* luciferase detection reagent, the plates were incubated 10 min at ambient temperature and then read by a ViewLux (Perkin Elmer) to detect luminescence. Raw luminescence reads for each titration point were first normalized relative to the positive control compound (-100%) and DMSO-only wells (0%) as follows: % Activity = $((V_{compound} - V_{DMSO})/(V_{pos} - V_{DMSO})) \times 100$, where $V_{compound}$ denotes the compound well values, V_{pos} denotes the median value of the positive control wells, and V_{DMSO} denotes the median values of the DMSO-only wells. Concentration–response titration points for each compound were then fitted to a four-parameter Hill equation (Hill, A. V., *J Physiol (London)*, **1910**, 40: 4-7) yielding concentrations of half-maximal inhibition (IC_{50}) and maximal response (efficacy) values (Wang, Y.; Jadhav, A.; Southall, N.; Huang, R.; Nguyen, D.T., *Curr Chem Genomics*, **2011**, 4: 57-66).

Nuclear receptors profiling:

Profiling of **4n(-)** in a panel of 20 nuclear receptors for antagonistic activity was done by Invitrogen Inc.

Table 2. List of tested nuclear receptors with IC_{50} values of **4n(-)**.

Nuclear Receptor	AR	ER-alpha	ER-beta	ERR-alpha	FXR	GR	LXR-alpha	LXR-beta	MR	PPAR-delta
IC50 (nM)	>20000	>20000	>20000	14100	>20000	>20000	10500	>20000	>20000	>20000
Nuclear Receptor	PPAR-gamma	PR	RAR-alpha	RAR-beta	RAR-gamma	RXR-alpha	RXR-beta	TR-alpha	TR-beta	VDR
IC50 (nM)	>20000	>20000	>20000	>20000	>20000	>20000	>20000	4480	12700	>20000

RORγ Binding assay:

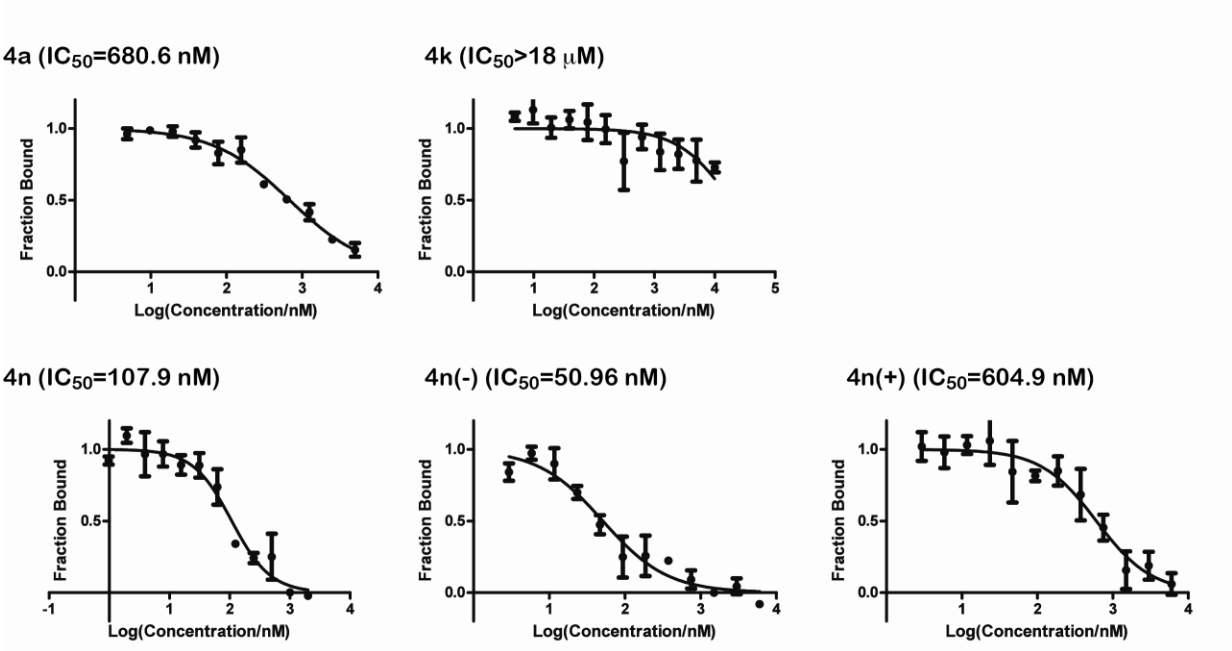


Figure 1. A series of diphenylpropanamides bound to human RORγ. Recombinant human RORγ LBD was loaded with fluorescently-labeled 25-hydroxycholesterol in the presence of the indicated concentrations of diphenylpropanamide compounds, and fluorescence polarization was measured.

***In vitro* binding and competition assay.** Human RORγ LBD (residues 262–518) with an N-terminal His-tag and a thrombin cleavage site, was expressed in BL21-CodonPlus(DE3)-RIL *E. coli* cells (Stratagene) using a pET46 Ek/Lic vector. The cells were induced with 0.5 mM IPTG at 16 °C for 12 h, then collected and lysed in 20 mM Tris-HCl (pH 8.0), 500 mM NaCl, 20 mM imidazole and 10% glycerol. The supernatant was loaded onto a HisTrap™ FF crude 5 mL column on an AKTAxpress HPLC system. The protein was eluted with a 20–500 mM imidazole gradient and dialyzed overnight against 20 mM Tris-HCl (pH 8.0), 200 mM NaCl and 5 mM DTT. The protein was further purified using a Q Sepharose™ Fast Flow anion exchange column (Amersham Biosciences). The flow-through fractions containing the RORγ LBD were pooled and concentrated. Subsequent size exclusion chromatography was performed on a HiLoad™ 16/60 Superdex™ 200 prep grade column. The final protein sample was in 20 mM Tris-HCl (pH 8.0), 200 mM NaCl and 10 mM DTT.

For fluorescence polarization competition assays, 104 nM RORγ LBD protein and 5 nM Fluorescein-labeled 25-hydroxycholesterol were incubated with various concentrations of testing compounds at 4 °C for 6 h. The fluorescence polarization signals were recorded using 96-well black polystyrene plates on a FlexStation 3. All the experiments were performed in triplicate and the data were later converted to fluorescence anisotropy values and normalized. The IC_{50} of each tested compound was calculated by fitting the curve in GraphPad Prism 5.

***In vitro* mouse T cell culture.** T cells were purified from the lymph nodes and spleens of six to eight week old C57BL/6 mice (Taconic) by magnetically depleting $B220^+$ cells (autoMACS, Miltenyi), and then sorted $CD8^-DAPI^-CD19^-CD4^+CD25^-CD62L^+CD44^{low/Int}$ naïve T cells on a FACSAria (BD). Cells were cultivated at 37 °C and 5% CO_2 in T cell media: RPMI 1640 (Invitrogen) supplemented with 10% (vol/vol) heat-inactivated FBS (Hyclone), 50 U penicillin-streptomycin (Invitrogen), 2 mM glutamine, and 50 μM b-mercaptoethanol. For T cell polarization, 200 μL cells were seeded at $0.4x10^5$ cells per μL in 96-well plates pre-coated with anti-CD3e (2.5 μg/mL) and anti-CD28 (10 μg/mL). Cells were cultured for 4–5 days in Th17 polarizing conditions (TGF-β (0.3 ng/mL), IL-6 (20

ng/mL), and anti-IFN-γ and anti-IL-4 (2 ng/mL)), Th1 conditions (IL-12 (2 ng/mL), IL-2 (10 U/mL) and anti-IL-4 (2 ng/mL)), or Treg conditions (TGF-β (5 ng/mL)). For cytokine analysis, cells were incubated for 5 h with phorbol 12-myristate 13-acetate (50 ng/mL; Sigma), ionomycin (500 ng/mL; Sigma) and GolgiStop (BD). Intracellular cytokine staining was performed according to the manufacturer's protocol (Cytofix/Cytoperm buffer set from BD or the FoxP3 staining buffer set from eBioscience) with alexa647-conjugated anti-IL-17a (eBioscience), PE-conjugated anti-IFN-γ, and PE-conjugated anti-FoxP3 (eBioscience). An LSR II (BD Biosciences) and FlowJo software (Tree Star) were used for flow cytometry and analysis. Dead cells were excluded using the Live/Dead fixable aqua dead cell stain kit (Invitrogen).

