Supporting Information

NOpiates^{TM}: Novel dual action neuronal nitric oxide synthase inhibitors with μ-opioid agonist activity.

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General Experimental:

All reactions were performed under an atmosphere of argon and stirred magnetically unless otherwise noted. Commercial reagents and anhydrous solvents were used as received without further purification. When necessary, Sure/Seal^{TM} anhydrous solvents were utilized. Reactions were monitored by analytical TLC using pre-coated silica gel aluminum plates (Sigma-Aldrich, 0.2 mm, 60 Å) and were visualized with UV light or stained appropriately. Flash column chromatography was performed using Silicycle Siliaflash F60 (40-63 μm) silica gel. The ^{1}H NMR spectra were performed on a Bruker 300 MHz spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) relative to $CDCl_3$ (7.26 ppm), CD_3OD (4.87 ppm) or DMSO-d_6 (2.50 ppm). Coupling constants (J-values) are given in hertz (Hz). Low and high resolution Mass Spectra were performed on an applied Biosystems/MDS Sciex QstarXL hybrid quadrupole/TOF instrument using electrospray ionization unless stated otherwise. Chemical purity was determined on an Agilent 1100 HPLC system using a Phenomenex Luna C18 reverse phase column and the purity was determined to be ≥95% unless specified otherwise. No attempts were made to optimize the yields.

Synthetic Procedures:

N'-(2,4-dinitro-phenyl)-N,N-diethyl-ethane-1,2-diamine (2):

Chloro-2,4-dinitrobenzene **1** (1.00g, 4.937 mmol) was dissolved in anhydrous EtOH (20 mL) in a small argon purged flask and warmed in an oil bath to 40 ºC. Addition of N, N-diethylethylenediamine (630 mg, 5.430 mmol) occurred dropwise. The reaction was stirred at reflux for 24 hours then cooled to room temperature. The mixture was basified by the addition of aqueous 1M ammonium hydroxide solution to adjust pH to 10-11. The solid that precipitated was

collected on a sintered glass funnel and briefly dried. Recrystallization of the crude solid from EtOH yielded a yellow powder. Yield: 940 mg (67.4%). ^{1}H NMR (DMSO-d_6) δ: 9.05 (br s, 1 H), 8.67 (d, 1H), 8.28 (dd, 1H, J = 9.5, 2.6 Hz), 7.19 (d, 1H, J = 9.7 Hz), 3.49 (q, 2H, J = 5.5 Hz), 2.70 (t, 2H, J = 6.1 Hz), 2.56 (m, 4H), 0.99 (t, 6H, J = 7.1 Hz). MS (APCI): 283 (MH^+, 100%).

N'-(2,4-dinitro-phenyl)-N,N-dimethyl-ethane-1,2-diamine (3):

Prepared as described for compound **2** using **1** and N,N-dimethylethylenediamine. Yield: 760 mg (60.4%). ^{1}H NMR (DMSO-d_6) δ: 8.94 (br s, 1 H), 8.87 (d, 1H, J = 2.7 Hz), 8.29 (dd, 1H, J = 9.6, 2.7 Hz), 7.21 (d, 1H, J = 9.6 Hz), 3.53 (q, 2H, J = 6.0 Hz), 2.57 (t, 2H, J = 6.1 Hz), 2.24 (s, 6H). MS (ESI): 255 (MH^+, 100%).

N-(2-(1-methylpyrrolidin-2-yl)ethyl)-2,4-dinitroaniline (4):

Prepared as described for compound **2** using **1** and 2-(1-methylpyrrolidin-2-yl)ethanamine. Yield: 979 mg (67.4%). ^{1}H NMR (DMSO-d_6) δ: 9.50 (br s, 1H). 8.86 (d, 1H, J = 2.8 Hz), 8.27 (dd, 1H, J = 9.7, 2.7 Hz), 7.18 (d, 1H, J = 9.6 Hz), 3.53-3.48 (m, 2H), 3.03-2.93 (m, 1H), 2.36-2.26 (m, 1H), 2.26 (s, 3H), 2.14-2.06 (m, 1H), 1.96-1.78 (2 x m, 3H), 1.71-1.51 (m, 3H). MS (ESI): 295 (MH^+, 100%).

N-(2,4-dinitrophenyl)-1-methylpiperidin-4-amine (5):

Prepared as described for compound **2** using **1** and 1-methyl-piperidin-4-ylamine. Yield: 1.50 g (54.2%). ^{1}H NMR (DMSO-d_6) δ: 8.85 (d, 1 H, J = 2.6 Hz), 8.45 (d, 1H, J = 7.7 Hz), 8.24 (dd, 1H, J = 9.7, 2.7 Hz), 7.31 (d, 1H, J = 9.7 Hz), 3.84-3.71 (m, 1H), 2.77-2.62 (m, 2H), 2.18 (s, 3H), 2.17-2.06 (m, 2H), 1.98-1.86 (m, 2H), 1.75-1.59 (m, 2H). MS (ESI): 281 (MH^+, 100%).

