

Supporting Information

Synthesis and Stability of an Oxetane Analog of Thalidomide and Lenalidomide

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GENERAL INFORMATION

All non-aqueous reactions were carried out using oven-dried (90 °C) or heat gun dried glassware under a positive pressure of dry argon unless otherwise noted. Acetonitrile, dichloromethane, diethyl ether, tetrahydrofuran, and toluene were purified by passage over activated alumina under an argon atmosphere (H₂O content < 30 ppm, Karl–Fischer titration). Triethylamine was distilled from KOH under an atmosphere of dry nitrogen. All other commercially available reagents were used without further purification. Except if indicated otherwise, reactions were magnetically stirred and monitored by thin layer chromatography using *Merck* Silica Gel 60 F254 plates and visualized by fluorescence quenching under UV light. In addition, TLC plates were stained using ceric ammonium molybdate, potassium permanganate, vanillin, 4-methoxybenzaldehyde, or ninhydrin stain. Chromatographic purification of products (flash chromatography) was performed on *Brunschwig* or *Fluka* silica 32-63, 60 Å using a forced flow of eluent at 0.3-0.5 bar. Concentration under reduced pressure was performed by rotary evaporation at 40 °C at the appropriate pressure. Purified compounds were further dried under high vacuum. Yields refer to chromatographically purified and spectroscopically pure compounds, unless otherwise stated.

Melting points: measured on a *Büchi* SMP-20 or B-545 apparatus. All melting points were measured in open capillaries and are uncorrected.

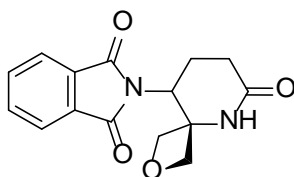
NMR spectra: NMR spectra were recorded on a *Varian* Mercury 300 spectrometer operating at 300 MHz and 75 MHz for ¹H and ¹³C acquisitions, respectively, or on *Bruker* DRX400 (or AV400) spectrometers operating at 400 MHz (¹H) and 101 MHz (¹³C). Chemical shifts (δ) are reported in ppm with the solvent resonance as the internal standard relative to chloroform (δ = 7.26) for ¹H, and chloroform (δ = 77.0) for ¹³C. All ¹³C spectra were measured with complete proton decoupling. Data are reported as follows: s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, m = multiplet, br = broad signal; coupling constants in Hz.

IR spectra: recorded on a *Perkin Elmer* Spectrum RX-I FT-IR (as thin film) or *Perkin Elmer* Spectrum BX FT-IR (neat) spectrometer. Absorptions are given in wavenumbers (cm⁻¹).

Mass spectra: recorded by the MS service at *ETH Zürich*. EI-MS (*m/z*): *Waters* Micromass AutoSpec Ultima spectrometer. ESI-MS (*m/z*): *Bruker* Daltonics maXis spectrometer. MALDI-MS (*m/z*): *Bruker* Daltonics UltraFlex II spectrometer.

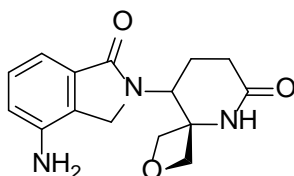
Chemical names: generated with ChemBioDraw Ultra 11.0 or 12.0 (*Cambridgesoft*) and modified where appropriate.

EXPERIMENTAL PROCEDURES



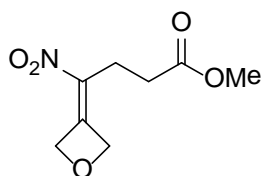
2-(6-Oxo-2-oxa-5-azaspiro[3.5]nonan-9-yl)isoindoline-1,3-dione (2). To a solution of phthalimide **12** (100 mg, 0.246 mmol, 1.0 equiv) in CH₃CN (3.5 ml), cooled to 0 °C, was added a solution of CAN (270 mg, 0.492 mmol, 2.0 equiv) in H₂O (1.2 ml), and the yellow clear solution was stirred at 0 °C for 30 min. It was allowed to warm to RT and stirring was continued for 45 min, when more CAN (67 mg, 0.143 mmol, 0.5 equiv) was added in one portion. Stirring was continued for another hour. At this point the mixture was partitioned between EtOAc (20 ml) and half-saturated aqueous NaCl (20 ml). The phases were separated, and the aqueous phase was extracted with EtOAc (20 ml), then with CH₂Cl₂ (2 × 20 ml). The combined organic phases were dried (Na₂SO₄), filtered, and concentrated *in vacuo*. The crude product was dissolved in CH₂Cl₂/MeOH (*ca.* 3:1) and silica gel was added, and the mixture was concentrated. The product on silica was added to a column and purified by FC (SiO₂; CH₂Cl₂ : MeOH 100:2 → 100:3) to afford the pure title compound. Yield: 38 mg (0.133 mmol, 54%). Colorless powder.

TLC: R_f = 0.21 (EtOAc; UV); **Melting Point:** >250 °C; **¹H NMR** (300 MHz, CDCl₃ : CD₃OD 5:2): δ = 7.78 – 7.67 (m, 2H), 7.67 – 7.54 (m, 2H), 4.80 (d, J =7.4, 1H), 4.59 – 4.35 (m, 4H), 2.79 – 2.52 (m, 1H), 2.42 – 2.11 (m, 2H), 1.91 – 1.72 (m, 1H); **¹³C NMR** (75 MHz, CDCl₃): δ = 169.9, 168.8, 134.5, 131.3, 123.6, 81.6, 81.3, 60.8, 50.9, 30.8, 22.2; **IR** (thin film): 3183, 3080, 2884, 1713, 1672, 1394, 1374, 1220, 966, 773, 719 cm⁻¹; **HRMS** (ESI): exact mass calculated for C₁₅H₁₄N₂NaO₄ ([M+Na]⁺), 309.0846; found 309.0844.



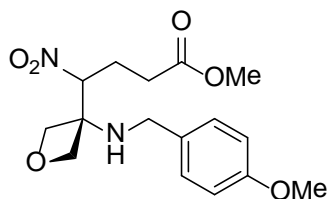
9-(4-Amino-1-oxoisindolin-2-yl)-2-oxa-5-azaspiro[3.5]nonan-6-one (4). To a solution of lactam **15** (69 mg, 0.22 mmol, 1.0 equiv) in MeOH (5 ml)/CH₂Cl₂ (5 ml) was added at RT palladium (10% on carbon; 43 mg, 0.04 mmol, 0.18 equiv), and a hydrogen atmosphere was built up (balloon). The mixture was stirred at RT for 50 min, when TLC analysis indicated full conversion of the starting material. An argon atmosphere was reinstalled, and the mixture was filtered over celite (washed with a 1:1 mixture of MeOH/CH₂Cl₂), and concentrated *in vacuo*. The residue was purified by FC (SiO₂; CH₂Cl₂ : MeOH 95:5 → 92:8 gradient) to afford the pure title compound. Yield: 62 mg (0.22 mmol, 99%). Colorless powder.

