Discovery of Benzimidazole Oxazolidinediones as Novel and Selective Nonsteroidal Mineralocorticoid Receptor Antagonists

Christine Yang,* Jaume Balsells, Hong D. Chu, Jason M. Cox, Alejandro Crespo, Xiuying Ma, Lisa Contino, Patricia Brown, Sheng Gao, Beata Zamlynny, Judyann Wiltsie, Joseph Clemas, JeanMarie Lisnock, Jack Gibson, Gaochao Zhou, Marga Garcia-Calvo, Thomas J. Bateman, Vincent Tong, Ling Xu, Martin Crook, Peter Sinclair, and Hong C. Shen*

Merck Research Laboratories, Merck & Co., Kenilworth, NJ 07033, USA

*Correspondence should be addressed to: Christine Yang, Merck Research Laboratories, Mail Code K15-A102-02, 2015 Galloping Hill Road, Kenilworth, New Jersey 07033. Tel: (908) 740-6593. E-mail: christine_yang@merck.com and hongshen@stanfordalumni.org

Supporting Information

Part I. The human MR NH Pro assay

The human MR NH Pro assay is a commercially available PathHunter™ Protein :Protein interaction assay (DiscoveRx; http://www.discoverx.com/nhrs/prod-nhrs.php) that measures the ability of compounds to antagonize full-length human Mineralocorticoid Receptor (MR) binding to a coactivator peptide. PathHunter™ CHO-K1 cells that overexpress human MR (Cat #93-0456C2, Lot No: 09B0913) were cultured in growth media (F12K w/ glutamine and phenol red (Gibco 11765-047)) supplemented with 10% HI FBS (Gibco 16000); 0.25mg/ml hygromycin in PBS (Invitrogen 10687-010, 50mg/ml); 100 I.U./mL and 100 ug/mL Pen/Strep (Gibco 15140-122); 0.6mg/mL Geneticin).

Compounds were assessed for MR antagonist activity by incubating the cells with a titrating dose of compound in F12K w/ glutamine and phenol red culture media (Invitrogen 11765-047) supplemented with 1 % charcoal/dextran treated FBS (Hyclone #SH30068.01) and aldosterone (0.3 nM) for 6 hours at 37^{o}C. Cells were then treated with DiscoveRx detection reagent for 1 hour at room temperature and read using an Envision luminescence plate reader. % activity was measured relative to cells treated with aldosterone alone and IP were calculated using ADA software.

1. Growth Media:
F12K w/ glutamine and phenol red (Gibco 11765-047)
10% HI FBS (Gibco 16000)
0.25mg/ml hygromycin in PBS (Invitrogen 10687-010, 50mg/ml)
100 I.U./mL and 100 ug/mL Pen/Strep (Gibco 15140-122)
0.6mg/mL Geneticin (Gibco 10131, 50mg/ml)

2. Assay media:
F12K w/ glutamine and phenol red (Invitrogen 11765-047)
1 % charcoal/dextran treated FBS (Hyclone #SH30068.01)

3. 3x PathHunter Detection Reagents (Cat# 93-0001) (need ~6ml/plate). Do not freeze and thaw the reagents more than 3 times.

19x PathHunter Cell Assay Buffer

5x Emerald II

1x Galacton Star

4. Control Agonist: Aldosterone: Sigma cat# A9477

Prepare stock solution- 10uM in DMSO kept at -20C

For assay, dilute in assay media to 1.8 nM (6x of final concentration=0.3 nM)

5. Cell line: PathHunter CHO-K1 MR cells Cat #93-0456C2, Lot No: 09B0913, from operation liquid nitrogen stock.

6. Control Antagonist: spironolactone: Sigma #S-3378 and eplerenone: Sigma #107724-20-9 (10mM stock concentration also prepared in DMSO and stored at -20°C)

Methods:

Assay Set up and Calculations:

1. Cells are grown in F12+FBS+hygromycin+pen/strep+Geneticin
2. Cells are collected with 0.05% trypsin and the cell suspension is spun and re-suspended in a volume of F12+1.5% CD-FBS and counted.
3. The cells are re-suspended to 4x105 cells /ml.
4. Cells are (25 ul/well) added to the wells of a 384 well plate.
5. The plate is then incubated at 37ºC over night in a humidified incubator with 5% CO2.
6. Test compounds are titrated starting at 4.4mM, 10-point titration in 1:3 dilution.
7. Aldosterone is diluted in assay media to 1.8 nM from 10 uM stock (final concentration to be 0.3 nM)

Protocol for 384 well plate format: 6 hr treatment:

1. Plate 10K exponentially growing cells/well (25 ul) re-suspended in assay media to each well using the Multidrop (Thermo Electron) (use white wall, clear bottom assay plates (Costar #3570) and incubate overnight at 37° C, 5% CO_2.
2. Add 0.25 ul 120 x test compound (final DMSO concentration should be < 1%) to each well n=2, 10 point titrations starting at 36.7 uM final concentration.
3. Add 5ul 6x agonist (final aldosterone concentration should be 0.3 nM) to all wells using the PlateMate Plus.

   (ThermoFisher) (Except those wells in columns 23 and 24)
4. Add 5 ul of assay media to all wells in column 23 and 24.
5. Incubate 6 hrs at 37° C, 5% CO_2.
6. Add 15 ul 3x DiscoveRx detection reagent to each well.
7. Incubate 1 hour at room temperature (keep plates stored in the dark).
8. Read plates on Envision (PerkinElmer) luminesence plate reader and analyze using ADA.

Part II. Synthesis procedures

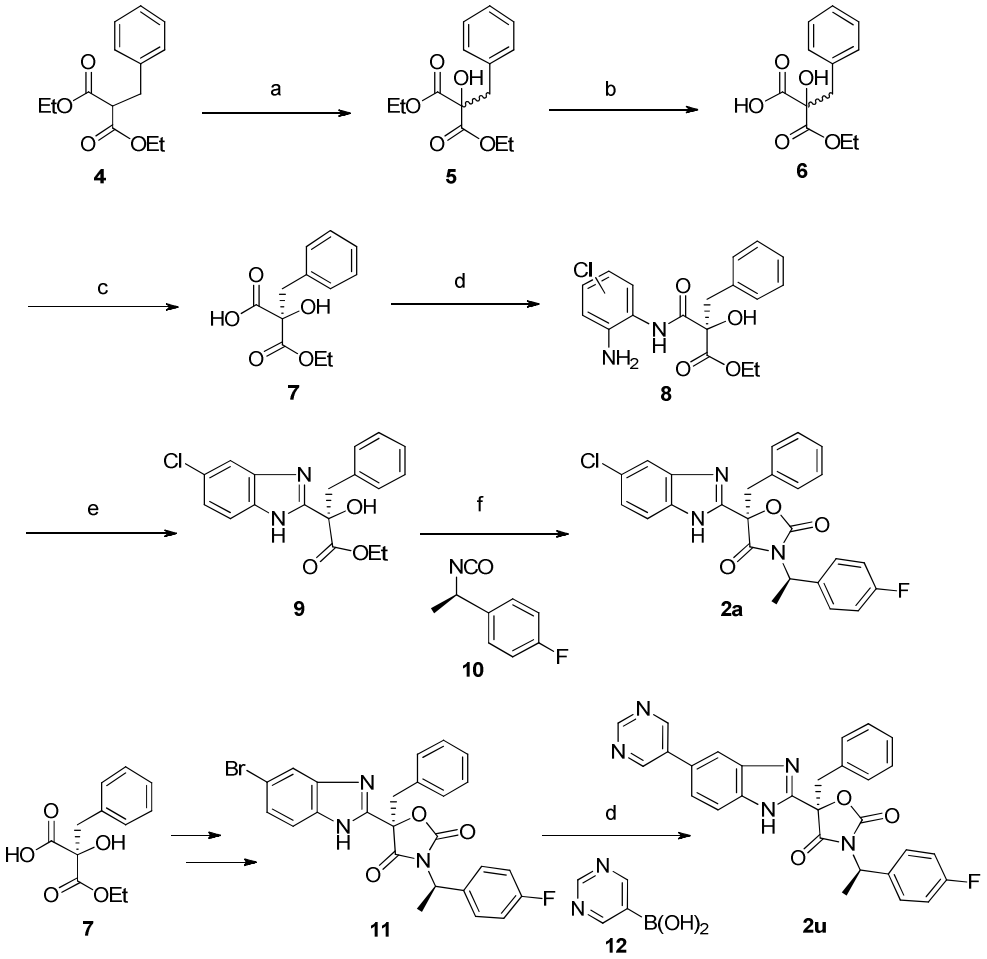
Nuclear magnetic resonance (NMR) spectra were recorded on a 500 MHz NMR spectrometer. Chemical shifts are given in parts per million (ppm) with the residual solvent signal used as reference. Coupling constants are reported in Hz. NMR abbreviations are used as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets, dt = doublet of triplets, bs = broad singlet.

