Supporting Information

The First Potent Inhibitor of Mammalian Group X Secreted Phospholipase A_2: Elucidation of Sites for Enhanced Binding

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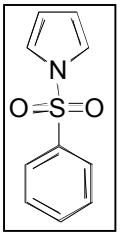
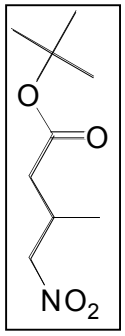
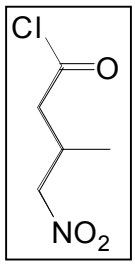
Chemistry



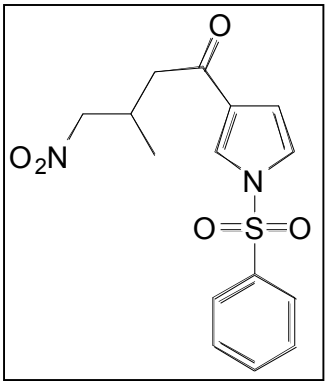
1-(phenylsulfonyl)-1H-pyrrole. To pyrrole (9.66 g, 144 mmol) in anhydrous 1,2-dichloroethane (100 mL) at room temperature was added powdered NaOH (17.30 g, 432 mmol) all at once and the reaction was left to stir under N_2 for 30 minutes. The reaction was then cooled to 0°C and benzenesulfonyl chloride (30.59 g, 172.8 mmol) was added dropwise over 30 minutes via addition funnel before removal of the ice bath. The mixture was allowed to stir under N_2 for 24 hours at room temperature before pouring onto 100 mL H_2O in a separatory funnel. The organic layer was separated and the aqueous layer was extracted 3 x 25 mL CH_2Cl_2. The combined organic layer was then poured onto 50 mL H_2O and portions of 1 M HCl were added until the aqueous layer was neutral. The organic layer was then dried over $MgSO_4$, filtered, and the solvent removed by rotary evaporation. The product was recrystallized from toluene to leave colorless plates (19.3 g, 65% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.32 (t, J = 2.1 Hz, 2H), 7.19 (t, J = 2.1 Hz, 2H), 7.52 (t, J = 7.2 Hz, 2H), 7.62 (t, J = 7.2 Hz, 1H), 7.88 (d, J = 7.2 Hz, 2H).



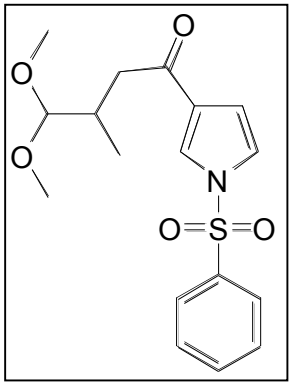
tert-butyl 3-methyl-4-nitropentanoate. To the t-butylcrotonic acid (44.5 g, 313 mmol) in anhydrous CH_3CN (200 mL) was added 18-Crown-6 (4.14 g, 15.65 mmol), KF (1.818 g, 31.29 mmol), and nitromethane (85 mL, 1.565 mol). The mixture was set to reflux under N_2 for 48 hours before cooling to room temperature. The solvent was removed from the bright yellow solution by rotary evaporation and the oil redissolved in 200 mL EtOAc and poured on 200mL H_2O. The organic phase was washed 3 x 50 mL H_2O, followed by 50 mL brine. The combined organic layer was dried over $MgSO_4$, filtered, and the solvent removed by rotary evaporation. The oil was purified by distillation (0.35 mmHg/69-72°C) to yield a colorless oil (58.5 g, 92% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.11 (d, J = 6.9 Hz, 3H), 1.47 (s, 9H), 2.24-2.40 (m, 2H), 2.71-2.82 (m, 1H), 4.40 (m, 2H).



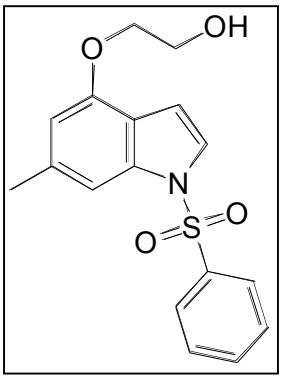
3-methyl-4-nitropentanoyl chloride (2). To the tert-butyl 3-methyl-4-nitropentanoate (11.02 g, 54.29 mmol) in $CHCl_3$ (30 mL) was added concentrated H_2SO_4 (0.50 mL) and the reaction was refluxed for 10 hours. The reaction was cooled to room temperature and poured onto 100 mL H_2O and the layers separated. The organic phase was washed with 100 mL portions of H_2O until the aqueous layer was neutral pH. The organic phase was then dried over $MgSO_4$, filtered, and the solvent removed by rotary evaporation. To the residue was added thionyl chloride (10 mL) and the reaction set to reflux for 1 hour. The excess thionyl chloride was removed by rotary evaporation and the residue was purified by distillation (0.35 mmHg, 60-65°C) to leave a colorless oil (6.52 g, 72% yield). (Note: High heat decomposes the acid chloride.) ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.17 (d, J = 6.9 Hz, 3H), 2.80-2.90 (m, 1H), 2.94-3.18 (m, 2H), 4.35-4.47 (m, 2H).



3-methyl-4-nitro-1-(1-(phenylsulfonyl)-1H-pyrrol-3-yl)butan-1-one (3). To the acyl chloride in 1,2-dichloroethane (50 mL) was added $AlCl_3$ (7.16 g, 53.72 mmol) all at once under N_2 at room temperature. After 5 minutes, the 1-(phenylsulfonyl)-1H-pyrrole (3.37 g, 16.28 mmol) was added all at once at room temperature and left to stir for 0.5 hours. The reaction was poured onto ice in a separatory funnel, followed by addition of 100 mL saturated $NaHCO_3$. The organic layer was separated and the aqueous layer was extracted 3 x 20 mL CH_2Cl_2 and the combined organic layer was dried over $MgSO_4$. The solvent was removed by rotary evaporation and the product was purified by column chromatography on silica gel (25% EtOAc/75% Hexanes). The product was precipitated from MeOH at 0°C to yield a white solid (3.68 g, 67% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.12 (d, J = 6.9 Hz, 3H), 2.75-2.80 (m, 1H), 2.89-2.97 (m, 2H), 4.36-4.50 (m, 2H), 6.67-6.69 (m, 1H), 7.15-7.17 (m, 1H), 7.56 (t, J = 7.5 Hz, 2H), 7.67 (t, J = 7.5 Hz, 1H), 7.75-7.77 (m, 1H), 7.928 (d, J = 7.5 Hz, 2 H).

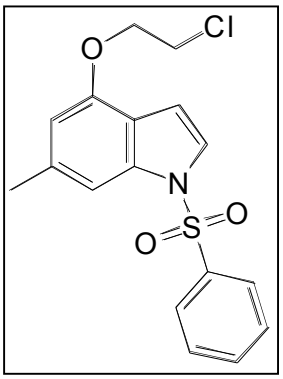


4,4-dimethoxy-3-methyl-1-(1-(phenylsulfonyl)-1H-pyrrol-3-yl)butan-1-one (4). To the nitro-compound (10.86 g, 30.77 mmol) at 0°C in MeOH (25 mL, not anhydrous) was added powdered NaOH (1.85 g, 46.15 mmol) and let stir 15 minutes. After 15 minutes, this solution was poured onto a 20% conc. H_2SO_4 in MeOH (25 mL) solution at -20°C. After 15 minutes, the entire solution was then poured onto a 1 M solution of NaOH (100 mL) in a separatory funnel. To the separatory funnel was added 300 mL EtOAc and the layers separated. The aqueous layer was extracted 3 x 25 mL EtOAc. The combined organic layer was dried over $MgSO_4$, filtered, and the solvent removed by rotary evaporation. The product was purified by column chromatography on silica gel (40% EtOAc/60% Hexanes) to yield a white solid (9.25 g, 86% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.95 (d, J = 6.9 Hz, 3H), 2.39-2.49 (m, 2H), 2.89-2.98 (m, 1H), 3.34 (s, 3H), 3.35 (s, 3H), 4.12 (d, J = 5.4 Hz, 1H), 6.69-6.70 (m, 1H), 7.13-7.15 (m, 1H), 7.54-7.58 (m, 2H), 7.64-7.66 (m, 1H), 7.76 (s, 1H), 7.92 (d, J = 7.2 Hz, 2H).