TLC: R_f = 0.17 (CH₂Cl₂ : MeOH 10:1; UV, ninhydrin); **Melting Point:** >200 °C; **¹H NMR** (300 MHz, CDCl₃ : CD₃OD 5:2): δ = 7.25 – 7.14 (m, 2H), 6.79 (dd, J =6.7, 2.1, 1H), 5.00 (dd, J =6.1, 3.8, 1H), 4.67 (d, J =6.9, 1H), 4.63 – 4.52 (m, 3H), 4.19 (q, J =16.5, 2H), 2.40 (dd, J =8.1, 6.2, 2H), 2.22 – 1.90 (m, 2H); **¹³C NMR** (101 MHz, CDCl₃ : CD₃OD 3:1): δ = 171.7, 170.9, 141.6, 131.3, 129.3, 125.8, 117.8, 112.8, 82.2, 79.2, 59.2, 49.6, 47.4, 28.1, 21.8; **IR** (neat): 3442, 3349, 3178, 2956, 2892, 1677, 1657, 1607, 1487, 1393, 1311, 1232, 1154, 986, 819, 742 cm⁻¹; **HRMS** (ESI): exact mass calculated for C₁₅H₁₈N₃O₃ ([M+H]⁺), 288.1343; found 288.1344.



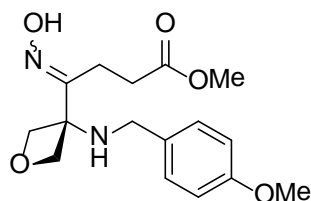
Methyl 4-nitro-4-(oxetan-3-ylidene)butanoate (6). To methyl 4-nitrobutanoate (1.03 g, 6.97 mmol, 1.0 equiv) was added oxetan-3-one (653 mg, 9.07 mmol, 1.3 equiv) and Et₃N (0.19 ml, 1.40 mmol, 0.2 equiv), and the slightly yellowish mixture was stirred at RT for 75 min. Then it was dissolved in CH₂Cl₂ (40 ml) and the solution was cooled to –78 °C. Et₃N (2.94 ml, 20.9 mmol, 3.0 equiv) was added followed by MsCl (1.36 ml, 17.4 mmol, 2.5 equiv), and the mixture was stirred at –78 °C for 30 min, when it was allowed to warm to –25 °C over *ca.* 45 min. After stirring at RT for 20 min, it was quenched with HCl (0.1 M in H₂O; 50 ml). The resulting mixture was diluted with CH₂Cl₂ (30 ml), and the phases were separated. The aqueous phase was extracted with CH₂Cl₂ (20 ml), and the combined organic layers were dried (MgSO₄), filtered, and concentrated *in vacuo*. Purification of the residue by FC (SiO₂; hexanes : EtOAc 3:1 → 2:1 → 3:2 gradient) afforded the pure title compound. Yield: 1.00 g (4.99 mmol, 72%). Colorless oil.

TLC: *R_f* = 0.26 (hexanes : EtOAc 3:1; UV, CAM); **¹H NMR** (300 MHz, CDCl₃): δ = 5.62 – 5.52 (m, 2H), 5.46 – 5.34 (m, 2H), 3.69 (s, 3H), 2.70 – 2.57 (m, 4H); **¹³C NMR** (75 MHz, CDCl₃): δ = 172.3, 152.1, 139.2, 79.4, 75.9, 51.9, 30.6, 22.6.



Methyl 4-(3-((4-methoxybenzyl)amino)oxetan-3-yl)-4-nitrobutanoate (8). To a cooled (0 °C) solution of nitro compound **6** (2.78 g, 13.8 mmol, 1.0 equiv) in THF (110 ml) was added 4-methoxybenzylamine (1.80 ml, 13.8 mmol, 1.0 equiv), and the mixture was stirred at 0 °C for 30 min. Then it was allowed to warm to RT and stirring was continued for another 10 min. At this point the mixture was concentrated *in vacuo* and the residue was purified by FC (SiO₂; hexanes : EtOAc 3:2 → 1:1 gradient) to afford the title compound in good purity (>95% by ¹H NMR). Yield: 4.34 g (12.8 mmol, 93%). Colorless oil.

TLC: *R_f* = 0.49 (hexanes : EtOAc 1:1; UV, CAM); **¹H NMR** (300 MHz, CDCl₃): δ = 7.24 (d, *J*=8.6, 2H), 6.86 (d, *J*=8.6, 2H), 5.06 (dd, *J*=10.4, 2.2, 1H), 4.76 (dd, *J*=7.4, 4.6, 2H), 4.61 (t, *J*=7.1, 2H), 3.95 – 3.79 (m, 2H), 3.79 (s, 3H), 3.72 (s, 3H), 2.70 – 2.23 (m, 4H), 1.79 (s, 1H); **¹³C NMR** (75 MHz, CDCl₃): δ = 172.4, 158.9, 131.4, 129.1, 113.9, 89.6, 77.0, 62.4, 55.2, 52.0, 46.3, 30.0, 23.8; **IR** (thin film): 3323, 2953, 2891, 2833, 1736, 1552, 1513, 1440, 1369, 1247, 1220, 1175, 1033, 983, 773 cm⁻¹; **HRMS** (MALDI): exact mass calculated for C₁₆H₂₃N₂O₆ ([M+H]⁺), 339.1551; found 339.1550.

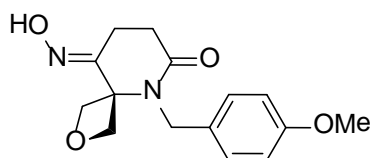


Methyl 4-(hydroxyimino)-4-(3-((4-methoxybenzyl)amino)oxetan-3-yl)butanoate (9). To a solution of nitro compound **8** (901 mg, 2.66 mmol, 1.0 equiv) in THF (20 ml) was added at RT tetrabutylammonium iodide

(49 mg, 0.13 mmol, 0.05 equiv), benzyl bromide (0.39 ml, 3.20 mmol, 1.2 equiv), and potassium hydroxide (179 mg, 3.20 mmol, 1.2 equiv). The mixture was stirred at RT for 3.5 h. Then it was diluted with Et₂O (40 ml) and washed with saturated aqueous NaCl (20 ml). Then it was dried (Na₂SO₄), filtered, and concentrated *in vacuo*. The pure title compound as a mixture of oxime isomers (ratio: 1:1.65) was obtained after purification by FC (SiO₂; hexanes : EtOAc 1:1). Yield: 652 mg (2.02 mmol, 76%). Colorless solid. Oxime isomers can also be separated by FC.