LC/MS data were recorded by the LC/MS system with an electrospray source. UV-Detector, monitoring at 254 nm, C18 column (5 •, 50 × 4.6 mm), using a gradient of 5% v/v CH_3CN (containing 0.05% v/v TFA) in H_2O (containing 0.05% v/v TFA) (t = 0.0 min) gradient to 100% v/v CH_3CN in H_2O (t = 20.0 min), 3.5 mL/min.

Flash chromatography was performed using Si 25 S/M cartridges. Analytical thin layer chromatography (TLC) was performed with 0.25 mm silica gel 60-F commercial silica gel plates. Visualization was accomplished with UV light or potassium

permanganate stain, followed by heating. Preparative HPLC was conducted on a reverse phase HPLC using a C18 column (10 μ, 250 × 20 mm), 5% (v/v) CH_3CN (containing 0.1% v/v TFA) in H_2O (containing 0.1% v/v TFA) gradient to 100% CH_3CN, 20-50 mL/min, λ = 254 nm.

Procedure for the synthesis of 2a:



The mixture of diethyl α-benzylmalonate **4** (41 g, 164 mmol) and CsF (49.8 g) in 90 mL of DMF was heated at 40 ^oC with vigorous air bubbling for 3 days. The resulting mixture was diluted with ethyl acetate (800 mL) and washed with water (1 L x 3). The organic layer was concentrated and then purified by silica gel chromatography (5-20% ethyl acetate in hexanes) to give the diethyl α-benzyl α-hydroxy malonate **5** (31 g, 117 mmol, 71%) as a colorless oil. ^1H NMR (300 MHz, CDCl3) δ: 7.22-7.26 (m, 5H), 4.23 (q, J = 7.2 Hz, 4H), 3.80 (br, 1H), 3.35 (s, 2H), 1.26 (t, J = 7.2 Hz, 6H).

To diethyl α-benzyl α-hydroxy malonate **5** (6.3 g, 24 mmol) in 50 mL of EtOH (anhydrous) was added KOH (1.5 g). The mixture was stirred at rt over night. After removing the solvent in vacuo, the residue was taken up with 100 mL of ethyl acetate and 150 mL of water. The organic layer was removed. To the aqueous layer containing the potassium salt of the desired product was added 0.2 N HCl until pH = 2. The mixture was extracted with 200 mL ethyl acetate. The organic layer was dried with magnesium sulfate and concentrated to give the acid **6** (2.6 g, 11 mmol, 46%) as a colorless oil. The racemic acid was then purified by chiral SFC (OJ-H, 4.6 x 100 mm, 5% MeOH/0.1% TFA/CO_2, 2.5 mL/min, 100 bar) to give (2*R*)-2-benzyl-3-ethoxy-2-hydroxy-3-oxopropanoic acid as a single enantiomer **7** (1.0 g, the first eluting peak). LC/MS 261.1 $[M + 23(Na)]^+$.

To the mixture of (2*R*)-2-benzyl-3-ethoxy-2-hydroxy-3-oxopropanoic acid **7** (450 mg, 1.89 mmol), HATU (790 mg, 2.08 mmol), 3,4-diamino-chlorobenzene (296 mg, 2.08 mmol), diisopropylethyl amine (269 mg, 2.08 mmol) was added 4 mL of DMF at rt. The mixture was stirred at rt overnight. To this mixture was added water and ethyl acetate. The organic layer was washed with water and then concentrated *in vacuo* after being dried with magnesium sulfate. The residue containing the crude product **8**, without purification, was dissolved in 5 mL of acetic acid and heated at 75 degrees for 2 h. The mixture was then concentrated and purified by silica gel chromatography (8-35% ethyl acetate in hexanes) to give ethyl (2*R*)-2-(6-chloro-1H-benzimidazole-2-yl)-2-hydroxy-3-phenylpropanoate **9** as a pale brown oil (380 mg, 1.10 mmol, 58% over 2 steps). LC/MS 347.0 $[M+1]^+$.

To a solution of ethyl (2*R*)-2-(6-chloro-1H-benzimidazole-2-yl)-2-hydroxy-3-phenylpropanoate **9** (0.52 g, 1.51 mmol) and isocyanate **10** in THF (5 ml) at rt was added NaOH (60 mg, 1.5 mmol). The mixture was heated to 70 °C for 25 min. The resulting mixture was filtered through a short plug of silica and concentrated. The filtrate was concentrated and the resulting residue was purified by silica gel chromatography (5-35% ethyl acetate in hexanes) to give the desired product **2a** (0.54 g, 1.16 mmol, 77%). ^{1}H NMR (Acetone-d_6, 500 MHz) δ 7.63 (s, 1H), 7.57 (d, *J* = 9.0 Hz, 1H), 7.28 (m, 1H), 7.21 (m, 5H), 6.93 (m, 2H), 6.87 (t, *J* = 8.5 Hz, 2H), 5.01 (q, *J* = 7.0 Hz,

1H), 3.96 (d, J = 14.0 Hz, 1H), 3.61 (d, J = 14.0 Hz, 1H), 1.47 (d, J = 7.0 Hz, 3H); LC/MS m/z 465.95 $[M+1]^+$.

The same procedure using properly substituted anilines was adopted to prepare analogs **2a-2j, 2o-p,** and **3a-3e**.

