Electronic Supplementary Information for

Development of subnanomolar-affinity serotonin 5-HT_4 receptor ligands based on quinoline structure

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Experimental details

Chemistry

All chemicals used were of reagent grade. Yields refer to purified products and are not optimized. Melting points were determined in open capillaries on a Gallenkamp apparatus and are uncorrected. Merck silica gel 60 (230-400 mesh) was used for column chromatography. Merck TLC plates, silica gel 60 F_{254} were used for TLC. ^{1}H NMR spectra were recorded by means of a Bruker AC 200 or a Bruker DRX 400 AVANCE spectrometers in the indicated solvents (TMS as internal standard); the values of the chemical shifts are expressed in ppm and the coupling constants (*J*) in Hz. Mass spectra were recorded on either a ThermoFinnigan LCQ-Deca or an Agilent 1100 LC/MSD. The purity of ligands **7c-k** and **9a-e** was assessed by RP-HPLC and was found to be higher than 95%. An Agilent 1100 Series system equipped with a Zorbax Eclipse XDB-C8 (4.6 x 150 mm, 5 μm) column was used in the HPLC analysis with the suitable mobile phase at a flow rate of 0.5 mL/min. UV detection was achieved at 254 nm.

General procedure for the synthesis of compounds 7h-k (Method A).

A mixture of the appropriate quinoline-4-carboxylic acid (**10a-d**, 1 equivalent) and CDI (1 equivalent) in dry DMF (5.0 mL) was stirred at 40 °C for 1 h. A solution of (1-butyl-4-piperidinyl)methanol (1.5 equivalent) and DBU (1 equivalent) in dry DMF (3.0 mL) was then added. The reaction mixture was stirred at 50 °C for 1 h and then concentrated under reduced pressure. The residue was diluted with dichloromethane and washed with water. The organic layer was dried over sodium sulfate and evaporated under reduced pressure. Purification of the residue by flash chromatography with ethyl acetate-triethylamine (9:1) as the eluent gave pure target compounds **7h-k**.

(1-Butylpiperidin-4-yl)methyl 2-methoxyquinoline-4-carboxylate (7h).

This compound was prepared from **10a** (50 mg, 0.25 mmol), CDI (40 mg, 0.25 mmol), (1-butyl-4-piperidinyl)methanol (63 mg, 0.37 mmol), and DBU (55 μL, 0.37 mmol) to obtain **7h** as an off-white

solid (68 mg, yield 76%, mp 68-70 °C). ^{1}H NMR (400 MHz, $CDCl_3$): 0.90 (t, *J* = 7.3, 3H), 1.29 (m, 2H), 1.44 (m, 4H), 1.79 (m, 3H), 1.91 (t, *J* = 11.0, 2H), 2.30 (t, *J* = 7.8, 2H), 2.95 (d, *J* = 11.6, 2H), 4.07 (s, 3H), 4.25 (d, *J* = 6.2, 2H), 7.40 (s, 1H), 7.43 (t, *J* = 7.7, 1H), 7.63 (t, *J* = 8.2, 1H), 7.87 (d, *J* = 8.4, 1H), 8.58 (d, *J* = 8.4, 1H). MS (ESI) *m*/*z*: $[M + H]^+$ Calcd for $C_{21}H_{29}N_2O_3$ 357.2; Found 356.9. HPLC: acetonitrile-(H_2O + 1% acetic acid)-methanol (6:2:2), retention time 3.2 min, purity 99%.

(1-Butylpiperidin-4-yl)methyl 2-chloroquinoline-4-carboxylate (7i).

