Supplementary material

Synthesis of *rac*-N-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]methyl}cyclopropanecarboxamide (*rac*-H-1), (*S*)- and (*R*)-N-{[2-((3-^{2}H)-2,6-dioxo-piperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]methyl}cyclopropanecarboxamide ((*S*)-D-1 and (*R*)-D-1)

*3-[(2,2-Dimethylhydrazono)methyl]-phthalic anhydride (**4**)*: A solution of 2-furaldehyde dimethylhydrazone **3** (30.0g, 200 mmol) in ethyl acetate (40 mL) was added over 10 minutes to a solution of maleic anhydride (25.0 g, 255 mmol) in ethyl acetate (100 mL) while stirring at room temperature. Trifluoroacetic acid (0.755 mL, 9.8 mmol) was added dropwise over 10 minutes, and the reaction was then warmed to 50°C. After about 30 min., a precipitate started to form. The reaction was stirred at 50°C for another 4 hours, after which no starting material was observed by HPLC. The reaction was cooled in a refrigerator for 6 hours. The product was isolated by filtration, washed with ethyl acetate, water and hexane, then dried in a warm oven (70°C) overnight to provide product **4** (40.54 g, 90%) as a bright yellow crystalline solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 (dd, J = 6.4, 2.4 Hz, 1H), 7.86 (br s, 1H), 7.72 (m, 2H), and 3.19 (s, 6 H). MS (ESI+) calc. for $[C_{11}H_9N_2O_3+H]^+$ 219.2, found 219.2.

*rac-3-{4-[(2,2-Dimethylhydrazono)methyl]-1,3-dioxoisoindol-2-yl}-piperidine-2,6-dione (rac-H-**5**)*: Compound **4** (3.0 g, 13.7 mmol), imidazole (7.8 g, 110 mmol), and *rac*-3-aminopiperidine-2,6-dione hydrochloride (1.89 g, 11.5 mmol) were dissolved in acetonitrile (22.8 mL) in a one-neck round-bottom flask. Acetic acid (6.51 mL) was added slowly. The flask was equipped with a Dean-Stark trap and heated at 77°C for 2 hours while stirring. The solution became homogeneous and a yellow precipitate formed after a few minutes. The reaction mixture was cooled to room temperature and diluted with water (40 mL). The resulting solid was collected by filtration, washed with water (3 X 50 mL), rinsed with hexanes, and then air dried to give product *rac*-H-**5** (3.19 g, 85%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.13 (s, 1H), 8.17 (d, J = 7.6 Hz, 1H), 7.94 (s, 1H), 7.73 (t, J = 7.6 Hz, 1H), 7.67 (dd, J = 7.6, 1.1 Hz, 1H), 5.13 (dd, J = 13.0, 5.4 Hz, 1H), 3.09 (s, 6H), 2.89 (m, 1H), 2.58 (m, 2H), and 2.05 (m, 1H). MS (ESI-) calc. for $[C_{16}H_{16}N_4O_4-H]^-$ 327.2, found 327.0.

*(R)-3-{4-[(2,2-Dimethylhydrazono)methyl]-1,3-dioxoisoindol-2-yl}-(3-^{2}H)-piperidine-2,6-dione ((R)-D-**5**)*: Intermediate **4** (0.50 g, 2.29 mmol), (*R*)-(3-^{2}H)-3-aminopiperidine-2,6-dione hydrochloride (97% ee, 317 mg, 1.91 mmol), and Hunig's base (833 μL, 4.79 mmol) were dissolved in tetrahydrofuran (13 mL) while stirring. The reaction mixture was warmed to 70°C until HPLC analysis showed disappearance of the starting material (2 hours). The reaction was then cooled to ambient temperature and partially concentrated by rotary evaporation. The residue was diluted with a saturated sodium bicarbonate solution (40 mL). The resulting yellow precipitate was collected by filtration, washed with water (3 mL), rinsed with hexanes, and air dried overnight to provide (*R*)-D-**5** (284 mg, 45%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.14 (s, 1H), 8.17 (d, J = 7.3 Hz, 1H), 7.95 (s, 1H), 7.74 (t, J = 7.5 Hz, 1 H), 7.68 (m, 1H), 5.13 (dd, J = 12.8, 5.2 Hz, 0.06H, 6% residual ^{1}H at chiral center), 3.09 (s, 6 H), 2.88 (m, 1H), 2.57 (m, 2H), 2.05 (m, 1H). MS (ESI+) calc. for $[C_{16}H_{15}{}^2HN_4O_4+H]^+$ 330.3, found 330.0.

*(S)-3-{4-[(2,2-Dimethylhydrazono)methyl]-1,3-dioxoisoindol-2-yl}-(3-^{2}H)-piperidine-2,6-dione ((S)-D-**5**)*: (*S*)-D-**5** was prepared as described for (*R*)-D-**5** in 60% yield (382 mg) from 500 mg of intermediate **4**. (*S*)-D-**5** was immediately used for the next reaction.