N^1-(2-(diethylamino)ethyl)-4-nitrobenzene-1,2-diamine (6):

N'-(2,4-dinitro-phenyl)-N,N-diethyl-ethane-1,2-diamine **2** (630 mg, 2.322 mmol) was dissolved in anhydrous EtOH (9 mL) in a 2 neck 100 mL argon purged flask. The reaction vessel was fitted with a condenser and dropping funnel and heated in an oil bath to 65 ºC. H_2O (7.5 mL), EtOH (15 mL) and aqueous $(NH_4)_2S$ (50wt%, 1.064g, 7.807 mmol) were charged to the dropping funnel and added to the hot reaction mixture dropwise over 30 minutes. The reaction was heated at 65-70 ºC for 2 hours then cooled to room temperature overnight. Mixture was acidified by the addition of aqueous 1M HCl to adjust pH to 0-1. The reaction mixture was filtered to remove any insoluble material and the filtrate was concentrated under reduced pressure to remove EtOH. The resulting aqueous solution was basified by the addition of aqueous 2M ammonium hydroxide solution to adjust pH to 9-10. The aqueous solution was diluted with dichloromethane and transferred to a separatory funnel and the organic layer collected. The aqueous layer was further extracted with dichloromethane and the combined organic layers were washed with H_2O, brine, and dried over magnesium sulphate, filtered and concentrated to afford a dark red oil. The product was purified using silica gel dry column chromatography with a solvent system of (5% 2M NH_3 in methanol/ 95% dichloromethane) to afford an orange oil which solidified upon drying. Yield: 476 mg (84.5%). ^{1}H NMR (DMSO-d_6) δ: 7.53 (dd, 1H, J = 8.9, 2.6 Hz), 7.42 (d, 1H, J = 2.6 Hz), 6.49 (d, 1H, J = 8.9 Hz), 5.82 (br s, 1H), 5.09 (br s, 2H), 3.24 (m, 2H), 2.62 (t, 2H, J = 6.8 Hz), 2.52 (m, 4H), 0.96 (t, 6H, J = 7.1 Hz). MS (ESI): 253 (MH^+, 100%).

N^1-(2-(dimethylamino)ethyl)-4-nitrobenzene-1,2-diamine (7):

Prepared from N'-(2,4-dinitro-phenyl)-N,N-dimethyl-ethane-1,2-diamine **(3)** as described for compound **6**. Yield: 465 mg (70.2%). ^{1}H NMR (DMSO-d_6) δ: 7.54 (dd, 1H, J = 8.7, 2.8 Hz),

7.41 (d, 1H, J = 2.8 Hz), 6.49 (d, 1H, J = 8.8 Hz), 5.79 (br s, 1H), 5.14 (br s, 2H), 3.26 (q, 2H, J = 6.0 Hz), 2.47 (m, 2H), 2.19 (s, 6H). MS (ESI): 225 (MH^+, 100%).

N^1-(2-(1-methylpyrrolidin-2-yl)ethyl)-4-nitrobenzene-1,2-diamine (8):

Prepared from N-(2-(1-methylpyrrolidin-2-yl)ethyl)-2,4-dinitroaniline **(4)** as described for compound **6**. Yield: 396 mg (45.1%). ^{1}H NMR (DMSO-d_6) δ: 7.52 (dd, 1H, J = 8.9, 2.5 Hz), 7.40 (d, 1H, J = 2.5 Hz), 6.46 (d, 1H, J = 8.8 Hz), 5.98-5.92 (m, 1H), 5.11 (br s, 2H), 3.24-3.16 (m, 2H), 2.98-2.91 (m, 1H), 2.22 (s, 3H), 2.16-2.10 (m, 1H), 2.10-2.01 (m, 1H), 1.98-1.86 (m, 2H), 1.69-1.39 (m, 5H). MS (ESI): 265 (MH^+, 100%).

N^1-(1-methylpiperidin-4-yl)-4-nitrobenzene-1,2-diamine (9):

Prepared from N-(2,4-dinitrophenyl)-1-methylpiperidin-4-amine **(5)** as described for compound **6**. Yield: 342 mg (25.5%). ^{1}H NMR (DMSO-d_6) δ: 7.49 (dd, 1H, J = 8.9, 2.7 Hz), 7.39 (d, 1H, J = 2.9 Hz), 6.53 (d, 1H, J = 9.0 Hz), 5.63 (br d, 1H, J = 7.4 Hz), 5.20 (br s, 2H), 3.42-3.30 (m, 1H), 2.81-2.70 (m, 2H), 2.17 (s, 3H), 2.05-1.97 (m, 2H), 1.93-1.89 (m, 2H), 1.55-1.42 (m, 2H). MS (ESI): 251 (MH^+, 100%).

N-(2-(2-(diethylamino)ethylamino)-5-nitrophenyl)-2-(4-ethoxyphenyl)acetamide (10):

N^1-(2-(diethylamino)ethyl)-4-nitrobenzene-1,2-diamine **6** (470 mg, 1.863 mmol) was dissolved in anhydrous dichloromethane (10 mL) in a small, argon purged flask fitted with an condenser and magnetic stirbar. 2-(4-ethoxyphenyl)acetic acid (352 mg, 1.956mmol) followed by 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (0.552g, 2.235 mmol) are added quickly as solids and the resulting solution heated in an oil bath at 35 ºC for 18 hours. After cooling to room temperature