This compound was prepared from **10b** (0.10 g, 0.48 mmol), CDI (0.078 g, 0.48 mmol), (1-butyl-4-piperidinyl)methanol (0.124 g, 0.72 mmol), and DBU (0.11 mL, 0.72 mmol) to obtain **7i** as a brown oil (0.113 g, yield 65%). ^{1}H NMR (400 MHz, $CDCl_3$): 0.88 (t, *J* = 7.3, 3H), 1.29 (m, 2H), 1.43 (m, 4H), 1.78 (m, 3H), 1.92 (t, *J* = 11.9, 2H), 2.28 (t, *J* = 7.8, 2H), 2.95 (d, *J* = 11.6, 2H), 4.27 (d, *J* = 6.2, 2H), 7.61 (t, *J* = 7.7, 1H), 7.74 (t, *J* = 7.7, 1H), 7.82 (s, 1H), 8.02 (d, *J* = 8.4, 1H), 8.67 (d, *J* = 8.5, 1H). MS (ESI) *m*/*z*: $[M + H]^+$ Calcd for $C_{20}H_{26}ClN_2O_2$ 361.2; Found 360.9. HPLC: acetonitrile-(H_2O + 1% acetic acid)-methanol (4:3:3), retention time 3.2 min, purity 97%.

(1-Butylpiperidin-4-yl)methyl 2-cyclopropylquinoline-4-carboxylate (7j).

This compound was prepared from **10c** (50 mg, 0.23 mmol), CDI (37 mg, 0.23 mmol), (1-butyl-4-piperidinyl)methanol (60 mg, 0.35 mmol), and DBU (52 μL, 0.35 mmol) to obtain **7j** as a yellow oil (64 mg, yield 76%). ^{1}H NMR (400 MHz, $CDCl_3$): 0.88 (t, *J* = 7.3, 3H), 1.10 (m, 2H), 1.20 (m, 2H), 1.29 (m, 2H), 1.44 (m, 4H), 1.79 (m, 3H), 1.93 (t, *J* = 11.6, 2H), 2.25 (m, 1H), 2.30 (t, *J* = 7.8, 2H), 2.95 (d, *J* = 11.2, 2H), 4.27 (d, *J* = 6.3, 2H), 7.49 (t, *J* = 7.7, 1H), 7.64 (t, *J* = 7.6, 1H), 7.69 (s, 1H), 7.97 (d, *J* = 8.4, 1H), 8.60 (d, *J* = 8.6, 1H). MS (ESI) *m*/*z*: $[M + H]^+$ Calcd for $C_{23}H_{31}N_2O_2$ 367.2; Found 367.0. HPLC: acetonitrile-(H_2O + 1% acetic acid)-methanol (4:3:3), retention time 3.3 min, purity 99%.

(1-Butylpiperidin-4-yl)methyl quinoline-4-carboxylate (7k).

This compound was prepared from **10d** (0.20 g, 1.15 mmol), CDI (0.19 g, 1.15 mmol), (1-butyl-4-piperidinyl)methanol (0.30 g, 1.73 mmol), and DBU (0.26 mL, 1.73 mmol) to obtain **7k** as a yellow oil (0.25 g, yield 67%). ^{1}H NMR (400 MHz, $CDCl_3$): 0.89 (t, *J* = 7.3, 3H), 1.30 (m, 2H), 1.45 (m, 4H), 1.83 (m, 3H), 1.93 (t, *J* = 11.7, 2H), 2.30 (t, *J* = 7.8, 2H), 2.95 (d, *J* = 11.6, 2H), 4.28 (d, *J* = 6.2, 2H), 7.63 (t, *J* = 8.4, 1H), 7.74 (t, *J* = 8.4, 1H), 7.87 (d, *J* = 4.4, 1H), 8.14 (d, *J* = 8.4, 1H), 8.74 (d, *J* = 8.5, 1H), 8.97 (d, *J* = 4.4, 1H). MS (ESI) *m*/*z*: $[M + H]^+$ Calcd for $C_{20}H_{27}N_2O_2$ 327.2; Found 326.9. HPLC: acetonitrile-(H_2O + 1% acetic acid)-methanol (4:3:3), retention time 2.9 min, purity 99%.