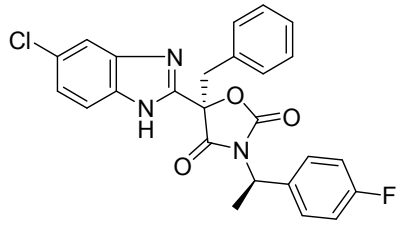
Procedure for the synthesis of 2u:

To the mixture of (5R)-5-benzyl-5-(5-bromo-1H-benzimidazol-2-yl)-3-[(1R)-1-(4-fluorophenyl)ethyl]-1,3-oxazolidine-2,4-dione **11** (25 mg, 0.049 mmol), obtained from a similar procedure as described from the synthesis of analog **2a**, $Pd(OAc)_2$ (1 mg, 0.0045 mmol), dppf (5.5 mg), K_3PO_4 (31 mg, 0.15 mmol), 3-pyrimidinyl boronic acid **12** (12 mg, 0.097 mmol) in 0.5 mL of ethanol was heated at 120 ^oC in microwave for 5 min. The reaction mixture was diluted with EtOAc, filtered, concentrated, and then purified via RP-HPLC (30-100% MeCN/water containing 0.1% TFA) to give compound **2u** (19 mg, 0.037 mmol, 76%) as a white solid after lyophilization. LC/MS 508.15 $[M + 1]^+$. ^1H NMR (Acetone-d_6, 500 MHz) δ 9.19 (s, 1H), 9.15 (s, 2H), 8.09 (s, 1H), 7.85 (d, J = 8.5 Hz, 1H), 7.74 (dd, J = 1.5, 8.5 Hz, 1H), 7.35 (m, 1H), 7.31 (m, 4H), 7.04 (m, 2H), 6.98 (m, 2H), 5.10 (q, J = 7.0 Hz, 1H), 4.07 (d, J = 14.0 Hz, 1H), 3.83 (d, J = 14.0 Hz, 1H), 1.56 (d, J = 7.0 Hz, 3H); LC/MS m/z 508.13 $[M+1]^+$.

The same Suzuki coupling condition was applied with various boronic acids/esters to give the corresponding products **2k-2o, 2q-2u, 3f-3k.**

Part III. Characterization of compounds

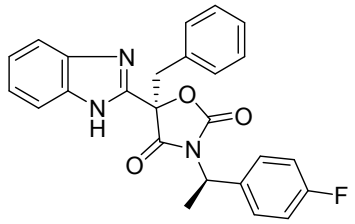
Compound **2a**



^1H NMR (Acetone-d_6, 500 MHz) δ 7.63 (s, 1H), 7.57 (d, J = 9.0 Hz, 1H), 7.28 (m, 1H), 7.21 (m, 5H), 6.93 (m, 2H), 6.87 (t, J = 8.5 Hz, 2H), 5.01 (q, J = 7.0

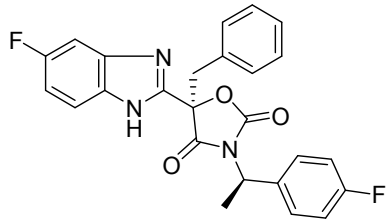
Hz, 1H), 3.96 (d, *J* = 14.0 Hz, 1H), 3.61 (d, *J* = 14.0 Hz, 1H), 1.47 (d, *J* = 7.0 Hz, 3H); LC/MS m/z 465.95 $[M+1]^+$. HPLC purity: >99%.

Compound **2b**



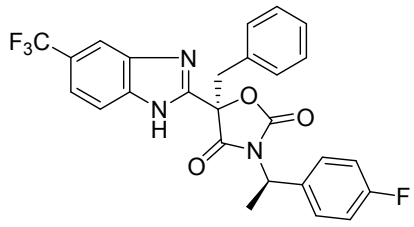
^{1}H NMR (Acetone-d_6, 500 MHz) δ 7.72 (m, 2H), 7.37 (m, 2H), 7.25 (m, 1H), 7.15 (m, 4H), 6.93 (m, 2H), 6.84 (m, 2H), 4.95 (q, *J* = 7.0 Hz, 1H), 3.98 (d, *J* = 14.0 Hz, 1H), 3.59 (d, *J* = 14.0 Hz, 1H), 1.43 (d, *J* = 7.0 Hz, 3H); LC/MS m/z 430.13 $[M+1]^+$. HPLC purity: >99%.

Compound **2c**



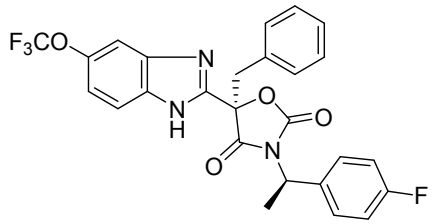
^{1}H NMR (Acetone-d_6, 500 MHz) δ 7.68 (m, 1H), 7.41 (d, *J* = 6.5 Hz, 1H), 7.36 (m, 1H), 7.33 (m, 4H), 7.15 (m, 1H), 7.11 (m, 2H), 6.96 (t, *J* = 7.0 Hz, 2H), 5.09 (q, *J* = 7.0 Hz, 1H), 4.01 (d, *J* = 14.5 Hz, 1H), 3.78 (d, *J* = 14.5 Hz, 1H), 1.54 (d, *J* = 7.5 Hz, 3H); LC/MS m/z 448.17 $[M+1]^+$. HPLC purity: >95%.

Compound **2d**



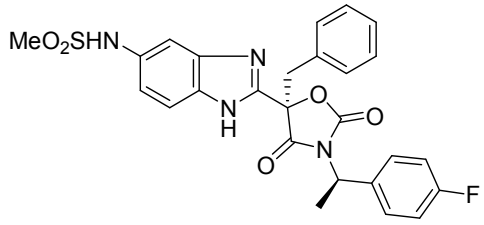
^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.04 (s, 1H), 7.83 (d, *J* = 8.5 Hz, 1H), 7.61 (d, *J* = 8.5 Hz, 1H), 7.31 (m, 5H), 7.01 (m, 2H), 6.96 (m, 2H), 5.09 (q, *J* = 7.0 Hz, 1H), 4.05 (d, *J* = 14.0 Hz, 1H), 3.81 (d, *J* = 14.0 Hz, 1H), 1.54 (d, *J* = 7.5 Hz, 3H); LC/MS m/z 498.07 $[M+1]^+$. HPLC purity: >95%.

Compound **2e**



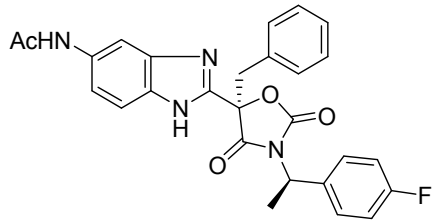
^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.70 (d, *J* = 8.5 Hz, 1H), 7.61 (s, 1H), 7.29 (m, 6H), 7.02 (m, 2H), 6.90 (t, *J* = 9.0 Hz, 2H), 5.04 (q, *J* = 7.5 Hz, 1H), 3.84 (d, *J* = 14.5 Hz, 1H), 3.61 (d, *J* = 14.5 Hz, 1H), 1.51 (d, *J* = 7.5 Hz, 3H); LC/MS m/z 514.07 $[M+1]^+$. HPLC purity: >99%.

Compound **2f**



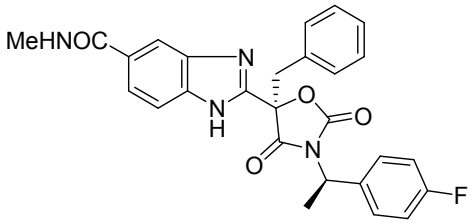
^{1}H NMR (Acetone-d_6, 500 MHz) δ 7.66 (bs, 1H), 7.30-7.36 (m, 7H), 7.04 (m, 2H), 6.97 (m, 2H), 6.70 (s, 1H), 5.08 (q, *J* = 7.0 Hz, 1H), 4.02 (d, *J* = 14.5 Hz, 1H), 3.78 (d, *J* = 14.5 Hz, 1H), 2.97 (s, 3H), 1.55 (d, *J* = 7.5 Hz, 3H); LC/MS m/z 523.14 $[M+1]^+$. HPLC purity: >99%.