the solvent was removed under reduced pressure and the resulting residue partitioned between H_2O and chloroform and 3M ammonium hydroxide solution added to adjust pH to 11-12. The mixture was transferred to a separatory funnel and the organic layer collected. The aqueous layer was further extracted with chloroform and the combined organic layers were washed with brine, dried over magnesium sulphate, filtered and concentrated to afford crude solid. Recrystallization of the crude solid from ethyl acetate yielded a yellow powder. Yield: 305 mg (39.5%). ^{1}H NMR (DMSO-d_6) δ: 9.54 (br s, 1H), 7.97 (m, 2H), 7.24 (d, 2H, J = 8.4 Hz), 6.87 (d, 2H, J = 8.4 Hz), 6.75 (d, 1H, J = 9.9 Hz), 6.21 (br s, 1H), 4.02 (q, 2H, J = 6.9 Hz), 3.55 (s, 2H), 3.26 (m, 2H), 2.59 (t, 2H, J = 6.5 Hz), 2.50 (m, 4H), 1.31 (t, 3H, J = 6.9 Hz), 0.94 (t, 6H, J = 7.1 Hz). MS (ESI): 415 (MH^+, 100%)

N-(2-(2-(dimethylamino)ethylamino)-5-nitrophenyl)-2-(4-ethoxyphenyl)acetamide (11):

Prepared from N^1-(2-(dimethylamino)ethyl)-4-nitrobenzene-1,2-diamine **7** as described for compound **10** except using anhydrous tetrahydrofuran as solvent. Yield: 280 mg (35.2%). ^{1}H NMR (DMSO-d_6) δ: 9.55 (br s, 1H); 8.01 (m, 1H), 7.97 (d, 1H, J = 9.1 Hz), 7.25 (d, 2H, J = 8.5 Hz), 6.87 (d, 2H, J = 8.5 Hz), 6.76 (d, 1H, J = 9.1 Hz), 6.21 (br s, 1H), 4.00 (q, 2H, J = 6.9 Hz), 3.61 (s, 2H), 3.26 (m, 2H), 2.50 (m, 2H), 2.20 (s, 6H), 1.31 (t, 3H, J = 6.9 Hz). MS (ESI): 387 (MH^+, 45%), 369 (MH^+ - H_2O, 100%).

2-(4-ethoxyphenyl)-N-(2-(2-(1-methylpyrrolidin-2-yl)ethylamino)-5-nitrophenyl)acetamide (12):

Prepared from N^1-(2-(1-methylpyrrolidin-2-yl)ethyl)-4-nitrobenzene-1,2-diamine **8** as described for compound **10**. Yield: 115 mg (18.0%). ^{1}H NMR (DMSO-d_6) δ: 9.43 (br s, 1H),

7.99 (d, 1H, J = 2.5 Hz), 7.94 (dd, 1H, J = 9.1, 2.6 Hz), 7.24 (d, 2H, J = 8.6 Hz), 6.88 (d, 2H, J = 8.4 Hz), 6.70 (d, 1H, J = 9.1 Hz), 6.57 (br, 1H), 4.00 (q, 2H, J = 6.9 Hz), 3.61 (s, 2H), 3.30-3.19 (m, 2H), 2.99-2.89 (m, 1H), 2.22 (s, 3H), 2.19-2.12 (m, 1H), 2.10-2.01 (m, 1H), 1.91-1.78 (m, 2H), 1.68-1.43 (2 x m, 4-5H), 1.31 (t, 3H, J = 7.0 Hz). MS (ESI): 427 (MH^+, 100%).

2-(4-ethoxyphenyl)-N-(2-(1-methylpiperidin-4-ylamino)-5-nitrophenyl)acetamide (13):

Prepared from N^1-(1-methylpiperidin-4-yl)-4-nitrobenzene-1,2-diamine **9** as described for compound **10**. Yield: 200 mg (36.8 %). ^{1}H NMR (DMSO-d_6) δ: 9.47 (br s, 1H), 8.10 (d, 1H, J = 2.3 Hz), 7.92 (dd, 1H, J = 9.3, 2.3 Hz), 7.26 (d, 2H, J = 8.3 Hz), 6.89 (d, 2H, J = 8.4 Hz), 6.81 (d, 1H, J = 9.3 Hz), 5.89 (d, 1H, J = 7.5 Hz), 4.00 (q, 2H, J = 7.0 Hz), 3.61 (s, 2H), 3.45-3.37 (m, 1H), 2.73-2.69 (m, 2H), 2.17 (s, 3H), 2.07-1.99 (m, 2H), 1.89-1.86 (m, 2H), 1.56-1.41 (m, 2H), 1.31 (t, 3H, J = 6.9 Hz). MS (ESI): 413 (MH^+, 100%).

2-(2-(4-ethoxybenzyl)-5-nitro-1H-benzo[d]imidazol-1-yl)-N,N-diethylethanamine (14):