***N*-[(1-Butylpiperidin-4-yl)methyl]-2-methoxyquinoline-4-carboxamide (7g) (Method B).**

A mixture of acid **10a** (0.25 g, 1.2 mmol), BOP[1] (0.54 g, 1.3 mmol), TEA (0.50 mL, 3.7 mmol) and (1-butyl-4-piperidinyl)methanamine (0.31 g, 1.84 mmol) in dry DMF (10 mL) was stirred at room temperature for 2 h. The solvent was evaporated under reduced pressure and the residue was diluted with CH_2Cl_2 and washed with water. The organic layer was dried over sodium sulfate and concentrated under reduced pressure. Purification of the residue by flash chromatography with ethyl acetate-triethylamine (9:1) gave amide **7g** as a white solid (0.24 g, yield 55%). An analytical sample was obtained by recrystallization from ethyl acetate by slow evaporation (mp 142-143 °C). ^{1}H NMR (200 MHz, $CDCl_3$): 0.89 (t, *J* = 7.0, 3H), 1.23-1.73 (m, 9H), 1.87 (t, *J* = 11.0, 2H), 2.32 (t, *J* = 7.6, 2H), 2.96 (d, *J* = 11.3, 2H), 3.41 (t, *J* = 6.0, 2H), 4.05 (s, 3H), 6.08 (br t, 1H), 6.92 (s 1H), 7.39 (t, *J* = 7.9, 1H), 7.62 (t, *J* = 7.2, 1H), 7.84 (d, *J* = 8.3, 1H), 8.02 (d, *J* = 8.2, 1H). MS (ESI) *m*/*z*: $[M + H]^+$ Calcd for $C_{21}H_{30}N_3O_2$ 356.2; Found 356.0. HPLC: acetonitrile-(H_2O + 1% acetic acid)-methanol (6:2:2), retention time 3.0 min, purity 99%.

2-Methoxy-*N*-[2-(piperidin-1-yl)ethyl]quinoline-4-carboxamide (7c) (Method C).

A mixture of acid **10a** (0.14 g, 0.69 mmol), EDC hydrochloride (0.27 g, 1.38 mmol), and DMAP (4.2 mg, 0.034 mmol) in dry CH_2Cl_2 (15 mL) was stirred at room temperature for 15 min. 1-(2-aminoethyl)piperidine (0.15 mL, 1.05 mmol) was then added and the reaction mixture was stirred at

room temperature overnight. The solvent was evaporated under reduced pressure and the residue was purified by flash chromatography with ethyl acetate-triethylamine (9:1) as the eluent to afford amide **7c** as a white solid (0.050 g, yield 23%). An analytical sample was obtained by recrystallization from *n*-hexane-diethyl ether by slow evaporation (mp 121-122 °C). ^{1}H NMR (200 MHz, $CDCl_3$): 1.50 (m, 6H), 2.41 (m, 4H), 2.54 (t, *J* = 6.0, 2H), 3.59 (q, *J* = 5.9, 2H), 4.07 (s, 3H), 6.67 (br t, 1H), 6.97 (s, 1H), 7.40 (t, *J* = 7.1, 1H), 7.64 (t, *J* = 7.2, 1H), 7.86 (d, *J* = 8.7, 1H), 8.12 (d, *J* = 7.8, 1H). MS (ESI) *m*/*z*: $[M + H]^+$ Calcd for $C_{18}H_{24}N_3O_2$ 314.2; Found 314.1. HPLC: acetonitrile-(H_2O + 1% acetic acid)-methanol (6:2:2), retention time 3.0 min, purity 99%.

2-Bromoethyl 2-methoxyquinoline-4-carboxylate (11).

