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Chemistry & Drug Discovery

Supporting Information

Structure-Guided Design of a Highly Selective Glycogen Synthase Kinase-3β Inhibitor: a Superior Neuroprotective Pyrazolone Showing Antimania Effects

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List of Contents

1. General Information

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker spectrometer at 400 and 100 MHz, respectively. Standard abbreviations indicating multiplicity were used as follows: s = singlet, br s = broad singlet, d = doublet, t = triplet, q = quadruplet, and m = multiplet. High-resolution mass spectra (HRMS) experiments were performed on LTO-FTICR, Q–TOF–2TM (Micromass) or Shimadzu IT-TOF mass spectrometers. Analytical thin layer chromatography (TLC) was performed with Merck 250-mm 60F254 silica gel plates. Column chromatography was performed using Merck silica gel (40-60 mesh). Analytical HPLC was carried out on an ACE 3AQ column (100 mm × 4.6 mm), with detection at 254 nM and 280 nM on a Shimadzu 10 VP Series HPLC; flow rate = 2.0 mL/min; from 10% MeOH in water to 100% MeOH with 0.05% (V/V) TFA. Compound **3a** was described in previous publication as a PKC inhibitor. [1]

2. Chemistry

2.1 Synthesis of the Acyl Chloride

Method A: To a solution of 1 mmol of substituted or unsubstituted 1-methyl-1*H*-indole-3-carboxylic acid or benzofuran-3-carboxylic acid (1 equiv) in $SOCl_2$ (15 mL), 5 drops of DMF were added, and the mixture was stirred for 16 h at room temperature. The reaction mixture was then evaporated and dried *in vacuo*. The crude product was directly used without further purification.

Method B: To a solution of 1-methyl-1*H*-indole-3-carboxylic acid (0.20 g, 1.14 mmol) in CH_2Cl_2 (5.0 ml), oxalyl chloride as a 2M solution in CH_2Cl_2 (1.30 ml, 2.63 mmol) was added at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 1 h. The solvent was evaporated and the resulting solid 1-methyl-1*H*-indole-3-carbonyl chloride was washed with cold Et_2O (10 ml), dried *in vacuo* and used in next step without further purification.

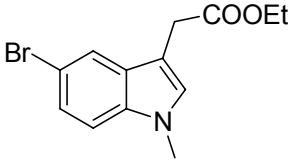
2.2 Synthesis of the Heterocycle-acetic acid ethyl ester counterpart

Benzofuran-3-yl-acetic acid ethyl ester. To a solution of benzofuran-3-one (1.00 g, 7.45 mmol) in toluene (25 mL) was added (carboxymethylene)triphenyl phosphorane (3.92 g, 11.2 mmol), and the mixture was refluxed for 24 h. The reaction mixture was cooled to room temperature and concentrated. The residue was purified by column chromatography (hexane, then ethyl acetate:hexane; 1:3) to give the title product (0.89 g, 58%). The spectral data for this compound is identical to that reported in the literature. [2]

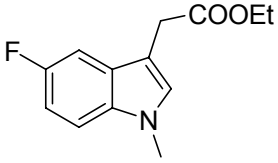
Substituted (1-Methyl-1*H*-indol-3-yl)-acetic acid ethyl ester [3]

A mixture of substituted 1-Methyl-1*H*-indole (2 mmol), ethyl diazoacetate (2.5 mmol), and $Cu(OTf)_2$ (0.2 mmol) in dichloromethane (10 mL) was stirred at room temperature for the appropriate time. After completion of the reaction, as indicated by TLC, the reaction mixture was diluted with water (10 mL), and extracted with dichloromethane (3×15 mL). The combined organic layers were washed with H_2O, brine, dried by Na_2SO_4

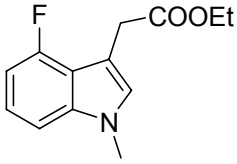
and evaporated *in vacuo*. The residue was purified by CombiFlash chromatography (ethyl acetate: hexane; 30%) to give the desired product (45-55%).



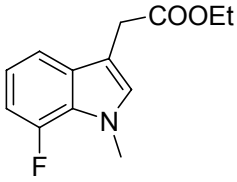
(5-Bromo-1-methyl-1*H*-indol-3-yl)-acetic acid ethyl ester (6a). Yield 52%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.75 (s, 1H), 7.31 (d, J = 8.8 Hz, 1H), 7.17 (d, J = 8.8 Hz, 1H), 7.06 (s, 1H), 4.19 (q, J = 7.2 Hz, 2H), 3.75 (s, 3H), 3.71 (s, 2H), 1.29 (t, J = 7.2 Hz, 3H).



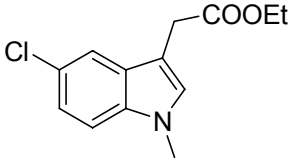
(5-Fluoro-1-methyl-1*H*-indol-3-yl)-acetic acid ethyl ester (6b). Yield 50%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.27 (dd, J = 9.6, 2.4 Hz, 1H), 7.21 (dd, J = 8.8, 4.4 Hz, 1H), 7.09 (s, 1H), 6,99 (td, J = 9.2, 2.4 Hz, 1H), 4.19 (q, J = 7.2 Hz, 2H), 3.76 (s, 3H), 3.72 (s, 2H), 1.29 (t, J = 7.2 Hz, 3H).