*rac-N-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1H-isoindol-4-yl]methyl}cyclopropanecarboxamide (rac-H-**1**)*: (a) Hydrazone cleavage and reduction: Compound *rac*-H-**5** (3.0g, 9.14 mmol) was dissolved in water (20 mL) and acetic acid (30 mL). Palladium on carbon (10%, 100mg) was added and the mixture was shaken for 16 hours under a hydrogen atmosphere (50 psi). The catalyst was filtered through a 1 cm celite pad which was rinsed with methanol (20 mL). The filtrate was partially concentrated by rotary evaporation then diluted with acetonitrile (20 mL). The solution was cooled to 0°C and 12 M aqueous HCl was added. Solvents were removed by rotary evaporation and the residue was redissolved in methanol (50 mL) with sonication. HCl in dioxane (4 M, 5.0 mL) was added dropwise and white crystals formed. Acetonitrile (50 mL) was added followed by ethyl acetate (50 mL). After 18h is the refrigerator, the slurry was filtered and the solid was air-dried to give the hydrochloric acid salt of *rac*-3-[4-(aminomethyl)-1,3-dioxo-2*H*-isoindol-2-yl]-2,6-piperidinedione (1.65 g, 57%) as a colorless crystalline solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.18 (br s, 1H), 8.65 (br s, 3H), 8.02 (m, 1H), 7.94 (m, 2H), 5.19 (dd, J = 12.7, 5.4 Hz, 1H), 4.50 (m, 2H), 2.91 (m, 1H), 2.58 (m, 2H), and 2.06 (m, 1H). MS (ESI+) calc. for $[C_{14}H_{13}N_3O_4+H]^+$ 288.3, found 288.1. (b) Amide formation: A solution of *rac*-3-[4-(aminomethyl)-1,3-dioxo-2*H*-isoindol-2-yl]-2,6-piperidinedione hydrochloride (1.0 g, 3.48 mmol) in acetonitrile (10.2 mL) was cooled to 0°C. Cyclopropanecarbonyl chloride (347 μL, 3.83 mmol) was added followed by Hunig's base (1.21 mL, 6.96 mmol). The reaction mixture was allowed to warm to room temperature. After 1 hour, the pale yellow solution was cooled to 0°C and neutralized with 2M HCl. The reaction was partially concentrated by rotary evaporation until a white precipitate started to form whereupon the mixture was placed in a refrigerator overnight. The solid was filtered, washed with water, and air dried to provide *rac*-N-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]methyl}cyclopropanecarboxamide *rac*-H-**1** (964 mg, 78%) as a colorless powder. Chiral purity (ChiralPak AD-H 150 x 2.1 mm, hexane:2-propanol 70:30 v/v, 1 mL/min, 210 nm): 1%ee. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.15 (s, 1H), 8.71 (t, J = 5.8 Hz, 1H), 7.84 (m, 2H), 7.69 (dd, J = 7.6, 1.4 Hz, 1H), 5.16 (dd, J = 12.9, 5.3 Hz, 1H), 4.73 (d, J = 5.8 Hz, 2H), 2.90 (m, 1H), 2.57 (m, 2H), 2.07 (m, 1 H), 1.67 (m, 1H), and 0.70 (m, 4H). MS (ESI+) calc. for $[C_{18}H_{17}N_3O_5+H]^+$ 356.3, found 356.0.

*(R)-N-{[2-((3-^{2}H)-2,6-dioxo-piperidin-3-yl)-1,3-dioxo-2,3-dihydro-1H-isoindol-4-yl]methyl}cyclopropanecarboxamide ((R)-D-**1**)*: (a) Hydrazone cleavage and reduction: Compound (*R*)-D-**5** (201 mg, 0.609 mmol) was dissolved in water (7 mL) and acetic acid (15 mL). Palladium on carbon (10%, 80 mg) was added and the mixture was shaken for 6 hours under a hydrogen atmosphere (50 psi). The crude reaction was filtered through a 1 cm celite pad which was rinsed with methanol. The filtrate was concentrated by rotary evaporation and the residue diluted with methanol (10 mL) and dissolved by sonication. HCl in dioxane (5.0 mL, 4M) was added dropwise until white crystals began to form. Acetonitrile (5 mL) was then added followed by ethyl acetate (15 mL) 30 minutes later. After 1 h in the refrigerator, the crystalline material was filtered, washed with ethyl acetate, and air dried to give the product (210 mg, 99%), which was carried on to the next step without characterization. (b) Amide formation: The reaction was performed as described for step (b) of the synthesis of *rac*-H-**1**. (*R*)-N-{[2-((3-^{2}H)-2,6-dioxo-piperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]methyl}cyclopropanecarboxamide (*R*)-D-**1** was isolated as a white powder (121 mg, 52%). Purity (HPLC, 210 nm): >99%. Chiral purity (ChiralPak AD-H 150 x 2.1 mm, hexane:2-propanol 70:30 v/v, 1 mL/min, 210 nm): 92.4%ee. Deuterium content (^{1}H NMR): 92%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.15 (s, 1H), 8.71 (t, J = 5.8 Hz, 1H), 7.84 (m, 2H), 7.69 (dd, J = 7.6, 1.4 Hz, 1H),

5.16 (dd, J = 12.9, 5.3 Hz, 0.08H, residual ^{1}H at chiral center), 4.73 (d, J = 5.8 Hz, 1.6H, overlaps with DMSO peak), 2.90 (m, 1H), 2.57 (m, 1H), 2.07 (m, 1 H), 1.67 (m, 1H), 1.25 (m, 1H), 0.70(m, 4H). MS (ESI+) calc. for $[C_{18}H_{16}{}^{2}HN_3O_5+H]^+$ 357.3, found 357.2.

*(S)-N-{[2-((3-^{2}H)-2,6-dioxo-piperidin-3-yl)-1,3-dioxo-2,3-dihydro-1H-isoindol-4-yl]methyl}cyclopropanecarboxamide ((S)-D-**1**):* (a) Hydrazone cleavage and reduction: The reaction was performed as described in step (a) of the synthesis of (*R*)-D-**1**. The hydrochloride salt (204 mg, 99%) was isolated as white crystals. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.18 (br s, 1H), 8.65 (br s, 3H), 8.02 (m, 1H), 7.94 (m, 2H), 5.19 (dd, J = 12.7, 5.4 Hz, 0.20H, residual ^{1}H at chiral center), 4.50 (m, 2H), 2.91 (m, 1H), 2.58 (m, 2H), 2.06 (m, 1H). (b) Amide formation: The reaction was performed as described for step (b) of the synthesis of *rac*-H-**1**. (*S*)-N-{[2-((3-^{2}H)-2,6-dioxo-piperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]methyl}cyclopropanecarboxamide (*S*)-D-**1** was isolated as a white powder (143 mg, 65%). Purity (HPLC, 210 nm): 98.5%. Chiral purity (ChiralPak AD-H 150 x 2.1 mm, hexane:2-propanol 70:30 v/v, 1 mL/min, 210 nm): 86%ee. Deuterium content (^{1}H NMR): 80%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.15 (s, 1H), 8.72 (t, J = 5.9 Hz, 1H), 7.84 (m, 2H), 7.69 (dd, J = 7.6, 1.4 Hz, 1H), 5.16 (dd, J = 12.9, 5.3 Hz, 0.21H, residual ^{1}H at chiral center), 4.73 (d, J = 5.8 Hz, 2H), 2.90 (m, 1H), 2.57 (m, 1.6H, overlaps with DMSO peak), 2.07 (m, 1 H), 1.67 (m, 1H), 1.25 (m, 0.5H), 0.70(m, 4H). MS (ESI+) calc. for $[C_{18}H_{16}{}^{2}HN_3O_5+H]^+$ 357.3, found 357.2.