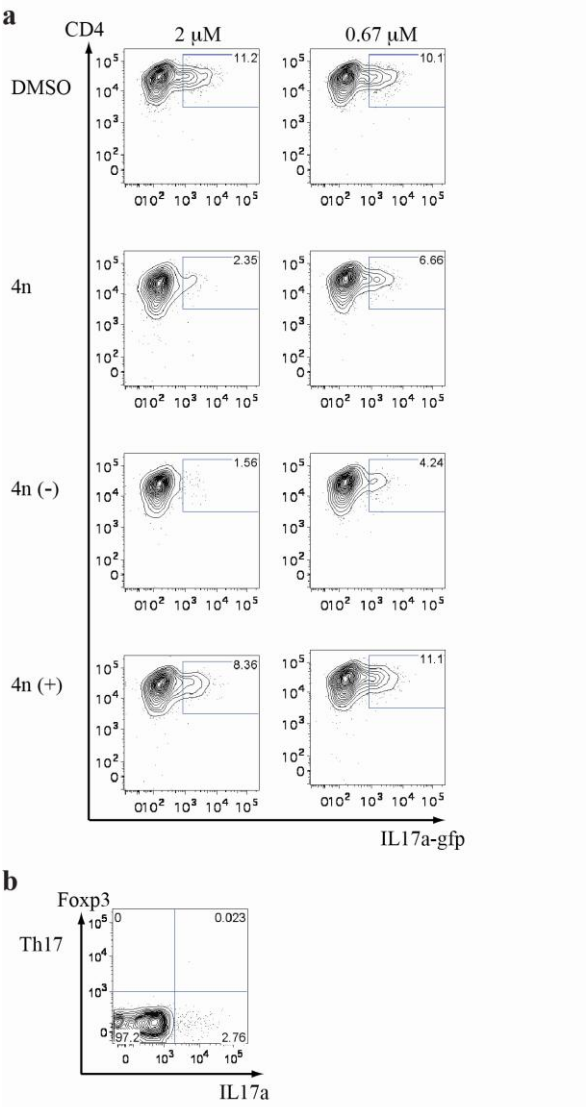
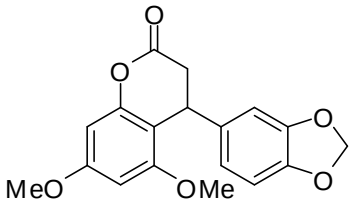


Figure 2. A series of diphenylpropanamides inhibit mouse Th17 cell differentiation. (a) Inhibition of mouse Th17 cell differentiation with **4n**, **4n(-)**, and **4n(+)**. Flow cytometry of surface staining for CD4 and GFP signal for IL-17a expression in sorted naïve T cell populations, isolated from a *il17a-ires-egfp* knock-in mouse line (Biocytogen). Cells were activated and expanded in Th17 polarizing culture conditions. DMSO or compounds (2 and 0.67 μM) were added on day 1 and analysis was done on day 5. **(b)** Flow cytometry of intracellular staining for IL-17a and FoxP3 in sorted naïve T cell populations activated and expanded in Th17 polarizing culture conditions.

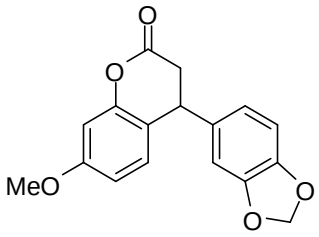
***In vitro* human T cell culture.** Blood samples were obtained from the New York Blood Center. Mononuclear cells were prepared from buffy coats from healthy adult donors or from cord blood, using FicollPAQUE gradients. $CD4^+$ T cells were magnetically selected (autoMACS, Miltenyi). Adult memory $CD4^+$ T cell subsets were further purified as $CD3^+CD4^+CD25^-$ $CD45RO^+CCR6^+CD161^+$ by cell sorting on a FACSAria (BD). Naïve $CD4^+$ T cells were isolated from cord blood as $CD3^+CD4^+CD45RA^+HLA\text{-}DR^-CD25^-DAPI^-$. Memory cells were activated with anti-CD3/CD28 coated beads (1 bead/cell) in T cell media (see above) supplemented with 100 U/mL IL-2 (Peprotech), 10 ng/mL IL-1b and 10 ng/mL IL-23 (eBioscience). Naïve cells were activated with anti-CD3/CD28 beads and cultured with the same cytokines, and TGF-b (0, 0.1, 1, or, 10 ng/mL) (Peprotech), in XVIVO-20 (Lonza) media. For ectopic expression experiments, naive $CD4^+$ T cells, cultured in XVIVO-20 media supplemented with100 U/mL IL-2 (Peprotech), were transduced with RORα-IRES-GFP or RORγt-IRES-GFP on day 1 and analyzed on day 6. GFP-expressing cells were gated for analysis. DMSO or compounds were added 6–8 h after the viral transduction. For flow cytometry and cell culture, the following human specific antibodies were used: CCR6-biotin (11A9 BD), CD3-Alexa750Cy7 (UCHT1 eBioscience), CD4-PacBlue (OKT4 eBioscience), CD25-APC (555434 BD), CD45RA-PE (HI100 eBioscience), CD45RO-APC (UCHL1 eBioscience), IL-17a-APC (eBio64CAP17 eBioscience), IFN-γ-PECy7 (45.B3 eBioscience), HLA-DR-FITC (555558 BD), CD3 purified (UCHT1 eBioscience), and CD28 purified (CD28.2 eBioscience).

Chemistry

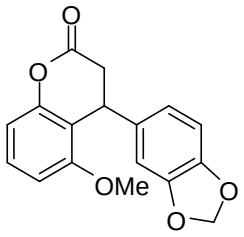
General materials and methods: All commercially available reagents and solvents were purchased and used without further purification. ^1H spectra were recorded using an Inova 400 MHz spectrometer (Varian). For compounds **4**, several peaks within the proton NMR spectrum existed as rotomers. Samples were analyzed for purity on an Agilent 1200 series LC/MS equipped with a Zorbax™ Eclipse XDB-C18 reverse phase (5 micron, 4.6 x 150 mm) column having a flow rate of 1.1 mL/min. The mobile phase was a mixture of acetonitrile and H_2O each containing 0.05% trifluoroacetic acid. A gradient of 5% to 100% acetonitrile over 8 minutes was used during analytical analysis. High-resolution mass spectroscopy measurements were performed on an Agilent 6210 Electrospray TOF mass spectrometer.



4-(Benzo[d][1,3]dioxol-5-yl)-5,7-dimethoxychroman-2-one (7a): A mixture of 3,5-dimethylphenol (1.54 g, 10.0 mmol) and (E)-3-(benzo[d][1,3]dioxol-5-yl)acrylic acid (1.92 g, 10.0 mmol) in TFA (30 mL) was heated at 70 °C for 4 h. The reaction mixture was concentrated under reduced pressure and purified via column chromatography on SiO_2 (ethyl acetate/hexane 7% - 60%) to afford **7a** (2.13 g, 65%) as a white powder. ^1H NMR ($CDCl_3$) δ 6.69 (d, J = 7.8 Hz, 1 H), 6.60 (d, J = 1.6 Hz, 1 H), 6.56 (dd, J = 7.8, 2.0 Hz, 1 H), 6.32 (d, J = 2.0 Hz, 1 H), 6.28 (d, J = 2.3 Hz, 1 H), 5.91 (s, 2 H), 4.48 (t, J = 4.5 Hz, 1 H), 3.82 (s, 3 H), 3.77 (s, 3 H), 2.97 (d, J = 4.3 Hz, 2 H); LC/MS: (electrospray +ve), m/z 329.1 $(MH)^+$, t_R = 5.83 min, UV_{254} = 100%.