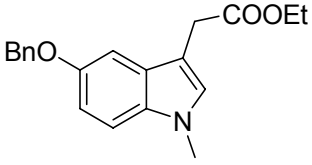
(4-Fluoro-1-methyl-1*H*-indol-3-yl)-acetic acid ethyl ester (6c). Yield 45%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.14-7.04 (m, 2H), 7.00 (s, 1H), 7.76 (ddd, J = 7.6, 11.2, 0.8 Hz, 1H), 4.21 (q, J = 7.2 Hz, 2H), 3.89 (s, 2H), 3.76 (s, 3H), 1.29 (t, J = 7.2 Hz, 3H).



(7-Fluoro-1-methyl-1*H*-indol-3-yl)-acetic acid ethyl ester (6d). Yield 55%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.38 (d, J = 8 Hz, 1H), 7.01-6.96 (m, 2H), 6.89-6.86 (m, 1H), 4.18 (q, J = 7.2 Hz, 2H), 3.97 (d, J = 1.6 Hz, 2H), 3.73 (s, 3H), 1.29 (t, J = 7.2 Hz, 3H).



(5-Chloro-1-methyl-1*H*-indol-3-yl)-acetic acid ethyl ester (6e). Yield 52%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.60 (s, 1H), 7.23-7.17 (m, 2H), 7.08 (s, 1H), 4.20 (q, J = 7.2 Hz, 2H), 3.75 (s, 3H), 3.72 (s, 2H), 1.30 (t, J = 7.2 Hz, 3H).



(5-Benzyloxy-1-methyl-1*H*-indol-3-yl)-acetic acid ethyl ester (6f). Yield 48%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.51-7.49 (m, 2H), 7.42-7.33 (m, 3H), 7.21 (d, J = 8.8 Hz, 1H), 7.17 (d, J = 2.4 Hz, 1H), 7.03 (s, 1H), 6.99 (dd, J = 8.8, 2.4 Hz, 1H), 5.13 (s, 2H), 4.16 (q, J = 7.2 Hz, 2H), 3.75 (s, 3H), 3.72 (s, 2H), 1.27 (t, J = 7.2 Hz, 3H).

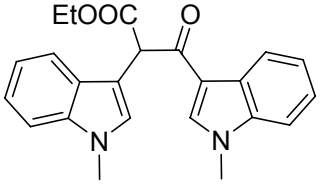
2.3 Synthesis of β-keto ester derivatives as cyclization precursors

Method A: To a solution of the above mentioned indole ester or pyridine ester (1 equiv.) in THF (~0.2 M) at -78 ^{o}C, a 2.0 M solution of LDA in THF (1.5 equiv.) was added dropwise. After 1 h, a solution of the acyl chloride (1.5 equiv.) in THF (~0.2 M) was added and the reaction mixture was stirred at room temperature for several hours until completion. The reaction mixture was quenched with water and diluted with EtOAc. The organic phase was washed with 10% sodium bicarbonate solution, water, brine, dried over Na_2SO_4 and evaporated *in vacuo*. The residue was purified by column chromatography (ethyl acetate/hexane; 1:2 to ethyl acetate/hexane; 1:1) to give the product. The yields were in the range of 28-60%.

Method B: To a solution of the indole ester (1.1 equiv.) in THF (~0.2 M) was added lithium diisopropylamide (2M solution in THF, 2.5 equiv.) dropwise at -78 °C under Ar. After 1 h at this temperature, the acid chloride (1.0 equiv.) in THF (~0.2 M) was added dropwise to the enolate at -78 °C. The resulting reaction mixture was stirred at -78 °C and allowed to warm to ambient temperature overnight. The reaction was diluted with CH_2Cl_2 (50 ml) and the organic layer was washed with 1N HCl, water, brine, and dried (Na_2SO_4). After evaporation *in vacuo*, the residue was purified by flash chromatography (ethyl acetate/hexane; 1:2 to ethyl acetate/hexane; 1:1) to give the product (45-60%).

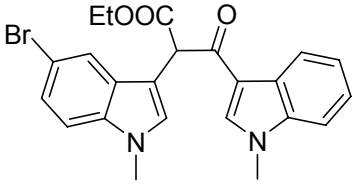
Method C: To a solution of ethyl 2-(benzofuran-3-yl) acetate (0.17 g, 0.83 mmol) in THF was added (5.0 ml) lithium diisopropylamide (LDA) as 2M solution in THF (0.83 ml, 1.65 mmol) at -78 °C under Ar. After 1h, the acid chloride (0.16 g, 0.83 mmol) in THF (1.0 ml) was added. The resulting reaction mixture was stirred for 1 h at -78 °C and allowed to warm to room temperature and stirred overnight. The reaction was diluted with CH_2Cl_2 (50 ml). The organic layer was washed with 1N HCl, saturated $NaHCO_3$, water and brine, dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by CombiFlash chromatography (ethyl acetate/hexane; 30%) to give the product (0.20 g, 66%).

Unless mentioned, the compounds are synthesized according to method A.