Synthesis of deuterated *rac*-3-(5-amino-2-methyl-4-oxoquinazolin-3(4*H*)-yl)-(3-^{2}H)-piperidine-2,6-dione (*rac*-D-2)

*2-methyl-5-nitro-4H-3,1-benzoxazin-4-one (**7**)*: 2-Amino-6-nitrobenzoic acid **6** (25.0 g, 137 mmol) was mixed with acetic anhydride (50 mL, 529 mmol) and heated to 120°C in an oil bath for 2 hours, while monitoring by HPLC. The reaction was cooled, and partially concentrated by evaporation under a stream of dry nitrogen. The reaction mixture was then diluted with 100 mL diethyl ether and cooled in a refrigerator overnight. The crystals were filtered and rinsed with diethyl ether (50 mL) to provide 2-methyl-5-nitro-4*H*-3,1-benzoxazin-4-one (**7**) (25.3 g, 123 mmol, 89%) as a tan crystalline solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (t, J = 8.0 Hz, 1H), 7.57 (dd, J = 7.8, 1.01 Hz, 1H), 7.53 (dd, J = 8.3, 1.0 Hz, 1H), 2.53 (s, 3H). MS (ESI+) calc. for $[C_9H_6N_2O_4+H]^+$ 207.2, found 207.2.

*2-acetamido-6-nitrobenzoic acid (**8**)*: The starting material (**7**, 24.7 g, 120 mmol) was dispersed in water (216 mL) and heated to reflux for 30 min. Upon cooling, crystallization began. The reaction mixture was placed in a refrigerator for 18 hours to complete crystallization. The crystalline material was isolated by filtration and dried under vacuum to give 2-acetamido-6-nitrobenzoic acid (**8**) (24.3 g, 108 mmol, 90.5%) as a pale yellow crystalline solid. ^{1}H NMR (300 MHz, MeOH-d_4) δ 8.49 (d, J = 8.1 Hz, 1H), 8.15 (d, J = 7.8 Hz, 1H), 8.01 (t, J = 8.2 Hz, 1 H), 2.53 (s, 3H). MS (ESI-) calc. for $[C_9H_8N_2O_5-H]^-$ 223.0, found 223.1

*rac-2-Acetamido-N-(2,6-dioxopiperidin-3-yl)-6-nitrobenzamide (**9**)*: Starting material nitrobenzoic acid **8** (3.10 g, 13.8 mmol) was mixed with hydroxybenzotriazole (HOBt, 2.12 g of the hydrate, 13.8 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC, 2.54 g, 13.3 mmol), under a nitrogen atmosphere. N,N-dimethylformamide (DMF, 21.4 mL) was added and the mixture was stirred for 30 minutes at room temperature. *rac*-3-Aminopiperidine-2,6-dione hydrochloride (5.01 g, 30.4 mmol) was added, followed by N,N-diisopropylethylamine (DIEA, 9.63 mL, 55.3 mmol). The reaction mixture was stirred at 20°C, while monitoring by HPLC. After 24 hours, the reaction mixture showed ca. 40% conversion to the desired product containing some remaining starting acid, but no amine. The reaction was slowly poured into 200 mL water with vigorous stirring. After 20 min, a white precipitate began to form. The mixture was placed in the refrigerator for 18 hours. The precipitate was then filtered. The filter cake was washed with 50 mL ether, and air dried to provide the desired product (1.60 g, 4.79 mmol, 35%) as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.16 (s, 1H), 9.40 (s, 1H), 9.34 (d, J = 8.0 Hz, 1H), 8.53 (d, J = 7.5 Hz, 1H), 7.89 (dd, J = 8.2, 0.98 Hz, 1H), 7.66 (t, J = 8.3 Hz, 1H), 4.79 (m, 1H), 2.85(m, 1H), 2.59 (m, 1H), 2.21 (m, 1H), 2.20 (s, 3H), 2.03 (m, 1H). MS (ESI-) calc. for $[C_{14}H_{14}N_4O_6-H]^-$ 333.3, found 333.2.

*rac-3-(2-Methyl-5-nitro-4-oxoquinazolin-3(4H)-yl)-(3-^{2}H)-piperidine-2,6-dione (**10**)*: *rac*-2-Acetamido-N-(2,6-dioxopiperidin-3-yl)-6-nitrobenzamide **9** (4.50 g, 13.5 mmol), was dispersed in anhydrous acetonitrile (70.3 mL) under a nitrogen atmosphere. Triethylamine (88.2 mL, 633 mmoles) was added via syringe, followed by the dropwise addition of chlorotrimethylsilane (25.6 mL, 202 mmol). The reaction mixture was warmed to 75°C and the reaction was monitored by HPLC. After 42 hours, 85% of the desired product was present. The reaction mixture was cooled to 20°C, stirred rapidly, and quenched with deuterium oxide (100 mL, 5.55 moles). The reaction mixture was stirred for an additional 20 min and a white precipitate formed. The reaction mixture was cooled in a refrigerator for 4 hours and filtered to provide

deuterated compound **10** (2.25 g, 7.1 mmol, 53%) as an off-white crystalline solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.07 (s, 1H), 7.98 (t, J = 7.6 Hz,1H), 7.83 (ddd, J = 14.4, 8.0, 1.2 Hz, 2H), 2,82 (m, 1H), 2.67 (s, 3H), 2.56 (m, 2H), 2.18 (m, 1H), MS (ESI-) calc. for $[C_{14}H_{11}[^{2}H]N_4O_5\text{-}H]^-$ 316.1, found 316.0.

*rac-3-(5-Amino-2-methyl-4-oxoquinazolin-3(4H)-yl)-(3-^{2}H)-piperidine-2,6-dione (rac-D-**2**)*: *rac*-3-(2-Methyl-5-nitro-4-oxoquinazolin-3(4*H*)-yl)-(3-^{2}H)-piperidine-2,6-dione **10** (2.25 g, 7.09 mmol) was dispersed in DMF (60 mL) in a Parr bottle and palladium hydroxide (500 mg, 20% active catalyst, 50 wt% water) was added. The bottle was then placed under 50 psi hydrogen pressure and shaken for 2 hours at 21°C. The reaction mixture was analyzed by HPLC, which showed a major peak for the desired product and no starting material. The reaction mixture was treated with activated carbon and filtered through a plug of silica gel overlain with 1 cm of sodium sulfate. The plug was washed with 50 mL acetonitrile and the filtrate was evaporated under high vacuum to give a black gum. This material was dissolved in 50 mL of acetonitrile and filtered through a Magnesol® plug, then washed with 100 mL of acetonitrile. The filtrate was then placed in a freezer for 48 hours then filtered to afford smoky grey crystals, which were air dried for 3 hours to give the desired product *rac*-D-**2** (1.63 g, 5.67 mmol, 80%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.99 (s, 1H), 7.37 (t, J = 8.1 Hz, 1H), 7.03 (br s, 1.85H, NH_2), 6.58 (m, 2H), 5.15 (m, 0.13H, residual C(3)H, ca. 87% D incorporation), 2.83 (m, 0.92H), 2.60 (m, 2H), 2.53 (s, 3H), 2.14 (m, 0.92H). MS (ESI+) calc. for $[C_{14}H_{13}[^{2}H]N_4O_3+H]^+$ 288.1, found 288.2.