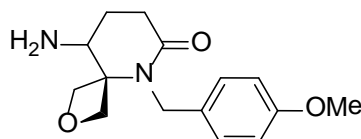
Isomer 1: Colorless oil. **TLC:** *R_f* = 0.15 (hexanes : EtOAc 3:2; UV, CAM); **¹H NMR** (400 MHz, CDCl₃): δ = 8.40 (br s, 1H), 7.23 (d, *J*=8.7, 2H), 6.86 (d, *J*=8.7, 2H), 4.91 (d, *J*=6.7, 2H), 4.49 (d, *J*=6.7, 2H), 3.79 (s, 3H), 3.67 (s, 3H), 3.52 (s, 2H), 2.79 – 2.53 (m, 4H), 1.95 (s, 1H); **¹³C NMR** (101 MHz, CDCl₃): δ = 173.2, 158.9, 158.0, 131.5, 129.3, 113.9, 79.3, 64.0, 55.3, 51.8, 47.1, 30.1, 20.8; **IR** (neat): 3240, 2949, 2876, 1731, 1612, 1512, 1439, 1244, 1175, 1029, 977, 824 cm⁻¹; **HRMS** (ESI): exact mass calculated for C₁₆H₂₃N₂O₅ ([M+H]⁺), 323.1601; found 323.1600.

Isomer 2: Colorless solid. **TLC:** *R_f* = 0.10 (hexanes : EtOAc 3:2; UV, CAM); **Melting Point:** 118-119 °C; **¹H NMR** (400 MHz, CDCl₃): δ = 8.90 (br s, 1H), 7.27 (d, *J*=8.2, 2H), 6.84 (d, *J*=8.2, 2H), 4.87 (d, *J*=7.4, 2H), 4.60 (d, *J*=7.4, 2H), 3.78 (s, 3H), 3.67 (s, 5H), 2.62 (s, 4H); **¹³C NMR** (101 MHz, CDCl₃): δ = 173.1, 158.8, 157.8, 131.7, 129.5, 113.9, 79.0, 62.7, 55.3, 51.8, 47.4, 29.7, 27.0; **IR** (neat): 3254, 3052, 2950, 2841, 1734, 1512, 1460, 1244, 1174, 1027, 976, 831 cm⁻¹; **HRMS** (ESI): exact mass calculated for C₁₆H₂₃N₂O₅ ([M+H]⁺), 323.1601; found 323.1604.



(E)-9-(Hydroxyimino)-5-(4-methoxybenzyl)-2-oxa-5-azaspiro[3.5]nonan-6-one (10). A solution of methyl ester **9** (mixture of oxime isomers; 366 mg, 1.14 mmol, 1.0 equiv) in xylenes (38 ml) was heated to 140 °C and stirred at that temperature for 23 h. Then it was cooled to RT and concentrated *in vacuo* (bath temperature: 60 °C). (On larger batches, upon cooling the solution to RT the title compound will precipitate as an off-white solid that can be collected and dried *in vacuo* to afford pure material.) The residue was purified by FC (SiO₂; hexanes : EtOAc 1:2 → 1:5 gradient) to afford the pure title compound as one oxime isomer. Yield: 276 mg (0.95 mmol, 84%). Off-white solid.

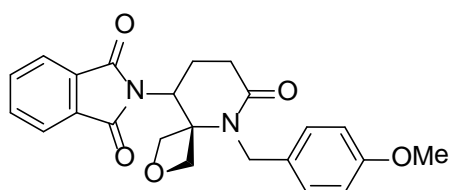
TLC: *R_f* = 0.09 (hexanes : EtOAc 1:1; UV, CAM); **Melting Point:** 154 °C; **¹H NMR** (300 MHz, CDCl₃): δ = 7.72 (br s, 1H), 7.07 (d, *J*=8.8, 2H), 6.83 (d, *J*=8.8, 2H), 4.97 (s, 2H), 4.93 (d, *J*=7.1, 2H), 4.81 (d, *J*=7.1, 2H), 3.78 (s, 3H), 2.86 (t, *J*=7.2, 2H), 2.47 (t, *J*=7.2, 2H); **¹³C NMR** (101 MHz, CDCl₃): δ = 171.4, 158.8, 154.9, 129.5, 127.6, 114.2, 78.2, 62.6, 55.2, 45.2, 29.7, 19.9; **IR** (neat): 3203, 3102, 2960, 2873, 1635, 1514, 1427, 1301, 1253, 1178, 991, 962, 809 cm⁻¹; **HRMS** (ESI): exact mass calculated for C₁₅H₁₈N₂NaO₄ ([M+Na]⁺), 313.1159; found 313.1156.



9-Amino-5-(4-methoxybenzyl)-2-oxa-5-azaspiro[3.5]nonan-6-one (11). Raney-Ni (50% slurry in H₂O; 15 ml, 5.7 mmol) was washed with EtOH (3 × 15 ml), then was added a solution of oxime **10** (1.65 g, 5.68 mmol, 1.0 equiv) in EtOH (60 ml), and the mixture was diluted with EtOH (60 ml). A hydrogen atmosphere (balloon) was built up, and the mixture was vigorously stirred at RT for 9 h. Then the atmosphere was changed to N₂ and the

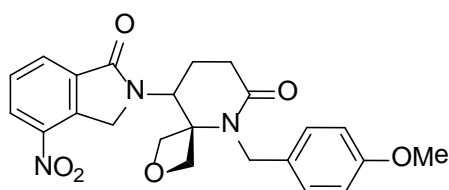
mixture was filtered over celite and washed with MeOH (*Caution*: new H₂-saturated Raney-Ni is very active – never leave it dry!). The filtrate was then concentrated *in vacuo*. The crude product was dissolved in CH₂Cl₂ and filtered again over celite. The filtrate was concentrated *in vacuo* to afford the pure title compound. Yield: 1.45 g (5.3 mmol, 93%). Colorless foam.

TLC: *R*_f = 0.68 (MeOH; UV, CAM, ninhydrin); **¹H NMR** (300 MHz, CDCl₃): δ = 7.13 (d, *J*=8.6, 2H), 6.83 (d, *J*=8.6, 2H), 5.16 (d, *J*=16.0, 1H), 4.82 (d, *J*=16.0, 1H), 4.75 (d, *J*=7.3, 2H), 4.63 (d, *J*=7.3, 1H), 4.48 (d, *J*=7.3, 1H), 3.77 (s, 3H), 3.52 (dd, *J*=6.2, 2.9, 1H), 2.81 – 2.58 (m, 1H), 2.58 – 2.36 (m, 1H), 2.05 – 1.85 (m, 1H), 1.85 – 1.62 (m, 1H), 1.37 (br s, 2H); **¹³C NMR** (75 MHz, CDCl₃): δ = 169.4, 158.2, 130.3, 127.1, 114.0, 79.4, 77.4, 65.5, 55.3, 51.2, 45.5, 28.3, 24.6; **IR** (neat): 3550, 2951, 2837, 1630, 1511, 1403, 1243, 1176, 1029, 979, 812 cm⁻¹; **HRMS** (ESI): exact mass calculated for C₁₅H₂₁N₂O₃ ([M+H]⁺), 277.1547; found 277.1539.