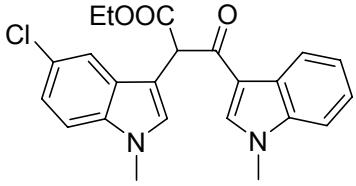


2,3-Bis-(1-methyl-1*H*-indol-3-yl)-3-oxo-propionic acid ethyl ester (9a). Yield 60%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.47-8.45 (m, 1H), 7.83 (s, 1H), 7.70 (d, J = 7.6 Hz, 1H), 7.34-

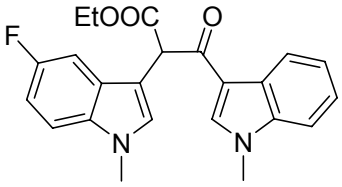
7.30 (m, 5H), 7.27-7.15 (m, 2H), 5.70 (s, 1H), 4.25 (q, *J* = 7.2 Hz, 2H), 3.79 (s, 3H), 3.77 (s, 3H), 1.28 (t, *J* = 7.2 Hz, 3H).



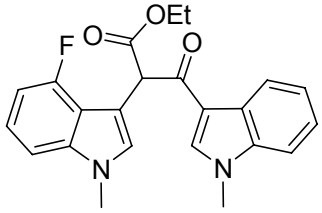
2-(5-Bromo-1-methyl-1*H*-indol-3-yl)-3-(1-methyl-1*H*-indol-3-yl)-3-oxo-propionic acid ethyl ester (9b). Yield 58%; ^{1}H NMR ($CDCl_3$, 400 MHz) *δ* 8.46-8.43 (m, 1H), 7.85 (s, 1H), 7.81 (d, *J* = 1.6 Hz, 1H), 7.37-7.30 (m, 5H), 7.19-7.16 (m, 1H), 5.62 (s, 1H), 4.24 (q, *J* = 6.8 Hz, 2H), 3.84 (s, 3H), 3.75 (s, 3H), 1.27 (t, *J* = 6.8 Hz, 3H).



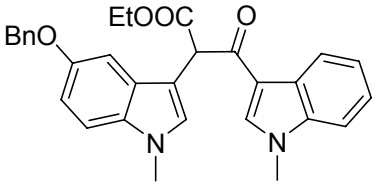
2-(5-Chloro-1-methyl-1*H*-indol-3-yl)-3-(1-methyl-1*H*-indol-3-yl)-3-oxo-propionic acid ethyl ester (9c). Yield 35%; ^{1}H NMR ($CDCl_3$, 400 MHz) *δ* 8.46-8.43 (m, 1H), 7.84 (s, 1H), 7.66 (d, *J* = 1.6 Hz, 1H), 7.41-7.31 (m, 4H), 7.23-7.18 (m, 2H), 5.62 (s, 1H), 4.24 (q, *J* = 7.2 Hz, 2H), 3.83 (s, 3H), 3.76 (s, 3H), 1.27 (t, *J* = 7.2 Hz, 3H).



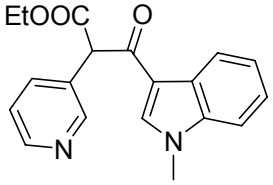
2-(5-Fluoro-1-methyl-1*H*-indol-3-yl)-3-(1-methyl-1*H*-indol-3-yl)-3-oxo-propionic acid ethyl ester (9d). Yield 38%; ^{1}H NMR ($CDCl_3$, 400 MHz) *δ* 8.47-8.43 (m, 1H), 7.83 (s, 1H), 7.40 (dd, *J* = 9.6, 2.4 Hz, 1H), 7.36 (s, 1H), 7.33-7.23 (m, 3H), 7.15 (dd, *J* = 8.8, 4.4 Hz, 1H), 6.95 (td, *J* = 7.2, 2.4 Hz, 1H), 5.64 (s, 1H), 4.26 (q, *J* = 7.2 Hz, 2H), 3.69 (s, 3H), 3.66 (s, 3H), 1.28 (t, *J* = 7.2 Hz, 3H).



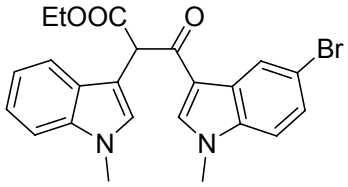
2-(4-Fluoro-1-methyl-1*H*-indol-3-yl)-3-(1-methyl-1*H*-indol-3-yl)-3-oxo-propionic acid ethyl ester (9e). Yield 28%; ^{1}H NMR ($CDCl_3$, 400 MHz) *δ* 8.47-8.45 (m, 1H), 7.94 (s, 1H), 7.35-7.25 (m, 4H), 7.13-7.04 (m, 2H), 6.82 (m, 1H), 6.04 (s, 1H), 4.27 (q, J = 7.2 Hz, 2H), 3.77 (s, 3H), 3,71 (s, 3H), 1.27 (t, J = 7.2, 3H).