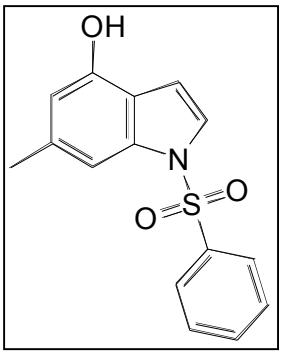


2-(6-methyl-1-(phenylsulfonyl)-1H-indol-4-yloxy)ethanol (5). To the dimethyl acetal (9.25 g, 26.32 mmol) in toluene (200 mL) was added ethylene glycol (47.41 g, 763 mmol) followed by p-toluenesulfonic acid monohydrate (1.502, 7.90 mmol). The reaction was set to reflux temperature for 5 hours under N_2 before cooling to room temperature and pouring onto 100 mL saturated $NaHCO_3$ and 100 mL EtOAc in a separatory funnel. The organic layer was washed 3 x 25 mL H_2O, dried over $MgSO_4$, filtered and the solvent removed on a rotary evaporator. Purification by column chromatography on silica gel (50% EtOAc/ 50% Hexanes) to produce a colorless oil (6.34 g, 73% yield). ^{1}H NMR

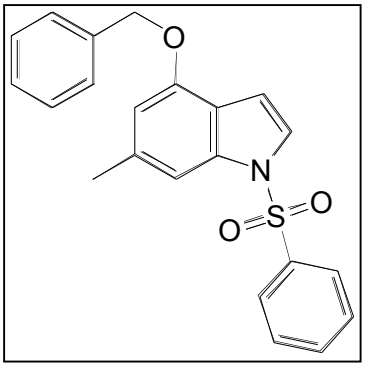
(300 MHz, $CDCl_3$) δ 2.03 (br s, 1H), 2.46 (s, 3H), 3.99 (t, J = 4.8 Hz, 2H), 4.16 (t, J = 4.8 Hz, 2H), 6.50 (s, 1H), 6.74 (d, J = 3.6 Hz, 1H), 7.41-7.46 (m, 4H), 7.54 (t, J = 7.5 Hz, 1H), 7.87 (d, J = 7.5 Hz, 2H).



4-(2-chloroethoxy)-6-methyl-1-(phenylsulfonyl)-1H-indole. To the 4-oxyethanol indole (6.34 g, 19.13 mmol) in CCl_4 (75 mL) at room temperature was added triphenylphosphine (7.54 g, 28.70 mmol) and the reaction set to reflux for 3 hours under N_2 before cooling to room temperature. The precipitate was filtered and the filtrate poured onto 100 mL H_2O and 100 mL CH_2Cl_2. The layers were separated and the aqueous layer extracted 2 x 25 mL CH_2Cl_2. The organic layer was dried over $MgSO_4$, filtered, and solvent removed by rotary evaporation. Purification by column chromatography on silica gel (25% EtOAc/75% Hexanes) yielded a white solid (5.22 g, 78% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.47 (s, 3H), 3.83 (t, J = 6 Hz, 2H), 4.28 (t, J = 6 Hz, 2H), 6.48 (s, 1H), 6.77 (d, 1H), 7.39-7.59 (m, 5H), 7.88 (J = 7.2 Hz).

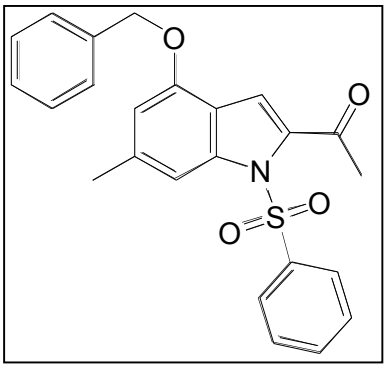


6-methyl-1-(phenylsulfonyl)-1H-indol-4-ol. To the 4-oxychloroethyl indole (5.22g, 14.91 mmol) in anhydrous THF (300 mL) at -78°C was added n-butyllithium (103.5 mL of 1.8 M in hexanes) slowly over 30 minutes. The reaction was left to stir at -78°C for 3 hours before pouring the cold solution onto ice in a separatory funnel. To the separatory funnel was added 300 mL EtOAc and the layers were separated. The aqueous layer was extracted 3 x 100 mL EtOAc, the organic layers combined, dried over $MgSO_4$, filtered, and the solvent removed by rotary evaporation. Purification by column chromatography on silica gel (30% EtOAc/70% Hexanes) provided a foamy tan oil (4.12 g, 96% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.37 (s, 3H), 6.06 (br s, 1H), 6.47 (s, 1H), 6.73 (d, J = 3.6 Hz, 1H), 7.39-7.50 (m, 4H), 7.51 (t, J = 7.5 Hz, 1H), 7.86 (d, J = 7.5 Hz, 2H).

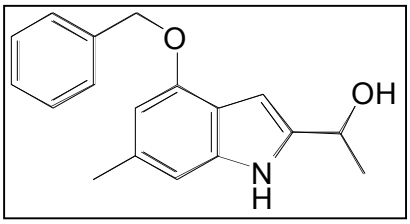


4-(benzyloxy)-6-methyl-1-(phenylsulfonyl)-1H-indole (6). To the hydroxyl indole (4.12 g, 14.34 mmol) in 1:1 anhydrous THF/DMF (50 mL) at 0°C was added 60% in NaH (0.69 g, 17.21 mmol) in mineral oil under N_2 and let stir 10 minutes. After 10 minutes, benzyl bromide (2.94 g, 17.21 mmol) was added by syringe, the cold bath removed, and the reaction left to stir under N_2 for 30 minutes. The reaction mixture was poured onto 50 mL saturated $NaHCO_3$ and 100 mL EtOAc and the organic layer was separated. The organic layer was then washed 3 x 100 mL H_2O, then the combined aqueous layer was extracted once with 20 mL EtOAc. The organic layers were combined and dried over $MgSO_4$, filtered, and the solvent removed by rotary evaporation. The tan solid was recrystallized from toluene to leave a white solid (4.25 g,

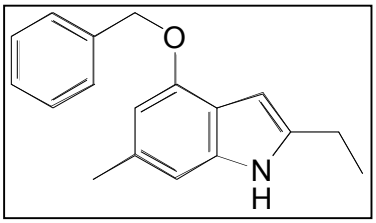
78% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.46 (s, 3H), 5.14 (s, 2H), 6.57 (s, 1H), 6.78 (d, J = 3.6 Hz, 1H), 7.36-7.47 (m, 9H), 7.50 (t, J = 7.5 Hz, 1H), 7.88 (d, J = 7.5 Hz, 2H).



1-(4-(benzyloxy)-6-methyl-1-(phenylsulfonyl)-1H-indol-2-yl)ethanone (7). To the 4-benzyloxy protected indole (1.00 g, 2.65 mmol) in anhydrous THF (25 mL) at -78^{o}C was added n-butyllithium (1.77 mL, 1.8 M in hexanes) by syringe over 5 minutes. The reaction was left to stir for 30 minutes under N_2 before being transferred by cannula onto acetic anhydride (0.351 g, 3.45 mmol) in THF (25 mL) at -78^{o}C. The reaction was then left to stir at this temperature for 30 minutes and then the cold bath removed and left to stir for 12 hours. The reaction mixture was poured onto 50 mL H_2O and 50 mL EtOAc in a separatory funnel and the organic layer separated. The aqueous layer was extracted 3 x 20 mL EtOAc and the combined organic layer was dried over $MgSO_4$, filtered, and the solvent removed on a rotary evaporator. Purification by column chromatography on silica gel (20% EtOAc/80%Hexanes) provided a white solid (0.944 g, 85% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.45 (s, 3H), 2.51 (s, 3H), 5.16 (s, 2H), 6.61 (s, 1H), 7.35-7.52 (m, 8H), 7.58 (t, J = 7.5 Hz, 1H), 7.63 (s, 1H), 8.02 (d, J = 7.5 Hz, 2H).