2-(5-(4-Methoxybenzyl)-6-oxo-2-oxa-5-azaspiro[3.5]nonan-9-yl)isoindoline-1,3-dione (12). To a cooled (0 °C) solution of amine **11** (578 mg, 2.09 mmol, 1.0 equiv) in THF (17 ml) was added triethylamine (0.88 ml, 6.28 mmol, 3.0 equiv) followed by dropwise addition of phthaloyl chloride (0.30 ml, 2.09 mmol, 1.0 equiv). Formation of a colorless precipitate was observed. The mixture was stirred at 0 °C for 15 min, then it was allowed to warm to RT and stirring was continued for 2 h. The suspension was diluted with THF (7 ml) and DBU (0.97 ml, 6.28 mmol, 3.0 equiv) was added. The mixture was heated at 75 °C for 2 h. Then it was cooled to RT and concentrated *in vacuo*. The residue was dissolved in CH₂Cl₂ (40 ml) and washed with saturated aqueous NH₄Cl (20 ml). The aqueous phase was extracted with CH₂Cl₂ (20 ml), and the combined organic phases were dried (MgSO₄), filtered, and concentrated *in vacuo*. The residue was purified by FC (SiO₂; hexanes : EtOAc 1:2 → 1:3 → 0:1 gradient) to afford the pure title compound. Yield: 700 mg (1.72 mmol, 82%). Colorless solid.

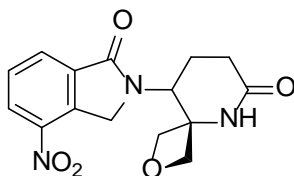
TLC: *R*_f = 0.45 (EtOAc; UV, CAM); **Melting Point:** 155-157 °C; **¹H NMR** (300 MHz, CDCl₃): δ = 7.94 – 7.82 (m, 2H), 7.82 – 7.71 (m, 2H), 7.16 (d, *J*=8.7, 2H), 6.84 (d, *J*=8.7, 2H), 5.34 (d, *J*=15.9, 1H), 5.09 (d, *J*=8.0, 1H), 4.90 – 4.60 (m, 4H), 4.42 (d, *J*=8.0, 1H), 3.78 (s, 3H), 2.98 – 2.46 (m, 3H), 2.16 – 1.96 (m, 1H); **¹³C NMR** (75 MHz, CDCl₃): δ = 169.3, 168.7, 158.5, 134.4, 131.2, 130.0, 127.5, 123.6, 114.1, 78.2, 77.7, 65.2, 55.3, 52.3, 45.8, 31.7, 21.6; **IR** (thin film): 2999, 1712, 1658, 1513, 1370, 1220, 1033, 773, 674 cm⁻¹; **HRMS** (MALDI): exact mass calculated for C₂₃H₂₃N₂O₅ ([M+H]⁺), 407.1602; found 407.1608.



5-(4-Methoxybenzyl)-9-(4-nitro-1-oxoisindolin-2-yl)-2-oxa-5-azaspiro[3.5]nonan-6-one (14). To a solution of amine **11** (0.79 g, 2.84 mmol, 1.0 equiv) and methyl 2-(bromomethyl)-3-nitrobenzoate (**13**) (1.39 g, 3.69 mmol, 1.3 equiv) in DMF (30 ml) was added triethylamine (1.20 ml, 8.52 mmol, 3.0 equiv), and the mixture was heated to 75 °C and stirred at that temperature for 20 h. Then it was cooled to RT and poured into LiCl (5% in H₂O; 70 ml). The mixture was extracted with EtOAc (2 × 60 ml), and the combined organic phases were washed with LiCl (5% in H₂O; 3 × 15 ml) and saturated aqueous NaCl (15 ml), dried (MgSO₄), filtered, and concentrated *in vacuo*. The pure title compound was obtained after purification by FC (SiO₂; EtOAc :

MeOH 1:0 → 20:1 gradient) and extraction (50 ml EtOAc / 2 × 20 ml 5% aqueous LiCl; to remove residual DMF). Yield: 0.90 g (2.07 mmol, 73%). Slightly yellowish foam.

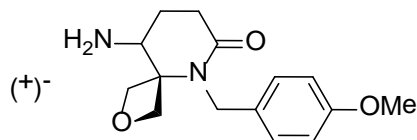
TLC: R_f = 0.28 (EtOAc; UV); **^1H NMR** (300 MHz, CDCl_3): δ = 8.41 (dd, J =7.9, 1.0, 1H), 8.20 (dd, J =7.9, 1.0, 1H), 7.72 (t, J =7.9, 1H), 7.29 (d, J =8.7, 2H), 6.88 (d, J =8.7, 2H), 5.29 – 5.12 (m, 2H), 5.04 – 4.82 (m, 4H), 4.73 (d, J =19.1, 1H), 4.64 (d, J =7.4, 1H), 4.58 (d, J =7.8, 1H), 3.77 (s, 3H), 2.69 (t, J =7.3, 2H), 2.38 – 2.05 (m, 2H); **^{13}C NMR** (101 MHz, CDCl_3): δ = 169.4, 167.2, 158.9, 143.4, 136.7, 134.6, 130.1, 130.0, 128.4, 127.2, 114.5, 79.9, 77.1, 64.3, 55.4, 52.4, 50.2, 46.0, 29.7, 22.0; **IR** (thin film): 2956, 1694, 1650, 1532, 1513, 1399, 1347, 1247, 1220, 1177, 1032, 985, 771, 733 cm^{-1} ; **HRMS** (MALDI): exact mass calculated for $\text{C}_{23}\text{H}_{23}\text{N}_3\text{NaO}_6$ ($[\text{M}+\text{Na}]^+$), 460.1479; found 460.1483.



9-(4-Nitro-1-oxoisindolin-2-yl)-2-oxa-5-azaspiro[3.5]nonan-6-one (15). To a solution of 4-methoxybenzyl-protected amide **14** (221 mg, 0.505 mmol, 1.0 equiv) in CH_3CN (8.5 ml), cooled to 0 °C, was added a solution of CAN (831 mg, 1.516 mmol, 3.0 equiv) in H_2O (2.8 ml), and the yellow clear solution was stirred at 0 °C for 2 h. Then it was diluted with EtOAc and quenched with half-saturated aqueous NaCl (10 ml) and diluted with EtOAc (50 ml) and H_2O (10 ml). The phases were separated, and the aqueous phase was extracted with EtOAc (2 × 30 ml) and CH_2Cl_2 (2 × 20 ml). The combined organic phases were dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The pure title compound was obtained after purification by FC (SiO_2 ; CH_2Cl_2 : MeOH 100:3 → 100:5 gradient). Yield: 69 mg (0.217 mmol, 43%). Colorless powder.