MS indicates 9% protonated, 75% mono-deuterated, and 15% bis-deuterated.

Separation of enantiomers of *rac*-N-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]methyl}cyclopropanecarboxamide (*rac*-H-1)

rac-N-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]methyl}cyclopropanecarboxamide, *rac*-H-**1** (98.5 mg, 0.277 mmol), was dissolved in 15 mL of acetonitrile : isopropanol : methanol (7:3:5 v/v/v) in the presence of 20 μL trifluoroacetic acid. The enantiomers were separated by chiral supercritical fluid chromatography on a ChiralPak AD-H column (21 x 250 mm) using a mobile phase of 34% isopropanol in carbon dioxide (flow rate: 70 mL/min; 1 mL injected per run). Compounds were detected by UV at 254 nm. Fractions containing the compounds were pooled and evaporated. Purity and enantiomeric excess were determined by analytical supercritical fluid chromatography on a ChiralPak AD-H column (4.6 x 100 mm) using the same eluent. The enantiomers, peaks 1 and 2, identified as (*R*)- and (*S*)-N-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]methyl}cyclopropanecarboxamide respectively ((*R*)-H-**1** and (*S*)-H-**1**) by comparison of their retention times with those of the deuterated analogs, were dried under vacuum and stored in the freezer. Yield: 92.2 mg overall (0.259 mmol, 93.6%) as 46.5 mg peak 1 (0.131 mmol, 100% purity and 100% ee) and 45.7 mg peak 2 (0.129 mmol, 100% purity and 100% ee).

Separation of enantiomers of *rac*-3-(5-amino-2-methyl-4-oxoquinazolin-3(4*H*)-yl)-piperidine-2,6-dione (*rac*-H-2) and of *rac*-3-(5-amino-2-methyl-4-oxoquinazolin-3(4*H*)-yl)-(3-^{2}H)-piperidine-2,6-dione (*rac*-D-2)

rac-3-(5-Amino-2-methyl-4-oxoquinazolin-3(4*H*)-yl)-piperidine-2,6-dione, *rac*-H-**2** (136 mg, 0.475 mmol), was dissolved in 10 mL of acetonitrile : isopropanol : methanol (5:3:2 v/v/v). The enantiomers were separated by chiral supercritical fluid chromatography on a ChiralPak AD-H column (21 x 250 mm) using a mobile phase of 37% isopropanol in carbon dioxide (flow rate: 70 mL/min; 1 mL injected per run). Compounds were detected by UV at 254 nm. Fractions containing the compounds were pooled and evaporated. Purity and enantiomeric excess were determined by analytical supercritical fluid chromatography on a ChiralPak AD-H column (4.6 x 100 mm) using the same eluent. The enantiomers, peaks 1 and 2, identified as (-)- and (+)-3-(5-amino-2-methyl-4-oxoquinazolin-3(4*H*)-yl)-piperidine-2,6-dione respectively, (-)-H-**2** and (+)-H-**2**, were dried under vacuum and stored in the freezer. Yield: 134.1 mg overall (0.468 mmol, 98.6%) as 67.3 mg peak 1 (0.235 mmol, 99.7% purity and 99.4% ee) and 66.8 mg peak 2 (0.233 mmol, 99.8% purity and 99.6% ee).

The enantiomers of *rac*-3-(5-amino-2-methyl-4-oxoquinazolin-3(4*H*)-yl)-(3-^{2}H)piperidine-2,6-dione, *rac*-D-**2**, were separated using the same chromatographic method. Thus separation of 0.948 g of *rac*-D-**2** (3.3 mmol) gave the two pure enantiomers in 726.7 mg (2.53 mmol, 77%) overall yield. Purity and enantiomeric excess were determined by supercritical fluid analytical chromatography as described above. Deuterium content was measured by LC/MS-MS and optical rotation was measured in N,N-dimethylformamide (DMF) at room temperature.

(-)-3-(5-amino-2-methyl-4-oxoquinazolin-3(4*H*)-yl)-(3-^{2}H)piperidine-2,6-dione, (-)-D-**2**: 340 mg (1.18 mmol); 99.8% purity; 99.6% ee; LC-MS: 288.3 (M+1) (90% deuterium); ^{1}H NMR (200 MHz, DMSO-d_6) δ 10.95 (s, 1H), 7.33 (t, J = 8 Hz, 1H), 6.99 (br s, 2H), 6.56 (d, J = 8 Hz, 1H), 6.52 (d, J = 6 Hz, 1H), 5.1 (m, 0.12H, residual C(3)H, ca. 88% deuterium incorporation), 2.75 (m, 6H), 2.11 (m, 1H); optical rotation $[\alpha]_D$ = -47.2°(c 1.0, 19.3°C, DMF)

(+)-3-(5-amino-2-methyl-4-oxoquinazolin-3(4*H*)-yl)-(3-^{2}H)piperidine-2,6-dione, (+)-D-**2**: 386.7 mg (1.35 mmol); 99.2% purity; 98.4% ee; LC-MS: 288.3 (M+1) (92% deuterium); ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.04 (s, 1H), 7.40 (t, J = 8.0 Hz, 1H), 7.0 (br s, 2H), 6.61 (d, J = 8.0, 2H), 5.2 (m, 0.14H, residual C(3)H, ca. 86% deuterium incorporation), 2.82 (m, 1H), 2.61 (m, 5H), 2.15 (m, 1H); optical rotation $[\alpha]_D$ = +43.35°(c 1.0, 19.3°C, DMF)

Kinetics of D/H exchange and enantiomerization as studied for compounds D-1 and D-2