N-(2-(2-(diethylamino)ethylamino)-5-nitrophenyl)-2-(4-ethoxyphenyl)acetamide **10** (295 mg, 0.712 mmol), Phosphorous pentachloride (148.3 mg, 0.712 mmol) were dissolved in anhydrous chloroform (10 mL) in a small, argon purged flask fitted with a condenser and magnetic stirbar. The solution was heated to reflux in an oil bath for 4 hours and cooled to room temperature overnight. The mixture was diluted with H_2O and chloroform and 2M ammonium hydroxide solution added to adjust pH to 9-10. The mixture was transferred to a separatory funnel and the organic layer collected. The aqueous layer was further extracted with chloroform and the combined organic layers were washed with brine, dried over magnesium sulphate, filtered and concentrated to afford crude. The product was purified using dry silica gel column

chromatography eluting with 25 mL portions of solvent system (2.5% 2M NH_3 in methanol/ 97.5% dichloromethane) to afford a yellow residue. Recrystallization from diethyl ether/hexanes at 0 ºC yielded a pale yellow solid. Yield: 177 mg (62.7%). ^{1}H NMR (DMSO-d_6) δ: 8.45 (d, 1H, J = 2.1 Hz), 8.14 (dd, 2H, J = 8.9, 2.1 Hz), 7.71 (d, 1H, J = 8.9 Hz), 7.19 (d, 2H, J = 8.4 Hz), 6.87 (d, 2H, J = 8.4 Hz), 4.32 (s, 2H), 4.25 (m, 2H), 3.98 (q, 2H, J = 6.9 Hz), 2.50 (m, 2H), 2.37 (q, 4H, J = 7.1 Hz), 1.29 (t, 3H, J = 7.0 Hz), 0.71 (t, 6H, J = 7.0 Hz). MS (ESI): 397 (MH^+, 100%).

2-(2-(4-ethoxybenzyl)-5-nitro-1H-benzo[d]imidazol-1-yl)-N,N-dimethylethanamine (15): Prepared from N-(2-(2-(dimethylamino)ethylamino)-5-nitrophenyl)-2-(4-ethoxyphenyl)acet -amide **11** as described for compound **14**. Yield: 112 mg (58.7%). ^{1}H NMR (DMSO-d_6) δ: 8.46 (d, 1H, J = 2.0 Hz), 8.14 (dd, 1H, J = 8.8, 2.1 Hz), 7.72 (d, 1H, J = 8.9 Hz), 7.20 (d, 2H, J = 8.5 Hz), 6.88 (d, 2H, J = 8.7 Hz), 4.31 (m, 2H + s, 2H), 3.97 (q, 2H, J = 6.9 Hz), 2.34 (m, 2H), 2.12 (s, 6H), 1.29 (t, 3H, J = 7.0 Hz). MS (ESI): 369 (MH^+, 100%).

2-(4-ethoxybenzyl)-1-(2-(1-methylpyrrolidin-2-yl)ethyl)-5-nitro-1H-benzo[d]imidazole (16): Prepared from 2-(4-ethoxyphenyl)-N-(2-(2-(1-methylpyrrolidin-2-yl)ethylamino)-5-nitrophenyl) acetamide **12** as described for compound **14**. Yield: 22 mg (38.3%). ^{1}H NMR (DMSO-d_6) δ: 8.47 (d, 1H, J = 2.1 Hz), 8.14 (dd, 1H, J = 8.9, 2.1 Hz), 7.70 (d, 1H, J = 9.0 Hz), 7.19 (d, 2H, J = 8.5 Hz), 6.87 (d, 2H, J = 8.5 Hz), 4.32 (s, 2H), 4.21 (t, 2H, J = 7.9 Hz), 3.97 (q, 2H, J = 7.0 Hz), 2.95-2.85 (m, 1H), 2.07 (s, 3H), 2.05-1.96 (m, 2H), 1.87-1.75 (m, 1H), 1.70-1.51 (m, 3H), 1.51-1.38 (m, 2H), 1.29 (t, 3H, J = 7.0 Hz). MS (ESI): 409 (MH^+, 100%). ESI-HRMS calculated for $C_{23}H_{29}N_4O_3$ (MH^+): 409.2234, Observed: 409.2235.

2-(4-ethoxybenzyl)-1-(1-methylpiperidin-4-yl)-5-nitro-1H-benzo[d]imidazole (17):

Prepared from 2-(4-ethoxyphenyl)-N-(2-(1-methylpiperidin-4-ylamino)-5-nitrophenyl)acetamide **13** as described for compound **14**. Yield: 127 mg (70.0%). ^{1}H NMR (DMSO-d_6) δ: 8.48 (d, 1H, J = 2.3 Hz), 8.07 (dd, 1H, J = 9.0, 2.4 Hz), 7.82 (d, 1H, J = 9.0 Hz), 7.17 (d, 2H, J = 8.4 Hz), 6.87 (d, 2H, J = 8.7 Hz), 4.37 (s, 2H), 4.37-4.29 (m, 1H), 3.96 (q, 2H, J = 7.0 Hz), 2.82 (br d, 2H, J = 11.2 Hz), 2.31-2.20 (m, 2H), 2.19 (s, 3H), 1.97-1.89 (m, 2H), 1.39 (br d, 2H, J = 9.8 Hz), 1.29 (t, 3H, J = 7.0 Hz). MS (ESI): 395 (MH^+, 100%).