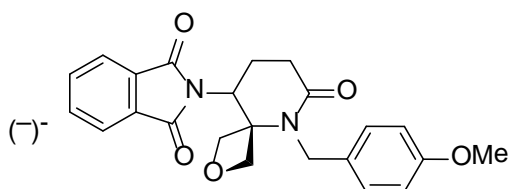
TLC: R_f = 0.44 (CH_2Cl_2 : MeOH 3:1; UV); **Melting Point:** ~240 °C (decomp.); **^1H NMR** (300 MHz, CDCl_3 : CD_3OD 5:2): δ = 8.32 (dd, J =7.9, 0.9, 1H), 8.09 (d, J =7.9, 1H), 7.64 (t, J =7.9, 1H), 4.87 (s, 2H), 4.82 (dd, J =8.6, 3.6, 1H), 4.68 – 4.47 (m, 4H), 2.38 (t, J =6.9, 2H), 2.24 – 2.05 (m, 1H), 2.05 – 1.85 (m, 1H); **^{13}C NMR** (101 MHz, CDCl_3 : CD_3OD 3:1): δ = 171.1, 167.7, 143.1, 136.6, 134.2, 129.9, 129.7, 127.0, 81.4, 79.4, 59.4, 50.5, 50.0, 28.5, 21.7; **IR** (neat): 3349, 2953, 2891, 1694, 1670, 1532, 1391, 1344, 1234, 977, 827, 735 cm^{-1} ; **HRMS** (ESI): exact mass calculated for $\text{C}_{15}\text{H}_{16}\text{N}_3\text{O}_5$ ($[\text{M}+\text{H}]^+$), 318.1084; found 318.1080.

SYNTHESIS OF ENANTIOMERICALLY ENRICHED COMPOUNDS
(AFTER CHIRAL SEPARATION OF AMINE **11**)



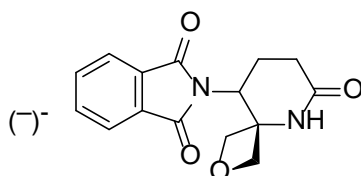
(+)-9-Amino-5-(4-methoxybenzyl)-2-oxa-5-azaspiro[3.5]nonan-6-one [(+)-11]. This compound was obtained from the racemate (4.87 g, 17.6 mmol) using preparative chiral HPLC (Chiralpak AD, 220 nm, 40% *i*PrOH in heptane, flow rate: 35 ml/min at 18 bar, t_R : 71 min). Yield: 1.80 g (6.5 mmol, 37%). Colorless foam.

Optical rotation: $[\alpha]_D^{24.4} +41.4$ ($c = 1.0$, CHCl_3).



(-)-2-(5-(4-Methoxybenzyl)-6-oxo-2-oxa-5-azaspiro[3.5]nonan-9-yl)isoindoline-1,3-dione [(-)-12]. To a cooled (0 °C) solution of amine (+)-**249** (0.88 g, 3.19 mmol, 1.0 equiv) in THF (23 ml) was added triethylamine (1.35 ml, 9.58 mmol, 3.0 equiv) followed by dropwise addition of phthaloyl chloride (0.46 ml, 3.19 mmol, 1.0 equiv). Formation of a colorless precipitate was observed. The mixture was stirred at 0 °C for 15 min, then it was allowed to warm to RT and stirring was continued for 2 h. Then the suspension was diluted with THF (10 ml) and DBU (1.47 ml, 9.58 mmol, 3.0 equiv) was added. The mixture was heated at 75 °C for 2 h. Then it was cooled to RT and concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 (40 ml) and washed with saturated aqueous NH_4Cl (20 ml). The aqueous phase was extracted with CH_2Cl_2 (20 ml), and the combined organic phases were dried (MgSO_4), filtered, and concentrated *in vacuo*. The pure title compound was obtained after purification by FC (SiO_2 ; hexanes : EtOAc 1:2 \rightarrow 1:3 \rightarrow 0:1 gradient). Yield: 1.18 g (2.91 mmol, 91%). Colorless foam.

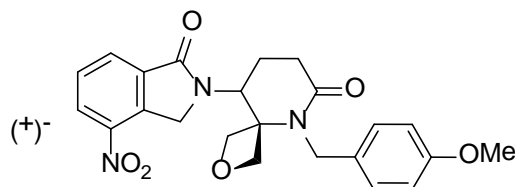
Optical rotation: $[\alpha]_D^{21.0} -14.8$ ($c = 0.99$, CHCl_3).



(-)-2-(6-Oxo-2-oxa-5-azaspiro[3.5]nonan-9-yl)isoindoline-1,3-dione [(-)-2]. To a solution of 4-methoxybenzyl-protected amide (-)-**248** (300 mg, 0.738 mmol, 1.0 equiv) in CH_3CN (10 ml), cooled to 0 °C, was added a solution of CAN (809 mg, 1.48 mmol, 2.0 equiv) in H_2O (3.3 ml), and the yellow clear solution was stirred at 0 °C for 30 min, then it was allowed to warm to RT and stirring was continued for 45 min, when more CAN (202 mg, 0.369 mmol, 0.5 equiv) was added in one portion. Stirring was continued for another hour. Then it was partitioned between EtOAc (50 ml) and half-saturated aqueous NaCl (50 ml). The phases were separated, and the aqueous phase was extracted with EtOAc (60 ml), then with CH_2Cl_2 (2 \times 60 ml). The

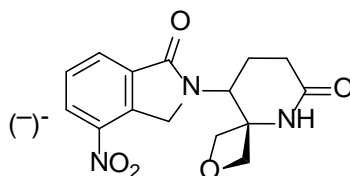
combined organic phases were dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The pure title compound was obtained after purification by FC (SiO_2 ; CH_2Cl_2 : MeOH 100:2 \rightarrow 100:3 gradient). Yield: 104 mg (0.363 mmol, 49%). Colorless powder.

Optical rotation: $[\alpha]_{\text{D}}^{23.0} -49.9$ ($c = 0.5$, CHCl_3 : MeOH 4:1); **Melting Point:** >200 °C.