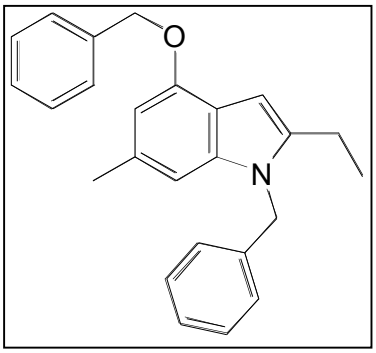


1-(4-(benzyloxy)-6-methyl-1H-indol-2-yl)ethanol (8). To the 2-acetyl indole (0.944 g, 2.25 mmol) in anhydrous THF (50 mL) was added lithium aluminum hydride (0.428 g, 11.26 mmol) and the reaction set to reflux for 24 hours under N2. After cooling to room temperature, the reaction was poured onto 100 mL ice cold saturated $NaHCO_3$ and 100 mL EtOAc in a separatory funnel. The organic layer was separated and the aqueous layer was extracted 3 x 25 mL EtOAc, dried over $MgSO_4$, filtered, and the solvent removed by rotary evaporation. The product was purified by column chromatography on silica gel (30% EtOAc/70% Hexanes) to give a white solid (0.42 g, 67% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.61 (d, J = 6.6 Hz, 3H), 2.24 (br s, 1H), 2.42 (s, 3H), 4.99-5.04 (m, 1H), 5.18 (s, 2H), 6.42 (s, 1H), 6.45 (s, 1H), 6.77 (s, 1H), 7.32-7.51 (m, 5H), 8.24 (br s, 1H).

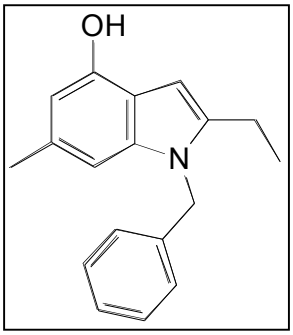


4-(benzyloxy)-2-ethyl-6-methyl-1H-indole (9). To $NaBH_4$ (0.57, 14.95 mmol) was added trifluoroacetic acid (10 mL) and the reaction left to stir 10 minutes. After all the solid dissolved, the indole alcohol (0.42 g, 1.49 mmol) was added dropwise in CH_2Cl_2 (10 mL) and the reaction left to stir under N_2 for 1 hour. The reaction was then poured onto 100 mL saturated $NaHCO_3$ and 25 mL CH_2Cl_2 and the organic layer was separated. The aqueous layer was extracted 3 x 10 mL CH_2Cl_2, dried over $MgSO_4$, filtered, and the solvent removed by rotary evaporation. Purification by column chromatography on silica gel (10% EtOAc/90% Hexanes) left a white solid (0.18 g, 45% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.29 (t, J = 7.5Hz, 3H), 2.40 (s, 3H), 2.70 (q, J =

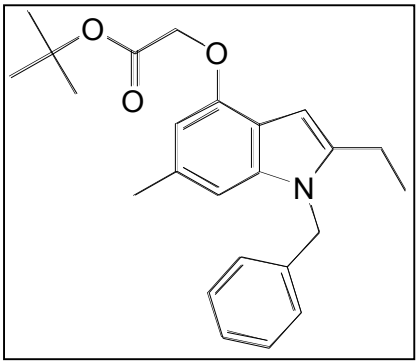
7.5 Hz, 2H), 5.18 (s, 2H), 6.33 (s, 1H), 6.41 (s, 1H), 6.70 (s, 1H), 7.28-7.51 (m, 5H), 7.67 (br s, 1H).



1-benzyl-4-(benzyloxy)-2-ethyl-6-methyl-1H-indole. To the 2-ethyl indole (0.18 g, 0.68 mmol) in anhydrous DMF (3 mL) at 0°C was added 60% NaH (32.6 mg, 0.82 mmol) in mineral oil and the reaction left to stir under N_2 for 5 minutes. After 5 minutes, benzyl bromide (0.14 g, 0.82 mmol) was added and the ice bath removed. The reaction stirred under N_2 for 2 hours before being poured onto 50 mL H_2O and 20 mL EtOAc. The layers were separated and the organic layer was washed 3 x 25 mL H_2O. The aqueous layer was extracted once with 10 mL EtOAc and the combined organic layers were dried over $MgSO_4$, filtered, and the solvent removed by rotary evaporation. Purification by column chromatography on silica gel (5%EtOAc/95% Hexanes) produced a white solid (0.195 g, 81% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.27 (t, J = 7.5Hz, 3H), 2.37 (s, 3H), 2.61 (q, J = 7.5 Hz, 2H), 5.20 (s, 2H), 6.43 (s, 1H), 6.44 (s, 1H), 6.65 (s, 1H), 6.93-7.53 (m, 10H).

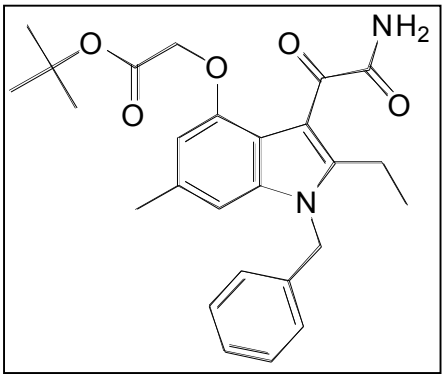


1-benzyl-2-ethyl-6-methyl-1H-indol-4-ol (10). The 4-benzyloxy indole (0.195 g, 0.55 mmol) was dissolved in MeOH (10 mL) and to it was added Pd/C (10 mol %) and hydrogenated (40 psi) using a Parr hydrogenator for 2 hours. The solvent was then removed and the residue dissolved in EtOAc and filtered through celite. Purification by column chromatography on silica gel (10% EtOAc/90% Hexanes) produced a foamy white solid (0.114 g, 78% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.29 (t, J = 7.5Hz, 3H), 2.35 (s, 3H), 2.63 (q, J = 7.5 Hz, 2H), 4.89 (br s, 1H), 5.24 (s, 2H), 6.31 (s, 1H), 6.35 (s, 1H), 6.61 (s, 1H), 6.95 (d, J = 7.2 Hz, 2H), 7.24-7.26 (m, 3H).

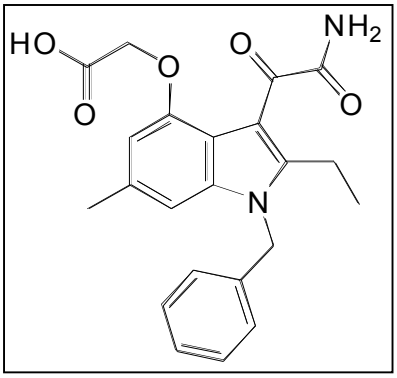


tert-butyl 2-(1-benzyl-2-ethyl-6-methyl-1H-indol-4-yloxy)acetate (11). To the 4-hydroxy indole (0.114 g, 0.43 mmol) in anhydrous DMF (1 mL) at room temperature was added 60% NaH (21 mg, 0.52 mmol) in mineral oil and let stir 5 minutes. After 5 minutes, the *tert*-butylbromoacetate (0.10 g, 0.52 mmol) was added and the reaction left to stir 30 minutes. The reaction mixture was poured onto 25 mL H_2O and 10 mL EtOAc in a separatory funnel and the layers separated. The organic layer was washed 3 x 25 mL H_2O and then the combined aqueous layer was extracted once with 10 mL EtOAc. The combined organic layer was dried over $MgSO_4$, filtered, and the solvent removed by rotary evaporation. Purification by column chromatography on silica gel (10% EtOAc/90% Hexanes) left a white solid (0.141 g,

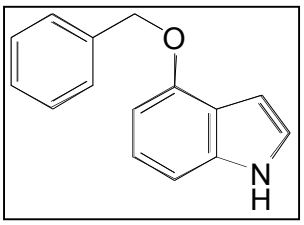
87% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.29 (t, J = 7.5Hz, 3H), 1.51 (s, 9H), 2.36 (s, 3H), 2.62 (q, J = 7.5 Hz, 2H), 4.65 (s, 2H), 5.24 (s, 2H), 6.24 (s, 1H), 6.46 (s, 1H), 6.65 (s, 1H), 6.93 (d, J = 6.3 Hz, 2H), 7.20-7.25 (m, 3H).



