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Synthesis of Quinoline Derivatives as 5-HT$_4$ Receptor Ligands

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A general and convenient synthesis of 6-methoxyquinoline-3-carboxamides commencing with a cyclization step that involves ρ-anisidine and diethyl (ethoxymethylene)malonate is described. An additional tetrahydroquinoline scaffold **19** is prepared from 6-methoxyquinoline-3-carboxamide and this represents a novel serotinergic lead structure. These compounds show reasonable affinity at 1×10^{-6} M, and docking experiments suggest that they may bind in a similar manner to serotonin.

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Introduction

Compounds that modulate serotonin (5-HT, **1**) (Fig. 1) transmission have many varied uses in clinical settings. In particular, clinical agents that activate 5-HT$_4$ receptors have use in gastro-esophageal reflux disease[1] and irritable bowel syndrome.[2] The endogenous ligand **1** acts on 5-HT$_4$ receptors in the smooth muscle of the gut and the bladder to regulate muscle tone.[3] While compounds like cisapride and tegaserod also act as 5-HT$_4$ receptor agonists, they have been restricted in their use because of cardiovascular side effects.[4,5] The need for compounds that have improved selectivity for the 5-HT$_4$ receptor and reduced toxicity is clear.

A range of molecular scaffolds have been used for 5-HT$_4$ ligands including benzamides, indoles, and benzimidazoles.[6] While various quinoline and quinolinone derivatives have been explored,[7–9] the methods employed to generate these compounds were not fully elucidated. Given this lack of detail and the desire to explore the use of quinolines as potential 5-HT$_4$ receptor ligands we have undertaken a more extensive synthetic campaign. Initial molecular docking studies using a 6-methoxyquinoline-3-carboxamide moiety showed that favourable interactions could occur between this molecule and the receptor. This result prompted our research and here we report the design and synthesis of novel 5-HT$_4$ compounds based on a 6-methoxyquinoline ring system as analogues of the agonist, 5-methoxytryptamine (**2**) (Fig. 1) as well as a novel tetrahydroquinoline that contains a basic side chain.

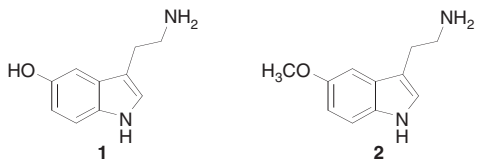

Fig. 1. Structures of serotonin **1** and 5-methoxytryptamine **2**.

Results and Discussion

The synthesis of the simplest carboxamide described in this study was carried out in a four step reaction sequence (Scheme 1) that involved cyclization of ρ-anisidine **3** and diethyl (ethoxymethylene)malonate **4** to afford ethyl 4-hydroxy-6-methoxyquinoline-3-carboxylate **5**.[10] The difficulty in the cyclization of this product was the requirement of high temperatures to achieve significant yields. Various measures were taken to maintain the high temperatures needed, without exceeding the maximum temperatures as this led to dimerization of the uncyclized intermediate. Hydrolysis of the ester **5** was easily achieved by both base and acid catalyzed hydrolysis; however, the efficiency of the latter (98% of **6**), and the ease of work up (where filtration was adequate to achieve a pure product), were factors in choosing this method over base catalysis. Acyl chloride formation and simultaneous chlorination were achieved by refluxing in phosphorus oxychloride in a one pot synthesis. This reaction was quenched in a solution of saturated ammonia gas in dichloromethane, to afford the equivalent amide **7** in quantitative yield. The 4-chloro adduct was treated with sodium borohydride in the presence of palladium chloride catalyst to give the dechlorinated species **8**.

Synthesis of further analogues employed an alternative route found in Scheme 2. The cyclized compound **5** was treated with phosphorus oxychloride to give the chlorinated species **9** to facilitate removal of this 4-position substituent in subsequent steps. The dechlorination step was achieved with a palladium chloride catalyst and sodium borohydride hydrogen donor in anhydrous methanol to give product **10**. As previously described for the ester hydrolysis of compound **5**, this was readily achieved in acidic conditions to yield **11**. The acid chloride intermediate **12** was synthesized using phosphorus oxychloride under reflux