N-(1-(2-(diethylamino)ethyl)-2-(4-ethoxybenzyl)-1H-benzo[d]imidazol-5-yl)thiophene-2-carboximidamide (24):

2-(2-(4-ethoxybenzyl)-5-nitro-1H-benzo[d]imidazol-1-yl)-N,N-diethylethanamine **14** (150 mg, 0.378 mmol) was dissolved in anhydrous ethanol (10 mL) in a dry argon purged flask. Palladium, 10 wt% on activated carbon (40.3 mg, 0.0378 mmol) is quickly added and the atmosphere from the flask evacuated by vacuum pump and replaced with hydrogen from a balloon. The atmosphere is evacuated from the flask and replaced with hydrogen twice more and the mixture stirred under a hydrogen atmosphere at room temperature. After 3 hours, thin layer chromatography in a solvent system of (5% 2M NH_3 in methanol/95% dichloromethane) shows complete consumption of starting material. The mixture is filtered through a pad of celite to remove insolubles, the pad washed with anhydrous ethanol (10 mL) and the ethanolic solution is charged to a small, argon purged flask fitted with a magnetic stirbar. Methyl thiophene-2-carbimidothioate hydroiodide[1] **18** (140 mg, 0.491 mmol) is added to the flask and the reaction was stirred under Argon at ambient temperature for 67 hours. The solution was diluted with

diethyl ether (80 mL) and cooled in and ice bath resulting in the formation of an off-white precipitate that was collected on a sintered glass funnel and washed with ether. The hygroscopic solid was solubilized on the funnel in methanol and the solvent collected and evaporated to yield crude solid. The solid was partitioned between H_2O and ethyl acetate and 1M sodium hydroxide solution added to adjust pH to 8. The mixture was transferred to a separatory funnel and the organic layer collected. The aqueous layer was further extracted with ethyl acetate and the combined organic layers were washed with brine (twice), dried over magnesium sulphate, filtered and concentrated to afford yellow solid. Yield: 94 mg (52.2 %). ^{1}H NMR (DMSO-d_6) δ: 7.72 (d, 1H, J = 3.4 Hz), 7.59 (d, 1H, J = 5.1 Hz), 7.35 (d, 1H, J = 8.4 Hz), 7.17 (d, 2H, J = 8.4 Hz), 7.11-7.08 (m, 1H), 6.99 (s, 1H), 6.88 (d, 2H, J = 8.7 Hz), 6.72 (d, 1H, J = 7.7 Hz), 6.31 (br s, 2H), 4.22 (s, 2H), 4.13-4.07 (m, 2H), 3.98 (q, 2H, J = 7.0 Hz), 2.50-2.45 (m, 2H), 2.45 (q, 4H, J = 7.0 Hz), 1.29 (t, 3H, J = 7.0 Hz), 0.82 (t, 6H, J = 7.1 Hz). MS (ESI): 476 (MH^+, 100%). ESI-HRMS calculated for $C_{27}H_{34}N_5OS$ (MH^+): 476.2478, Observed: 476.2475.

N-(1-(2-(dimethylamino)ethyl)-2-(4-ethoxybenzyl)-1H-benzo[d]imidazol-5-yl)thiophene-2-carboximidamide (25):

Prepared from 2-(2-(4-ethoxybenzyl)-5-nitro-1H-benzo[d]imidazol-1-yl)-N,N-dimethylethan-amine **15** as described for compound **24**. Yield: 67 mg (61.3 %). ^{1}H NMR (DMSO-d_6) δ: 8.17 (d, 1H, J = 4.7 Hz), 8.11 (d, 1H, J = 2.8 Hz), 7.81-7.70 (2 x m, 1H + 1H), 7.42-7.36 (m, 1H), 7.31 (d, 1H, J = 8.7 Hz), 7.24 (d, 2H, J = 8.5 Hz), 6.90 (d, 2H, J = 8.5 Hz), 4.65-4.48 (m, 2H), 4.31 (s, 2H), 4.00 (q, 2H, J = 7.0 Hz), 2.84 (s, 6H), 1.31 (t, 3H, J = 7.0 Hz). MS (ESI): 448 (MH^+, 100%). ESI-HRMS calculated for $C_{25}H_{30}N_5OS$ (MH^+): 448.2165, Observed: 448.2166.

N-(2-(4-ethoxybenzyl)-1-(2-(1-methylpyrrolidin-2-yl)ethyl)-1H-benzo[d]imidazol-5-yl)thiophene-2-carboximidamide (26):

Prepared from 2-(4-ethoxybenzyl)-1-(2-(1-methylpyrrolidin-2-yl)ethyl)-5-nitro-1H-benzo[d]-imidazole **16** as described for compound **24** except purified using silica gel dry column chromatography with a solvent system of (2.5% 2M NH_3 in methanol/ 97.5% dichloromethane). Yield: 48 mg (35.9%). ^{1}H NMR (DMSO-d_6) δ: 7.73-7.74 (m, 1H), 7.59 (d, 1H, J = 5.4 Hz), 7.33 (d, 1H, J = 8.4 Hz), 7.19 (d, 2H, J = 8.6 Hz), 7.09 (dd, 1H, J = 5.2, 3.8 Hz), 7.00 (d, 1H, J = 1.6 Hz), 6.87 (d, 2H, J = 8.8 Hz), 6.73 (dd, 1H, J = 8.7, 1.6 Hz), 6.33 (br s, 2H), 4.20 (s, 2H), 4.06 (t, 2H, J = 7.7 Hz), 3.98 (q, 2H, J = 6.9 Hz), 2.95-2.84 (m, 1H), 2.08 (s, 3H), 2.05-1.95 (m, 2H), 1.90-1.77 (m, 1H), 1.69-1.54 (m, 3H), 1.49-1.35 (m, 2H), 1.29 (t, 3H, J = 6.9 Hz). MS (ESI): 488 (MH^+, 60%), 244 (100%).