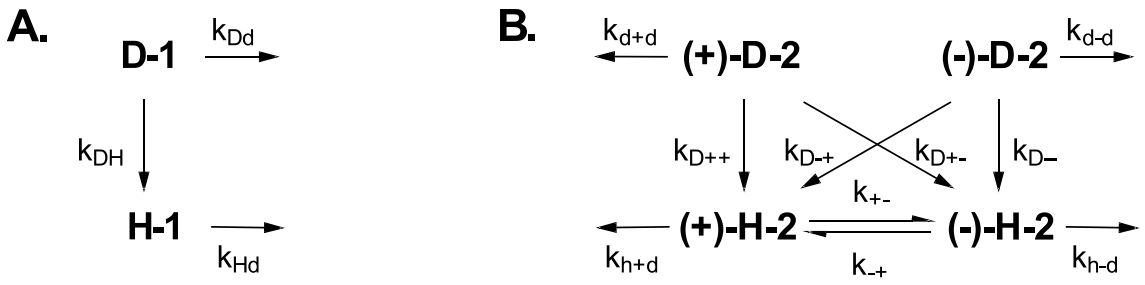

Figure S1: Kinetics of deuterium/hydrogen exchange, enantiomerization and degradation, **A**: for *rac*-**1**, (*R*)-**1** or (*S*)-**1** where D-**1** and H-**1** represent deuterated and protonated isotopomers, k_{DH}, k_{Dd}, and k_{Hd} are the rate constants for deuterium/hydrogen exchange, deuterium isotopomer degradation, and hydrogen isotopomer degradation respectively, and **B**: for *rac*-D-**2** where k_{id} (where i = h+, h-, d+, and d- stand for (+)-H-**2**, (-)-H-**2**, (+)-D-**2**, or (-)-D-**2**) are the degradation rate constants, k_{+-} and k_{-+} are the enantiomerization rate constants ((+)-H-**2** to (-)-H-**2** and (-)-H-**2** to (+)-H-**2** respectively), and k_{Dij} are the deuterium/proton exchange rate constants (where i and j = + or – independently, i represents the deuterated enantiomer and j is the protonated enantiomer, eg +- stands for (+)-D-**2** to (-)-H-**2**)

Human stability of (*S*)- and (*R*)-N-{[2-((3-^{2}H)-2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]methyl}cyclopropanecarboxamide, (*R*)-D-1 and (*S*)-D-1, and of the protonated (*S*)-enantiomer, (*S*)-H-1

The stability of enantiomers (*S*)-D-**1**, (*R*)-H-**1**, and of isotopomer (*S*)-H-**1** in human plasma was evaluated and data were analyzed by fitting experimental data to equations 1 through 4 as appropriate as described in the Materials and Methods section. Graphs in Figure S2 below show the experimental data and fitted lines. The calculated rate constants are presented in Table S1 below.

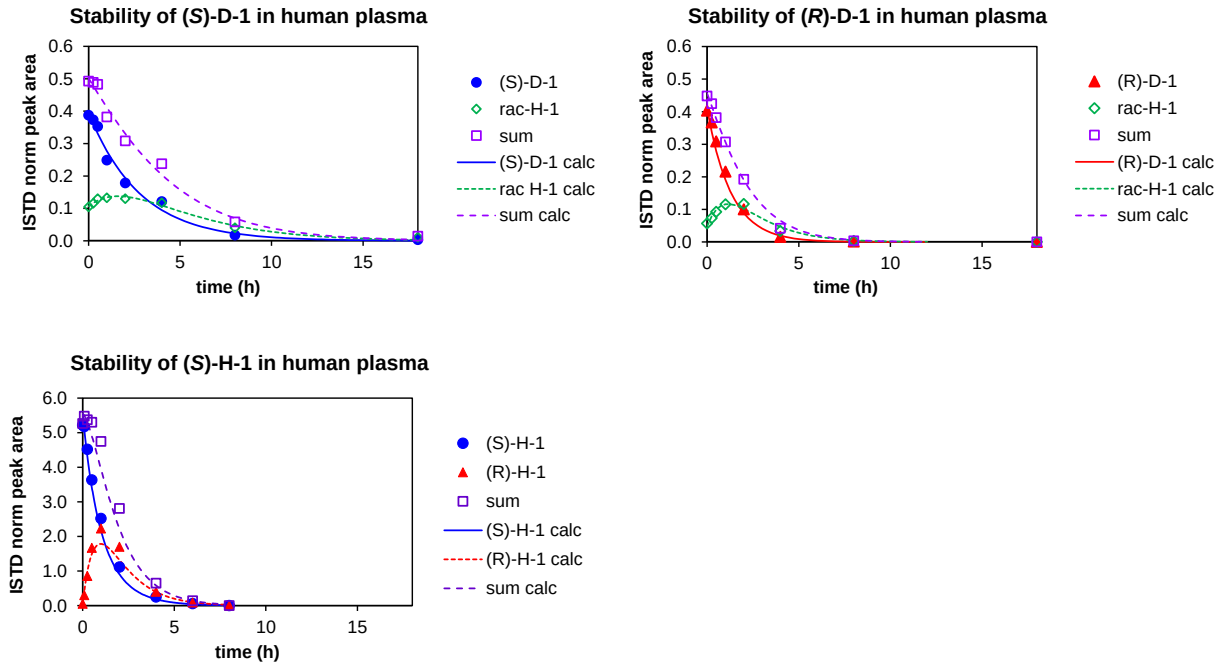

Figure S2: *In vitro* stability of (*S*)-D-**1** (top left), (*R*)-D-**1** (top right), and (*S*)-H-**1** (bottom) in human plasma at 37°C; experimental data points and results from fitting to kinetic equations 1 through 4 (see Materials and methods)

Table S1: Rate constants and calculated half-lives ($t_{1/2}$) for the *in vitro* stability of (*S*)-H-**1**, (*R*)-D-**1**, and (*S*)-D-**1**, in human plasma at 37°C

Compound	(*S*)-D-**1**		(*R*)-D-**1**		(*S*)-H-**1**	
Parameter	**k (h^{-1})**	**$t_{1/2}$ (h)**	**k (h^{-1})**	**$t_{1/2}$ (h)**	**k (h^{-1})**	**$t_{1/2}$ (h)**
DH	0.21	3.3	0.45	1.5	-	-
Dd	0.14	4.8	0.31	2.2	-	-
Hd	0.35	2.0	0.66	1.0	-	-
RS	-	-	-	-	< 0.058	> 12
SR	-	-	-	-	0.84	0.83
hSd	-	-	-	-	< 0.02	> 35
hRd	-	-	-	-	1.10	0.63

Human and mouse plasma stability of (+)- and (-)-3-(5-amino-2-methyl-4-oxoquinazolin-3(4*H*)-yl)-piperidine-2,6-dione, (+)- and (-)-H-2, and of *rac*-3-(5-amino-2-methyl-4-oxoquinazolin-3(4*H*)-yl)-(3-^{2}H)-piperidine-2,6-dione, *rac*-D-2

The stability of enantiomers (+)-H-**2** and (-)-H-**2** and of racemate *rac*-D-**2** was evaluated as described in the Materials and Methods section. Data were analyzed by fitting experimental data to equations 6 and 7 for the enantiomers and 8 to 11 for the racemate. Graphs in Figures S3 and S4 show the experimental data and fitted lines. The calculated rate constants are presented in Table S2 below.