A mixture of acid **10a** (0.50 g, 2.46 mmol) in dry THF (15 mL) containing 1,2-dibromoethane (2.1 mL, 24.6 mmol) was heated under reflux for 10 min, and DBU (0.36 mL, 2.46 mmol) was then added dropwise. The reaction mixture was refluxed for 5 h and then concentrated under reduced pressure. The residue was partitioned between dichloromethane and water. The organic layer was dried over sodium sulfate and evaporated under reduced pressure. Purification of the residue by flash chromatography with dichloromethane as the eluent gave pure compound **11** (0.48 g, yield 63%) as a pale yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$): 3.65 (t, *J* = 5.9, 2H), 4.07 (s, 3H), 4.68 (t, *J* = 5.9, 2H), 7.43 (m, 2H), 7.64 (t, *J* = 7.3, 1H), 7.88 (d, *J* = 8.4, 1H), 8.58 (d, *J* = 8.2, 1H). MS (ESI) *m*/*z*: $[M + H]^+$ Calcd for $C_{13}H_{13}BrNO_3$ 310.0; Found 309.9.

General procedure for the synthesis of compounds 7d-f.

A mixture of bromide **11** in dry CH_3CN containing DIPEA and the suitable piperidine derivative was heated under reflux for the appropriate time (the reaction was monitored by TLC). The reaction mixture was concentrated under reduced pressure; the residue was diluted with CH_2Cl_2 and washed with brine. The organic layer was dried over sodium sulfate and concentrated under reduced pressure. Purification

of the residue by flash chromatography with the appropriate eluent gave the corresponding ester compounds **7d-f**.

2-(Piperidin-1-yl)ethyl 2-methoxyquinoline-4-carboxylate (7d).

This compound was prepared from bromide **11** (0.15 g, 0.48 mmol), DIPEA (0.14 mL, 0.82 mmol), and piperidine (71 μL, 0.72 mmol, reaction time 5 h) and purified by flash chromatography with ethyl acetate as the eluent to give ester **7d** as a yellow oil which crystallized on standing (85 mg, yield 56%). ^{1}H NMR (200 MHz, $CDCl_3$): 1.23-1.65 (m, 6H), 2.51 (m, 4H), 2.76 (t, J = 5.9, 2H), 4.08 (s, 3H), 4.53 (t, J = 6.0, 2H), 7.41 (m, 2H), 7.64 (m, 1H), 7.87 (d, J = 7.9, 1H), 8.60 (d, J = 8.8, 1H). MS (ESI) m/z: $[M + H]^+$ Calcd for $C_{18}H_{23}N_2O_3$ 315.2; Found 315.1. HPLC: acetonitrile-(H_2O + 1% acetic acid)-methanol (5:3:2), retention time 4.6 min, purity 97%.

2-[4-(Hydroxymethyl)piperidin-1-yl]ethyl 2-methoxyquinoline-4-carboxylate (7e).

This compound was prepared from bromide **11** (0.15 g, 0.48 mmol), DIPEA (0.14 mL, 0.82 mmol) and piperidin-4-yl-methanol (83 mg, 0.72 mmol, reaction time 6 h) and purified by flash chromatography with ethyl acetate-triethylamine (8:2) as the eluent to give ester **7e** as a white solid (0.13 g, yield 79%). An analytical sample was obtained by recrystallization from *n*-hexane-diethyl ether by slow evaporation (mp 98-99 °C). ^{1}H NMR (400 MHz, $CDCl_3$): 1.31 (m, 2H), 1.50 (m, 1H), 1.75 (d, J = 12.9, 2H), 1.80 (br s, 1H), 2.13 (t, J = 11.7, 2H), 2.80 (t, J = 5.9, 2H), 3.03 (d, J = 11.4, 2H), 3.49 (d, J = 6.4, 2H), 4.08 (s, 3H), 4.54 (t, J = 5.8, 2H), 7.39 (s, 1H), 7.44 (t, J = 7.7, 1H), 7.64 (t, J = 7.6, 1H), 7.88 (d, J = 8.4, 1H), 8.60 (d, J = 8.5, 1H). MS (ESI) m/z: $[M + H]^+$ Calcd for $C_{19}H_{25}N_2O_4$ 345.2; Found 345.1. HPLC: acetonitrile-(H_2O + 1% acetic acid)-methanol (5:3:2), retention time 3.1 min, purity 97%.