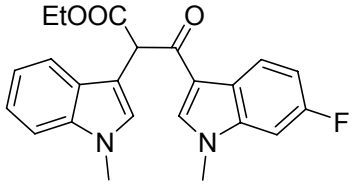
2-(5-Benzyloxy-1-methyl-1*H*-indol-3-yl)-3-(1-methyl-1*H*-indol-3-yl)-3-oxo-propionic acid ethyl ester (9f). Yield 60%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.50-8.47 (m, 1H), 7.78 (s, 1H), 7.51 (d, J = 7.2 Hz, 2H), 7.41 (t, J = 7.2 Hz, 2H), 7.36-7.31 (m, 4H), 7.29-7.27 (m, 2H), 7.19 (d, J = 8.8 Hz, 1H), 6.99 (dd, J = 8.8, 2.4 Hz, 1H), 5.64 (s, 1H), 5.14 (s, 2H), 4.26 (q, J = 7.2 Hz, 2H), 3.70 (s, 3H), 3.69 (s, 3H), 1.29 (t, J = 7.2 Hz, 3H).



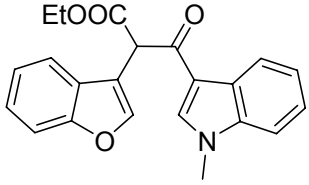
3-(1-Methyl-1*H*-indol-3-yl)-3-oxo-2-pyridin-3-yl-propionic acid ethyl ester (9g). Yield 47%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.67 (d, J = 2 Hz, 1H), 8.54 (dd, J = 4.4, 1.2 Hz, 1H), 8.39 (t, J = 4 Hz, 1H), 8.04 (d, J = 7 Hz, 1H), 7.81 (s, 1H), 7.33-7.28 (m, 4H), 5.44 (s, 1H), 4.23 (q, J = 7.2 Hz, 2H), 3.78 (s, 3H), 1.25 (t, J = 7.2 Hz, 3H).



3-(5-Bromo-1-methyl-1*H*-indol-3-yl)-2-(1-methyl-1*H*-indol-3-yl)-3-oxo-propionic acid ethyl ester (9h). (Method B) Yield 60%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.63 (s, 1H), 7.77 (s, 1H), 7.67 (d, J = 7.6 Hz, 1H), 7.39 (dd, J = 8.4, 2.0 Hz, 1H), 7.35-7.29 (m, 2H), 7.25-7.13 (m, 3H), 5.62 (s, 1H), 4.25 (q, J = 7.2 Hz, 2H), 3.77 (s, 3H), 3.75 (s, 3H), 1.26 (t, J = 7.2 Hz, 3H); LRMS $[M+H]^+$: 453.1 (97), 454.1 (30), 455.1 (100), 456.1 (29).



3-(6-Fluoro-1-methyl-1*H*-indol-3-yl)-2-(1-methyl-1*H*-indol-3-yl)-3-oxo-propionic acid ethyl ester (9i). (Method B) Yield 45%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.40–8.35 (m, 1H), 7.79 (s, 1H), 7.68 (d, J = 7.6 Hz, 1H), 7.26–7.12 (m, 3H), 7.04 (t, J = 8.8 Hz, 1H), 6.95 (t, J = 8.0 Hz, 1H), 6.88 (d, J = 8.4 Hz, 1H), 5.66 (s, 1H), 4.24 (q, J = 6.8 Hz, 2H), 3.74 (s, 3H), 3.69 (s, 3H), 1.27 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) 187.6, 169.3, 161.3, 158.9, 137.3, 137.2, 136.3, 136.0, 135.9, 128.5, 126.8, 123.6, 123.5, 122.8, 121.4, 119.1, 118.0, 114.7, 111.1, 110.8, 109.1, 107.0, 96.0, 95.8, 61.2, 52.9, 33.3, 32.5, 13.8; LRMS $[M+H]^+$: 393.1 (100).



2-Benzofuran-3-yl-3-(1-methyl-1*H*-indol-3-yl)-3-oxo-propionic acid ethyl ester (9j). (Method C) Yield 66%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.44 (m, 1H), 7.94 (s, 1H), 7.89 (s, 1H), 7.68 (d, J = 7.4 Hz, 1H), 7.48 (d, J = 7.4 Hz, 1H), 7.30 (m, 5H), 5.50 (s, 1H), 4.25 (q,

J = 7.1 Hz, 2H), 3.82 (s, 3H), 1.26 (t, *J* = 7.1, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) 186.0, 168.6, 155.0, 143.9, 137.3, 136.2, 127.9, 126.7, 124.4, 123.8, 123.0, 122.7, 120.1, 114.8, 114.2, 111.5, 109.6, 61.9, 53.1, 33.6, 14.0.

2.4 Synthesis of substituted indole-based pyrazolones

Method A: *p*-TsOH (0.3 equiv.) and hydrazine hydrate (6 equiv.) was added to a suspension of the appropriate β-ketoester **9** (1 equiv.) in EtOH (10 mL/mmol). Then the reaction was irradiated in a Biotage microwave reactor (high absorption) at 140 ºC for 4.5 h. The reaction mixture was separated by ethyl acetate and water. The aqueous layer was extracted by ethyl acetate. Then the combined organic layers were washed with H_2O, brine, dried (Na_2SO_4) and evaporated *in vacuo*. The residue was purified by flash chromatography ($CHCl_2$/ MeOH; 90:10) and further by HPLC to afford the desired pyrazolone (19–40%).