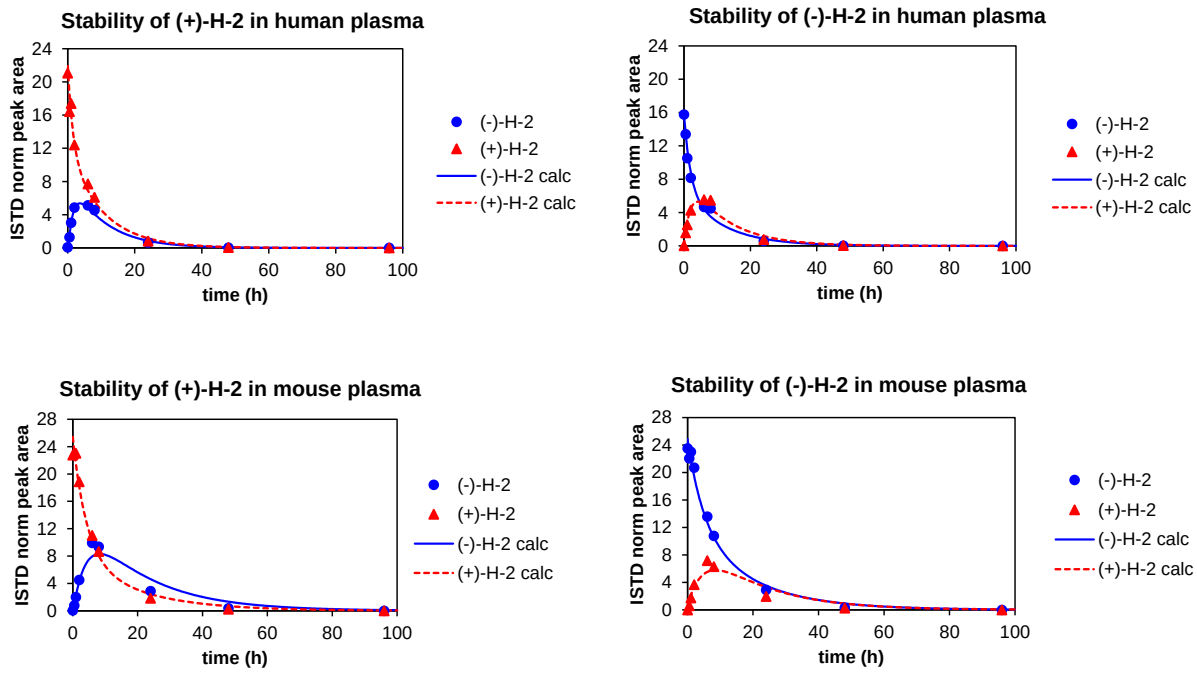

Figure S3: *In vitro* stability of (+)-H-**2** and (-)-H-**2** in human (top) and mouse (bottom) plasma at 37°C; experimental data points and results from fitting to kinetic differential equations 6 and 7

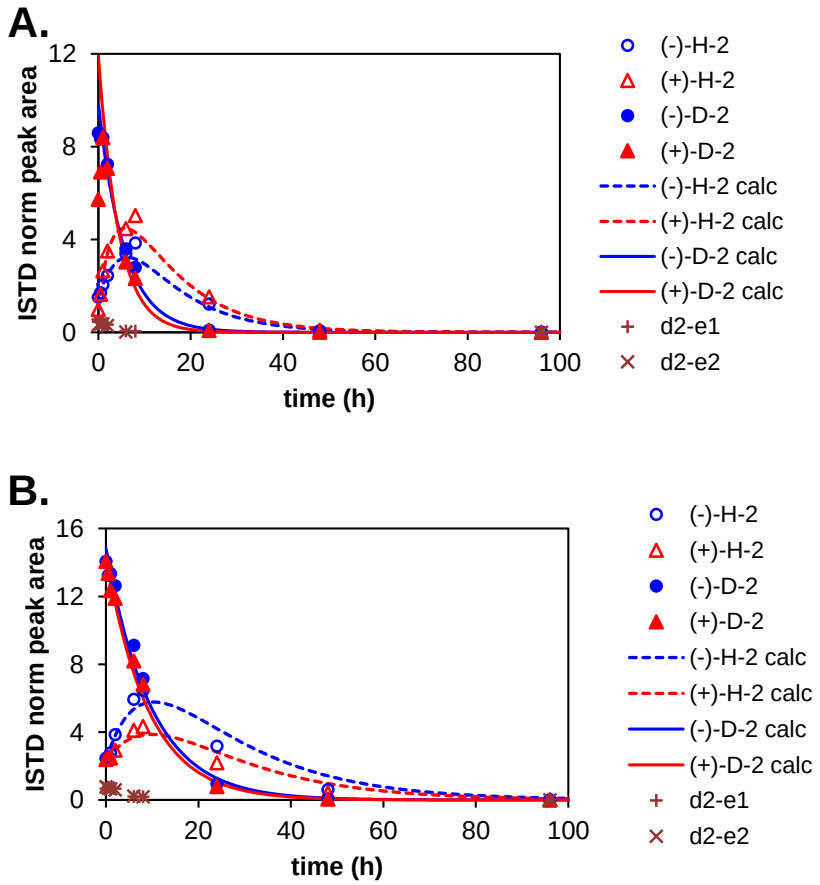

Figure S4: *In vitro* stability of *rac*-D-**2** (1:1 mixture of (+)-D-**2** and (-)-D-**2** enantiomers) in (A) human plasma and (B) mouse plasma; experimental results and fitting to kinetic differential equations 8 to 11 (d2-e1 and d2-e2 represent negligible amounts of the enantiomers of the bis-deuterated isotopomer)