2-[4-(*Tert*-butoxycarbonylamino)piperidin-1-yl]ethyl 2-methoxyquinoline-4-carboxylate (7f).

This compound was prepared from bromide **11** (0.98 g, 3.2 mmol), DIPEA (0.90 mL, 5.2 mmol), and 4-(*N*-Boc-amino)piperidine (0.49 g, 2.45 mmol, reaction time 24 h) and purified by flash chromatography with ethyl acetate as the eluent to give **7f** as a white solid (0.82 g, yield 78%, mp 113-114 °C). ^{1}H NMR (400 MHz, $CDCl_3$): 1.44-1.50 (m, 11H), 1.94 (d, J = 12.0, 2H), 2.24 (t, J = 10.1, 2H), 2.82 (t, J = 5.9, 2H), 2.94 (d, J = 11.4, 2H), 3.48 (m, 1H), 4.09 (s, 3H), 4.43 (br s, 1H), 4.54 (t, J = 5.9, 2H), 7.41 (s, 1H), 7.46 (t, J = 7.9, 1H), 7.68 (t, J = 7.6, 1H), 7.92 (d, J = 8.5, 1H), 8.62 (d, J = 8.5, 1H). MS (ESI) *m*/*z*: $[M + H]^+$ Calcd for $C_{23}H_{32}N_3O_5$ 430.2; Found 430.1. HPLC: acetonitrile-(H_2O + 1% acetic acid)-methanol (5:3:2), retention time 7.8 min, purity 98%.

General procedure for the synthesis of compounds 9a-c and 13.

A mixture of compound **12**[2] in $SOCl_2$ (3.0 mL) containing a catalytic amount of DMF (5 drops) was heated under reflux for 3 h. The excess of $SOCl_2$ was then evaporated under reduced pressure and the yellow residue was dissolved in CH_2Cl_2 (6.0 mL). To this solution, the suitable amine was added and the resulting mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with dichloromethane and washed with water. The organic layer was dried over sodium sulfate and evaporated under reduced pressure. Purification of the residue by flash chromatography with the appropriate eluent gave pure compounds **9a-c** and *N*-Boc derivative **13**.

4-Chloro-*N*-[3-(piperidin-1-yl)propyl]quinoline-2-carboxamide (9a).

This compound was prepared from **12**[2] (0.10 g, 0.48 mmol) and 1-(3-aminopropyl)piperidine (0.16 mL, 1.0 mmol) and purified by flash chromatography with ethyl acetate-triethylamine (9:1) as the eluent to obtain **9a** as a white solid (0.13 g, yield 82%). An analytical sample was obtained by recrystallization from n-hexane-diethyl ether by slow evaporation (colorless prisms, mp 68-69 °C). ^{1}H NMR (400 MHz, $CDCl_3$): 1.50 (m, 2H), 1.67 (m, 4H), 1.85 (m, 2H), 2.37-2.53 (m, 6H), 3.62 (q, J = 6.2, 2H), 7.68 (t, J = 7.1, 1H), 7.80 (t, J = 7.4, 1H), 8.10 (d, J = 7.8, 1H), 8.27 (d, J = 7.5, 1H), 8.38 (s, 1H), 9.05 (br s, 1H).

MS (ESI) *m*/*z*: $[M + H]^+$ Calcd for $C_{18}H_{23}ClN_3O$ 332.2; Found 332.1. HPLC: acetonitrile-(H_2O + 1% acetic acid)-methanol (4:3:3), retention time 3.3 min, purity 99%.

4-Chloro-*N*-[2-(piperidin-1-yl)ethyl]quinoline-2-carboxamide (9b).