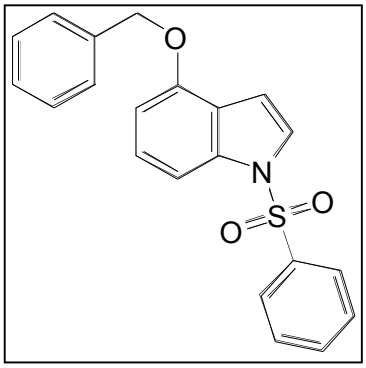
tert-butyl 2-(3-(2-amino-2-oxoacetyl)-1-benzyl-2-ethyl-6-methyl-1H-indol-4-yloxy)acetate. To oxalyl chloride (0.54 g, 0.42 mmol) in anhydrous CH_2Cl_2 (100 mL) was added the 4-oxyethanoic acid ester indole (0.141 g, 0.37 mmol) dropwise over 1 hour in CH_2Cl_2 (50 mL) at room temperature. The reaction was left to stir under N_2 for another hour before bubbling ammonia gas into the reaction for 10 minutes. The reaction was then poured onto 25 mL H_2O in a separatory funnel. The layers were separated and the aqueous layer extracted 2 x 10 mL CH_2Cl_2. The combined organic layer was dried over $MgSO_4$, filtered, and the solvent removed by rotary evaporation. A silica gel plug (60% EtOAc/40% Hexanes) afforded a yellow solid (0.187 g, 99% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ ^{1}H NMR (CD_3OD) δ 1.19 (t, J = 7.5Hz, 3H), 1.54 (s, 9H), 2.36 (s, 3H), 2.95 (q, J = 7.5 Hz, 2H), 4.75 (s, 2H), 5.46 (s, 2H), 6.42 (s, 1H), 6.83 (s, 1H), 7.09 (d, J = 7.2 Hz, 2H), 7.25-7.39 (m, 3H), 7.65 (br s, 1H).



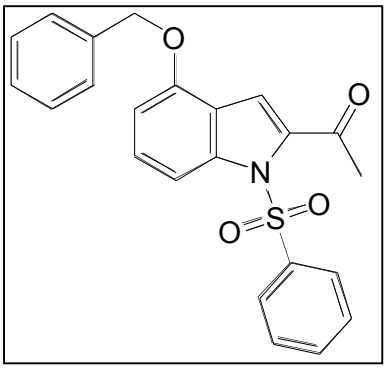
2-(3-(2-amino-2-oxoacetyl)-1-benzyl-2-ethyl-6-methyl-1H-indol-4-yloxy)acetic acid (A). To the ester protected indole (0.187 g, 0.416 mmol) in CH_2Cl_2 (10 mL) was added trifluoroacetic acid (2 mL) and the reaction left to stir and monitored by thin layer chromatography for completion. After 1 hour, the reaction solution was poured onto 20 mL saturated $NaHCO_3$ and 10 mL CH_2Cl_2 in a separatory funnel. The layers were separated and the aqueous layer extracted 2 x 10 mL CH_2Cl_2. The combined organic layer was dried over $MgSO_4$, filtered, and the solvent removed by rotary evaporation. The crude yellow solid was purified by HPLC on a C-18 reverse phase column eluting with 30% MeOH/70% H_2O (0.08% TFA in each) for 5 minutes, followed by increasing MeOH to 70% over 30 minutes. The product eluted at 18.2 minutes and after removal of solvent left a white solid (0.111 g, 68% yield). ^{1}H NMR (CD_3OD) δ 1.17 (t, J = 7.5Hz, 3H), 2.35 (s, 3H), 2.98 (q, J = 7.5 Hz, 2H), 4.76 (s, 2H), 5.46 (s, 2H), 6.45 (s, 1H), 6.80 (s, 1H), 7.05 (d, J = 7.2 Hz, 2H), 7.24-7.35 (m, 3H), 7.61 (br s, 1H).



4-(benzyloxy)-1H-indole. To 4-hydroxy indole (1.00 g, 7.52 mmol) in anhydrous acetone (50 mL) was added anhydrous K_2CO_3 (3.11 g, 22.56 mmol) and benzyl bromide (1.54 g, 9.02 mmol) and set to reflux under N_2 for 72 hours. After cooling to room temperature, the reaction mixture was poured onto 100 mL H_2O and 100 mL EtOAc in a separatory funnel and the layers separated. The aqueous layer was extracted 3 x 20 mL EtOAc and the combined organic layer was dried over $MgSO_4$, filtered, and the solvent removed by rotary evaporation. Purification by column chromatography on silica gel (20% EtOAc/80% Hexanes) afforded a pale yellow oil (1.576 g, 94% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.23 (s, 2H), 6.58 (d, J = 7.5 Hz, 1H), 6.72 (s, 1H), 7.01-7.12 (m, 3H), 7.30-7.42 (m, 3H), 7.51 (d, J = 7.2 Hz, 2H), 8.15 (br s, 1H).

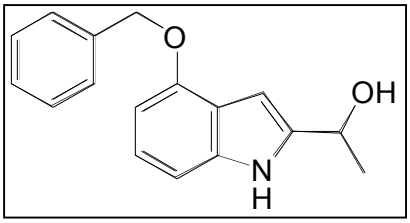


4-(benzyloxy)-1-(phenylsulfonyl)-1H-indole. To the 4-benzyloxy protected indole (1.576 g, 7.07 mmol) in anhydrous DMF (15 mL) at 0^{o}C was added 60% NaH (0.339 g, 8.48 mmol) in mineral oil and the reaction left to stir under N_2 for 5 minutes. After 5 minutes, benzenesulfonyl chloride was added by syringe and left to stir at room temperature for 30 minutes. The reaction mixture was then poured onto 100 mL H_2O and 50 mL EtOAc in a separatory funnel. The layers were separated and the organic layer was washed 5 x 50 mL H_2O. The combined aqueous layer was then extracted once with 25 mL EtOAc. The combined organic layer was then dried over $MgSO_4$, filtered, and the solvent removed on a rotary evaporator. The crude solid was purified by column chromatography on silica gel (15% EtOAc/85% Hexanes) to afford a white solid (2.05g, 80% yield). (Note: this material decomposes quickly to a pink color in air at room temperature). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.15 (s, 2H), 6.71 (d, J = 7.8 Hz, 1H), 6.83 (d, J = 3.6 Hz, 1H), 7.18-7.63 (m, 11H), 7.88 (d, J = 7.5 Hz, 2H).

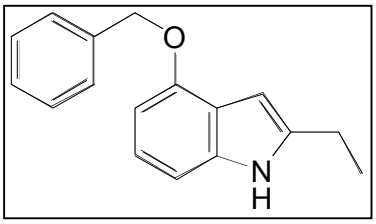


1-(4-(benzyloxy)-1-(phenylsulfonyl)-1H-indol-2-yl)ethanone. To the benzenesulfonyl protected indole (2.05 g, 5.65 mmol) in anhydrous THF (20 mL) at -78^{o}C was added n-butyllithium (3.76 mL, 1.8 M in hexanes) by syringe. The cold bath was removed and the reaction allowed to warm to room temperature over 2 hours. The reaction was then cannulated onto acetic anhydride (0.749 g, 7.34 mmol) in THF (30 mL) at -78^{o}C and the reaction let stir at this temperature for 30 minutes. The cold bath was then removed and the reaction allowed to warm to room temperature and stir for 12 hours under N_2. The reaction was then poured onto 50 mL saturated $NaHCO_3$ and 50 mL EtOAc in a separatory funnel. The layers were separated and the aqueous layer extracted 3 x 20 mL EtOAc. The combined organic layer was dried over $MgSO_4$, filtered, and the solvent removed by rotary evaporation. The crude residue was purified by column

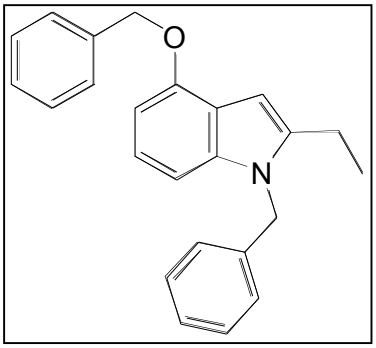
chromatography on silica gel (20% EtOAc/80% Hexanes) to afford a white solid (2.04 g, 89% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.59 (s, 3H), 5.22 (s, 2H), 6.75 (d, J = 8.1 Hz, 1H), 7.34-7.58 (m, 10H), 7.78 (d, J = 8.7 Hz, 1H), 8.01 (d, J = 7.8 Hz, 2H).