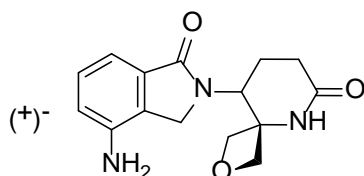
(+)-5-(4-Methoxybenzyl)-9-(4-nitro-1-oxoisindolin-2-yl)-2-oxa-5-azaspiro[3.5]nonan-6-one [(+)-14]. To a solution of amine (+)-**11** (0.79 g, 2.84 mmol, 1.0 equiv) and methyl 2-(bromomethyl)-3-nitrobenzoate (**13**) (1.39 g, 3.69 mmol, 1.3 equiv) in DMF (30 ml) was added triethylamine (1.20 ml, 8.52 mmol, 3.0 equiv), and the mixture was heated to 75 °C and stirred for 20 h. Then it was cooled to RT and poured into LiCl (5% in H_2O ; 70 ml). It was extracted with EtOAc (2×60 ml), and the combined organic phases were washed with LiCl (5% in H_2O ; 3×15 ml) and saturated aqueous NaCl (15 ml), dried (MgSO_4), filtered, and concentrated *in vacuo*. The pure title compound was obtained after purification by FC (SiO_2 ; EtOAc : MeOH 1:0 \rightarrow 20:1 gradient) and extraction (50 ml EtOAc / 2×20 ml 5% aqueous LiCl; to remove residual DMF). Yield: 1.16 g (2.65 mmol, 93%). Slightly yellowish foam.

Optical rotation: $[\alpha]_{\text{D}}^{21.0} +88.4$ ($c = 1.03$, CHCl_3).



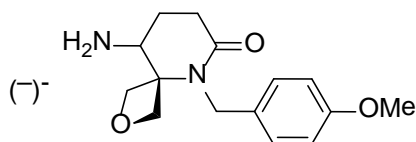
(-)-9-(4-Nitro-1-oxoisindolin-2-yl)-2-oxa-5-azaspiro[3.5]nonan-6-one [(-)-15]. To a solution of 4-methoxybenzyl-protected amide (+)-**14** (200 mg, 0.457 mmol, 1.0 equiv) in CH_3CN (6.7 ml), cooled to 0 °C, was added a solution of CAN (752 mg, 1.37 mmol, 3.0 equiv) in H_2O (2.23 ml), and the yellow clear solution was stirred at 0 °C for 2 h. Then it was partitioned between EtOAc (50 ml) and half-saturated aqueous NaCl (50 ml). The phases were separated, and the aqueous phase was extracted with EtOAc (60 ml), then CH_2Cl_2 (2×60 ml). The combined organic phases were dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The pure title compound was obtained after purification of the residue together with the residue of a second equivalent batch by FC (SiO_2 ; CH_2Cl_2 : MeOH 100:3 \rightarrow 100:5 gradient). Yield: 130 mg (0.41 mmol, 45%). Colorless powder.

Optical rotation: $[\alpha]_{\text{D}}^{21.1} -0.3$ ($c = 0.51$, CHCl_3 : MeOH 4:1).



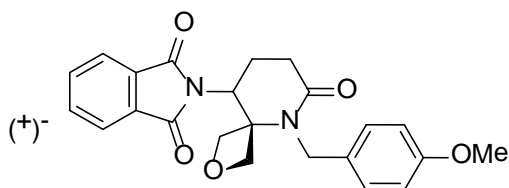
(+)-9-(4-Amino-1-oxoisindolin-2-yl)-2-oxa-5-azaspiro[3.5]nonan-6-one [(+)-4]. To a solution of lactam (–)-**15** (212 mg, 0.668 mmol, 1.0 equiv) in MeOH (10 ml)/CH₂Cl₂ (10 ml) was added at RT palladium (10% on carbon; 142 mg, 0.134 mmol, 0.2 equiv), and a hydrogen atmosphere was built up (balloon). The mixture was stirred at RT for 60 min, when TLC analysis indicated full conversion of the starting material. An argon atmosphere was reinstalled, and the mixture was filtered over celite (washed with a 1:1 mixture of MeOH/CH₂Cl₂), and concentrated *in vacuo*. The residue was purified by FC (SiO₂; CH₂Cl₂ : MeOH 95:5 → 92:8 gradient) to afford the pure title compound. Yield: 182 mg (0.633 mmol, 95%). Colorless powder.

Optical rotation: $[\alpha]_D^{21.9} +3.4$ (c = 0.5, CHCl₃ : MeOH 4:1); **Melting Point:** 182 °C (decomp.).



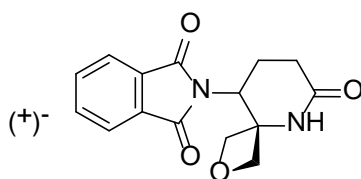
(–)-9-Amino-5-(4-methoxybenzyl)-2-oxa-5-azaspiro[3.5]nonan-6-one [(–)-11]. This compound was obtained from the racemate (4.87 g, 17.6 mmol) using preparative chiral HPLC (Chiralpak AD, 220 nm, 40% *i*PrOH in heptane, flow rate: 35 ml/min at 18 bar, *t*_R: 86 min). Yield: 1.70 g (6.2 mmol, 35%). Colorless foam.

Optical rotation: $[\alpha]_D^{25.3} -38.0$ (c = 1.0, CHCl₃).



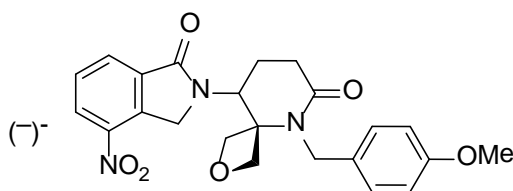
(+)-2-(5-(4-Methoxybenzyl)-6-oxo-2-oxa-5-azaspiro[3.5]nonan-9-yl)isoindoline-1,3-dione [(+)-12]. To a cooled (0 °C) solution of amine (–)-**11** (0.84 g, 3.05 mmol, 1.0 equiv) in THF (23 ml) was added triethylamine (1.29 ml, 9.14 mmol, 3.0 equiv) followed by dropwise addition of phthaloyl chloride (0.44 ml, 3.05 mmol, 1.0 equiv). Formation of a colorless precipitate was observed. The mixture was stirred at 0 °C for 15 min, then it was allowed to warm to RT and stirring was continued for 2 h. Then the suspension was diluted with THF (10 ml) and DBU (1.41 ml, 9.14 mmol, 3.0 equiv) was added. The mixture was heated at 75 °C for 2 h. Then it was cooled to RT and concentrated *in vacuo*. The residue was dissolved in CH₂Cl₂ (40 ml) and washed with saturated aqueous NH₄Cl (20 ml). The aqueous phase was extracted with CH₂Cl₂ (20 ml), and the combined organic phases were dried (MgSO₄), filtered, and concentrated *in vacuo*. The pure title compound was obtained after purification by FC (SiO₂; hexanes : EtOAc 1:2 → 1:3 → 0:1 gradient). Yield: 1.15 g (2.83 mmol, 93%). Colorless foam.