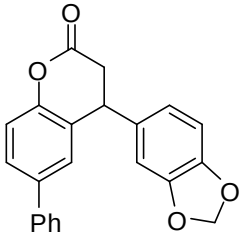


7b

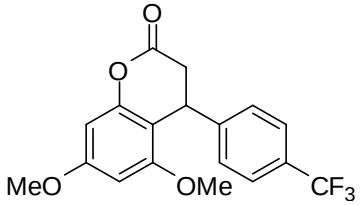


7c

4-(Benzo[d][1,3]dioxol-5-yl)-7-methoxychroman-2-one (7b) and **4-(benzo[d][1,3]dioxol-5-yl)-5-methoxychroman-2-one (7c):** A mixture of 3-methoxyphenol (248 mg, 2.00 mmol) and (E)-3-(benzo[d][1,3]dioxol-5-yl)acrylic acid (384 mg, 2.00 mmol) in TFA (15 mL) was heated at 60 °C for 3 h. The reaction mixture was concentrated under reduced pressure, dissolved in DCM (20 mL), and washed with a saturated $NaHCO_3$ (20 mL) aqueous solution. The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Preparative TLC purification on SiO_2 (ethyl acetate/DCM 5%) afforded **7c** (11 mg, 2%) and **7b** (78 mg, 13%). **7b**: ^{1}H NMR ($CDCl_3$) δ 6.91 (d, *J* = 8.6 Hz, 1 H); 6.77 (d, *J* = 8.6 Hz, 1 H), 6.55 - 6.71 (m, 4 H), 5.96 (s, 2 H), 4.22 (dd, *J* = 7.0, 6.6 Hz, 1 H), 3.81 (s, 3 H), 3.04 (dd, *J* = 15.6, 5.9 Hz, 1 H), 2.93 (dd, *J* = 15.6, 7.8 Hz, 1 H); LC/MS: (electrospray +ve), *m*/*z* 316.1 $(MH)^+$, t_R = 5.56 min, UV_{254} > 95%. **7c**: ^{1}H NMR ($CDCl_3$) δ 7.28 (t, *J* = 8.2 Hz, 1 H), 6.78 (d, *J* = 8.2 Hz, 1 H), 6.68 (d, *J* = 8.6 Hz, 1 H), 6.67 (d, *J* = 7.8 Hz, 1 H), 6.60 (d, *J* = 1.6 Hz, 1 H), 6.56 (dd, *J* = 8.2, 1.6 Hz, 1 H), 5.90 (s, 2 H), 4.57 (dd, *J* = 4.7, 3.9 Hz, 1 H), 3.80 (s, 3 H), 3.02 - 2.93 (m, 2 H); LC/MS: (electrospray +ve), *m*/*z* 316.1 $(MH)^+$, t_R = 5.54 min, UV_{254} = 100%.

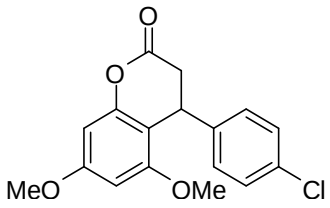


4-(Benzo[d][1,3]dioxol-5-yl)-6-phenylchroman-2-one (7d): A mixture of 4-phenylphenol (0.170 g, 1.00 mmol) and (E)-3-(benzo[d][1,3]dioxol-5-yl)acrylic acid (0.192 g, 1.00 mmol) in TFA (10 mL) was heated at 80 °C for 4 h. The reaction mixture was concentrated under reduced pressure and purified via column chromatography on SiO_2 (ethyl acetate/hexane 7% - 60%) to afford **7d** (0.154 g, 45%) as a white powder. ^{1}H NMR ($CDCl_3$) δ 7.45 - 7.58 (m, 3 H), 7.41 (t, *J* = 7.4 Hz, 2 H), 7.30 - 7.37 (m, 1 H), 7.24 (d, *J* = 1.6 Hz, 1 H), 7.21 (d, *J* = 8.6 Hz, 1 H), 6.79 (d, *J* = 8.6 Hz, 1 H), 6.62 - 6.70 (m, 2 H), 5.96 (s, 2 H), 4.34 (dd, *J* = 7.0, 6.5 Hz, 1 H), 3.09 (dd, *J* = 16.0, 5.9 Hz, 1 H) 3.01 (dd, *J* = 16.0, 7.4 Hz, 1 H); LC/MS: (electrospray +ve), *m*/*z* 345.1 $(MH)^+$, t_R = 6.56 min, UV_{254} = 100%.

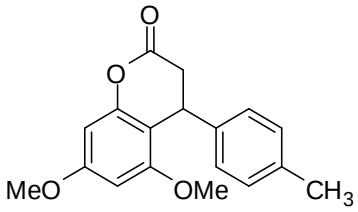


5,7-Dimethoxy-4-(4-(trifluoromethyl)phenyl)chroman-2-one (7e): A suspension of 5,7-dimethoxy-2H-chromen-2-one (103 mg, 0.500 mmol), 4-(trifluoromethyl)phenylboronic acid (475 mg, 2.50 mmol), 2,2'-bipyridine (62 mg, 0.40 mmol), and $Pd(OAc)_2$ (22 mg, 0.10 mmol) in dimethylacetamide (2.0 mL) and acetic acid (0.5 mL) was heated at 110 °C for 24 h. The reaction mixture was concentrated under reduced pressure and purified via column

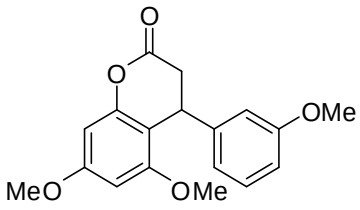
chromatography on SiO_2 (ethyl acetate/DCM 0% - 20%) to afford **7e** (64 mg, 36% yield) . ^{1}H NMR ($CDCl_3$) δ 7.53 (d, *J* = 8.2 Hz, 2 H), 7.24 (d, *J* = 8.2 Hz, 2 H), 6.35 (d, *J* = 2.3 Hz, 1 H), 6.29 (d, *J* = 2.0 Hz, 1 H), 4.61 (dd, *J* = 6.7, 2.0 Hz, 1 H), 3.83 (s, 3 H), 3.76 (s, 3 H), 3.04 (d, *J* = 6.7 Hz, 1 H), 3.02 (d, *J* = 2.3 Hz, 1 H); LC/MS: (electrospray +ve), *m*/*z* 353.1 $(MH)^+$, t_R = 6.50 min, UV_{254} = 100%.



4-(4-Chlorophenyl)-5,7-dimethoxychroman-2-one (7f): A suspension of 5,7-dimethoxy-2H-chromen-2-one (103 mg, 0.50 mmol), 4-chlorophenylboronic acid (391 mg, 2.51 mmol), 2,2'-bipyridine (62 mg, 0.40 mmol), and $Pd(OAc)_2$ (22 mg, 0.10 mmol) in dimethylacetamide (2.0 mL) and acetic acid (0.5 mL) was heated at 110 °C for 24 h. The reaction mixture was concentrated under reduced pressure and purified via column chromatography on SiO_2 (ethyl acetate/DCM 0% - 20%) to afford **7f** (70 mg, 44% yield). ^{1}H NMR ($CDCl_3$) δ 7.24 (d, *J* = 8.6 Hz, 2 H), 7.05 (d, *J* = 8.6 Hz, 2 H), 6.33 (d, *J* = 2.3 Hz, 1 H), 6.28 (d, *J* = 2.3 Hz, 1 H), 4.53 (dd, *J* = 6.3, 2.7 Hz, 1 H), 3.82 (s, 3 H), 3.76 (s, 3 H), 3.00 (d, *J* = 6.7, Hz, 1 H), 2.98 (d, *J* = 3.0 Hz, 1 H); LC/MS: (electrospray +ve), *m*/*z* 319.0 $(MH)^+$, t_R = 6.39 min, UV_{254} > 95%.

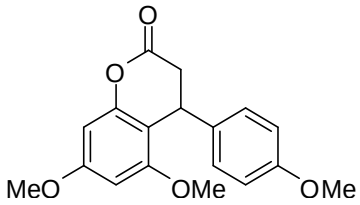


5,7-Dimethoxy-4-p-tolylchroman-2-one(7g): A mixture of 3,5-dimethoxylphenol (0.154 g, 1.00 mmol) and (E)-3-p-tolyacrylic acid (0.162 g, 1.00 mmol) in TFA (10 mL) was heated at 70 °C for 12 h. The reaction mixture was concentrated under reduced pressure and purified via column chromatography on SiO_2 (ethyl acetate/hexane 7% - 60%) to afford **7g** (0.162 g, 55%) as a white powder. ^{1}H NMR ($CDCl_3$) δ 7.07 (d, *J* = 7.8 Hz, 2 H), 7.00 (d, *J* = 8.2 Hz, 2 H), 6.33 (d, *J* = 2.0 Hz, 1 H), 6.28 (d, *J* = 2.3 Hz, 1 H), 4.53 (t, *J* = 4.3 Hz, 1 H), 3.82 (s, 3 H), 3.75 (s, 3 H), 2.99 (d, *J* = 4.7 Hz, 2 H), 2.29 (s, 3 H); LC/MS: (electrospray +ve), *m*/*z* 299.1 $(MH)^+$, t_R = 6.27 min, UV_{254} = 100%.