Compound **2g**



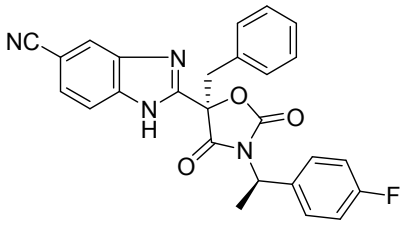
^{1}H NMR (Acetone-d_6, 500 MHz) δ 9.59 (s, 1H), 8.46 (s, 1H), 7.76 (d, *J* =8.5 Hz, 1H), 7.52 (d, *J* = 9.0 Hz, 1H), 7.35 (m, 1H), 7.30 (m, 4H), 7.01 (m, 2H), 6.96 (m, 2H), 5.11 (q, *J* = 7.5 Hz, 1H), 4.02 (d, *J* = 14.5 Hz, 1H), 3.90 (d, *J* = 14.5 Hz, 1H), 2.17 (s, 3H), 1.54 (d, *J* = 7.0 Hz, 3H); LC/MS m/z 487.16 $[M+1]^+$. HPLC purity: >99%.

Compound **2h**



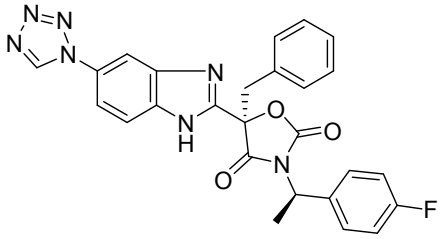
^{1}H NMR (Acetone-d_6, 500 MHz) δ 8.37 (s, 1H), 8.10 (d, *J* = 8.5 Hz, 1H), 8.05 (bs, 1H), 7.94 (d, *J* =8.5 Hz, 1H), 7.48 (m, 1H), 7.30 (m, 4H), 7.02 (m, 2H), 6.98 (m, 2H), 5.11 (q, *J* = 7.5 Hz, 1H), 4.03 (d, *J* = 14.5 Hz, 1H), 3.99 (s, 3H), 3.93 (d, *J* = 14.5 Hz, 1H), 1.54 (d, *J* = 7.0 Hz, 3H); LC/MS m/z 487.21 $[M+1]^+$. HPLC purity: >95%.

Compound **2i**



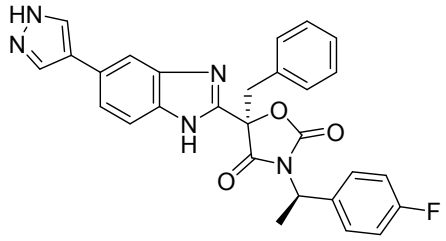
^{1}H NMR (Acetone-d_6, 500 MHz) δ 8.18 (s, 1H), 7.82 (m, 2H), 7.65 (dd, *J* = 1.5, 8.5 Hz, 2H), 7.26 (m, 3H), 7.00 (m, 4H), 5.11 (q, *J* = 7.5 Hz, 1H), 4.00 (d, *J* = 14.5 Hz, 1H), 3.78 (d, *J* = 14.5 Hz, 1H), 1.51 (d, *J* = 7.0 Hz, 3H); LC/MS m/z 473.15 $[M+1]^+$. HPLC purity: >90%.

Compound **2j**



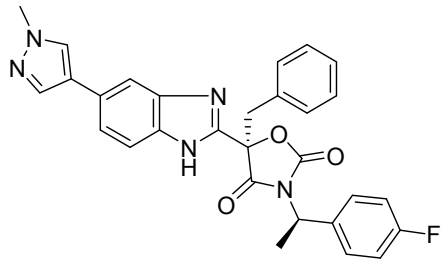
^{1}H NMR (Acetone-d_6, 500 MHz) δ 9.75 (s, 1H), 8.20 (s, 1H), 7.89 (d, *J* = 8.5 Hz, 1H), 7.83 (dd, *J* = 2.0, 8.5 Hz, 1H), 7.31-7.37 (m, 5H), 7.22 (m, 1H), 7.04 (m, 2H), 6.98 (m, 2H), 5.11 (q, *J* = 7.0 Hz, 1H), 4.06 (d, *J* = 14.5 Hz, 1H), 3.82 (d, *J* = 14.5 Hz, 1H), 1.55 (d, *J* = 7.0 Hz, 3H); LC/MS m/z 498.17 $[M+1]^+$. HPLC purity: >99%.

Compound **2k**



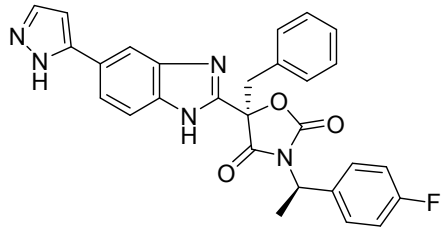
^{1}H NMR (Acetone-d_6, 500 MHz) δ 8.04 (s, 2H), 7.72 (bs, 1H), 7.58 (m, 2H), 7.35 (m, 1H), 7.31 (m, 5H), 7.04 (m, 2H), 6.96 (m, 2H), 5.10 (q, *J* = 7.0 Hz, 1H), 4.06 (d, *J* = 14.5 Hz, 1H), 3.82 (d, *J* = 14.5 Hz, 1H), 1.56 (d, *J* = 7.0 Hz, 3H); LC/MS m/z 496.15 $[M+1]^+$. HPLC purity: >99%.

Compound **2l**



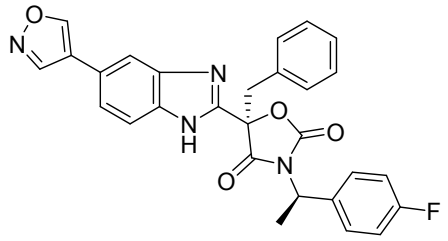
^{1}H NMR (Acetone-d_6, 500 MHz) δ 8.00 (s, 1H), 7.82 (s, 1H), 7.79 (s, 1H), 7.63 (d, *J* = 8.5 Hz, 1H), 7.53 (dd, *J* = 1.5, 8.0 Hz, 1H), 7.34 (m, 1H), 7.30 (m, 4H), 7.03 (m, 2H), 6.98 (m, 2H), 5.09 (q, *J* = 7.0 Hz, 1H), 4.03 (d, *J* = 14.5 Hz, 1H), 3.93 (s, 3H), 3.79 (d, *J* = 14.5 Hz, 1H), 1.55 (d, *J* = 7.0 Hz, 3H); LC/MS m/z 510.16 $[M+1]^+$. HPLC purity: >99%.

Compound **2m**



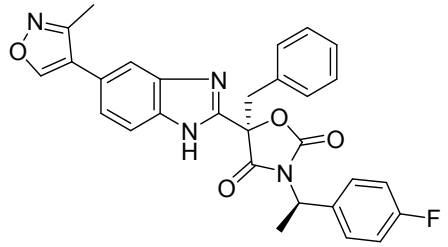
^{1}H NMR (Acetone-d_6, 500 MHz) δ 8.13 (s, 1H), 7.87 (d, *J* =8.5 Hz, 1H), 7.77 (s, 1H), 7.70 (d, *J* = 9.0 Hz, 1H), 7.32 (m, 1H), 7.31 (m, 4H), 7.03 (m, 2H), 6.99 (m, 2H), 6.77 (s, 1H), 5.11 (q, *J* = 7.0 Hz, 1H), 4.05 (d, *J* = 14.5 Hz, 1H), 3.81 (d, *J* = 14.5 Hz, 1H), 1.56 (d, *J* = 7.0 Hz, 3H); LC/MS m/z 496.07 $[M+1]^+$. HPLC purity: >99%.