Optical rotation: $[\alpha]_D^{27.0} +15.0$ (c = 0.98, CHCl₃).



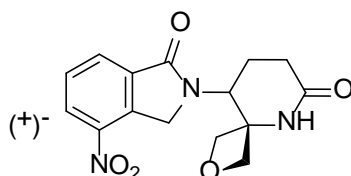
(+)-2-(6-Oxo-2-oxa-5-azaspiro[3.5]nonan-9-yl)isoindoline-1,3-dione [(+)-2]. To a solution of 4-methoxybenzyl-protected amide (+)-**12** (350 mg, 0.861 mmol, 1.0 equiv) in CH₃CN (11.6 ml), cooled to 0 °C, was added a solution of CAN (944 mg, 1.72 mmol, 2.0 equiv) in H₂O (3.9 ml), and the yellow clear solution was stirred at 0 °C for 30 min, then it was allowed to warm to RT and stirring was continued for 45 min, when more CAN (237 mg, 0.431 mmol, 0.5 equiv) was added in one portion. Stirring was continued for another hour. Then it was partitioned between EtOAc (50 ml) and half-saturated aqueous NaCl (50 ml). The phases were separated, and the aqueous phase was extracted with EtOAc (60 ml), then with CH₂Cl₂ (2 × 60 ml). The combined organic phases were dried (Na₂SO₄), filtered, and concentrated *in vacuo*. The pure title compound was obtained after purification by FC (SiO₂; CH₂Cl₂ : MeOH 100:2 → 100:3 gradient). Yield: 152 mg (0.531 mmol, 62%). Colorless powder.

Optical rotation: [α]_D^{23.6} +47.4 (c = 0.51, CHCl₃ : MeOH 4:1); **Melting Point:** >200 °C.



(-)-5-(4-Methoxybenzyl)-9-(4-nitro-1-oxoisindolin-2-yl)-2-oxa-5-azaspiro[3.5]nonan-6-one [(-)-14]. To a solution of amine (-)-**11** (0.86 g, 3.12 mmol, 1.0 equiv) and methyl 2-(bromomethyl)-3-nitrobenzoate (**13**) (1.52 g, 4.06 mmol, 1.3 equiv) in DMF (30 ml) was added triethylamine (1.32 ml, 9.37 mmol, 3.0 equiv), and the mixture was heated to 75 °C and stirred for 20 h. Then it was cooled to RT and poured into LiCl (5% in H₂O; 70 ml). It was extracted with EtOAc (2 × 60 ml), and the combined organic phases were washed with LiCl (5% in H₂O; 3 × 15 ml) and saturated aqueous NaCl (15 ml), dried (MgSO₄), filtered, and concentrated *in vacuo*. The pure title compound was obtained after purification by FC (SiO₂; EtOAc : MeOH 1:0 → 20:1 gradient) and extraction (50 ml EtOAc / 2 × 20 ml 5% aqueous LiCl; to remove residual DMF). Yield: 1.36 g (2.90 mmol, 93%). Slightly yellowish foam.

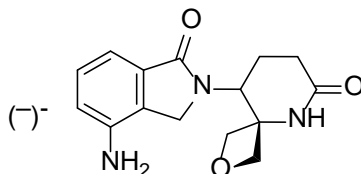
Optical rotation: [α]_D^{21.0} -89.9 (c = 1.0, CHCl₃).



(+)-9-(4-Nitro-1-oxoisindolin-2-yl)-2-oxa-5-azaspiro[3.5]nonan-6-one [(+)-15]. To a solution of 4-methoxybenzyl-protected amide (-)-**14** (200 mg, 0.457 mmol, 1.0 equiv) in CH₃CN (6.7 ml), cooled to 0 °C, was added a solution of CAN (752 mg, 1.37 mmol, 3.0 equiv) in H₂O (2.23 ml), and the yellow clear solution was stirred at 0 °C for 2 h. Then it was partitioned between EtOAc (50 ml) and half-saturated aqueous NaCl (50 ml). The phases were separated, and the aqueous phase was extracted with EtOAc (60 ml), then CH₂Cl₂ (2 ×

60 ml). The combined organic phases were dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The pure title compound was obtained after purification of the residue together with the residue of a second equivalent batch by FC (SiO_2 ; CH_2Cl_2 : MeOH 100:3 \rightarrow 100:5 gradient). Yield: 102 mg (0.32 mmol, 35%). Colorless powder.

Optical rotation: $[\alpha]_{\text{D}}^{21.1} +1.9$ (c = 0.5, CHCl_3 : MeOH 4:1).



(-)-9-(4-Amino-1-oxoisindolin-2-yl)-2-oxa-5-azaspiro[3.5]nonan-6-one [(-)-4]. To a solution of lactam (+)-**15** (222 mg, 0.700 mmol, 1.0 equiv) in MeOH (10 ml)/ CH_2Cl_2 (10 ml) was added at RT palladium (10% on carbon; 149 mg, 0.140 mmol, 0.2 equiv), and a hydrogen atmosphere was built up (balloon). The mixture was stirred at RT for 90 min, when TLC analysis indicated full conversion of the starting material. An argon atmosphere was reinstalled, and the mixture was filtered over celite (washed with a 1:1 mixture of MeOH/ CH_2Cl_2), and concentrated *in vacuo*. The residue was purified by FC (SiO_2 ; CH_2Cl_2 : MeOH 95:5 \rightarrow 92:8 gradient) to afford the pure title compound. Yield: 183 mg (0.637 mmol, 91%). Colorless powder.

Optical rotation: $[\alpha]_{\text{D}}^{22.7} -5.8$ (c = 0.5, CHCl_3 : MeOH 4:1); **Melting Point:** 182 °C (decomp.).

Physico-Chemical Assays

Determination of lipophilicities (logD pH=7.4)

The high-throughput assay method is derived from the conventional 'shake flask' method:

The compound of interest is distributed between a 50mM aqueous TAPSO buffer at pH 7.4 and 1-octanol. The distribution coefficient is then calculated from the difference in concentration in the aqueous phase before and after partitioning and the volume ratio of the two phases. To measure logD values within the range of -1 to 3.5, it is necessary to carry out the procedure at four different octanol/water ratios. The "one-phase-analysis" experiment starts with 2 or 9 μL of a pure DMSO-solution of the compound, which is dispensed into, respectively, 38 or 171 μL of the aqueous buffer solution, bringing the compound concentration to approximately $c = 0.5 \text{ mM}$. A small part of this solution is then analyzed by UV. The observed optical density corresponds to the concentration of the substance before partitioning. To a measured aliquot of the aqueous solution a matching aliquot of 1-octanol is added, and the mixture is incubated by quiet shaking for 2 hours at $23 \pm 1^\circ\text{C}$. The emulsion is allowed to stand over night at the same temperature to ensure that the partition equilibrium is reached. Then, thorough centrifugation at 3000 rpm for 10 min is applied to separate the layers, and the concentration of the compound in the aqueous phase is determined again by measuring the UV-absorption under the same conditions as the reference.