5,7-Dimethoxy-4-(3-methoxyphenyl)chroman-2-one (7h): A suspension of 5,7-dimethoxy-2H-chromen-2-one (103 mg, 0.50 mmol), 3-methoxyphenylboronic acid (380 mg, 2.5 mmol), 2,2'-bipyridine (62 mg, 0.40 mmol), and $Pd(OAc)_2$ (22 mg, 0.10 mmol) in dimethylacetamide (2.0 mL) and acetic acid (0.5 mL) was heated at 110 °C for 24

h. The reaction was concentrated under reduced pressure and the crude product was purified via column chromatography on SiO_2 (ethyl acetate/DCM 0% - 20%) to afford **7h** (58 mg, 37% yield). ^{1}H NMR ($CDCl_3$) δ 7.19 (t, *J* = 7.8 Hz, 1 H), 6.75 (dd, *J* = 8.2, 2.3 Hz, 1 H), 6.71 (d, *J* = 7.4 Hz, 1 H), 6.64 - 6,67 (m, 1 H), 6.33 (d, *J* = 2.3 Hz, 1 H), 6.28 (d, *J* = 2.3 Hz, 1 H), 4.53 (dd, *J* = 5.5, 3.5 Hz, 1 H), 3.82 (s, 3 H), 3.76 (s, 3 H), 3.75 (s, 3 H), 2.97 - 3.03 (m, 2 H); LC/MS: (electrospray +ve), *m*/*z* 315.1 $(MH)^+$, t_R = 5.93 min, UV_{254} = 100%.



5,7-Dimethoxy-4-(4-methoxyphenyl)chroman-2-one (7i): A mixture of 3,5-dimethoxyphenol (0.154 g, 1.00 mmol) and (E)-3-(4-methoxyphenyl)acrylic acid (0.178 g, 1.00 mmol) in TFA (10 mL) was heated at 60 °C for 4 h. The reaction mixture was concentrated under reduced pressure and purified via column chromatography on SiO_2 (ethyl acetate/hexane 7% - 60%) to afford **7i** (0.198 g, 63% yield). ^{1}H NMR ($CDCl_3$) δ 7.03 (d, *J* = 8.6 Hz, 2 H), 6.80 (d, *J* = 8.6 Hz, 2 H), 6.33 (d, *J* = 2.3 Hz, 1 H), 6.28 (d, *J* = 2.3 Hz, 1 H), 4.52 (t, *J* = 4.3 Hz, 1 H), 3.82 (s, 3 H), 3.76 (s, 6 H), 2.98 (d, *J* = 4.3 Hz, 2 H); LC/MS: (electrospray +ve), *m*/*z* 315.1 $(MH)^+$, t_R = 5.93 min, UV_{254} = 100%.

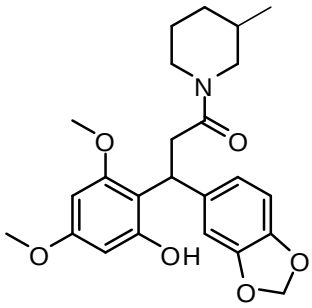
General procedures for the synthesis of **4:**

Method A

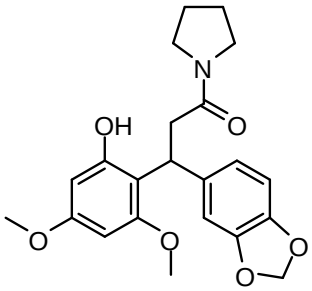
A solution of **7** (0.157 mmol, 1.0 equiv) in DMA (3.0 mL) was treated with amine NHR^3R^4 (2-10 equiv; for ammonium salts, 10 equiv of Hünig's base was also added). The mixture was heated at 80 °C for 8 h, concentrated under reduced pressure and dissolved in DCM (20 mL). The solution was washed with saturated $NaHCO_3$ (2x10 mL). The organic layer was dried ($MgSO_4$), filtered, concentrated under reduced pressure and purified either by HPLC or *via* column chromatography on SiO_2 (0 - 30% ethyl acetate/DCM) to afford the desired product.

Method B

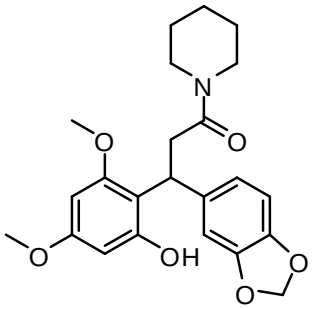
A solution of NHR^3R^4 (0.30 mmol, 2.0 equiv) in toluene (1.0 mL) was treated with Me_3Al (0.15 mL, 2.0 M in hexanes). The reaction mixture was stirred at r.t. for 30 min, treated with a solution of **7** (0.1 mmol) in toluene (1 mL) and heated at 80 °C for 6 h. The reaction mixture was cooled to r.t., quenched with ice water (20 mL) and extracted with DCM (2x15 mL). The combined organic layers were dried ($MgSO_4$), filtered, concentrated under reduced pressure and purified by column chromatography on SiO_2 (0 - 30% ethyl acetate/DCM) to afford the desired product.



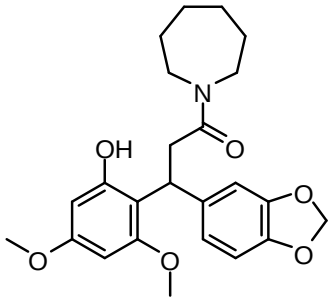
3-(Benzo[d][1,3]dioxol-5-yl)-3-(2-hydroxy-4,6-dimethoxyphenyl)-1-(3-methylpiperidin-1-yl)propan-1-one (4a): 4a was prepared by method A as a mixture of diastereomers. ^{1}H NMR (DMSO-d_6) δ 9.37 (br. s., 2 H), 6.74 - 6.85 (m, 2 H), 6.68 (s, 4 H), 6.00 (s, 4 H), 5.87 (s, 4 H), 4.78 - 4.94 (m, 2 H), 3.98 - 4.28 (m, 2 H), 3.56 - 3.82 (m, 14 H), 2.79 - 3.29 (m, 5 H), 2.50 - 2.64 (m, 1 H), 2.41 (d, *J* = 11.7 Hz, 1 H), 2.06 - 2.29 (m, 1 H), 1.59 - 1.77 (m, 2 H), 1.44 - 1.59 (m, 2 H), 0.94 - 1.40 (m, 6 H), 0.78 (t, *J* = 5.9 Hz, 6 H); HPLC: t_R = 6.00 min, UV_{254} = 100%; HRMS (ESI):m/z calcd for $C_{24}H_{29}NO_6$ $[M+H]^+$ 428.2071, found 428.2074.



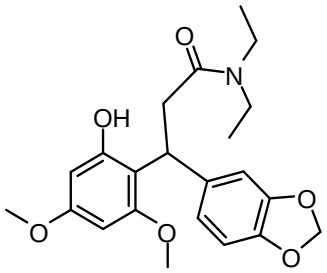
3-(Benzo[d][1,3]dioxol-5-yl)-3-(2-hydroxy-4,6-dimethoxyphenyl)-1-(pyrrolidin-1-yl)propan-1-one (4b): Prepared by method A. ^{1}H NMR (DMSO-d_6) δ 9.37 (s, 1 H), 6.79 (s, 1 H), 6.70 (br.s., 2 H), 6.01 (s, 2 H), 5.89 (s, 2 H), 4.92 (dd, *J* = 8.4, 6.1 Hz, 1 H), 3.66 (s, 3 H), 3.63 (s, 3 H), 3.30-3.41 (m, 2 H), 3.15 - 3.27 (m, 3 H), 2.83 (dd, *J* = 15.9, 5.7 Hz, 1 H), 1.76 - 1.87 (m, 2 H), 1.66 - 1.76 (m, 2 H); HPLC: t_R = 5.24 min, UV_{254} > 95%; HRMS (ESI):m/z calcd for $C_{22}H_{25}NO_6$ $[M+H]^+$ 400.1749, found 400.1755.



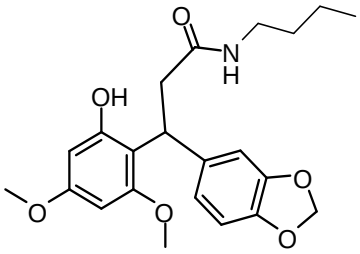
3-(Benzo[d][1,3]dioxol-5-yl)-3-(2-hydroxy-4,6-dimethoxyphenyl)-1-(piperidin-1-yl)propan-1-one (4c): Prepared by method A. ^{1}H NMR (DMSO-d_6) δ 9.35 (s, 1 H), 6.78 (s, 1 H), 6.68 (br.s., 2 H), 5.99 (s, 2 H), 5.87 (s, 2 H), 4.86 (t, *J* = 7.2 Hz, 1 H), 3.66 (s, 3 H), 3.63 (s, 3 H), 3.25-3.40 (m, 3 H), 3.19 (dd, *J* = 15.3, 8.2 Hz, 1 H), 2.85 - 3.02 (m, 2 H), 1.45 - 1.61 (m, 2 H), 1.24 - 1.40 (m, 4 H); HPLC: t_R = 5.71 min, UV_{254} > 90%; HRMS (ESI):m/z calcd for $C_{23}H_{27}NO_6$ $[M+H]^+$ 414.1911, found 414.1912.