N-(2-(4-ethoxybenzyl)-1-(1-methylpiperidin-4-yl)-1H-benzo[d]imidazol-5-yl)thiophene-2-carboximidamide (27):

Prepared from 2-(4-ethoxybenzyl)-1-(1-methylpiperidin-4-yl)-5-nitro-1H-benzo[d]imidazole **17** as described for compound **24** except purified using silica gel dry column chromatography with a solvent system of (5% 2M NH_3 in methanol/ 95% dichloromethane). Yield: 80 mg (55.5%). ^{1}H NMR (DMSO-d_6) δ: 7.75-7.73 (m, 1H), 7.59 (d, 1H, J = 5.3 Hz) 7.46 (d, 1H, J = 8.5 Hz), 7.17 (d, 2H, J = 8.5 Hz), 7.11-7.08 (m, 1H), 7.00 (d, 1H, J = 1.5 Hz), 6.85 (d, 2H, J = 8.7 Hz), 6.69 (dd, 1H, J = 8.7, 1.6 Hz), 6.36 (br s, 2H), 4.32-4.16 (s, 2H, + m, 1H), 3.96 (q, 2H, J = 7.0 Hz), 2.81 (m, 2H), 2.35-2.19 (m, 2H), 2.19 (s, 3H), 1.91 (br t, 2H, J = 11.0 Hz), 1.42-1.31 (m, 2H), 1.28 (t, 3H, J = 6.9 Hz). MS (ESI): 474 (MH^+, 80%), 377 (100%). ESI-HRMS calculated for $C_{27}H_{32}N_5OS$ (MH^+): 474.2322, Observed: 474.2312.

N-(1-(2-(diethylamino)ethyl)-2-(4-ethoxybenzyl)-1H-benzo[d]imidazol-5-yl)furan-2-carboximidamide (28):

Prepared from 2-(2-(4-ethoxybenzyl)-5-nitro-1H-benzo[d]imidazol-1-yl)-N,N-diethylethanamine **14** (108 mg, 0.273 mmol) and benzyl furan-2-carbimidothioate hydrobromide[2] **19** as described for compound **24** except purified using silica gel dry column chromatography with a solvent system of (5% 2M NH_3 in methanol/ 95% dichloromethane). Yield: 125mg (100%). ^{1}H NMR (DMSO-d_6) δ: 7.78 (s, 1H), 7.34 (d, 1H, J = 8.6 Hz), 7.17 (d, 2H, J = 8.6 Hz), 7.10 (d, 1H, J = 3.1 Hz), 6.99 (br s, 1H), 6.86 (d, 2H, J = 8.5 Hz), 6.72 (d, 1H, J = 7.9 Hz), 6.63-6.58 (m, 1H), 6.10 (br s, 2H), 4.21 (s, 2H), 4.09 (t, 2H, J = 6.8 Hz), 3.97 (q, 2H, J = 6.9 Hz), 2.50-2.39 (2 x m, 6H), 1.27 (t, 3H, J = 6.9 Hz), 0.83 (t, 6H, J = 7.1 Hz). MS (ESI): 460 (MH^+, 100%). ESI-HRMS calculated for $C_{27}H_{34}N_5O_2$ (MH^+): 460.2707, Observed: 460.2711.

N-(1-(2-(diethylamino)ethyl)-2-(4-ethoxybenzyl)-1H-benzo[d]imidazol-5-yl)thiophene-3-carboximidamide (29):

Prepared from 2-(2-(4-ethoxybenzyl)-5-nitro-1H-benzo[d]imidazol-1-yl)-N,N-diethylethanamine **14** (108 mg, 0.273 mmol) and benzyl thiophene-3-carbimidothioate hydrobromide **20** as described for compound **24** except purified using silica gel dry column chromatography with a solvent system of (5% 2M NH_3 in methanol/ 95% dichloromethane). Yield: 40 mg (30.8%). ^{1}H NMR (DMSO-d_6) δ: 8.13 (d, 1H, J = 3.0 Hz), 7.66-7.61 (m, 1H), 7.55-7.53 (m, 1H), 7.34 (d, 1H, J = 8.4 Hz), 7.19 (d, 2H, J = 8.6 Hz), 6.97 (d, 1H, J = 1.8 Hz), 6.86 (d, 2H, J = 8.4 Hz), 6.73 (d, 1H, J = 8.3 Hz), 6.12 (br, 2H), 4.22 (s, 2H), 4.10 (t, 2H, J = 6.5 Hz), 3.97 (q, 2H, J = 7.0 Hz), 2.50-2.38 (2 x m, 6H), 1.30 (t, 3H, J = 6.9 Hz), 0.83 (t, 6H, J = 7.0 Hz). MS (ESI): 476 (MH^+, 25%), 377 (100%).