This compound was prepared from **12**[2] (0.10 g, 0.48 mmol) and 1-(2-aminoethyl)piperidine (0.15 mL, 1.05 mmol) and purified by flash chromatography with ethyl acetate-triethylamine (9:1) as the eluent to obtain **9b** as a yellow glassy solid (0.067 g, yield 44%). ^{1}H NMR (400 MHz, $CDCl_3$): 1.46 (m, 2H), 1.63 (m, 4H), 2.48 (m, 4H), 2.61 (t, *J* = 6.4, 2H), 3.61 (q, *J* = 6.1, 2H), 7.68 (t, *J* = 7.1, 1H), 7.78 (t, *J* = 7.4, 1H), 8.12 (d, *J* = 8.0, 1H), 8.22 (d, *J* = 7.6, 1H), 8.35 (s, 1H), 8.54 (br s, 1H). MS (ESI) *m*/*z*: $[M + H]^+$ Calcd for $C_{17}H_{21}ClN_3O$ 318.1; Found 318.1. HPLC: acetonitrile-(H_2O + 1% acetic acid)-methanol (4:3:3), retention time 3.3 min, purity 96%.

***N*-[(1-Butylpiperidin-4-yl)methyl]-4-chloroquinoline-2-carboxamide (9c).**

This compound was prepared from **12**[2] (0.20 g, 0.96 mmol) and (1-butyl-4-piperidinyl)methanamine (0.31 g, 1.84 mmol) and purified by flash chromatography with ethyl acetate-triethylamine (9:1) as the eluent to obtain **9c** as a white solid (0.29 g, yield 84%, mp 84-85 °C). ^{1}H NMR (400 MHz, $CDCl_3$): 0.88 (t, *J* = 7.2, 3H) 1.31 (m, 2H), 1.43 (m, 4H), 1.62-1.93 (m, 5H), 2.28 (t, *J* = 7.8, 2H), 2.94 (d, *J* = 11.5, 2H), 3.42 (t, *J* = 6.3, 2H), 7.70 (t, *J* = 7.8, 1H), 7.79 (t, *J* = 7.8, 1H), 8.10 (d, *J* = 8.1, 1H), 8.26 (m, 2H), 8.37 (s, 1H). MS (ESI) *m*/*z*: $[M + H]^+$ Calcd for $C_{20}H_{27}ClN_3O$ 360.2; Found 359.9. HPLC: acetonitrile-(H_2O + 1% acetic acid)-methanol (4:3:3), retention time 3.4 min, purity 99%.

***tert*-Butyl 4-[(4-chloroquinoline-2-carboxamido)methyl]piperidine-1-carboxylate (13).**

This compound was prepared from **12**[2] (0.58 g, 2.8 mmol) and 1-Boc-4-(aminomethyl)piperidine (1.2 g, 5.6 mmol) and purified by flash chromatography with ethyl acetate as the eluent to obtain **13** as a pale yellow oil (0.80 g, yield 71%). ^{1}H NMR (200 MHz, $CDCl_3$): 1.10-1.34 (m, 2H), 1.44 (s, 9H), 1.52-1.95 (m, 3H), 2.70 (t, *J* = 12.2, 2H), 3.43 (t, *J* = 6.5, 2H), 4.13 (d, *J* = 11.4, 2H), 7.67-7.85 (m, 2H), 8.11 (d, *J*

= 8.1, 1H), 8.29 (m, 2H), 8.37 (s, 1 H). MS (ESI) *m*/*z*: $[M + Na]^+$ Calcd for $C_{21}H_{26}ClN_3NaO_3$ 426.2; Found 426.0.