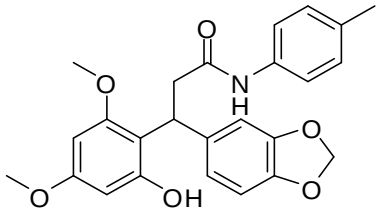
1-(Azepan-1-yl)-3-(benzo[d][1,3]dioxol-5-yl)-3-(2-hydroxy-4,6-dimethoxyphenyl)propan-1-one (4d): Prepared by method A. ^{1}H NMR (DMSO-d_6) δ 9.36 (s, 1 H), 6.79 (s, 1 H), 6.69 (br.s., 2 H), 6.01 (s, 2 H), 5.89 (s, 2 H), 4.94 (dd, *J* = 8.2, 6.3 Hz, 1 H), 3.68 (s, 3 H), 3.65 (s, 3 H), 3.16 - 3.57 (m, 5 H), 2.90 (dd, *J* = 15.9, 6.1 Hz, 1 H), 1.55 - 1.67 (m, 2 H), 1.45 - 1.55 (m, 2 H), 1.41 (d, *J* = 2.4 Hz, 4 H); HPLC: t_R = 5.82 min, UV_{254} = 100%; HRMS (ESI):m/z calcd for $C_{24}H_{29}NO_6$ $[M+H]^+$ 428.2071, found 428.2073.



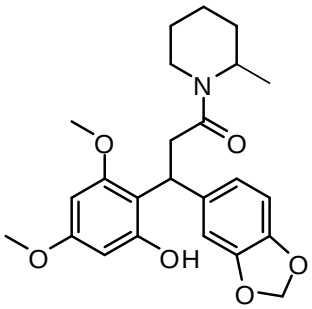
3-(Benzo[d][1,3]dioxol-5-yl)-N,N-diethyl-3-(2-hydroxy-4,6-dimethoxyphenyl)propanamide (4e): Prepared by method A. ^{1}H NMR (DMSO-d_6) δ 9.36 (s, 1 H), 6.77 (s, 1 H), 6.63 - 6.73 (m, 2 H), 6.01 (s, 2 H), 5.89 (s, 2 H), 4.92 (t, *J* = 7.2 Hz, 1 H), 3.68 (s, 3 H), 3.66 (s, 3 H), 3.10 - 3.32 (m, 5 H), 2.81 - 2.98 (m, 1 H), 1.06 (t, *J* = 6.9 Hz, 3 H), 0.91 (t, *J* = 6.9 Hz, 3 H); HPLC: t_R = 5.51 min, UV_{254} = 100%; HRMS (ESI):m/z calcd for $C_{22}H_{27}NO_6$ $[M+H]^+$ 402.1911, found 402.1912.



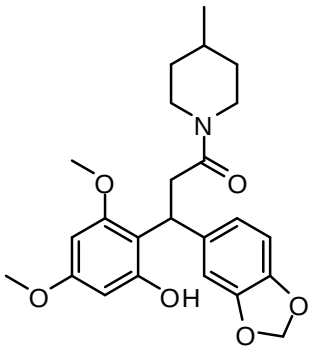
3-(Benzo[d][1,3]dioxol-5-yl)-N-butyl-3-(2-hydroxy-4,6-dimethoxyphenyl)propanamide (4f): Prepared by method A. ^{1}H NMR (DMSO-d_6) δ 9.34 (s, 1 H), 7.68 (t, *J* = 5.5 Hz, 1 H), 6.83 (s, 1 H), 6.68 (s, 2 H), 6.00 (s, 2 H), 5.88 (s, 2 H), 4.89 (dd, *J* = 9.8, 5.5 Hz, 1 H), 3.69 (s, 3 H), 3.65 (s, 3 H), 3.18 (dd, *J* = 14.7, 10.0 Hz, 1 H), 2.85 - 3.04 (m, 2 H), 2.44-2.50 (m, 1H), 1.19 - 1.30 (m, 2 H), 1.10-1.15 (m, 2 H), 0.79 (t, *J* = 7.4 Hz, 3 H); HPLC: t_R = 5.40 min, UV_{254} = 100%; HRMS (ESI):m/z calcd for $C_{22}H_{27}NO_6$ $[M+H]^+$ 402.1911, found 402.1909.



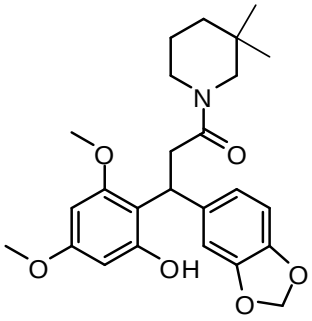
3-(Benzo[d][1,3]dioxol-5-yl)-3-(2-hydroxy-4,6-dimethoxyphenyl)-N-p-tolylpropanamide (4g): Prepared by method B. ^{1}H NMR (DMSO-d_6) δ 9.72 (s, 1 H), 9.34 (s, 1 H), 7.36 (d, *J* = 8.2 Hz, 2 H), 6.98 (d, *J* = 8.2 Hz, 2 H), 6.81 (s, 1 H), 6.60 - 6.71 (m, 2 H), 5.95 (s, 2 H), 5.82 (s, 2 H), 4.94 (dd, *J* = 9.4, 5.5 Hz, 1 H), 3.65 (s, 3 H), 3.59 (s, 3 H), 3.37 (dd, *J* = 15.3, 9.8 Hz, 1 H), 2.69 (dd, *J* = 15.3, 5.5 Hz, 1 H), 2.15 (s, 3 H); HPLC: t_R = 5.90 min, UV_{254} = 100%; HRMS (ESI):m/z calcd for $C_{25}H_{25}NO_6$ $[M+H]^+$ 436.1755, found 436.1749.



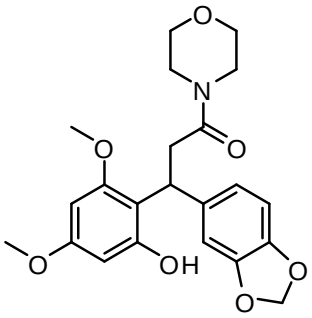
3-(Benzo[d][1,3]dioxol-5-yl)-3-(2-hydroxy-4,6-dimethoxyphenyl)-1-(2-methylpiperidin-1-yl)propan-1-one (4h): 4h was prepared by method B as a mixture of diastereomers. ^{1}H NMR (DMSO-d_6) δ 9.37 (s, 2 H), 6.80 (s, 2 H), 6.70 (s, 4 H), 6.01 (s, 4 H), 5.89 (s, 4 H), 4.88 (d, *J* = 5.5 Hz, 2 H), 4.59 - 4.70 (m, 2 H), 4.11 - 4.28 (m, 2 H), 3.68 (s, 6 H), 3.65 (s, 6 H), 2.84 - 3.26 (m, 6 H), 1.29 - 1.66 (m, 10 H), 0.86 - 1.27 (m, 8 H); HPLC: t_R = 5.89 min, UV_{254} = 100%; HRMS (ESI):m/z calcd for $C_{24}H_{29}NO_6$ $[M+H]^+$ 428.2071, found 428.2068.



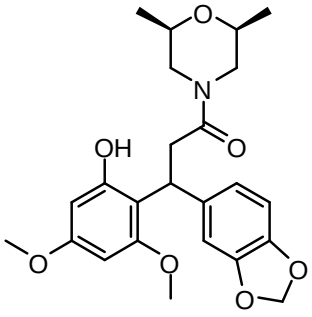
3-(Benzo[d][1,3]dioxol-5-yl)-3-(2-hydroxy-4,6-dimethoxyphenyl)-1-(4-methylpiperidin-1-yl)propan-1-one (4i): Prepared by method A. ^{1}H NMR (DMSO-d_6) δ 9.37 (br.s., 1 H), 6.79 (dd, *J* = 8.8, 5.3 Hz, 1 H), 6.63 - 6.74 (m, 2 H), 6.01 (s, 2 H), 5.89 (s, 2 H), 4.82 - 4.92 (m, 1 H), 3.68 (s, 3 H), 3.65 (s, 3 H), 3.10 - 3.29 (m, 2 H), 2.78 - 3.01 (m, 3 H), 2.35 - 2.46 (m, 1 H), 1.53 (d, *J* = 9.4 Hz, 3 H), 0.75 - 0.93 (m, 5 H); HPLC: t_R = 6.01 min, UV_{254} >90%; HRMS (ESI):m/z calcd for $C_{24}H_{29}NO_6$ $[M+H]^+$ 428.2071, found 428.2066.