1-(4-(benzyloxy)-1H-indol-2-yl)ethanol. To the 2-acetyl indole (2.04g, 5.04 mmol) in anhydrous THF (50 mL) was added LAH (0.96 g, 25.2 mmol) and the reaction mixture set to reflux for 12 hours under N_2. After cooling to room temperature, 1 mL H_2O was added followed by 2 mL 1 M NaOH. To this mixture was then added enough $MgSO_4$ to dry the solution and the solids were filtered. The filtrate was poured onto 50 mL H_2O and 50 mL EtOAc in a separatory funnel. The layers were separated and the aqueous layer extracted 3 x 10 mL EtOAc. The combined organic layer was dried over $MgSO_4$, filtered, and the solvent removed by rotary evaporation. The crude residue was purified by column chromatography on silica gel (40% EtOAc/60% Hexanes) to afford a white solid (0.834 g, 62% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.59, (d, J = 6.6 Hz, 3H), 2.78 (br s, 1H), 4.97-5.03, (m, 1H), 5.19 (s, 2H), 6.50 (s, 1H), 6.56 (d, J = 7.8 Hz, 1H), 6.95 (d, J = 8.1 Hz, 1H), 7.05 (t, J = 7.8 Hz, 1H), 7.31-7.50 (m, 5H), 8.54 (br s, 1H).

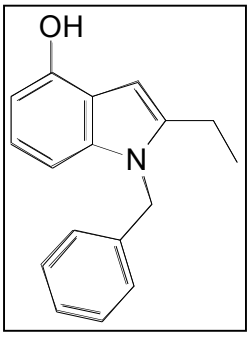


4-(benzyloxy)-2-ethyl-1H-indole. To $NaBH_4$ (0.591 g, 15.62 mmol) was added trifluoroacetic acid (20 mL) and the reaction left to stir 10 minutes until the solid dissolved. To this solution was add the indole alcohol (0.834 g, 3.12 mmol) in CH_2Cl_2 (80 mL) dropwise over 10 minutes at room temperature under N_2. The reaction was left to stir for 30 minutes before being poured onto 100 mL saturated $NaHCO_3$ in a separatory funnel. The layers were separated and the aqueous layer was extracted 2 x 20 mL CH_2Cl_2. The combined organic layer was dried over $MgSO_4$, filtered, and the solvent removed by rotary evaporation. The crude residue was purified by column chromatography on silica gel (20% EtOAc/80% Hexanes) to afford a white solid (0.603 g, 77% yield). ^{1}H NMR ($CDCl_3$) δ 1.32, (t, J = 7.5 Hz, 3H), 2.75 (q, 7.5 Hz, 2H), 5.21 (s, 2H), 6.40 (s, 1H), 6.56 (d, J = 7.8 Hz, 1H), 6.92 (d, J = 8.1 Hz, 1H), 7.01 (t, J = 7.8 Hz, 1H), 7.28-7.41 (m, 3H), 7.50 (d, J = 6.9 Hz, 2H), 7.86 (br s, 1H).

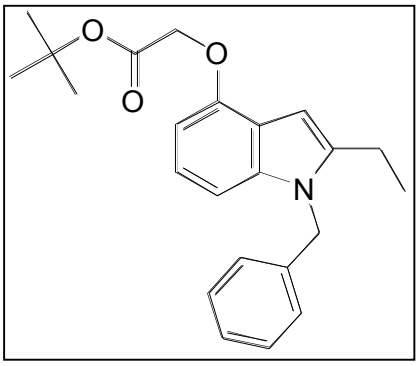


1-benzyl-4-(benzyloxy)-2-ethyl-1H-indole. To the 2-ethyl indole (0.603 g, 2.40 mmol) in anhydrous DMF (10 mL) at 0^{o}C was added 60% NaH (115 mg, 2.88 mmol) in mineral oil and the reaction left to stir under N_2 for 5 minutes. After 5 minutes, benzyl bromide (0.14 g, 0.82 mmol) was added and the ice bath removed. The reaction stirred under N_2 for 2 hours before being poured onto 50 mL H_2O and 20 mL EtOAc. The layers were separated and the organic layer was washed 3 x 30 mL H_2O. The aqueous layer was extracted once with 10 mL EtOAc and

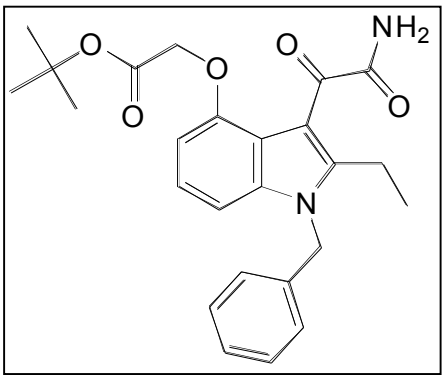
the combined organic layers were dried over $MgSO_4$, filtered, and the solvent removed by rotary evaporation. Purification by column chromatography on silica gel (5%EtOAc/95% Hexanes) produced a white solid (0.679 g, 83% yield). ^{1}H NMR ($CDCl_3$) δ 1.31, (t, J = 7.5 Hz, 3H), 2.66 (q, 7.5 Hz, 2H), 5.23 (s, 2H), 5.29 (s, 2H), 6.52 (s, 1H), 6.58 (d, J = 7.8 Hz, 1H), 6.85 (d, J = 8.1 Hz, 1H), 6.94-7.00 (m, 3H), 7.20-7.42 (m, 6H), 7.53 (d, J = 7.2 Hz, 2H).



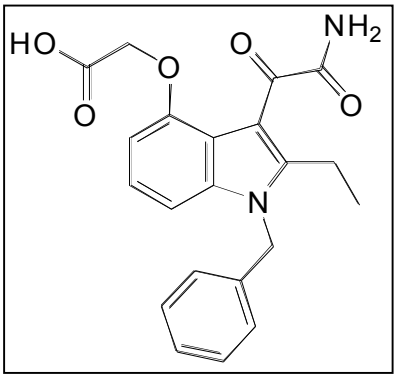
1-benzyl-2-ethyl-1H-indol-4-ol. The 4-benzyloxy indole (0.679 g, 1.99 mmol) was dissolved in MeOH (20 mL) and to it was added Pd/C (10 mol %) and hydrogenated (40 psi) using a Parr hydrogenator for 2 hours. The solvent was then removed and the residue dissolved in EtOAc and filtered through celite. Purification by column chromatography on silica gel (10% EtOAc/90% Hexanes) produced a foamy white solid (0.340 g, 68%). ^{1}H NMR ($CDCl_3$) δ 1.32, (t, J = 7.5 Hz, 3H), 2.68 (q, 7.5 Hz, 2H), 4.94 (br s, 1H), 5.29 (s, 2H), 6.39 (s, 1H), 6.51 (d, J = 7.5 Hz, 1H), 6.82 (d, J = 8.4 Hz, 1H), 6.94-6.99 (m, 3H), 7.21-7.27 (m, 3H).