Method B: A solution of β-ketoester (1.0 equiv) in DMF/EtOH (4:1 v/v, ~0.2 M) was treated with hydrazine monohydrate (2.5 equiv.) and the reaction was irradiated in a Biotage microwave reactor (normal absorption) at 140 ºC for 10 min. Additional hydrazine monohydrade (2.5 equiv.) was added and the reaction was reirradiated in the microwave reactor at 140 ºC for another 10 min. This cycle was repeated for a total of 6 times after which the starting material was totally consumed as evidenced by TLC. The reaction was diluted with ethyl acetate and water. The organic layer was separated and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with H_2O, brine, dried (Na_2SO_4) and evaporated *in vacuo*. The residue was purified by flash chromatography (MeOH/ethyl acetate; 5:95, Rf ~0.2) and further by HPLC to afford the desired pyrazolone (15–21%).

Method C: A solution of β-ketoester **13a** (0.20 g, 0.55 mmol), hydrazine monohydrate (65%, 0.04 ml, 0.55 mmol), and acetic acid (99%, 0.04 ml, 0.67 mmol) in dioxane (3.0 ml) was refluxed for 16 h. Additional hydrazine monohydrade (65%, 0.04 ml, 0.55 mmol) was added and the reaction mixture was refluxed for 4 h. The solvent was evaporated, EtOAc (50 ml) was added and the organic phase was washed with 1N HCl, saturated $NaHCO_3$, water and brine, dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by HPLC to afford the pyrazolone **4a** (0.105 g, 58%).

Unless mentioned, the compounds are synthesized according to method A.

4,5-Bis-(1-methyl-1*H*-indol-3-yl)-1,2-dihydro-pyrazol-3-one (3a). Yield 40%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.4 (br s, 1H), 7.50 (d, *J* = 8 Hz, 1H), 7.31-7.26 (m, 2H), 7.24-7.17 (m, 3H), 7.09-7.06 (m, 3H), 6.93 (t, *J* = 7.6 Hz, 1H), 3.73 (s, 3H), 3.59 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 158.4, 142.3, 136.9, 136.7, 130.7, 129.1, 126.6, 124.9, 122.7, 121.7, 121.1, 120.6, 119.9, 119.3, 109.7, 109.3, 102.5, 102.4, 97.3, 33.0, 32.8; ESI-MS m/z 343.2 (M^+); HRMS calcd. for $C_{21}H_{18}N_4O$ $[M + H]^+$: 343.1553; found: 343.1550. HPLC purity: 99.3%.

5-Benzofuran-3-yl-4-(1-methyl-1*H*-indol-3-yl)-1,2-dihydro-pyrazol-3-one (3b). Yield 19%; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.81 (d, J = 7.2 Hz, 1H), 7.69 (d, J = 7.6 Hz, 1H), 7.48-7.44 (m, 2H), 7.36 (s, 1H), 7.31-7.24 (m, 2H), 7.18-7.12 (m, 2H), 6.92 (td, J = 7.6, 0.8 Hz, 1H), 3.86 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 158.5, 158.1, 139.2, 138.7, 136.7, 129.7, 127.7, 124.5, 124.4, 123.4, 122.3, 121.1, 120.4, 119.8, 118.8, 117.1, 114.2, 109.8, 103.9, 32.6; ESI-MS m/z 330.1 (M^+); HRMS calcd. for $C_{20}H_{15}N_3O_2$ $[M + H]^+$: 330.1237; found: 330.1244. HPLC purity: 98.1%.

4-(5-Bromo-1-methyl-1*H*-indol-3-yl)-5-(1-methyl-1*H*-indol-3-yl)-1,2-dihydro-pyrazol-3-one (3c). Yield 32%; ^{1}H NMR (MeOD, 400 MHz) δ 7.44-7.36 (m, 2H), 7.30-7.24 (m, 2H), 7.21-7.13 (m, 4H), 6.98 (t, J = 7.2 Hz, 1H), 3.78 (s, 3H), 3.75 (s, 3H); ^{13}C NMR (MeOD, 100 MHz) δ 161.1, 137.0, 135.6, 129.9, 129.1, 128.9, 127.9, 125.6, 123.7, 122.5, 121.9, 119.8, 119.6, 111.8, 110.5, 109.3, 104.3, 104.1, 96.9, 31.7, 31.6; ESI-MS m/z 421.1 (M^+); HRMS calcd. for $C_{21}H_{17}N_4OBr$ $[M + H]^+$: 421.0658; found: 421.0640. HPLC purity: 98.2%.

5-(5-Bromo-1-methyl-1*H*-indol-3-yl)-4-(1-methyl-1*H*-indol-3-yl)-1,2-dihydro-pyrazol-3-one (3d). (Method B) Yield 15%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.53 (br s, 1H), 7.25–7.14 (m, 2H), 7.11–6.99 (m, 4H), 6.92 (s, 1H), 6.86–6.82 (m, 1H), 3.64 (s, 3H), 3.47 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.7, 138.9, 136.4, 134.8, 129.9, 128.2, 126.4, 126.4, 124.6, 122.5, 121.1, 120.2, 118.5, 113.4, 110.5, 108.6, 103.8, 103.4, 99.6, 32.6, 32.3; LRMS $[M+H]^+$: 421.1 (97), 422.1 (27), 423.1 (100), 424.1 (28); FAB-HRMS calcd. for $C_{21}H_{17}BrN_4O$ $[M+H]^+$: 421.0659, found: 421.0649; HPLC purity: 99.1%.