Compound **2n**



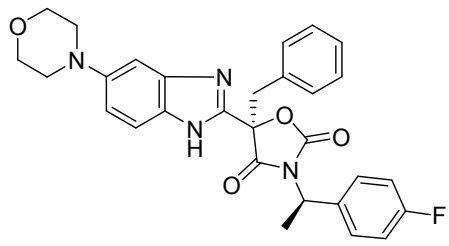
^{1}H NMR (Acetone-d_6, 500 MHz) δ 9.23 (s, 1H), 8.99 (s, 1H), 8.00 (bs, 1H), 7.76 (bs, 1H), 7.65 (s, 1H), 7.36 (m, 1H), 7.32 (m, 4H), 7.05 (m, 2H), 6.98 (m, 2H), 5.11 (q, *J* = 7.0 Hz, 1H), 4.03 (d, *J* = 14.5 Hz, 1H), 3.80 (d, *J* = 14.5 Hz, 1H), 1.56 (d, *J* = 7.5 Hz, 3H); LC/MS m/z 497.13 $[M+1]^+$. HPLC purity: >95%.

Compound **2o**



^{1}H NMR (Acetone-d_6, 500 MHz) δ 8.87 (s, 1H), 7.78 (s, 1H), 7.73 (d, *J* =8.5 Hz, 1H), 7.45 (dd, *J* = 1.5, 8.5 Hz, 1H), 7.35 (m, 1H), 7.31 (m, 6H), 7.06 (m, 2H), 6.98 (t, *J* =8.5 Hz, 2H), 5.10 (q, *J* = 7.0 Hz, 1H), 4.05 (d, *J* = 14.5 Hz, 1H), 3.80 (d, *J* = 14.5 Hz, 1H), 1.55 (d, *J* = 7.0 Hz, 3H); LC/MS m/z 511.15 $[M+1]^+$. HPLC purity: >95%.

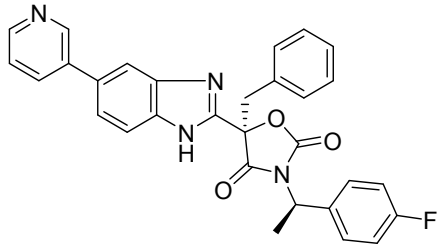
Compound **2p**



^{1}H NMR (Acetone-d_6, 500 MHz) δ 7.76 (d, *J* = 9.0 Hz, 1H), 7.61 (d, *J* = 2.5 Hz, 1H), 7.47 (dd, *J* = 2.0, 9.0 Hz, 1H), 7.28-7.36 (m, 5H), 7.04 (m, 2H), 6.98 (m, 2H), 5.08 (q, *J* = 7.0 Hz, 1H), 4.05 (d, *J* = 14.0 Hz, 1H), 3.96 (m, 4H), 3.82 (d, *J*

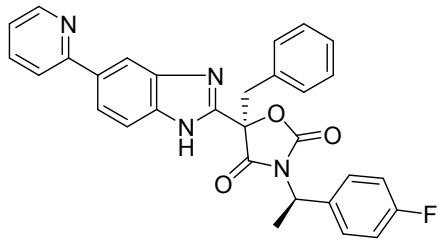
= 14.0 Hz, 1H), 3.43 (m, 4H), 1.54 (d, *J* = 7.5 Hz, 3H); LC/MS m/z 515.16 $[M+1]^+$. HPLC purity: >99%.

Compound **2q**



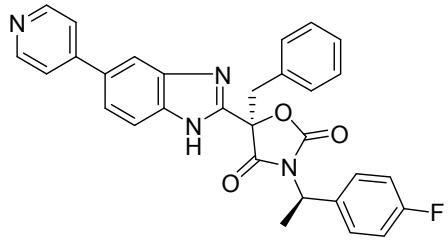
^{1}H NMR (Acetone-d_6, 500 MHz) δ 9.30 (s, 1H), 8.94 (d, *J* = 4.5 Hz, 1H), 8.84 (d, *J* = 9.0 Hz, 1H), 8.14 (m, 2H), 7.86 (d, *J* = 8.5 Hz, 1H), 7.80 (dd, *J* = 1.5, 8.5 Hz, 1H), 7.37 (m, 5H), 7.32 (m, 2H), 7.04 (m, 2H), 5.10 (q, *J* = 7.0 Hz, 1H), 4.07 (d, *J* = 14.0 Hz, 1H), 3.82 (d, *J* = 14.0 Hz, 1H), 1.55 (d, *J* = 7.5 Hz, 3H); LC/MS m/z 507.18 $[M+1]^+$. HPLC purity: >99%.

Compound **2r**



^{1}H NMR (Acetone-d_6, 500 MHz) δ 8.80 (s, 1H), 8.51 (s, 1H), 8.08-8.16 (m, 3H), 7.85 (s, 1H), 7.57 (s, 1H), 7.34 (m, 1H), 7.31 (m, 4H), 7.03 (m, 2H), 6.97 (m, 2H), 5.10 (q, *J* = 7.0 Hz, 1H), 4.03 (d, *J* = 14.0 Hz, 1H), 3.84 (d, *J* = 14.0 Hz, 1H), 1.58 (d, *J* = 7.0 Hz, 3H); LC/MS m/z 507.19 $[M+1]^+$. HPLC purity: >99%.

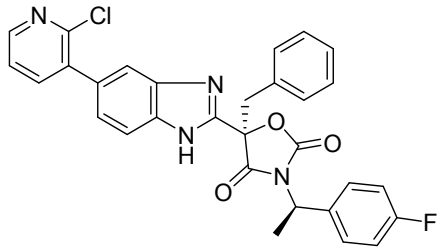
Compound **2s**



^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.89 (d, *J* = 6.0 Hz, 2H), 8.16 (s, 1H), 8.07 (d, *J* = 6.0 Hz, 2H), 7.85 (d, *J* = 8.5 Hz, 1H), 7.72 (d, *J* = 8.5 Hz, 1H), 7.30 (m, 5H),

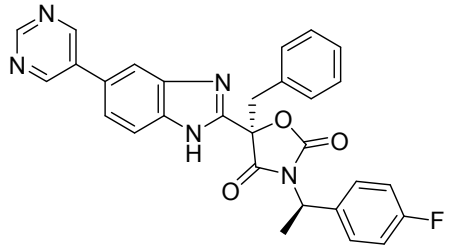
7.03 (m, 2H), 6.90 (m, 2H), 5.08 (q, *J* = 7.0 Hz, 1H), 3.90 (d, *J* = 14.5 Hz, 1H), 3.64 (d, *J* = 14.5 Hz, 1H), 1.54 (d, *J* = 7.0 Hz, 3H); LC/MS m/z 507.18 $[M+1]^+$. HPLC purity: >99%.