N-(1-(2-(diethylamino)ethyl)-2-(4-ethoxybenzyl)-1H-benzo[d]imidazol-5-yl)furan-3-carboximidamide (30):

Prepared from 2-(2-(4-ethoxybenzyl)-5-nitro-1H-benzo[d]imidazol-1-yl)-N,N-diethylethanamine **14** (108 mg, 0.273 mmol) and benzyl furan-3-carbimidothioate hydrobromide **21** as described for compound **24** except purified using silica gel dry column chromatography with a solvent system of (5% 2M NH_3 in methanol/ 95% dichloromethane). Yield: 62.8 mg (50.0%). ^{1}H NMR (DMSO-d_6) δ: 8.23 (s, 1H), 7.73-7.68 (m, 1H), 7.33 (d, 1H, J = 8.5 Hz), 7.18 (d, 2H, J = 8.5 Hz), 6.96 (br, 1H), 6.92-6.88 (m, 1H), 6.86 (d, 2H, J = 8.7 Hz), 6.71 (d, 1H, J = 9.6 Hz), 5.98-6.09 (br, 1-2H), 4.21 (s, 2H), 4.10 (t, 2H, J = 6.8 Hz), 3.99 (q, 2H, J = 6.9 Hz), 2.50-2.37 (2 x m, 6H), 1.29 (t, 3H, J = 7.0 Hz), 0.83 (t, 6H, J = 7.0 Hz). MS (ESI): 460 (MH^+, 95%). ESI-HRMS calculated for $C_{27}H_{34}N_5O_2$ (MH^+): 460.2707, Observed: 460.2709.

N-(1-(2-(diethylamino)ethyl)-2-(4-ethoxybenzyl)-1H-benzo[d]imidazol-5-yl)acetimidamide (31):

Prepared from 2-(2-(4-ethoxybenzyl)-5-nitro-1H-benzo[d]imidazol-1-yl)-N,N-diethylethanamine **14** (100 mg, 0.252 mmol) and naphthalen-2-ylmethyl ethanimidothioate hydrobromide[3] **22** as described for compound **24** except purified using silica gel dry column chromatography with a solvent system of (10% 2M NH_3 in methanol/ 90% dichloromethane). Yield: 65 mg (63.2%). ^{1}H NMR (MeOD-d_4) δ: 8.30 (d, 1H, J = 8.8 Hz), 7.81 (d, 1H, J = 1.7 Hz), 7.61 (dd, 1H, J = 8.8, 1.8 Hz), 7.40 (d, 2H, J = 8.6 Hz), 7.01 (d, 2H, J = 8.6 Hz), 5.13-5.03 (m, 2H), 4.73 (s, 2H), 4.06 (q, 2H, J = 7.0 Hz), 3.57-3.49 (m, 2H), 3.40-3.30 (m, 4H), 2.47 (s, 3H), 1.40 (t, 3H, J = 5.4 Hz), 1.38 (t, 6H, J = 7.1 Hz). MS (ESI): 408 (MH^+, 100%).

1-(1-(2-(diethylamino)ethyl)-2-(4-ethoxybenzyl)-1H-benzo[d]imidazol-5-yl)-3-nitro-guanidine (32):

Prepared from 2-(2-(4-ethoxybenzyl)-5-nitro-1H-benzo[d]imidazol-1-yl)-N,N-diethylethanamine **14** (120 mg, 0.303 mmol) and 1-methyl-3-nitro-1-nitrosoguanidine[4] **23** as described for compound **24** except mixture heated at reflux after addition of **23** and purified using silica gel dry column chromatography with a solvent system of (5% 2M NH_3 in methanol/ 95% dichloromethane). Yield: 35 mg (31%). ^{1}H NMR (DMSO-d_6) δ: 9.89-9.60 (br, 1H), 8.30-7.94 (br, 2H), 7.52-7.44 (m, 2H), 7.15 (d, 2H, J = 8.4 Hz), 7.06 (dd, 1H, J = 8.8, 1.7 Hz), 6.85 (d, 2H, J = 8.6 Hz), 4.26 (s, 2H), 4.13 (t, 2H, J = 6.1 Hz), 3.98 (q, 2H, J = 6.9 Hz), 2.50-2.40 (2 x m, 6H), 1.29 (t, 3H, J = 7.0 Hz), 0.79 (t, 6H, J = 7.0 Hz). MS (ESI): 454 (MH^+, 100%).

N-(1-(2-(diethylamino)ethyl)-2-(4-ethoxybenzyl)-1H-benzo[d]imidazol-6-yl)thiophene-2-carboximidamide (34):

Prepared from 2-(2-(4-ethoxybenzyl)-6-nitro-1H-benzo[d]imidazol-1-yl)-N,N-diethylethanamine **33** (90 mg, 0.227 mmol) and **18** as described for compound **24** except purified using silica gel column chromatography eluting with a solvent system of (5% methanol/ 95% dichloromethane). Yield: 38 mg (35.2 %). ^{1}H NMR (DMSO-d_6) δ: 7.73 (d, 1H, J = 3.6 Hz), 7.60 (d, 1H, J = 5.2 Hz), 7.46 (d, 1H, J = 8.5 Hz), 7.17 (d, 2H, J = 8.7 Hz), 7.11-7.08 (m, 1H), 6.88 (d, 2H, J = 8.6 Hz), 6.88-6.85 (m, 1H), 6.70-6.67 (m, 1H), 6.42 (br s, 2H), 4.21 (s, 2H), 4.07 (t, 2H, J = 6.7 Hz), 3.97 (q, 2H, J = 7.0 Hz), 2.50-2.35 (2 x m, 6H), 1.28 (t, 3H, J = 7.0 Hz), 0.81 (t, 6H, J = 7.0 Hz). MS (ESI): 476 (MH^+, 100%).

General procedure for the conversion of the free base to the dihydrochloride salt:

To a solution of the free base (1.0 equiv) in dichloromethane or methanol was added 1 M HCl in diethyl ether (3.0 equiv). The solution was stirred at room temperature for ~10 minutes then concentrated to dryness. The residue was dried under reduced pressure to obtain the dihydrochloride salt as a solid.