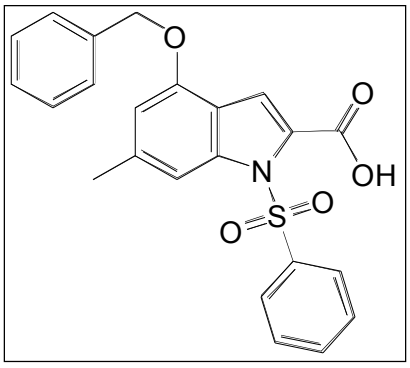
tert-butyl 2-(1-benzyl-2-ethyl-1H-indol-4-yloxy)acetate. To the 4-hydroxy indole (0.340 g, 1.35 mmol) in anhydrous DMF (5 mL) at room temperature was added 60% NaH (65 mg, 1.63 mmol) in mineral oil and let stir 5 minutes. After 5 minutes, the *tert*-butylbromoacetate (0.317 g, 1.63 mmol) was added and the reaction left to stir 30 minutes. The reaction mixture was poured onto 50 mL H_2O and 20 mL EtOAc in a separatory funnel and the layers separated. The organic layer was washed 3 x 50 mL H_2O and then the combined aqueous layer was extracted once with 20 mL EtOAc. The combined organic layer was dried over $MgSO_4$, filtered, and the solvent removed by rotary evaporation. Purification by column chromatography on silica gel (10% EtOAc/90% Hexanes) left a white solid (0.428 g, 87% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.31, (t, J = 7.5 Hz, 3H), 1.51 (s, 9H), 2.66 (q, 7.5 Hz, 2H), 4.67 (s, 2H), 5.29 (s, 2H), 6.40 (d, J = 7.8 Hz, 1H), 6.54 (s, 1H), 6.86 (d, J = 8.1 Hz, 1H), 6.90-7.11 (m, 3H), 7.20-7.26 (m, 3H).



tert-butyl 2-(3-(2-amino-2-oxoacetyl)-1-benzyl-2-ethyl-1H-indol-4-yloxy)acetate. To oxalyl chloride (0.446 g, 3.52 mmol) in anhydrous CH_2Cl_2 (200 mL) was added the 4-oxyethanoic acid ester indole (0.428 g, 1.17 mmol) dropwise over 1 hour in CH_2Cl_2 (100 mL) at room temperature. The reaction was left to stir under N_2 for another hour before bubbling ammonia gas into the reaction for 10 minutes. The reaction was then poured onto 50 mL H_2O in a separatory funnel. The layers were separated and the aqueous layer extracted 2 x 20 mL CH_2Cl_2. The combined organic layer was dried over $MgSO_4$, filtered, and the solvent removed by rotary evaporation. A silica gel plug (60% EtOAc/40% Hexanes) afforded a yellow solid (0.505 g, 99% yield). ^{1}H NMR ($CDCl_3$) δ 1.19, (t, J = 7.5 Hz, 3H), 1.47 (s, 9H), 2.94 (q, 7.5 Hz, 2H), 4.62 (s, 2H), 5.35 (s, 2H), 5.73 (br s, 1H), 6.52 (d, J = 7.8 Hz, 1H), 6.84 (d, J = 8.4 Hz, 1H), 7.01-7.09 (m, 3H), 7.26-7.30 (m, 3H).

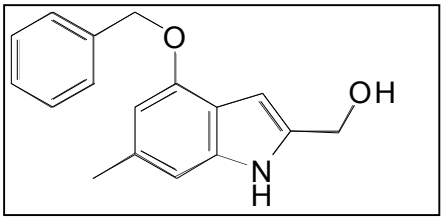


2-(3-(2-amino-2-oxoacetyl)-1-benzyl-2-ethyl-1H-indol-4-yloxy)acetic acid (B). To the ester protected indole (0.505 g, 1.16 mmol) in CH_2Cl_2 (20 mL) was added trifluoroacetic acid (5 mL) and the reaction left to stir and monitored by thin layer chromatography for completion. After 1 hour, the reaction solution was poured onto 50 mL saturated $NaHCO_3$ and 10 mL CH_2Cl_2 in a separatory funnel. The layers were separated and the aqueous layer extracted 2 x 10 mL CH_2Cl_2. The combined organic layer was dried over $MgSO_4$, filtered, and the solvent removed by rotary evaporation. The crude yellow solid was purified by HPLC on a C-18 reverse phase column eluting with 30% MeOH/70% H_2O (0.08% TFA in each) for 5 minutes, followed by increasing MeOH to 70% over 30 minutes. The product eluted at 16.1 minutes and after removal of solvent left a white solid. ^{1}H NMR ($CDCl_3$) δ 1.25, (t, J = 7.5 Hz, 3H), 2.99 (q, 7.5 Hz, 2H), 4.81 (s, 2H), 5.42 (s, 2H), 6.52 (d, J = 7.8 Hz, 1H), 6.84 (d, J = 8.4 Hz, 1H), 7.03-7.09 (m, 3H), 7.22-7.33 (m, 3H).

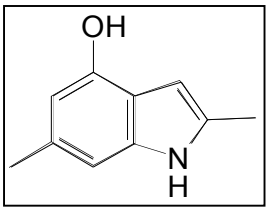


4-(benzyloxy)-6-methyl-1-(phenylsulfonyl)-1H-indole-2-carboxylic acid. To the benzenesulfonyl protected indole (0.707 g, 1.88 mmol) in anhydrous THF (25 mL) at -78^{o}C was added n-butyllithium (1.25 mL, 1.8 M in hexanes) by syringe. The cold bath was removed and the reaction allowed to warm to room temperature over 2 hours. To the cooled reaction was added powdered dry ice (~5 g) and the reaction was left to stir for 1 hour. The cold bath was then removed and the reaction allowed to warm to room temperature and

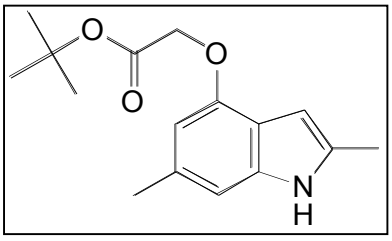
stir for 2 hours under N_2. The reaction was then poured onto 50 mL saturated $NaHCO_3$ and 50 mL EtOAc in a separatory funnel. The layers were separated and the aqueous layer extracted 3 x 20 mL EtOAc. The combined organic layer was dried over $MgSO_4$, filtered, and the solvent removed by rotary evaporation. The crude residue was purified by column chromatography on silica gel (60% EtOAc/40% Hexanes) to afford a white solid (0.35 g, 44% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.49 (s, 3H), 5.14 (s, 2H), 6.60 (s, 1H), 7.31-7.62 (m, 10H), 8.02 (d, J = 7.5 Hz, 2H), 9.51 (br s, 1H).



(4-(benzyloxy)-6-methyl-1H-indol-2-yl)methanol. To the 2-methyl alcohol indole (0.35g, 0.83 mmol) in anhydrous THF (20 mL) was added LAH (0.158 g, 4.16 mmol) and the reaction mixture set to reflux for 12 hours under N_2. After cooling to room temperature, 1 mL H_2O was added followed by 2 mL 1 M NaOH. To this mixture was then added enough $MgSO_4$ to dry the solution and the solids were filtered. The filtrate was poured onto 50 mL H_2O and 50 mL EtOAc in a separatory funnel. The layers were separated and the aqueous layer extracted 3 x 10 mL EtOAc. The combined organic layer was dried over $MgSO_4$, filtered, and the solvent removed by rotary evaporation. The crude residue was purified by column chromatography on silica gel (40% EtOAc/60% Hexanes) to afford a white solid (0.165 g, 83% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.42 (s, 3H), 4.76, (s, 2H), 5.19 (s, 2H), 6.43 (s, 1H), 6.52 (s, 1H), 6.78 (s, 1H), 7.32-7.53 (m, 5H), 8.22 (br s, 1H).