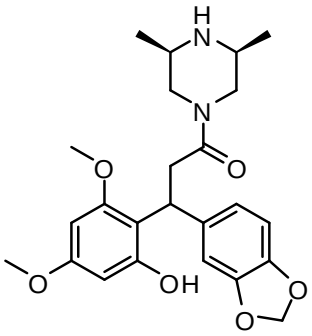
3-(Benzo[d][1,3]dioxol-5-yl)-1-(3,3-dimethylpiperidin-1-yl)-3-(2-hydroxy-4,6-dimethoxyphenyl)propan-1-one (4j): Prepared by method A. ^{1}H NMR (DMSO-d_6) δ 9.33 - 9.42 (m, 1 H), 6.77 - 6.85 (m, 1 H), 6.67 - 6.73 (m, 2 H), 6.00 (s, 2 H), 5.89 (s, 2 H), 4.82 - 4.97 (m, 1 H), 3.68 (s, 3 H), 3.65 (s, 3 H), 2.88 - 3.48 (m, 6 H), 1.20 - 1.45 (m, 4 H), 0.68 - 0.97 (m, 6 H); HPLC: t_R = 6.03 min, UV_{254} = 98%; HRMS (ESI):m/z calcd for $C_{25}H_{31}NO_6$ $[M+H]^+$ 442.2224, found 442.2224.



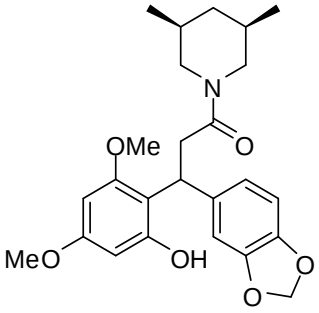
3-(Benzo[d][1,3]dioxol-5-yl)-3-(2-hydroxy-4,6-dimethoxyphenyl)-1-morpholinopropan-1-one (4k): Prepared by method A. ^{1}H NMR (DMSO-d_6) δ 9.39 (s, 1 H), 6.81 (s, 1 H), 6.66 - 6.74 (m, 2 H), 6.01 (s, 2 H), 5.90 (s, 2 H), 4.88 (t, J = 7.4 Hz, 1 H), 3.68 (s, 3 H), 3.65 (s, 3 H), 3.34 - 3.53 (m, 6 H), 3.11 - 3.22 (m, 2 H), 3.03 - 3.10 (m, 1 H), 2.99 (dd, J = 15.3, 6.7 Hz, 1 H); HPLC: t_R = 4.92 min, UV_{254} = 95%; HRMS (ESI):m/z calcd for $C_{22}H_{25}NO_7$ $[M+H]^+$ 416.1715, found 416.1714.



3-(Benzo[d][1,3]dioxol-5-yl)-1-(*cis*-2,6-dimethylmorpholino)-3-(2-hydroxy-4,6-dimethoxyphenyl)propan-1-one (4l): Prepared by method A. ^{1}H NMR (DMSO-d_6) δ 9.38 (m, 1 H), 6.74 - 6.86 (m, 1 H), 6.61 - 6.73 (m, 2 H), 5.96 - 6.02 (m, 2 H), 5.88-5.91 (m, 2 H), 4.74 - 4.92 (m, 1 H), 4.15-4.22 (m, 1 H), 3.56 - 3.73 (m, 6 H), 3.11 - 3.27 (m, 3 H), 2.82 - 3.08 (m, 2 H), 2.51 - 2.69 (m, 1 H), 1.97 - 2.18 (m, 1 H), 0.90 - 1.12 (m, 6 H); HPLC: t_R = 5.27 min, UV_{254} = 95%; HRMS (ESI):m/z calcd for $C_{24}H_{29}NO_7$ $[M+H]^+$ 444.2020, found 442.2020.



3-(Benzo[d][1,3]dioxol-5-yl)-1-(*cis*-3,5-dimethylpiperazin-1-yl)-3-(2-hydroxy-4,6-dimethoxyphenyl)propan-1-one (4m): Prepared by method A. ^{1}H NMR (DMSO-d_6) δ 9.36 - 9.51 (m, 1 H), 8.42 (br. s., 1 H), 6.61 - 6.94 (m, 3 H), 6.02 (br. s., 2 H), 5.90-5.92 (m, 2 H), 4.86 (t, *J* = 7.2 Hz, 1 H), 4.40 - 4.48 (m, 1 H), 3.91 - 4.06 (m, 1 H), 3.69 (s, 3 H), 3.66 (s, 3 H), 2.74 - 3.25 (m, 5 H), 2.28 - 2.47 (m, 1 H), 1.18 (d, *J* = 6.3 Hz, 6 H); HPLC: t_R = 4.04 min, UV_{254} = 98%; HRMS (ESI):m/z calcd for $C_{24}H_{30}N_2O_6$ $[M+H]^+$ 443.2188, found 443.2182.

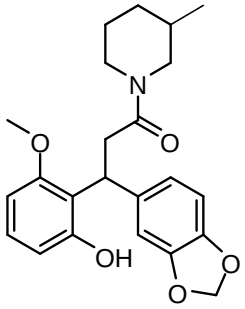


3-(Benzo[d][1,3]dioxol-5-yl)-1-(*cis*-3,5-dimethylpiperidin-1-yl)-3-(2-hydroxy-4,6-dimethoxyphenyl)propan-1-one (4n): Prepared by method A. ^{1}H NMR (DMSO-d_6, 25 °C) δ 9.39 (s, 1 H), 6.82 (d, *J* = 15.3 Hz, 1 H), 6.63 - 6.75 (m, 2 H), 6.01 (s, 2 H), 5.90 (s, 2 H), 4.79 - 4.91 (m, 1 H), 4.28 - 4.38 (m, 1 H), 3.71 - 3.90 (m, 1 H), 3.68 (m, 3 H), 3.65 (s, 3 H), 2.87 - 3.29 (m, 2 H), 2.40 (q, *J* = 11.7 Hz, 1 H), 1.86 (t, *J* = 11.9 Hz, 1 H), 1.62 - 1.74 (m, 1 H), 1.10 - 1.38 (m, 2 H), 0.79 (d, *J* = 6.3 Hz, 6 H), 0.56 - 0.75 (m, 1 H); LC/MS: (electrospray +ve), *m/z* 442.2 $(MH)^+$, t_R = 6.14 min, UV_{254} = 98%. Chiral separation of **4n** by HPLC (Column: IA Preparatory 5 cm x 50 cm; Run Time: 40 minutes; Flow Rate: 35 mL/min; Mobile Phase: 60/40 EtOH/Hexane; Detectors: DAD (220 and 254 nm)) gave **4n(-)** and **4n(+)**.

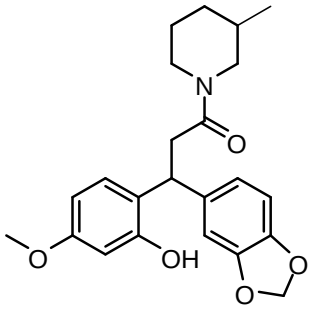
(-) 3-(Benzo[d][1,3]dioxol-5-yl)-1-*cis*-3,5-dimethylpiperidin-1-yl)-3-(2-hydroxy-4,6-dimethoxyphenyl)propan-1-one (4n(-)): ^{1}H NMR (DMSO-*d6*, 60 °C) δ 9.21 (s, 1 H), 6.82 (s, 1 H), 6.67 - 6.73 (m, 2 H), 6.02 - 6.04 (m, 2 H), 5.88 - 5.89 (m, 2 H), 4.88 (t, *J* = 7.2 Hz, 1 H), 4.28 - 4.38 (m, 1 H), 3.72 - 3.90 (m, 1 H), 3.69 (s, 3 H), 3.67 (s, 3 H), 2.92 - 3.20 (m, 2 H), 2.34 - 2.50 (m, 1 H), 1.80 - 1.94 (m, 1 H), 1.64 - 1.74 (m, 1 H), 1.20 - 1.38 (m, 2 H), 0.81 (d, *J* = 6.3 Hz, 6 H), 0.65 - 0.74 (m, 1 H); LC/MS: t_r = 4.39 min; Purity: UV_{220} > 95%, UV_{254} > 95%; (Column: IA analytical, 0.46 cm x 25 cm; Run time: 15 min; Flow rate: 1 mL/min; Mobile phase; 60/40 EtOH/Hexane; Detectors: DAD (220 and 254 nm) and PDR chiral detector); $[\alpha]_D^{23}$ = -129.1 (c 1.0, $CHCl_3$); HRMS (ESI): *m/z* calcd for $C_{25}H_{31}NO_6[M+H]^+$442.2224, found 442.2221.