Table S2: Rate constants (see Figure 3, panel B) and calculated half-lives ($t_{1/2}$) for the *in vitro* stability of *rac*-D-**2**, (+)-H-**2**, and (-)-H-**2** in human and mouse plasma at 37°C

Species	Compound		D++	D+-	D-+	D--	+-*	-+*	d**
human	*rac*-D-2	k (h^{-1})	0.084	0	0	0.134	0.264	0.195	0.0905
		$t_{1/2}$ (h)	8.3		5.2		2.6	3.6	7.8
	(-)-H-2	k (h^{-1})	-	-	-	-	0.231	0.169	0.0845
		$t_{1/2}$ (h)	-		-		3.0	4.1	8.2
	(+)-H-2	k (h^{-1})	-	-	-	-	0.297	0.220	0.0965
		$t_{1/2}$ (h)	-		-		2.3	3.2	7.2
mouse	*rac*-D-2	k (h^{-1})	0.0466	0.00109	0.0166	0.0380	0.0662	0.0959	0.0533
		$t_{1/2}$ (h)	14.5		12.7		10.5	7.2	13.0
	(-)-H-2	k (h^{-1})	-	-	-	-	0.0722	0.0730	0.0544
		$t_{1/2}$ (h)	-		-		9.6	9.5	12.7
	(+)-H-2	k (h^{-1})	-	-	-	-	0.0603	0.119	0.0523
		$t_{1/2}$ (h)	-		-		11.5	5.8	13.3

*: enantiomerization rate constants used in analysis of stability of deuterated racemate, k_{+-} or k_{-+} = average of corresponding enantiomerization rate constants obtained by fitting data for stability of (+)-H-**2** and (-)-H-**2**

: degradation rate constant used in the analysis of stability of deuterated racemate *rac*-D-2** = average of degradation rate constants for (+)-H-**2** and (-)-H-**2**

Pharmacokinetics of compound 2 in CB.17 SCID mice

The pharmacokinetics of enantiomers (+)-D-**2** and (-)-D-**2** and racemate *rac*-H-**2** were evaluated as described in the Materials and Methods section. Graphs in Figure S5 below show the experimental data for the four enantiomeric isotopomers. Calculated pharmacokinetic parameters are summarized in Table 2.

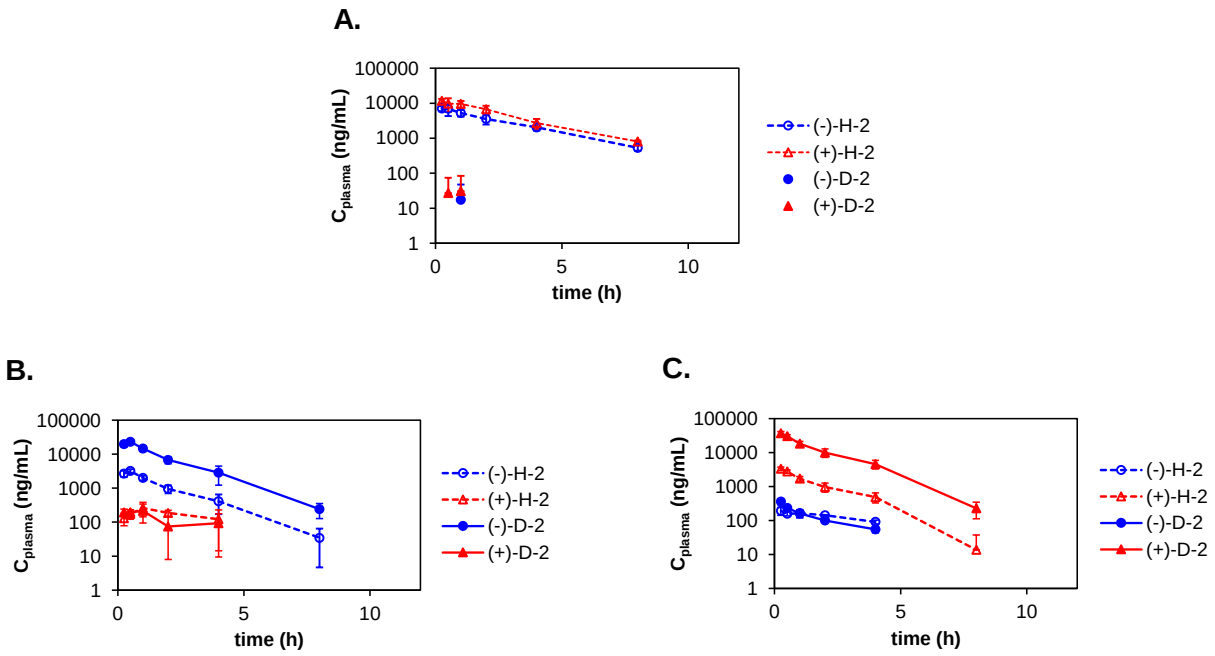

Figure S5: Pharmacokinetics of **A**: *rac*-H-**2**, **B**: (-)-D-**2**, and **C**: (+)-D-**2** administered orally at 30, 15, and 15 mg/kg respectively to female CB.17 SCID mice

Table S3: Time (T_{max}) to maximum plasma concentration (C_{max}) for (-)-H-**2**, (+)-H-**2**, (-)-D-**2**, and (+)-D-**2** (h-, h+, d-, and d+) in female CB.17 SCID mice orally administered protonated racemate, *rac*-H-**2** (h-rac, 30 mg/kg) or deuterated enantiomers, (-)-D-**2** and (+)-D-**2** (15 mg/kg each)

	Enantiomer Observed							
Compound Dosed	(-)-H-**2**		(+)-H-**2**		(-)-D-**2**		(+)-D-**2**	
	T_{max} (h)	C_{max} (ng/mL)	T_{max} (h)	C_{max} (ng/mL)	T_{max} (h)	C_{max} (ng/mL)	T_{max} (h)	C_{max} (ng/mL)
***rac*-H-2** (30 mg/kg)	0.25	7,070	0.25	11,700	-	-	-	-
(-)-D-2 (15 mg/kg)	0.50	3,210	1.00	261	0.50	23,100	1.00	216
(+)-D-2 (15 mg/kg)	0.25	193	0.25	3,330	0.25	360	0.25	36,600