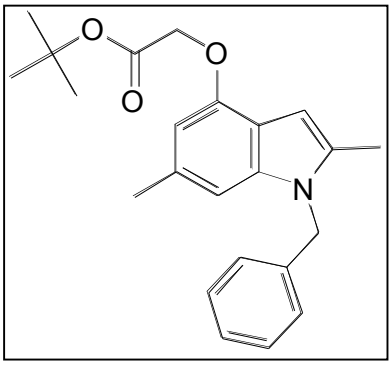


2,6-dimethyl-1H-indol-4-ol. The 4-benzyloxy-2-methyl alcohol indole (0.165 g, 0.618 mmol) was dissolved in MeOH (5 mL) and to it was added a catalytic amount of Pt black (5 mg). This mixture was hydrogenated at 40 psi H_2 gas for 5 days. The solution was then filtered through celite and purified on column chromatography on silica gel (30% EtOAc/70% Hexanes) to leave a foamy white solid (50 mg, 50% yield). The remainder of material was the deprotection of the benzyloxy with the 2-methanol intact. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.39 (s, 3H), 2.40 (s, 3H), 4.85 (br s, 1H), 6.17 (s, 1H), 6.32 (s, 1H), 6.68 (s, 1H), 7.72 (br s, 1H).

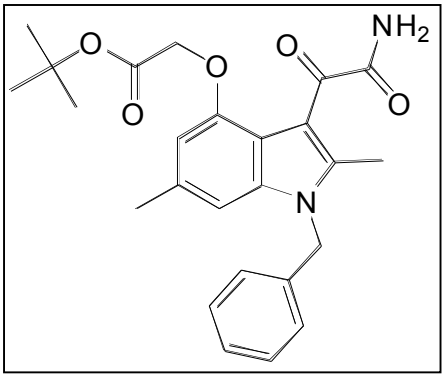


tert-butyl 2-(2,6-dimethyl-1H-indol-4-yloxy)acetate. To the 4-hydroxy indole (50 mg, 0.31 mmol) in anhydrous acetone (15 mL) was added K_2CO_3 (128 mg, 0.93 mmol) followed by *tert*-butylbromoacetate (72 mg, 0.372 mmol) and the reaction set to reflux for 12 hours. After cooling to room temperature, the reaction was poured onto 50 mL saturated H_2O and 50 mL EtOAc in a separatory funnel. The layers were separated and the aqueous layer extracted 3 x 10 mL

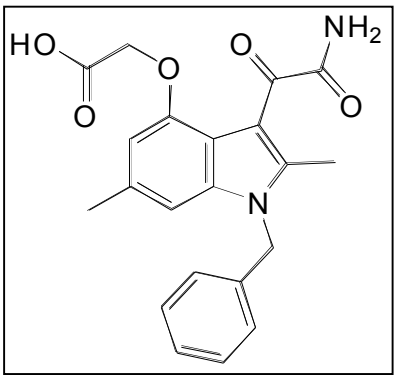
EtOAc. The combined organic layer was dried over $MgSO_4$, filtered, and the solvent removed by rotary evaporation. The crude residue was purified by column chromatography on silica gel (20% EtOAc/80% Hexanes) to afford a white solid (75 mg, 88% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.49 (s, 9H), 2.38 (s, 3H), 2.41 (s, 3H), 4.62 (s, 2H), 6.22 (s, 1H), 6.30-6.31 (m, 1H), 6.73 (s, 1H), 7.72 (br s, 1H).



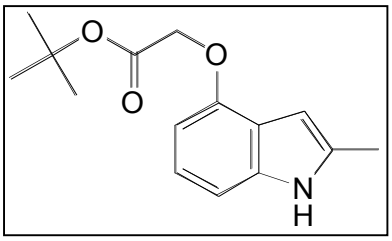
tert-butyl 2-(1-benzyl-2,6-dimethyl-1H-indol-4-yloxy)acetate. To the ester protected indole (75 mg, 0.27 mmol) in anhydrous DMF (2 mL) was added 60% NaH (13 mg, 0.324 mmol) in mineral oil and left to stir at room temperature for 5 minutes. After 5 minutes, benzyl bromide (55 mg, 0.324 mmol) was added by syringe and the reaction let stir 1 hour. The reaction mixture was poured onto 30 mL H_2O and 20 mL EtOAc in a separatory funnel and the layers separated. The organic layer was washed 3 x 30 mL H_2O and then the combined aqueous layer was extracted once with 20 mL EtOAc. The combined organic layer was dried over $MgSO_4$, filtered, and the solvent removed by rotary evaporation. Purification by column chromatography on silica gel (10% EtOAc/90% Hexanes) left a white solid (64 mg, 64% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.53 (s, 9H), 2.33 (s, 3H), 2.38 (s, 3H), 4.66 (s, 2H), 5.27 (s, 2H), 6.26 (s, 1H), 6.44-6.45 (s, 1H), 6.95-7.00 (m, 2H), 7.22-7.32 (m, 3H).



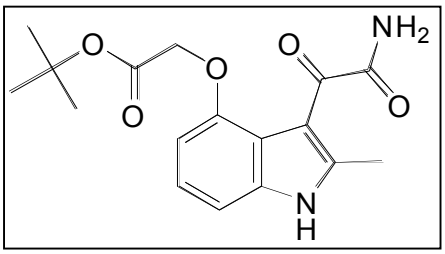
tert-butyl 2-(3-(2-amino-2-oxoacetyl)-1-benzyl-2,6-dimethyl-1H-indol-4-yloxy)acetate. To oxalyl chloride (66 mg, 0.526 mmol) in anhydrous CH_2Cl_2 (50 mL) was added the 4-oxyethanoic acid ester indole (64 mg, 0.175 mmol) dropwise over 1 hour in CH_2Cl_2 (50 mL) at room temperature. The reaction was left to stir under N_2 for another hour before bubbling ammonia gas into the reaction for 10 minutes. The reaction was then poured onto 20 mL H_2O in a separatory funnel. The layers were separated and the aqueous layer extracted 2 x 20 mL CH_2Cl_2. The combined organic layer was dried over $MgSO_4$, filtered, and the solvent removed by rotary evaporation. A silica gel plug (60% EtOAc/40% Hexanes) afforded a yellow solid (68 mg, 89% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.50 (s, 9H), 2.38 (s, 3H), 2.54 (s, 3H), 4.61 (s, 2H), 5.31 (s, 2H), 6.38 (s, 1H), 6.73 (s, 1H), 7.02-7.06 (m, 2H), 7.23-7.33 (m, 3H).



2-(3-(2-amino-2-oxoacetyl)-1-benzyl-2,6-dimethyl-1H-indol-4-yloxy)acetic acid (C). To the ester protected indole (68 mg, 0.156 mmol) in CH_2Cl_2 (4 mL) was added trifluoroacetic acid (1 mL) and the reaction left to stir and monitored by thin layer chromatography for completion. After 1 hour, the reaction solution was poured onto 20 mL saturated $NaHCO_3$ and 10 mL CH_2Cl_2 in a separatory funnel. The layers were separated and the aqueous layer extracted 2 x 10 mL CH_2Cl_2. The combined organic layer was dried over $MgSO_4$, filtered, and the solvent removed by rotary evaporation. The crude yellow solid was purified by HPLC on a C-18 reverse phase column eluting with 30% MeOH/70% H_2O (0.08% TFA in each) for 5 minutes, followed by increasing MeOH to 70% over 30 minutes. The product eluted at 18.9 minutes and after removal of solvent left a white solid. ^{1}H NMR ($CDCl_3$) δ 2.42 (s, 3H), 2.57 (s, 3H), 4.80 (s, 2H), 5.35 (s, 2H), 6.48 (s, 1H), 6.81 (s, 1H), 7.02-7.06 (m, 2H), 7.28-7.34 (m, 3H).