5-(6-Fluoro-1-methyl-1*H*-indol-3-yl)-4-(1-methyl-1*H*-indol-3-yl)-1,2-dihydro-pyrazol-3-one (3e). (Method B) Yield 21%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.7 (br s, 2H), 7.34–7.27 (m, 2H), 7.19–7.15 (m, 2H), 7.07 (s, 2H), 7.00–6.85 (m, 2H), 6.75 (t, J = 8.0 Hz, 1H), 3.73 (s, 3H), 3.53 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.8, 158.4, 157.7, 141.2, 136.6, 136.5, 130.5, 128.7, 126.1, 121.5, 121.0, 120.7, 120.6, 120.1, 119.0, 109.4, 109.2, 108.9, 102.5, 101.8, 97.3, 96.0, 95.7, 32.8, 32.5; LRMS $[M+H]^+$: 361.1 (100); FAB-HRMS calcd. for $C_{21}H_{17}FN_4O$ $[M+H]^+$: 361.1459, found: 361.1452; HPLC purity: 96.1%.

4-(5-Fluoro-1-methyl-1*H*-indol-3-yl)-5-(1-methyl-1*H*-indol-3-yl)-1,2-dihydro-pyrazol-3-one (3f). Yield 34%; ^{1}H NMR (MeOD, 400 MHz) δ 7.41-7.34 (m, 3H), 7.25-7.18 (m, 3H), 7.03-6.99 (m, 1H), 6.92 (td, J = 10.2, 2.4 Hz, 1H), 6.83 (dd, J = 10.2, 2.4 Hz, 1H), 3.78 (s, 3H), 3.69 (s, 3H); ^{13}C NMR (MeOD, 100 MHz) δ 158.0, 157.4 (d, J = 232 Hz), 142.1, 136.6, 133.4, 130.7, 129.7, 127.5, 127.4, 124.8, 121.9, 119.9, 119.0, 109.8, 109.7, 109.3, 109.2, 109.0, 103.7, 103.4, 102.2, 102.1, 102.0, 96.3, 31.43, 31.37; ESI-MS m/z 361.1 (M^+); HRMS calcd. for $C_{21}H_{17}FN_4O$ $[M + H]^+$: 361.1459; found: 361.1455. HPLC purity: 98.1%.

4-(5-Chloro-1-methyl-1*H*-indol-3-yl)-5-(1-methyl-1*H*-indol-3-yl)-1,2-dihydro-pyrazol-3-one (3g). Yield 37%; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.45-7.40 (m, 3H), 7.29-7.27 (m,

2H), 7.16-7.12 (m, 2H), 7.07 (dd, *J* = 10.4, 2.0 Hz, 1H), 6.93 (t, *J* = 7.6 Hz, 1H), 3.75 (s, 3H), 3.73 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 158.5, 136.4, 134.9, 130.5, 129.3, 128.1, 125.4, 123.3, 121.8, 120.8, 119.8, 119.6, 119.2, 114.0, 111.2, 110.1, 104.4, 103.9, 95.8, 32.7, 32.6; ESI-MS m/z 377.1 (M^+); HRMS calcd. for $C_{21}H_{17}N_4OCl$ $[M + H]^+$: 377.1164; found: 377.1173. HPLC purity: 98.5%.

4-(4-Fluoro-1-methyl-1*H*-indol-3-yl)-5-(1-methyl-1*H*-indol-3-yl)-1,2-dihydro-pyrazol-3-one (3h). Yield 24%; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.44 (d, *J* = 4.8 Hz, 1H), 7.38 (d, *J* = 8.4 Hz, 1H), 7.24-7.22 (m, 2H), 7.15 (s, 1H), 7.13-7.03 (m, 2H), 6.91 (t, *J* = 7.4 Hz, 1H), 6.64 (dd, J_1 = 9.4 Hz, J_2 = 8 Hz, 1H), 3.75 (s, 3H), 3.68 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 159.8, 156.3 (d, *J* = 245 Hz), 139.5, 139.4, 136.3, 129.9, 128.0, 125.6, 121.5, 121.4, 120.2, 119.3, 116.9, 116.7, 109.9, 106.1, 104.1, 103.9, 103.3, 99.6, 32.8, 32.6; ESI-MS m/z 361.1 (M^+); HRMS calcd. for $C_{21}H_{17}FN_4O$ $[M + H]^+$: 361.1459; found: 361.1457. HPLC purity: 99.3%.

4-(7-Fluoro-1-methyl-1*H*-indol-3-yl)-5-(1-methyl-1*H*-indol-3-yl)-1,2-dihydro-pyrazol-3-one (3i). Yield 32%; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.40 (d, *J* = 8 Hz, 1H), 7.33-7.30 (m, 2H), 7.19 (s, 1H), 7.12 (t, *J* = 7.4 Hz, 1H), 6.95-6.89 (m, 2H), 6.84-6.64 (m, 2H), 3.92 (s, 3H), 3.73 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 159.6, 149.7 (d, *J* = 240.1 Hz), 136.4, 131.8, 131.7, 130.4, 128.5, 125.6, 124.0, 123.9, 121.6, 120.1, 119.4, 118.6, 118.5, 116.71, 116.68, 109.9, 107.0, 106.4, 106.2, 105.2, 95.3, 35.3, 32.6; ESI-MS m/z 361.1 (M^+); HRMS calcd. for $C_{21}H_{17}FN_4O$ $[M + H]^+$: 361.1459; found: 361.1453. HPLC purity: 98.3%.