HPLC Purity:

Compound	Retention time (min.)	Purity
16		NA
24	8.45	99.1 %
25	7.97	95.1 %
26	7.52	97.1 %
27	8.07	97.5 %
28	8.19	97.4%
29	7.80	94.6 %
30	9.60	96.6 %
31	7.30	94.3 %
32	7.80	97.7 %
34	7.79	93.6 %

HPLC Method:

Solvent A: Acetonitrile:TFA (100:0.1)
Solvent B: Water:TFA (100:0.1)
Column: Phenomenex Luna C18, 2.5µM, 3.0 x 100 mm
Flow rate: 0.5 mL/min.
Concentration: 1 mg/mL in MeOH
Injection volume: 5µL
Wavelength: 254 nM

Time, min	Solvent A %	Solvent B %
0	0	100
5-7	30	70
7.5-10	50	50
12	30	70
14-15	0	100

Biological Protocols:

NOS inhibition assay:

Recombinant human nNOS, eNOS and iNOS were produced in Baculovirus-infected Sf9 cells (ALEXIS). In a radiometric method, NOS activity is determined by measuring the conversion of [^{3}H]L-arginine to [^{3}H]L-citrulline. To measure nNOS or eNOS, 10 μL of enzyme is added to 100 μL of 40 mM HEPES, pH = 7.4, containing 2.4 mM $CaCl_2$, 1mM $MgCl_2$, 1mg/mL BSA, 1mM EDTA, 1 mM dithiothreitol, 1μM FMN, 1μM FAD, 10 μM tetrahydrobiopterin, 1mM NADPH, and 1.2 μM CaM. To measure iNOS, 10 μL of enzyme is added to 100 μL of 100 mM HEPES, pH = 7,4, containing 1mM $CaCl_2$, 1mM EDTA, 1mM dithiothreitol, 1 μM FMN, 1 μM FAD, 10 μM tetrahydrobiopterin, 120 μM NADPH, and 100 nM CaM.

To measure enzyme inhibition, a 15 μL solution of a test substance is added to the enzyme assay solution, followed by a pre-incubation time of 15 min at RT. The reaction is initiated by addition of 20 μL L-arginine containing 0.25 μCi of [^{3}H] arginine/mL and 24 μM L-arginine. The total volume of the reaction mixture is 150 μL in every well. The reactions are carried out at 37 °C for 45 min. The reaction is stopped by adding 20 μL of ice-cold buffer containing 100 mM HEPES, 3 mM EGTA, 3 mM EDTA, pH = 5.5. [^{3}H]L-citrulline is separated by DOWEX (ion-exchange resin DOWEX 50 W X 8-400, SIGMA) and the DOWEX is removed by spinning at 12,000 *g* for 10 min in the centrifuge. A 70 μL aliquot of the supernatant is added to 100 μL of scintillation fluid and the samples are counted in a liquid scintillation counter (1450 Microbeta Jet, Wallac). Specific NOS activity is reported as the difference between the activity

recovered from the test solution and that observed in a control sample containing 240 mM of the inhibitor L-NMMA. All assays are performed at least in duplicate.

μ opioid binding assay:

The human μ opioid[5,6] binding assay was performed in transfected HEK-293 cells. Briefly, cell membrane homogenates (60 μg protein) are incubated for 120 min at 22 °C with 0.5 nM [^{3}H]DAMGO in the absence or presence of the test compound in a buffer containing 50 mM Tris-HCl (pH 7.4) and 5 mM $MgCl_2$. Nonspecific binding is determined in the presence of 10 μM naloxone. Following incubation, the samples are filtered rapidly under vacuum through glass fiber filters (GF/B, Packard) presoaked with 0.3% PEI and rinsed several times with ice-cold 50 mM Tris-HCl using a 96-sample cell harvester (Unifilter, Packard). The filters are dried then counted for radioactivity in a scintillation counter (Topcount, Packard) using a scintillation cocktail (Microscint 0, Packard). The results are expressed as a percent inhibition of the control radioligand specific binding.

μ opioid cellular functional assay (agonist effect):

Evaluation of the agonist activity of the compounds was performed using the human μ opioid[5,6] receptor expressed in transfected CHO cells, by measuring their effects on cAMP modulation using the HTRF detection method. Briefly, cells are suspended in HBSS buffer (Invitrogen) complemented with 20 mM HEPES (pH 7.4) and 500 μM IBMX, then distributed in microplates at a density of 7.10^3 cells/well in the presence of either of the following: HBSS (basal control), the reference agonist at 300 nM (stimulated control) or various concentrations (EC_{50} determination), of the test compounds. Thereafter, the adenylyl cyclase activator forskolin

(final concentration, 20 μM) or NKH 477 (a water soluble analog of forskolin, final concentration, 1 μM) is added. Following 10 min incubation at 37 °C, the cells are lysed and the fluorescence acceptor (D2-labeled cAMP) and fluorescence donor (anti-cAMP antibody labeled with europium cryptate) are added. After 60 min at room temperature, the fluorescence transfer is measured at λex=337 nm and λem=620 and 665 nm using a microplate reader (Rubystar, BMG). The cAMP concentration is determined by dividing the signal measured at 665 nm by that measured at 620 nm (ratio). The results are expressed as a percent of the control response to 300 nM DAMGO.

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