(+) 3-(Benzo[d][1,3]dioxol-5-yl)-1-*cis*-3,5-dimethylpiperidin-1-yl)-3-(2-hydroxy-4,6-dimethoxyphenyl)propan-1-one (4n(+)): ^{1}H NMR (DMSO-d_6, 60 °C) δ 9.21 (s, 1 H), 6.82 (br. s., 1 H), 6.64 - 6.75 (m, 2 H), 6.02 - 6.04 (m, 2 H), 5.88-5.89 (m, 2 H), 4.88 (t, *J* = 7.2 Hz, 1 H), 4.24 - 4.38 (m., 1 H), 3.72 - 3.90 (m, 1 H), 3.69 (s, 3 H), 3.67 (s, 3 H), 2.92 - 3.24 (m, 2 H), 2.31 - 2.50 (m, 1 H), 1.82 - 1.93 (m, 1 H), 1.65 - 1.71 (m., 1 H), 1.22 - 1.38 (m, 2 H), 0.81 (d, *J* = 6.5 Hz, 6 H), 0.65-0.74 (m, 1 H); LC/MS: t_r = 4.83 min; Purity: UV_{220} > 90%, UV_{254} > 95%; (Column: IA analytical, 0.46 cm x 25 cm; Run time: 15 min; Flow rate: 1 mL/min; Mobile phase; 60/40 EtOH/Hexane; Detectors:

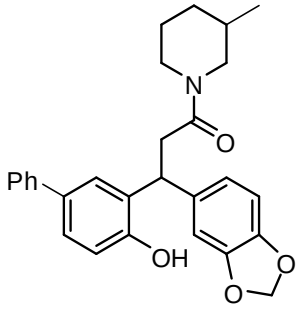
DAD (220 and 254 nm) and PDR chiral detector); $[\alpha]_D^{23}$ = +127.9 (c 1.0, $CHCl_3$); HRMS (ESI): *m/z* calcd for $C_{25}H_{31}NO_6[M+H]^+$ 442.2224, found 442.2220.



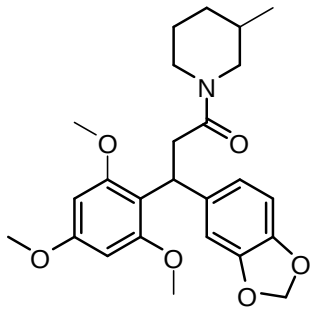
3-(Benzo[d][1,3]dioxol-5-yl)-3-(2-hydroxy-6-methoxyphenyl)-1-(3-methylpiperidin-1-yl)propan-1-one (4o): 4o was prepared by method A as a mixture of diastereomers. ^{1}H NMR (DMSO-d_6) δ 9.35 (br. s., 2 H), 6.94 (t, *J* = 8.0 Hz, 2 H), 6.78 - 6.89 (m, 2 H), 6.72 (s, 4 H), 6.41 (dd, *J* = 8.0, 3.7 Hz, 4 H), 5.90 (s, 4 H), 4.93 - 5.06 (m, 2 H), 3.96 - 4.27 (m, 2 H), 3.70 (s, 6 H), 3.65 - 3.78 (m, 2 H), 2.82 - 3.31 (m, 5 H), 2.54 - 2.63 (m, 1 H), 2.40 - 2.43 (m, 1 H), 2.09 - 2.30 (m, 1 H), 1.46 - 1.76 (m, 4 H), 0.92 - 1.41 (m, 6 H), 0.73 - 0.86 (m, 6 H); HPLC: t_R = 5.91 min, UV_{254} = 100%; HRMS (ESI):m/z calcd for $C_{23}H_{27}NO_5$ $[M+H]^+$ 398.1965, found 398.1963.



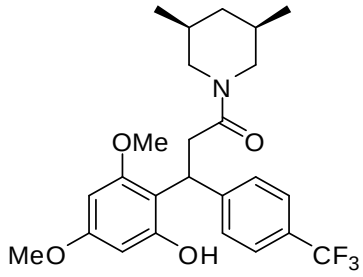
3-(Benzo[d][1,3]dioxol-5-yl)-3-(2-hydroxy-4-methoxyphenyl)-1-(3-methylpiperidin-1-yl)propan-1-one (4p): 4p was prepared by method A as a mixture of diastereomers. ^{1}H NMR (DMSO-d_6) δ 9.34 - 9.41 (m, 2 H), 7.00 - 7.11 (m, 2 H), 6.64 - 6.86 (m, 6 H), 6.26 - 6.36 (m, 4 H), 5.92 (s, 2 H), 5.91 (s, 2 H), 4.63 (t, *J* = 7.2 Hz, 2 H), 3.98 - 4.22 (m, 2 H), 3.79 - 3.93 (m, 2 H), 3.64 (s, 6 H), 2.70 - 3.14 (m, 5 H), 2.52 - 2.66 (m, 1 H), 2.37 - 2.48 (m, 1 H), 2.11 - 2.24 (m, 1 H), 1.45 - 1.75 (m, 4 H), 0.94 - 1.46 (m, 6 H), 0.71 - 0.91 (m, 6 H); HPLC: t_R = 5.83 min, UV_{254} = 98%; HRMS (ESI):m/z calcd for $C_{23}H_{27}NO_5$ $[M+H]^+$ 398.1965, found 398.1960.



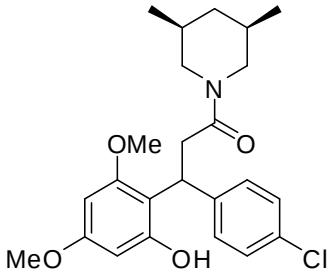
3-(Benzo[d][1,3]dioxol-5-yl)-3-(4-hydroxybiphenyl-3-yl)-1-(3-methylpiperidin-1-yl)propan-1-one (4q): 4q was prepared by method A as a mixture of diastereomers. ^{1}H NMR (DMSO-d_6) δ 9.52 (s, 2 H), 7.45 - 7.62 (m, 6 H), 7.40 (t, *J* = 7.24 Hz, 4 H), 7.22 - 7.32 (m, 4 H), 6.95 (br. s., 2 H), 6.72 - 6.86 (m, 6 H), 5.92 (s, 2 H), 5.91 (s, 2 H), 4.74 - 4.87 (m, 2 H), 4.05 - 4.24 (m, 2 H), 3.87 - 4.00 (m, 2 H), 2.81 - 3.27 (m, 5 H), 2.53 - 2.66 (m, 1 H), 2.34 - 2.49 (m, 1 H), 2.13 - 2.27 (m, 1 H), 1.44 - 1.74 (m, 4 H), 0.94 - 1.45 (m, 6 H), 0.82 (dd, *J* = 6.3, 3.9 Hz, 3 H), 0.76 (dd, *J* = 6.5, 2.9 Hz, 3 H); LC/MS: (electrospray +ve), *m*/*z* 444.2 $(MH)^+$, t_R = 6.55 min, UV_{254} = 100%.



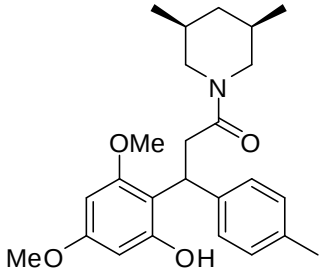
3-(Benzo[d][1,3]dioxol-5-yl)-1-(3-methylpiperidin-1-yl)-3-(2,4,6-trimethoxyphenyl)propan-1-one (4r): A solution of **4a** (42 mg, 0.10 mmol) in DMA (2 mL) was treated with iodomethane (43 mg, 0.30 mmol) and Cs_2CO_3 (0.328 g, 1.01 mmol). The reaction mixture was heated in a microwave at 150 °C for 10 min, concentrated under reduced pressure, dissolved in DCM (10 mL) and washed with water (10 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated under reduced pressure. The crude product was purified via column chromatography on SiO_2 (ethyl acetate/DCM 0% - 30%) to afford **4r** (32 mg, 72% yield) as a mixture of diastereomers. ^{1}H NMR (DMSO-d_6) δ 6.62 - 6.81 (m, 6 H), 6.19 (s, 4 H), 5.90 (s, 2 H), 5.89 (s, 2 H), 4.89 - 4.99 (m, 2 H), 4.09 - 4.24 (m, 2 H), 3.60 - 3.80 (m, 20 H), 2.84 - 3.25 (m, 5 H), 2.52 - 2.66 (m, 1 H), 2.37 - 2.48 (m, 1 H), 2.10 - 2.30 (m, 1 H), 1.62 - 1.73 (m, 2 H), 1.47 - 1.62 (m, 2 H), 0.96 - 1.42 (m, 6 H), 0.75 - 0.85 (m, 6 H); LC/MS: (electrospray +ve), *m*/*z* 442.2 $(MH)^+$, t_R = 6.34 min, UV_{254} = 100%.