4-(5-Benzyloxy-1-methyl-1*H*-indol-3-yl)-5-(1-methyl-1*H*-indol-3-yl)-1,2-dihydro-pyrazol-3-one (3j). Yield 20%; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.45-7.40 (m, 2H), 7.36-7.12 (m, 8H), 7.14 (t, *J* = 7.2 Hz, 1H), 6.96 (t, *J* = 7.6 Hz, 1H), 6.70 (dd, *J* = 5.4, 2.4 Hz, 1H), 6.48 (d, *J* = 2.4 Hz, 1H), 4.37(s, 2H), 3.73 (s, 3H), 3.70 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 161.0, 151.9, 137.5, 136.4, 131.9, 129.0, 128.9, 128.3, 127.6, 127.4, 127.0, 126.1, 121.7, 120.1, 119.5, 111.7, 110.1, 109.9, 105.5, 105.3, 103.4, 96.9, 69.2, 32.62, 32.58; ESI-MS m/z 449.2 (M^+); HRMS calcd. for $C_{28}H_{24}N_4O_2$ $[M+H]^+$: 449.1972; found: 449.1956. HPLC purity: 99.0%.

4-(Benzofuran-3-yl)-5-(1-methyl-1*H*-indol-3-yl)-1,2-dihydro-pyrazol-3-one (4a). (Method C) Yield 58%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.56 (br s, 2H), 7.44 (d, *J* = 8.3 Hz, 1H), 7.42 (s, 1H), 7.35 (d, *J* = 8.3 Hz, 1H), 7.14 (m, 4H), 6.96 (m, 3H), 3.53 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) 159.4, 155.1, 143.7, 141.8, 136.7, 130.2, 126.2, 124.9, 124.3, 122.8, 122.4, 121.4, 121.1, 119.7, 111.3, 109.9, 109.8, 102.2, 94.1, 33.0; FAB-HRMS calcd. for $C_{20}H_{15}N_3O_2$ + H^+: 330.1237; found: 330.1244. HPLC purity: 98.0%.

5-(1-Methyl-1*H*-indol-3-yl)-4-pyridin-3-yl-1,2-dihydro-pyrazol-3-one (4b). Yield 34%; ^{1}H NMR (MeOD, 400 MHz) δ 8.84 (s, 1H), 8.50 (d, *J* = 8.4 Hz, 1H), 8.45 (s, 1H), 7.78 (td, *J* = 7.2, 2.0 Hz, 1H), 7.57 (s, 1H), 7.53 (d, *J* = 8.4 Hz, 1H), 7.27 (td, *J* = 8.2, 1.2 Hz, 1H), 7.09-7.00 (m, 2H), 3.93 (s, 3H); ^{13}C NMR (MeOD, 100 MHz) δ 160.5, 141.9, 138.3,

137.1, 136.3, 128.9, 128.0, 126.0, 125.1, 124.5, 122.0, 119.9, 118.7, 109.6, 102.4, 96.1, 31.5; ESI-MS m/z 291.1 (M^+); HRMS calcd. for $C_{17}H_{14}N_4O_1$ $[M + H]^+$: 291.1240; found: 291.1250. HPLC purity: 98.0%.

3. GSK-3β *in Vitro* Kinase Assay

For GSK-3β, EMBL # L33801, performed at Reaction Biology Corporation, Malvern, PA (www.reactionbiology.com), recombinant human protein (aa 2-end, H350L), 6X His tagged, purified from insect cells is used at a kinase final concentration in the assay of 4 nM. The substrate is the phospho-glycogen synthase peptide sequence: [YRRAAVPPSPSLSRHSSPHQ(pS)EDEEE], at a final concentration of 20 μM. No additional cofactors are added to the reaction mixture. The general reaction procedure consists of preparing the indicated substrate in freshly prepared Base Reaction Buffer consisting of 20 mM Hepes (pH 7.5), 10 mM $MgCl_2$, 1 mM EGTA, 0.02% Brij35, 0.02 mg/ml BSA, 0.1 mM Na_3VO_4, 2 mM DTT, and 1% DMSO. The kinase is then delivered into the substrate solution and gently mixed. The inhibitors are then added in DMSO into the kinase reaction mixture, and ^{32}P-ATP (specific activity 0.01 μCi/μl final) is added into the reaction mixture to initiate the reaction. The kinase reaction is incubated for 120 min at rt, the reactions are then spotted onto P81 ion exchange paper (Whatman # 3698-915), and the filters washed extensively in 0.75% phosphoric acid. The P81 paper is transferred into 4 mL scintillation tubes, scintillation cocktail added, and the samples counted in a scintillation counter. Compounds were dissolved in DMSO and tested in 10-dose IC_{50} mode with 3-fold serial dilution starting at 50 μM. Control Compound staurosporine was tested in a 10-dose IC_{50} with 3-fold serial dilution starting at 20 μM. IC_{50} values were extracted by curve-fitting the dose/response slopes.