Compound **2t**



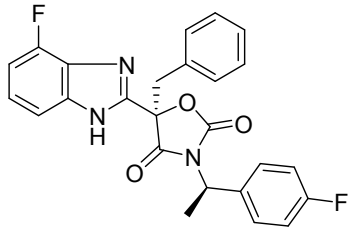
^{1}H NMR (Acetone-d_6, 500 MHz) δ 8.44 (dd, *J* = 2.0, 4.5 Hz, 1H), 7.92 (dd, *J* = 2.0, 7.5 Hz, 1H), 7.77 (m, 2H), 7.52 (m, 1H), 7.44 (m, 1H), 7.36 (m, 1H), 7.33 (m, 4H), 7.05 (m, 2H), 6.99 (m, 2H), 5.10 (q, *J* = 7.0 Hz, 1H), 4.07 (d, *J* = 14.5 Hz, 1H), 3.82 (d, *J* = 14.5 Hz, 1H), 1.57 (d, *J* = 7.0 Hz, 3H); LC/MS m/z 541.13 $[M+1]^+$. HPLC purity: >90%.

Compound **2u**



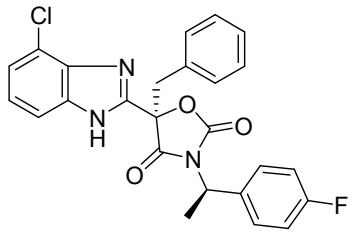
^{1}H NMR (Acetone-d_6, 500 MHz) δ 9.19 (s, 1H), 9.15 (s, 2H), 8.09 (s, 1H), 7.85 (d, *J* = 8.5 Hz, 1H), 7.74 (dd, *J* = 1.5, 8.5 Hz, 1H), 7.35 (m, 1H), 7.31 (m, 4H), 7.04 (m, 2H), 6.98 (m, 2H), 5.10 (q, *J* = 7.0 Hz, 1H), 4.07 (d, *J* = 14.0 Hz, 1H), 3.83 (d, *J* = 14.0 Hz, 1H), 1.56 (d, *J* = 7.0 Hz, 3H); LC/MS m/z 508.13 $[M+1]^+$. HPLC purity: >99%.

Compound **3a**



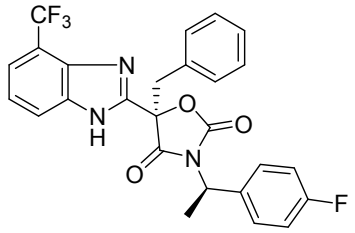
^{1}H NMR (Acetone-d_6, 500 MHz) δ 7.43 (d, *J* = 8.0 Hz, 1H), 7.34 (m, 1H), 7.31 (m, 5H), 7.06 (m, 3H), 6.97 (t, *J* = 8.5 Hz, 2H), 5.08 (q, *J* = 7.0 Hz, 1H), 4.05 (d, *J* = 14.5 Hz, 1H), 3.78 (d, *J* = 14.5 Hz, 1H), 1.55 (d, *J* = 7.5 Hz, 3H); LC/MS m/z 448.14 $[M+1]^+$. HPLC purity: >99%.

Compound **3b**



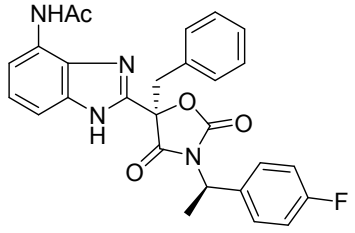
^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.71 (d, *J* = 7.5 Hz, 1H), 7.40 (d, *J* = 7.0 Hz, 1H), 7.33 (t, *J* = 8.0 Hz, 1H), 7.28 (m, 7H), 7.01 (m, 2H), 6.90 (t, *J* = 8.5 Hz, 2H), 5.05 (q, *J* = 7.5 Hz, 1H), 3.86 (d, *J* = 14.5 Hz, 1H), 3.64 (d, *J* = 14.5 Hz, 1H), 1.52 (d, *J* = 7.0 Hz, 3H); LC/MS m/z 464.06 $[M+1]^+$. HPLC purity: >99%.

Compound **3c**



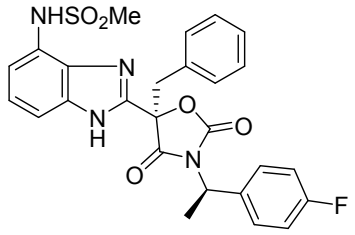
^{1}H NMR (Acetone-d_6, 500 MHz) δ 7.92 (d, *J* = 8.0 Hz, 1H), 7.66 (d, *J* = 8.0 Hz, 1H), 7.48 (t, *J* = 8.0 Hz, 1H), 7.34 (m, 5H), 7.07 (m, 2H), 6.99 (m, 2H), 5.09 (q, *J* = 7.0 Hz, 1H), 4.07 (d, *J* = 14.5 Hz, 1H), 3.82 (d, *J* = 14.5 Hz, 1H), 1.53 (d, *J* = 7.5 Hz, 3H); LC/MS m/z 498.11 $[M+1]^+$. HPLC purity: >99%.

Compound **3d**



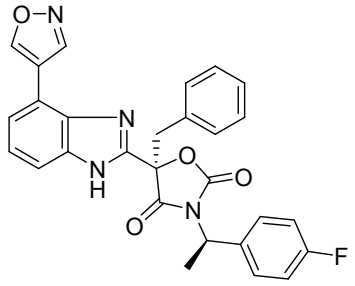
^{1}H NMR (Acetone-d_6, 500 MHz) δ 9.61 (s, 1H), 7.93 (d, *J* = 8.0 Hz, 1H), 7.34 (m, 7H), 7.01 (m, 2H), 6.99 (m, 2H), 5.09 (q, *J* = 7.0 Hz, 1H), 4.00 (d, *J* = 14.5 Hz, 1H), 3.81 (d, *J* = 14.5 Hz, 1H), 2.28 (s, 3H), 1.54 (d, *J* = 7.5 Hz, 3H); LC/MS m/z 487.16 $[M+1]^+$. HPLC purity: >99%.

Compound **3e**



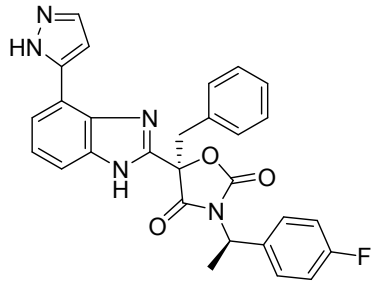
^{1}H NMR (Acetone-d_6, 500 MHz) δ 8.63 (s, 1H), 7.41 (d, *J* = 8.0 Hz, 1H), 7.33 (m, 8H), 7.04 (m, 2H), 6.99 (m, 2H), 5.09 (q, *J* = 7.0 Hz, 1H), 4.02 (d, *J* = 14.5 Hz, 1H), 3.80 (d, *J* = 14.5 Hz, 1H), 3.16 (s, 3H), 1.54 (d, *J* = 7.0 Hz, 3H); LC/MS m/z 523.15 $[M+1]^+$. HPLC purity: >99%.