High-throughput measurement of ionization constants (pKa)

Ionization constants are determined at $23 \pm 1^\circ\text{C}$ by spectrophotometry using a ProfilerSGA SIRIUS instrument in buffered water solution at ionic strength of 150 mM. To this end the UV-spectrum of a compound is measured at different pH values. The solution of the sample is injected at constant flow rate into a flowing pH gradient. Changes in UV absorbance are monitored as a function of the pH gradient. The pKa values are found and determined where the rate of change of absorbance is at a maximum. The pH gradient is established by proportionally mixing two flowing buffer solutions. The buffer solutions contain mixtures of weak acids and bases that are UV-spectroscopically transparent above 240 nm. It is necessary to calibrate the gradient in order to know exactly the pH at any given time. This is achieved by introducing standard compounds with known pKa values.

Solubility : Lyophilisation Solubility Assay (LYSA)

Samples were prepared in duplicate from 10 mM dimethylsulfoxide stock solutions. After evaporation (1h) of dimethylsulfoxide with a centrifugal vacuum evaporator (Genevac Technologies), the compounds were dissolved in 0.05 M phosphate buffer (pH 6.5), stirred for one hour and shaken two hours. After one night, the solutions were filtered using a microtiter filter plate (Millipore MSDV N65) and the filtrate and its 1/10 dilution were analyzed by direct UV measurement or by HPLC-UV. In addition a four point calibration curve is prepared from the 10 mM stock solutions and used for the solubility determination of the compounds. The results are expressed in $\mu\text{g/ml}$. Starting from a 10 mM stock solution, the measurement range for MW 500 was 0- 666 $\mu\text{g/ml}$. In case the percentage of sample measured in solution after evaporation divided by the calculated maximum of sample amount was larger than 80% the solubility was reported as larger than this value.

Permeability determination (PAMPA)

Permeabilities are determined via PAMPA (Parallel Artificial Membrane Permeation Assay). The small intestine, being the major site of oral absorption, is simulated by a three compartment model. Drugs permeate by passive diffusion from the donor compartment (320µl, Roche Teflon plate), which is separated by a phospholipid coated filter (4.5µl, Millipore MAIPN4550), to the acceptor compartment (280µl, Millipore MAIPN4550). Donor buffer: 0.05M MOPSO at pH 6.5 + 0.5% (w/v) Glycocholic acid. Acceptor buffer: 0.05M MOPSO at pH 6.5. Membrane: 10% (w/v) Egg Lecithin in Dodecane + 0.5% (w/v) Cholesterol. The permeation constant P_e [10^{-6} * cm/s] as well as the sample distribution can be retrieved by UV analytics of donor (t_{start}), donor (t_{end}) and acceptor (t_{end}) using the pION PAMPA Evolution software.

Determination of metabolic stability in liver microsomes

Microsomal incubations were carried out in 96-well plates in 200 µL of liver microsome incubation medium containing potassium phosphate buffer (50mM, pH 7.4), $MgCl_2$ (10mM), EDTA (1mM), NADP⁺ (2mM), glucose-6-phosphate * 2 H₂O (20 mM), glucose-6-phosphate dehydrogenase (4 units/ml) with 0.1mg of liver microsomal protein per mL. Test compounds were incubated at 2 µM for up to 30 min at 37°C under vortexing at 500 rpm. The reaction was stopped by transferring 30 L incubation aliquots to 90 µL of ice-cold methanol. Levels of un-metabolized drug were determined by high-performance liquid chromatography (HPLC) coupled with tandem mass spectrometry (LC/MS/MS). The system consisted of a Shimadzu binary gradient HPLC system, a Waters XTerra ® MS C18 column (1mm * 50mm) and a Sciex API 2000 mass spectrometer. A two component mobile phase pumped at 0.15 mL/min contained the following solvents: solvent A (1% aqueous formic acid and MeOH 80:20) and solvent B (MeOH). An initial isocratic step of 0.5 min solvent A was followed by a gradient of 0 to 80% solvent B within 1 min. Detection was performed in positive mode. The intrinsic clearance (CL_{int}) was determined in semilogarithmic plots of compound concentrations versus time.

Determination of metabolic stability in Hepatocytes

The hepatocyte stability assay measures the rate of disappearance of a test compound from incubations with hepatocytes suspension cultures of human or animal origin. The assay is primarily used for the assessment of clearance rates for establishing IVIV correlations (first PBPK/PD M&S efforts) and estimation of variability/biological variance for M&S efforts for PBPK/PD driven recommendation of dose & regimen.

For animals, hepatocyte suspension cultures in a Williams E medium supplemented with 10 % FCS are either freshly prepared by liver perfusion studies or prepared from cryopreserved hepatocyte batches. For human, commercially available, pooled (5-20 donors), cryopreserved human hepatocytes from non-transplantable liver tissues are mainly used. Incubations of a test compound at either 1 microM test concentration for animals and three different concentrations for human (0.1, 0.3 and 1 microM) in suspension cultures of 1 Mio cells/mL (~1 mg/mL protein concentration) are performed in 96 well plates and shaken at 900 rpm for up to 3 hours in a 5 % CO₂ atmosphere and 37°C. After, 2, 10, 20, 40, 60, 120 and 180 minutes, 100 µL cell suspension in each well is quenched with 200 µL methanol containing an internal standard. Samples are then cooled and centrifuged before analysis by LC-MS/MS.

Log peak area ratios (test compound peak area / internal standard peak area) or concentrations are plotted against incubation time and a linear fit made to the data with emphasis upon the initial rate of compound disappearance. The slope of the fit is then used to calculate the intrinsic clearance:

$$Cl_{int} (\mu\text{L}/\text{min}/1 \times 10^6 \text{ cells}) = -\text{slope} (\text{min}^{-1}) * 1000 / [1 \times 10^6 \text{ cells}]$$

Plasma Stability Testing

The plasma stability assay measures the rate of disappearance of a test compound from incubations with plasma of human or animal origin. The assay is primarily used for the assessment of plasma clearance.

Incubations are performed in deep-well plates with a final incubation volume of 700 μL . Incubations contain (finally) 1 μM test compound. 50 μL aliquots are removed after 15, 60, 120, 180 and 300 minutes and quenched in 150 μL acetonitrile containing internal standard. Samples are then cooled and centrifuged before analysis by LC-MS/MS.

Peak area ratios (test compound peak area / internal standard peak area) are plotted against incubation time and percentages remaining are calculated.

COPIES OF NMR SPECTRA OF NEW COMPOUNDS