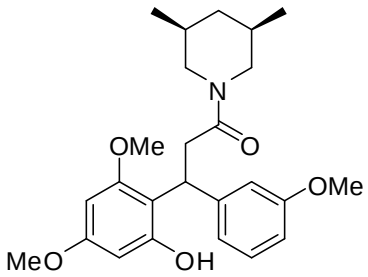
1-(*cis*-3,5-Dimethylpiperidin-1-yl)-3-(2-hydroxy-4,6-dimethoxyphenyl)-3-(4-(trifluoromethyl)phenyl)propan-1-one (4s): Prepared by method A. ^{1}H NMR (DMSO-d_6) δ 9.48 (br.s, 1 H), 7.54 (d, *J* = 8.2 Hz, 2 H), 7.37 - 7.45 (m, 2 H), 6.02 (s, 1 H), 6.01 (s, 1 H), 5.03 (t, *J* = 6.8 Hz, 1 H), 4.31 (d, *J* = 12.1 Hz, 1 H), 3.77 (d, *J* = 12.9 Hz, 1 H), 3.61 - 3.71 (m, 6 H), 2.84 - 3.52 (m, 2 H), 2.31 - 2.48 (m, 1 H), 1.89 (t, *J* = 12.1 Hz, 1 H), 1.68 (t, *J* = 14.5 Hz, 1 H), 1.10 - 1.46 (m, 2 H), 0.76 - 0.90 (m, 6 H), 0.60 - 0.76 (m, 1 H); LC/MS: (electrospray +ve), *m*/*z* 466.2 $(MH)^+$, t_R = 6.87 min, UV_{254} = 98%.



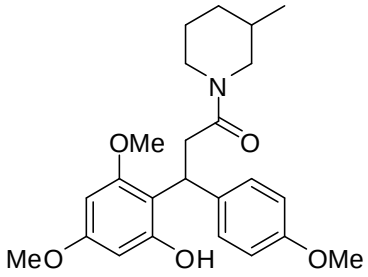
3-(4-Chlorophenyl)-1-(*cis*-3,5-dimethylpiperidin-1-yl)-3-(2-hydroxy-4,6-dimethoxyphenyl)propan-1-one (4t): Prepared by method A. ^{1}H NMR (DMSO-d_6) δ 9.44 (br. s., 1 H), 7.23 (d, *J* = 3.5 Hz, 4 H), 6.02 (s, 2 H), 4.94 (t, *J* = 7.2 Hz, 1 H), 4.32 (d, *J* = 12.5 Hz, 1 H), 3.70 - 3.86 (m, 1 H), 3.59 - 3.71 (m, 6 H), 2.84 - 3.36 (m, 2 H), 2.38-2.42 (m, 1 H), 1.87 (t, *J* = 12.1 Hz, 1 H), 1.58 - 1.77 (m, 1 H), 1.10 - 1.47 (m, 2 H), 0.73 - 0.87 (m, 6 H), 0.60-0.74 (m, 1 H); LC/MS: (electrospray +ve), *m*/*z* 432.2 $(MH)^+$, t_R = 6.75 min, UV_{254} = 98%.



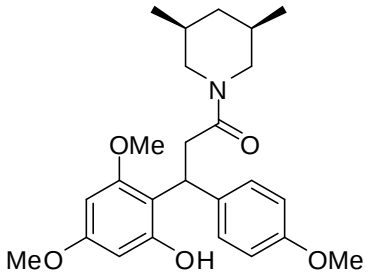
1-(*cis*-3,5-Dimethylpiperidin-1-yl)-3-(2-hydroxy-4,6-dimethoxyphenyl)-3-p-tolylpropan-1-one (4u): Prepared by method A. ^{1}H NMR (DMSO-d_6) δ 9.34 (s, 1 H), 7.10 (d, *J* = 7.4 Hz, 2 H), 6.97 (d, *J* = 7.8 Hz, 2 H), 6.00 (br.s, 2 H), 4.84 - 4.93 (m, 1 H), 4.29 - 4.32 (m, 1 H), 3.70 - 3.80 (m, 1 H), 3.65 (s, 6 H), 2.89 - 3.27 (m, 2 H), 2.30 - 2.46 (m, 1 H), 2.20 (s, 3 H), 1.86 (t, *J* = 12.1 Hz, 1 H), 1.58 - 1.75 (m, 1 H), 1.08 - 1.39 (m, 2 H), 0.74 - 0.84 (m, 6 H), 0.57 - 0.74 (m, 1 H); LC/MS: (electrospray +ve), *m*/*z* 412.2 $(MH)^+$, t_R = 6.64 min, UV_{254} = 98%.



1-(*cis*-3,5-Dimethylpiperidin-1-yl)-3-(2-hydroxy-4,6-dimethoxyphenyl)-3-(3-methoxyphenyl)propan-1-one (4v): Prepared by method A. ^{1}H NMR (DMSO-d_6) δ 9.37 (s, 1 H), 7.08 (t, *J* = 8.0 Hz, 1 H), 6.75 - 6.85 (m, 2 H), 6.59 - 6.69 (m, 1 H), 6.01 (s, 2 H), 4.86 - 4.96 (m, 1 H), 4.32 (d, *J* = 12.1 Hz, 1 H), 3.71 - 3.82 (m, 1 H), 3.65 (s, 6 H), 3.63 (s, 3 H), 2.88 - 3.30 (m, 2 H), 2.33 - 2.46 (m, 1 H), 1.87 (t, *J* = 11.9 Hz, 1 H), 1.67 (t, *J* = 12.1 Hz, 1 H), 1.11 - 1.38 (m, 2 H), 0.80 (d, *J* = 6.7 Hz, 6 H), 0.60 - 0.75 (m, 1 H); LC/MS: (electrospray +ve), *m*/*z* 428.2 $(MH)^+$, t_R = 6.30 min, UV_{254} = 95%.



3-(2-Hydroxy-4,6-dimethoxyphenyl)-3-(4-methoxyphenyl)-1-(3-methylpiperidin-1-yl)propan-1-one (4w): 4w was prepared by method A as a mixture of diastereomers. ^{1}H NMR (DMSO-d_6) δ 9.33 (br. s., 2 H), 7.12 - 7.16 (m, 4 H), 6.73 (d, *J* = 8.6 Hz, 4 H), 6.00 (s, 4 H), 4.85 - 4.89 (m, 2 H), 3.98 - 4.28 (m, 2 H), 3.77-3.55 (m, 20 H), 2.86 - 3.29 (m, 5 H), 2.50 - 2.64 (m, 1 H), 2.33 - 2.44 (m, 1 H), 2.08 - 2.28 (m, 1 H), 1.62 - 1.70 (m, 2 H), 1.49 - 1.60 (m, 2 H), 0.99 - 1.40 (m, 6 H), 0.79 - 0.84 (m, 6 H); HPLC: t_R = 6.64 min, UV_{254} = 95%; HRMS (ESI):m/z calcd for $C_{24}H_{31}NO_5$ $[M+H]^+$ 414.2278, found 414.2279.



1-(*cis*-3,5-Dimethylpiperidin-1-yl)-3-(2-hydroxy-4,6-dimethoxyphenyl)-3-(4-methoxyphenyl)propan-1-one (4x): Prepared by method A. ^{1}H NMR (DMSO-d_6) δ 9.35 (s, 1 H), 7.15 (d, *J* = 8.0 Hz, 2 H), 6.74 (d, *J* = 8.6 Hz, 2 H), 6.02 (s, 2 H), 4.81 - 4.95 (m, 1 H), 4.32 (d, *J* = 12.1 Hz, 1 H), 3.71 - 3.81 (m, 1 H), 3.67 (s, 6 H), 3.65 (s, 3 H), 2.92 - 3.29 (m, 2 H), 2.39 (q, *J* = 12.1 Hz, 1 H), 1.86 (t, *J* = 12.1 Hz, 1 H), 1.67 (t, *J* = 13.1 Hz, 1 H), 1.10 - 1.39 (m, 2 H), 0.80 (d, *J* = 5.5 Hz, 6 H), 0.58 - 0.74 (m, 1 H); HPLC: t_R = 6.29 min, UV_{254} = 95%; HRMS (ESI):m/z calcd for $C_{25}H_{33}NO_5$ $[M+H]^+$ 428.2435, found 428.2428.