Compound **3f**



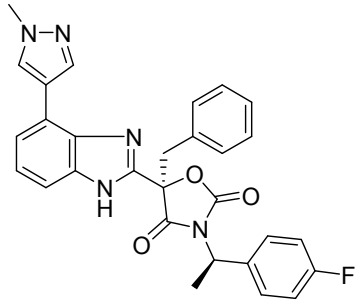
^{1}H NMR (Acetone-d_6, 500 MHz) δ 9.68 (s, 1H), 9.30 (s, 1H), 7.69 (d,, *J* = 7.0 Hz, 1H), 7.55 (d,, *J* = 8.0 Hz, 1H), 7.39 (m, 1H), 7.32 (m, 5H), 7.09 (m, 3H), 6.98 (m, 2H), 5.11 (q, *J* = 7.0 Hz, 1H), 4.13 (d, *J* = 14.0 Hz, 1H), 3.87 (d, *J* = 14.5 Hz, 1H), 1.56 (d, *J* = 7.0 Hz, 3H); LC/MS m/z 497.13 $[M+1]^+$. HPLC purity: >95%.

Compound **3g**



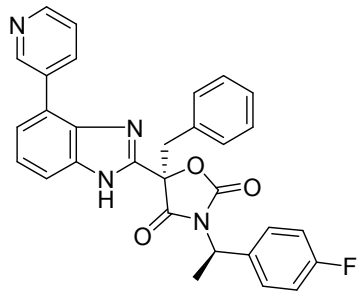
^{1}H NMR (Acetone-d_6, 500 MHz) δ 7.81 (s, 1H), 7.79 (s, 1H), 7.62 (d,, J = 8.0 Hz, 1H), 7.36 (m, 6H), 7.06 (m, 3H), 6.98 (t, J = 8.5 Hz, 2H), 5.12 (q, J = 7.0 Hz, 1H), 4.08 (d, J = 14.5 Hz, 1H), 3.87 (d, J = 14.5 Hz, 1H), 1.56 (d, J = 7.5 Hz, 3H); LC/MS m/z 496.16 $[M+1]^+$. HPLC purity: >95%.

Compound **3h**



^{1}H NMR (Acetone-d_6, 500 MHz) δ 8.59 (s, 1H), 8.28 (s, 1H), 7.56 (d,, J = 7.5 Hz, 1H), 7.39 (m, 1H), 7.031 (m, 6H), 7.05 (m, 2H), 6.98 (t, J = 8.5 Hz, 2H), 5.11 (q, J = 7.0 Hz, 1H), 4.11 (d, J = 14.5 Hz, 1H), 3.98 (s, 3H), 3.87 (d, J = 14.5 Hz, 1H), 1.57 (d, J = 7.5 Hz, 3H); LC/MS m/z 510.15 $[M+1]^+$. HPLC purity: >99%.

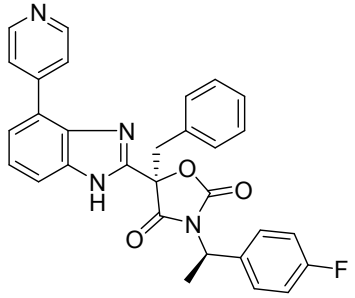
Compound **3i**



^{1}H NMR (Acetone-d_6, 500 MHz) δ 8.71 (bs, 1H), 7.70 (m, 3H), 7.48 (t, J = 7.5 Hz, 1H), 7.36 (m, 1H), 7.32 (m, 4H), 7.05 (m, 2H), 6.97 (t, J = 9.0 Hz, 2 H),

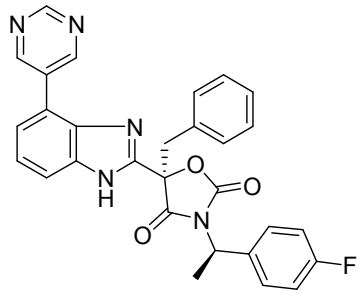
5.09 (q, J = 7.0 Hz, 1H), 4.08 (d, J = 14.5 Hz, 1H), 3.86 (d, J = 14.5 Hz, 1H), 1.54 (d, J = 7.5 Hz, 3H); LC/MS m/z 507.14 $[M+1]^+$. HPLC purity: >95%.

Compound **3j**



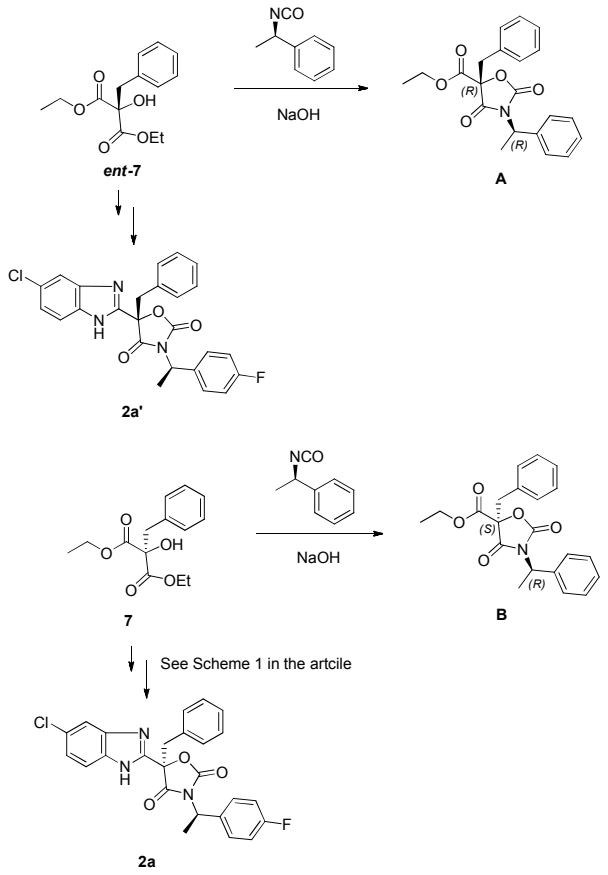
^{1}H NMR (Acetone-d_6, 500 MHz) δ 9.01 (bs, 2H), 8.94 (bs, 2H), 7.97 (d, J = 7.5 Hz, 1H), 7.87 (d, J = 8.0 Hz, 1H), 7.56 (t, J = 8.5 Hz, 1H), 7.28 (m, 1H), 7.33 (m, 4H), 7.04 (m, 2H), 6.96 (t, J = 8.5 Hz, 2H), 5.11 (q, J = 7.0 Hz, 1H), 4.09 (d, J = 14.5 Hz, 1H), 3.88 (d, J = 14.0 Hz, 1H), 1.58 (d, J = 7.5 Hz, 3H); LC/MS m/z 507.14 $[M+1]^+$. HPLC purity: >95%.

Compound **3k**



^{1}H NMR (Acetone-d_6, 500 MHz) δ 9.53 (s, 2H), 9.20 (s, 1H), 7.71 (m, 2H), 7.50 (t, J = 8.0 Hz, 1H), 7.37 (m, 1H), 7.33 (m, 4H), 7.06 (m, 2H), 6.99 (m, 2H), 5.09 (q, J = 7.0 Hz, 1H), 4.07 (d, J = 14.0 Hz, 1H), 3.85 (d, J = 14.0 Hz, 1H), 1.53 (d, J = 7.0 Hz, 3H); LC/MS m/z 508.15 $[M+1]^+$. HPLC purity: >95%.

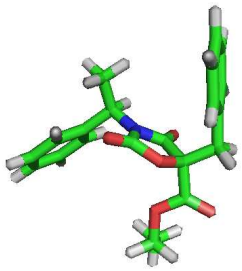
Scheme 1. Determination of absolute configuration of **2a**.