***tert*-Butyl 4-[(4-methoxyquinoline-2-carboxamido)methyl]piperidine-1-carboxylate (14).**

To a mixture of compound **13** (0.50 g, 1.24 mmol) in dry methanol (10 mL), NaH (0.11 g, 4.6 mmol) was cautiously added and the resulting mixture was refluxed for 24 h. The reaction mixture was then concentrated under reduced pressure and the residue was diluted with dichloromethane and washed with water. The organic layer was dried over sodium sulfate and evaporated under reduced pressure. Purification of the residue by flash chromatography with petroleum ether-ethyl acetate (6:4) as the eluent gave compound **14** as a colorless oil (0.43 g, yield 87%). ^{1}H NMR (200 MHz, $CDCl_3$): 1.06-1.30 (m, 2H), 1.38 (s, 9H), 1.67-1.96 (m, 3H), 2.62 (t, *J* = 12.2, 2H), 3.35 (t, *J* = 6.3, 2H), 4.02 (m, 5H), 7.47 (t, *J* = 7.3, 1H), 7.64 (m, 2H), 7.94 (d, *J* = 8.4, 1H), 8.13 (d, *J* = 7.9, 1H), 8.41 (br t, 1H). MS (ESI) *m*/*z*: $[M + Na]^+$ Calcd for $C_{22}H_{29}N_3NaO_4$ 422.2; Found 421.9.

4-Methoxy-*N*-(piperidin-4-ylmethyl)quinoline-2-carboxamide (15).

A mixture of compound **14** (0.13 g, 0.325 mmol) in ethanol (8.0 mL) containing concentrated HCl (0.25 mL) was stirred at room temperature under inert atmosphere for 3 h. The reaction mixture was then concentrated under reduced pressure to obtain pure compound **15** hydrochloride as a yellow glassy solid (0.10 g, yield 92%), which was used in the subsequent step without any further purification. ^{1}H NMR (200 MHz, D_2O): 1.17-1.35 (m, 2H), 1.77-1.99 (m, 3H), 2.76 (t, *J* = 12.5, 2H), 3.22 (m, 4H), 4.12 (s, 3H), 7.51 (s, 1H), 7.63 (m, 1H), 7.82-7.97 (m, 2H), 8.18 (d, *J* = 8.4, 1H). MS (ESI) *m*/*z*: $[M + H]^+$ Calcd for $C_{17}H_{22}N_3O_2$ 300.2; Found 300.1.

***N*-[(1-Butylpiperidin-4-yl)methyl]-4-methoxyquinoline-2-carboxamide (9d).**

A mixture of **15** hydrochloride (0.10 g, 0.30 mmol) and Na_2CO_3 (0.10 g, 0.94 mmol) in ethanol (10 mL) was stirred at room temperature for 30 min. Then *n*-butyl iodide (0.034mL, 0.30 mmol) was added

dropwise and the reaction mixture was heated under reflux in an inert atmosphere for 4 h. The solvent was evaporated under reduce pressure and the residue was diluted with dichloromethane and washed with water. The organic layer was dried over sodium sulfate and evaporated under reduced pressure. Purification of the residue by flash chromatography with ethyl acetate-triethylamine (9:1) as the eluent gave compound **9d** as a white solid (0.054 g, yield 51%, mp 82-85 °C). ^{1}H NMR (400 MHz, $CDCl_3$): 0.90 (t, *J* = 7.3, 3H) 1.29 (m, 2H), 1.38-1.55 (m, 4H), 1.68 (m, 1H), 1.80 (d, *J* = 12.6, 2H), 1.93 (m, 2H), 2.31 (t, *J* = 7.7, 2H), 2.96 (d, *J* = 11.3, 2H), 3.42 (t, *J* = 6.5, 2H), 4.11 (s, 3H), 7.54 (t, *J* = 7.9, 1H), 7.72 (m, 2H), 8.01 (d, *J* = 8.5, 1H), 8.21 (d, *J* = 8.6, 1H), 8.45 (br t, 1H). MS (ESI) *m*/*z*: $[M + H]^+$ Calcd for $C_{21}H_{30}N_3O_2$ 356.2; Found 356.1. HPLC: acetonitrile-(H_2O + 1% acetic acid)-methanol (4:3:3), retention time 3.4 min, purity 99%.