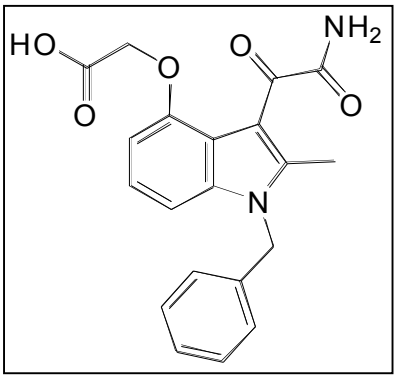


***tert*-Butyl [(2-methyl-1*H*-indol-4-yl)oxy]acetate.** The commercially available 4-hydroxy-2-methylindole (0.20 g, 1.36 mmol) and *t*-butylbromoacetate (0.32 g, 1.63 mmol) were dissolved in acetone (25 mL) and K_2CO_3 (0.56 g, 4.08 mmol) was added. The stirred mixture brought to reflux for 12 hours and the solid was removed by filtration and washed with two 20 mL portions of acetone. The solvent was removed by rotary evaporation to leave an off-white solid. This solid was dissolved in 50 mL EtOAc and washed with 50 mL of a brine solution in a separatory funnel. The aqueous phase was extracted twice with 20 mL portions of EtOAc and the organic layers combined, dried over $MgSO_4$ and evaporated to leave an off-white solid. The product was purified by flash column chromatography on silica gel using 20% EtOAc/hexanes as eluant to give a white powder (0.27g, 77% yield). This compound decomposes quickly in solution when not under argon. The product of decomposition is a bright green color. Therefore, it is imperative to work quickly, including the purification step. ^{1}H-NMR (300 MHz, $CDCl_3$, δ): 1.51 (s, 9H), 2.42 (s, 3H), 4.65 (s, 2H), 6.40-6.47 (m, 2H), 6.85 (d, 1H), 7.07 (t, 1H), 7.91 (br s, 1H).



***tert*-butyl ({3-[amino(oxo)acetyl]-2-methyl-1*H*-indol-4-yl}oxy)acetate.** To oxalyl chloride (0.39 g, 3.09 mmol) in anhydrous CH_2Cl_2 (100 mL) was added the 4-oxyethanoic acid ester indole (270 mg, 1.03 mmol) dropwise over 1 hour in CH_2Cl_2 (100 mL) at room temperature. The reaction was left to stir under N_2 for another hour before bubbling ammonia gas into the reaction for 10 minutes. The reaction was then poured onto 50 mL H_2O in a separatory funnel. The layers were separated and the aqueous layer extracted 2

x 20 mL CH_2Cl_2. The combined organic layer was dried over $MgSO_4$, filtered, and the solvent removed by rotary evaporation. A silica gel plug (60% EtOAc/40% Hexanes) afforded a yellow solid (283 mg, 83% yield). ^{1}H-NMR (200 MHz, CD_3OD, δ): 1.46 (s, 9H), 2.58 (s, 3H), 4.61 (s, 2H), 6.50 (dd, 1H), 6.98-7.12 (m, 2H).



2-(3-(2-amino-2-oxoacetyl)-1-benzyl-2-methyl-1H-indol-4-yloxy)acetic acid (D). To the ester protected indole (25 mg, 0.075 mmol) in anhydrous DMF (2 mL) was added 60% NaH (3.6 mg, 0.09 mmol) in mineral oil and left to stir at room temperature for 5 minutes. After 5 minutes, benzyl bromide (15.4 mg, 0.09 mmol) was added by syringe and the reaction let stir 1 hour. The reaction mixture was poured onto 20 mL H_2O and 20 mL EtOAc in a separatory funnel and the layers separated. The organic layer was washed 3 x 20 mL H_2O and then the combined aqueous layer was extracted once with 20 mL EtOAc. The combined organic layer was dried over $MgSO_4$, filtered, and the solvent removed by rotary evaporation. The residue was re-dissolved in CH_2Cl_2 (4 mL) and trifluoroacetic acid (1 mL) was added. The reaction was monitored by TLC for completion and after 30 minutes was poured onto 20 mL saturated $NaHCO_3$ and 10 mL CH_2Cl_2. The layers were separated and the aqueous layer was extracted 2 x 10 mL CH_2Cl_2. The combined organic layer was dried over $MgSO_4$, filtered, and the solvent removed by rotary evaporation. The crude yellow solid was purified by HPLC on a C-18 reverse phase column eluting with 30% MeOH/70% H_2O (0.08% TFA in each) for 5 minutes, followed by increasing MeOH to 70% over 30 minutes. The product eluted at 15.7 minutes and after removal of solvent left a white solid. ^{1}H NMR ($CDCl_3$) δ 2.35 (s, 3H), 4.84 (s, 2H), 5.31 (s, 2H), 6.50 (d, J = 7.8 Hz, 1H), 6.80 (d, J = 8.4 Hz, 1H), 7.01-7.10 (m, 3H), 7.22-7.30 (m, 3H).

Microtiter Plate Assay of sPLA$_2$s for Inhibition Analysis. To each well of a 96-well microtiter plate was added 100 μL of solution A in assay buffer (27 μM bovine serum albumin, 50 mM KCl, 1 mM $CaCl_2$, 50 mM Tris-HCl, pH 8.0) followed by the desired concentration of sPLA$_2$ inhibitor ($\leq$3 μL in DMSO from serial diluted stock solutions) or 3 μL of DMSO only for control reactions. Solution B was prepared immediately prior to each set of assays, which consisted of only eight wells, to avoid loss of enzymatic activity due to sticking to the walls of the container. Solution B was delivered in 100 μL portions to all eight wells except the first well and had the same composition as Solution A plus 5-2000 ng of sPLA$_2$ (depending on specific enzymatic activity).[1] In place of Solution B was added an additional 100 μL portion of Solution A as a minus enzyme control to the first of the eight wells in the assay. Quickly after the addition of Solution B, the assay was initiated by the addition of 100 μL of Solution C (4.2 μM 1-hexadecanoyl-2-(10-pyrenedecanoyl)-*sn*-glycero-3-phosphoglycerol (Molecular Probes) vesicles in assay buffer) with a repeating pipettor to all eight wells. The fluorescence (excitation = 342 nm, emission = 395 nm) was read with a microtiter plate spectrophotometer

(Fluorocount, Packard Instruments). Control reactions without enzyme or inhibitor were run with each assay of eight wells and the percent inhibition calculated from the initial slopes of fluorescence versus time. The amount of enzyme used per well are as follows: hGIB, 0.5 ng; hGIIA, 0.5 ng; hGIID, 200 ng; hGIIE, 25 ng; hGIIF, 30 ng; hGIII, 5 ng; hGV, 1 ng; hGX, 1 ng; hGXIIA, 1,500 ng; mGIB, 1.6 ng; mGIIA, 8 ng; mGIIC, 12 ng; mGIID, 2,000 ng; mGIIE, 5 ng; mGIIF, 100 ng; mGV, 6 ng; mGX, 3.5 ng. All of the recombinant mouse and human $sPLA_2$s were prepared as described.[2]

Molecular Modeling

Docking was performed using the program FLO99 (McMartin, C., Bohacek, R. S. QXP: Powerful, rapid computer algorithms for structure based drug design. *J. Comput.-Aided Mol. Des*. 1997, 11, 333-344.). Specifically, compound **A** was docked into the Me-indoxam-hGX co-structure (Smart, B. et al. *Bioorg. Med. Chem.* 12. (2004) pp.1737) by removing the o-phenyl group from the o-phenyl benzyl at the 1 position, changing the 2-methyl to 2-ethyl, and adding a methyl group at the 6-position. Energy minimization was then initialized for the docked ligand to obtain the lowest energy conformation. The enzyme was held fixed during the minimization runs. The energy minimized compound **A**-hGX enzyme structure was saved from FLO99 as a pdb file. Figure 2 was created using pymol (DeLano, W.L. The PyMOL Molecular Graphics System (2002) DeLano Scientific, San Carlos, CA, USA. http://www.pymol.org).

References

(1) Smart, B. P.; Pan, Y. H.; Weeks, A. K.; Bollinger, J. G.; Bahnson, B. J. et al. Inhibition of the complete set of mammalian secreted phospholipases A(2) by indole analogues: a structure-guided study. *Bioorg Med Chem* **2004***, 12*, 1737-1749.

(2) Singer, A. G.; Ghomashchi, F.; Le Calvez, C.; Bollinger, J.; Bezzine, S. et al. Interfacial kinetic and binding properties of the complete set of human and mouse groups I, II, V, X, and XII secreted phospholipases A2. *J Biol Chem* **2002***, 277*, 48535-48549.