Body weight measurements in the subcutaneous NCI-H929 SCID mouse xenograft model

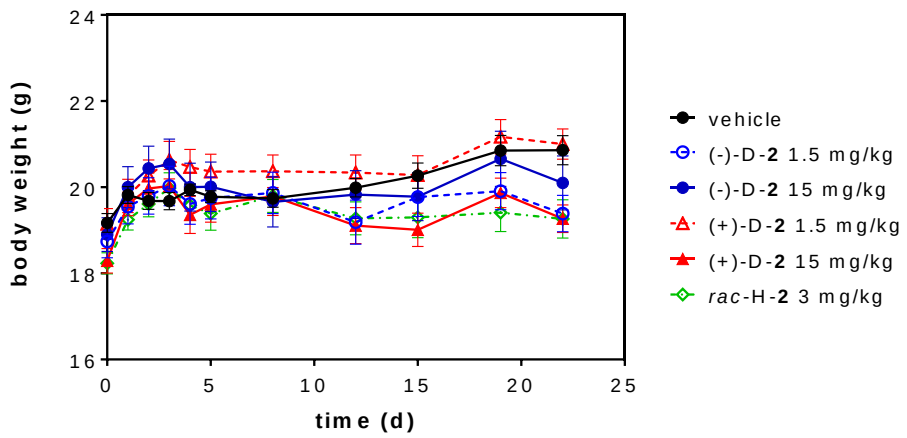

Figure S6: Average body weight (± SEM) as function of time in H929 xenograft-bearing female CB.17 SCID mice treated daily by oral gavage with vehicle (filled black circles), *rac*-3-(5-amino-2-methyl-4-oxoquinazolin-3(4*H*)-yl)-piperidine-2,6-dione (*rac*-H-**2**, 3 mg/kg, hollow green diamonds), (+)- and (-)-3-(5-amino-2-methyl-4-oxoquinazolin-3(4*H*)-yl)-(3-^{2}H)-piperidine-2,6-dione ((+)-D-**2**, 1.5 mg/kg, hollow red triangles, 15 mg/kg, filled red triangles, and (-)-D-**2**, 1.5 mg/kg, hollow blue circles, 15 mg/kg, filled blue circles)

Body weight measurements in the subcutaneous NCI-H929 SCID mouse xenograft model (repeat)

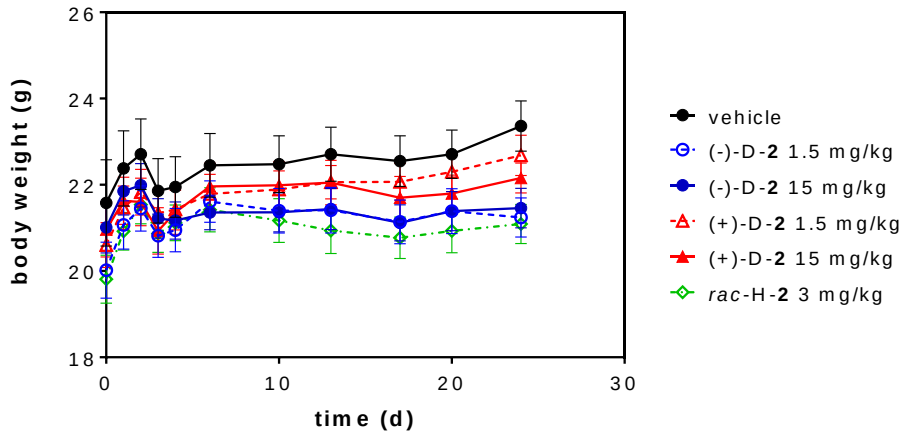

Figure S7: Average body weight (± SEM) as function of time in H929 xenograft-bearing female CB.17 SCID mice treated daily by oral gavage with vehicle (filled black circles), *rac*-3-(5-amino-2-methyl-4-oxoquinazolin-3(4*H*)-yl)-piperidine-2,6-dione (*rac*-H-**2**, 3 mg/kg, hollow green diamonds), (+)- and (-)-3-(5-amino-2-methyl-4-oxoquinazolin-3(4*H*)-yl)-(3-^{2}H)-piperidine-2,6-dione ((+)-D-**2**, 1.5 mg/kg, hollow red triangles, 15 mg/kg, filled red triangles, and (-)-D-**2**, 1.5 mg/kg, hollow blue circles, 15 mg/kg, filled blue circles)

Tumor volumes in the subcutaneous NCI-H929 SCID mouse xenograft model (repeat)

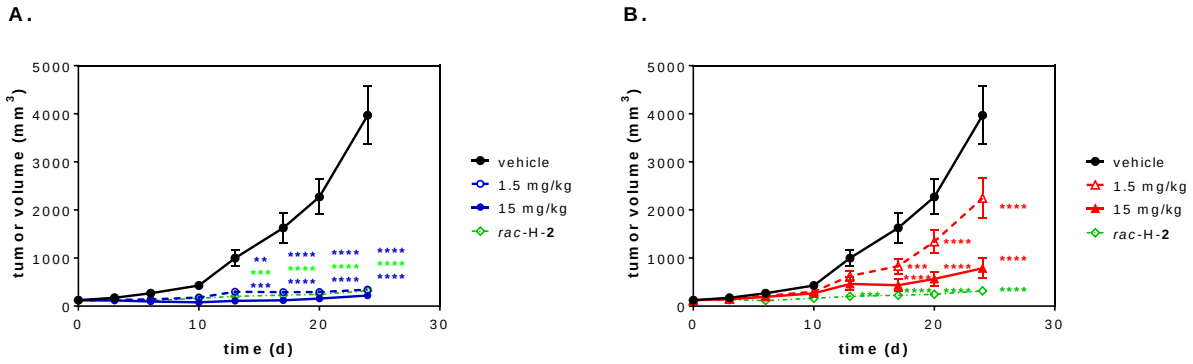

Figure S8: Average H929 tumor volume (±SEM) as function of time in H929 xenograft-bearing female CB.17 SCID mice (n = 10 per group) treated daily by oral gavage with vehicle (filled black circles), *rac*-H-**2** (h-rac, 3 mg/kg, hollow green diamonds), and (A) (-)-D-**2** (1.5 mg/kg, hollow blue circles, 15 mg/kg, filled blue circles) and (B) (+)-D-**2** (1.5 mg/kg, hollow red triangles, 15 mg/kg, filled red triangles) [2-way ANOVA of tumor volume vs. time with Bonferroni's multiple comparisons post-test against vehicle group, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$] – repeat study