4. PDSP screening

This work was performed by the NIMH Psychoactive Drug Screening Program. Compound **3a** was screened against 44 cloned human and rodent CNS receptors, channels, and transporters. The results revealed that compound **3a** was only active at 4 receptors: $5HT_{1a}$ (5HT = 5-hydroxytryptamine), $5HT_{2b}$, $5HT_7$, and alpha-2A (Alpha-2-adrenergic). The secondary test indicated that just three of them ($5HT_{1a}$, $5HT_{2b}$, $5HT_7$) bind compound **3a** with the corresponding IC_{50} values of 704, 6114, and 3836 nM.

5. Neuroprotection Assays

Primary cortical neuron cultures were obtained from the cerebral cortex of fetal Sprague-Dawley rats (embryonic day 17) as described previously. All experiments were initiated 24 h after plating. Under these conditions, the cells are not susceptible to glutamate-mediated excitotoxicity. For cytotoxicity studies, cells were rinsed with warm PBS and then placed in minimum essential medium (Invitrogen) containing 5.5 g/liter glucose, 10% fetal calf serum, 2 mM L-glutamine, and 100 μM cystine. Oxidative stress was induced by the addition of the glutamate analog homocysteate (HCA; 5 mM) to the media. HCA was diluted from 100-fold concentrated solutions that were adjusted to pH 7.5. In combination with HCA, neurons were treated with either compound **3a** or SB-

216763 at the indicated concentrations. Viability was assessed after 24 h by MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method.

6. Behavioral assays

Animals: Male C57BL/6J mice from the Jackson Laboratory (Bar Harbor, ME) were housed in groups of four in a colony room on a 12h light/dark cycle. Room temperature was maintained between 20 and 23 °C with relative humidity between 30% and 70%. Chow and water were provided *ad libitum* for the duration of the study. Prior to testing, all mice were examined on a regular basis, handled, and weighed to assure adequate health and suitability. In each test, animals were randomly assigned across treatment groups. All procedures were approved by PsychoGenics' Institutional Animal Care and Use Committee.

Locomotor Activity Apparatus: Locomotor activity was measured in a photocell apparatus (Med Associates Inc., St Albans, VT). The apparatus consisted of Plexiglas square chambers (27.3 x 27.3 x 20.3 cm) surrounded by 16 beam infrared photobeam sources. Distance traveled was measured as the index for activity.

Drugs: All compounds were administered intraperitoneally (i.p.) in a volume of 10mL/kg. Compound **3a** and sodium valproate (Sigma, St.:ouis, MO) were dissolved in sterile injectable water. Both d-amphetamine sulfate (Sigma) and chlordiazepoxide HCl (Sigma) were dissolved in water as well.

Procedure: C57BL/6J mice (approximately 8 weeks of age) were all placed in individual holding cages for 30 min. Mice administered either vehicle, valproate (400 mg/kg; 5min cohort) or **3a** (150 mg/kg) were injected after 25 min and placed back in their holding cage for 5 additional min. A separate cohort of mice was administered valproate (400mg/kg; 30min cohort) upon being placed in the holding cages, for a total pretreatment time of 30 min. Mice were then injected with amphetamine (Amph), amphetamine (Amph) + chlordiazepoxide (CDP) (amphetamine: 4 mg/kg; CDP 2.5 mg/kg), or vehicle and placed in the open field for a period of 1 h. The number of mice tested for each group was as follows**:** Veh-Veh (n=11); Valproate (30 min)- Veh (n=12); Valproate (5 min)-Veh (n=12); **3a**-Veh (n=11); Veh-Amph (n=11); Valproate (30 min)-Amph (n=11); Valproate (5 min)-Amph (n=11); **3a**-Amph (n=12); Veh-Amph+CDP (n=11); Valproate (30 min)-Amph+CDP (n=11); Valproate (5 min)-Amph+CDP (n=11); **3a**- Amph+CDP (n=11).

Analyses: Locomotor activity data were analyzed using analysis of variance (ANOVA) followed by Fisher's PLSD post doc test when appropriate. An effect was considered significant if $p<0.05$.

7. Docking

All molecular modeling studies were performed on a Linux computer using the SYBYL 7.0[4] and CHARMM[5] (version 31) software packages as we reported before.[6]

The coordinates for the kinase domain of GSK-3β were extracted from the co-crystal structure between GSK-3β and compound **2b**. The backbone atoms were fixed and the complex was subjected to a short MD relaxation of the sidechains followed by minimization using CHARMM. The active site was designated consisting of the amino

acids within the 6.5 Å radius of the original ligand. Val135, Asp133, Leu132, Lys85, Glu97, Arg141 were set as a core subpocket. The following FlexX-Pharm settings were used to restrict the binding of the ligands to the ATP binding site of GSK: Asp133 is an optional acceptor, Val135 is an optional donor, and a minimum of one optional criterion has to be satisfied. Then, the ligand docking was performed using the FlexX module. The binding modes of the docked ligands were found to be consistent with those expected for compounds that are structurally related to staurosporine.[7]

8. References:

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