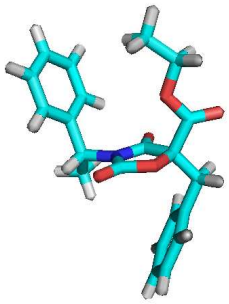
The absolute configuration of compound **2a** was indirectly established using the VCD (vibrational circular dichroism) spectroscopy of compounds, **A** and **B**, which were derived from intermediates *ent*-**7** and **7**, respectively. Compound **2a** was prepared from intermediate **10** (See **Scheme 1** from the article), and compound **B** was also prepared from intermediate **10**. Therefore, the absolute configuration of **2a** should match that of **B**.

Absolute configurations of **A** and **B** were assigned to be (*RR*) and (*SR*), respectively, as shown in **Scheme 1** using the VCD spectroscopy. The confidence level of the assignment was 100% on current Biotool's example database that includes 89 previous correct assignments for different chiral structures. The chiral center which is labeled as *R* in diastereomers **A** and **B** was known from the commercially available chiral starting material, namely, the chiral isocyanate. Assignment of the other absolute configuration relies on this initial identification of *R*.

Global Minimized Structures:



RR



SR

VCD Background: Vibrational circular dichroism is a method useful for determining the AC of chiral molecules. Similar to a circular dichroism experiment, but in the IR region, differences in the absorbance signal of left and right polarized light passing through the sample are exploited to determine the AC. The method relies on comparing the experimentally determined IR and VCD spectra to the calculated spectra. Calculations are performed to exhaustively sample conformation space, determine structural minima, and Boltzmann-weight the calculated spectra when relevant. Use of Biotool's CompareVOA application allows the measured and calculated spectra to be matched and goodness metrics are output describing how confident the AC assignment is. Figures output show the superposition of curves, and report the enantiomeric similarity index (ESI) which quantifies how well the IR and VCD spectra for one enantiomer match (experimental vs. calculated) versus the opposite enantiomer. The ESI value is used to calculate a confidence percentage, a confidence percent >60 indicates good agreement of the spectra.

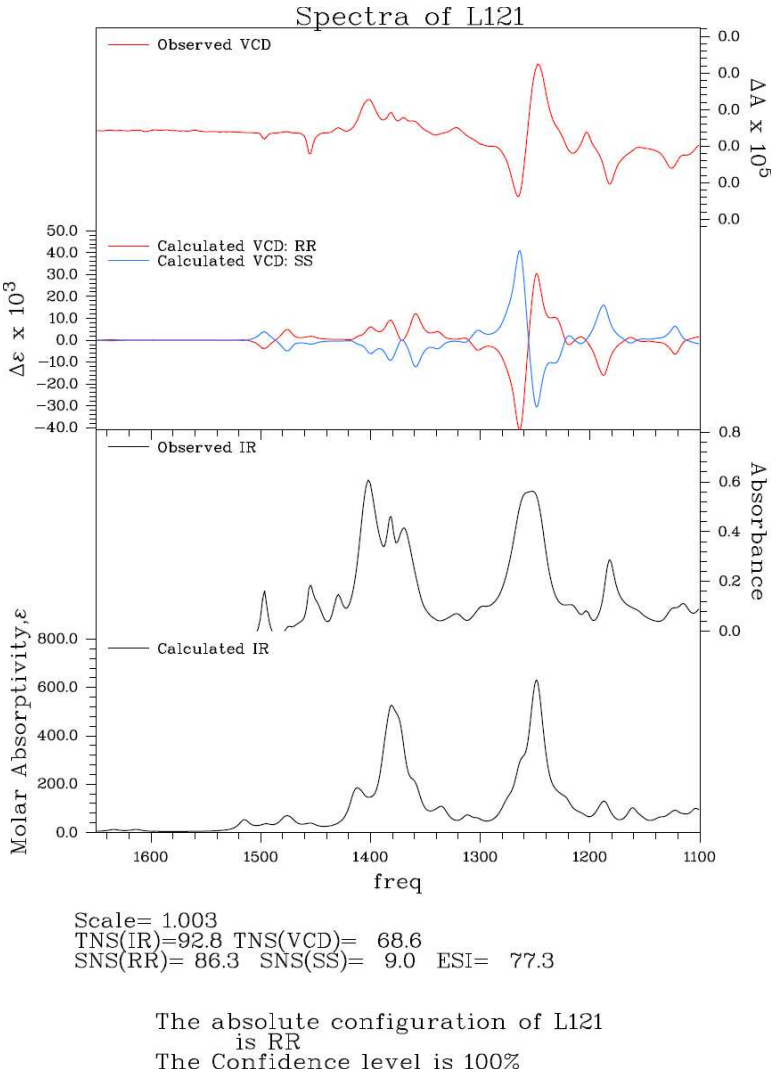


Figure 1. IR (lower frame) and VCD (upper frame) spectra of **A** in $CDCl_3$. VCD spectrum of **A** compares well with the Boltzmann-population-weighted calculated spectra of the (*RR*)-configuration.

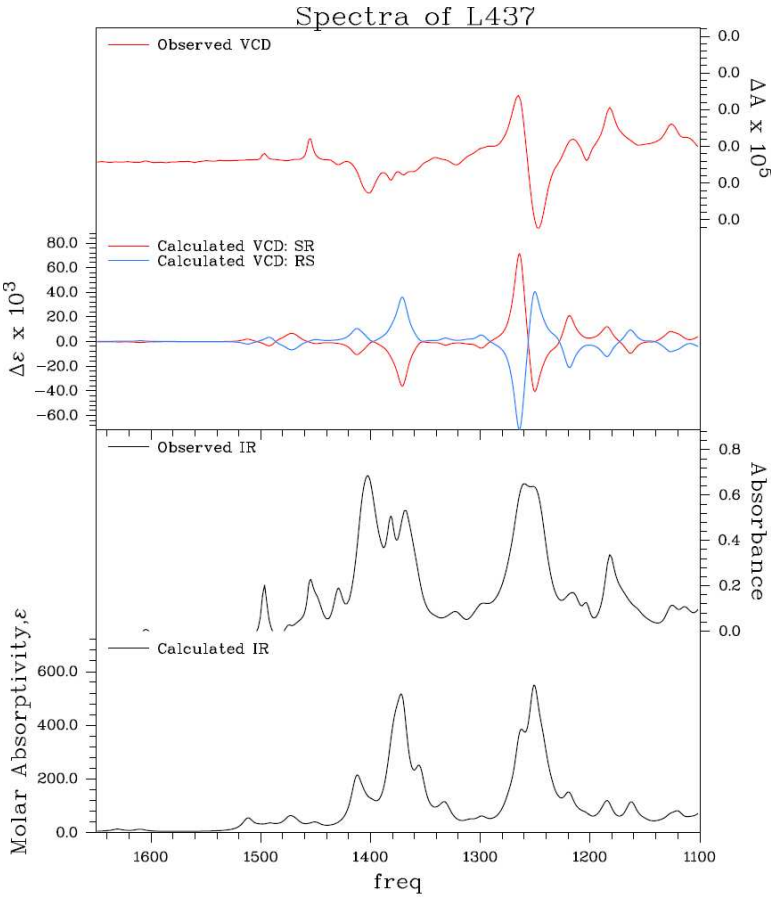


Figure 2. IR (lower frame) and VCD (upper frame) spectra of **B** in $CDCl_3$. VCD spectrum of **B** compares well with the Boltzmann-population-weighted calculated spectra of the (SR)-configuration.