***N*-[(1-Butylpiperidin-4-yl)methyl]-8-hydroxyquinoline-2-carboxamide (9e).**

A mixture of acid **16** (Sigma-Aldrich, 0.20 g, 1.06 mmol) in dry DMF (5.0 mL) containing BOP (0.47 g, 1.06 mmol), (1-butylpiperidin-4-yl)methanamine (0.25 g, 1.5 mmol) and TEA (0.44 mL, 3.2 mmol) was stirred at room temperature under an inert atmosphere for 3 h. The reaction mixture was then concentrated under reduced pressure and the residue was diluted with dichloromethane and washed with water. The organic layer was dried over sodium sulfate and evaporated under reduced pressure to give **9e** as a white solid (0.32 g, yield 88%). An analytical sample was obtained by recrystallization from n-hexane-diethyl ether by slow evaporation (colorless prisms, mp 68-69 °C). ^{1}H NMR (400 MHz, $CDCl_3$): 0.89 (t, *J* = 7.3, 3H), 1.29 (m, 2H), 1.43 (m, 4H), 1.58-1.80 (m, 3H), 1.89 (t, *J* = 11.6, 2H), 2.29 (t, *J* = 7.4, 2H), 2.93 (d, *J* = 10.6, 2H), 3.42 (t, *J* = 5.4, 2H), 7.23 (d, *J* = 7.7, 1H), 7.40 (d, *J* = 8.2, 1H), 7.53 (t, *J* = 7.9, 1H), 8.10 (br s, 1H), 8.32 (m, 2H). MS (ESI) *m*/*z*: $[M + H]^+$ Calcd for $C_{20}H_{28}N_3O_2$ 342.2; Found 342.1. HPLC: acetonitrile-(H_2O + 1% acetic acid)-methanol (4:3:3), retention time 3.1 min, purity 99%.

X-Ray crystallography. Single crystal of compounds **9a** and **9e** were submitted to X-ray data collection on an Oxford-Diffraction Xcalibur Sapphire 3 diffractometer with a graphite monochromated

Mo-Kα radiation (λ = 0.71073 Å) at 293 K. The structure was solved by direct methods implemented in SHELXS-97 program.[3] The refinement was carried out by full-matrix anisotropic least-squares on F^2 for all reflections for non-H atoms by means of the SHELXL-97 program.[4] Crystallographic data (excluding structure factors) have been deposited with the Cambridge Crystallographic Data Centre as supplementary publications no. CCDC 1841095 (**9a**) and 1841096 (**9e**). Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; (fax: + 44 (0) 1223 336 033; or e-mail: deposit@ccdc.cam.ac.uk).

Binding assays

Male Dunkin-Hartley guinea pigs (Charles River Italia, Calco, CO, Italy) weighing 300-400 g were used. Animal care and handling throughout the experimental procedures were in accordance with both the Italian legislative decree DL 26/2014 and the European Communities Council Directive of 22 September 2010 (2010/63 UE).

Binding assays on striatal nuclei membrane preparations were performed according to Grossman et al. with slight modification.[5] Crude membranes were diluted in the binding buffer (Hepes 50 mM, pH 7.4) in order to obtain the final protein concentration. The incubation was performed in polystyrene 24-well multiwell plate at 37 °C for 30 min. The bound radioligand was separated by rapid filtration on glass fiber Unifilter GF/B 24w plate pre-treated with polyethyleneimine 0.1% in buffer. Filtrates were washed two times with 2 mL cold binding buffer, plates were dried for 30 min at room temperature, then 0.2 mL/well MICROSCINT-20 (Perkin Elmer Life and Analytical Sciences) and radioactivity measured after at least 2 h of stabilization. The specific binding of [^{3}H]GR113808 (final concentration 0.2 nM), defined as the difference between the total binding and the nonspecific binding determined in the presence of 30 μM 5-HT, represented about 70-80% of the total binding.

Competition experiments were analyzed by the GraphPad Prism software (version 6 for Windows) to obtain the concentration of unlabelled drug that caused 50% inhibition of [^{3}H]GR113808 specific

binding (IC_{50}). Apparent affinity constants (K_i) were derived from the IC_{50} values according to the Cheng and Prusoff equation [$K_i = IC_{50}/(1+L/K_d)$].[6]

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