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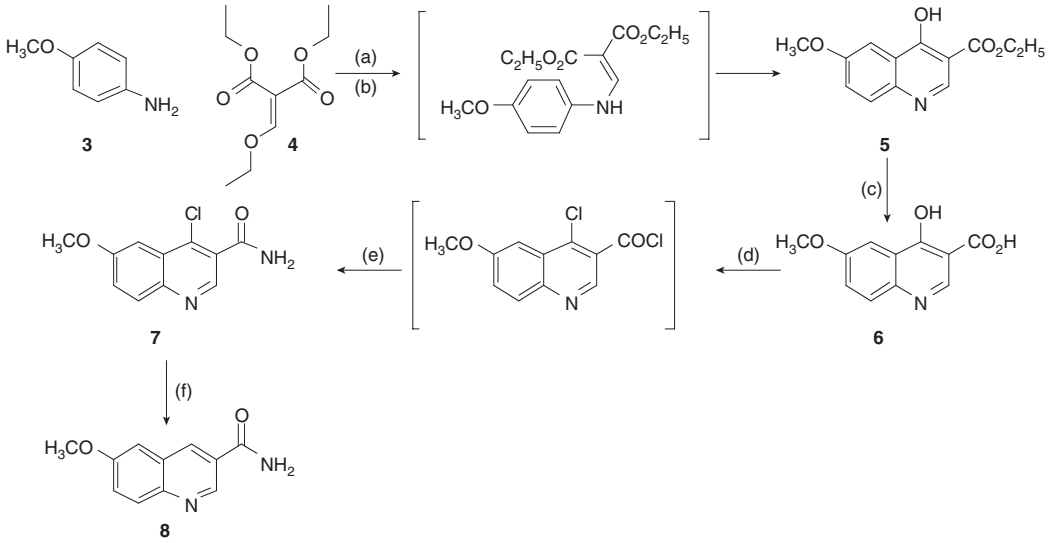
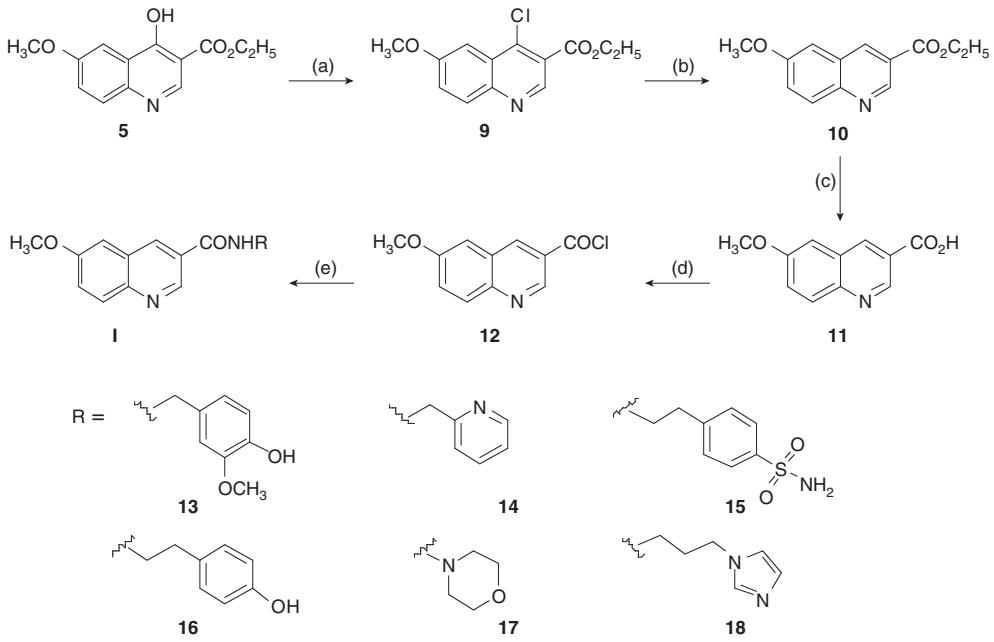


Scheme 1. (a) 130°C neat 4 h. (b) Dowtherm A, reflux, 1 day. (c) 20% HCl, reflux, 3 h. (d) $POCl_3$, reflux, 3 h. (e) Sat. NH_3 in DCM, 0°C, 0.5 h→rt. (f) $PdCl_2$, $NaBH_4$, anhydrous MeOH, 30°C, 3 h.



Scheme 2. (a) $POCl_3$, reflux, 3 h. (b) $PdCl_2$, $NaBH_4$, anhydrous MeOH, 30°C, 3 h. (c) 20% HCl, reflux, 3 h. (d) $POCl_3$, reflux, 3 h. (e) RNH_2, DCM, reflux, 4–24 h.

conditions. Analogues of **I** (Scheme 2) were synthesised in situ with the acid chloride using an excess of a base.

Docking

Docking studies were performed on analogues **8**, **10**, **11**, and **13**–**19** using a homology model of the human 5-HT$_4$ receptor. This model was built based on the crystal structure of bovine rhodopsin and was optimized in a lipid bilayer environment as well as using information from docking experiments and site-directed mutagenesis studies.[11–13] Using the Glide Package (Schrodinger, NY, USA) and appropriate charge states for both the active site residues and the ligands, **1** and **2** were successfully docked into the protein in agreement with the previous model (Fig. 2a).[11] For each analogue, five poses were evaluated for both their docking score and a visual analysis of the binding mode relative to both **1** and **2**. Table 1 gives the G-score of the best pose for each compound, which shows that there was no relationship with their biological activity. This result was not unexpected and it should be remembered that this exercise was oriented to idea generation and to facilitate future design work. One noticeable observation was that the analogues synthesized in this study lacked a basic amine and as such they were unable to form a strong charge-assisted hydrogen bond with residue D100. Among the binding modes there were cases where the quinoline ring behaved in a similar manner to the indole of **1** where the amide nitrogen had an interaction with D100 and the quinoline ring stacked with F201 (Fig. 2b). In other cases, however (e.g., **16**

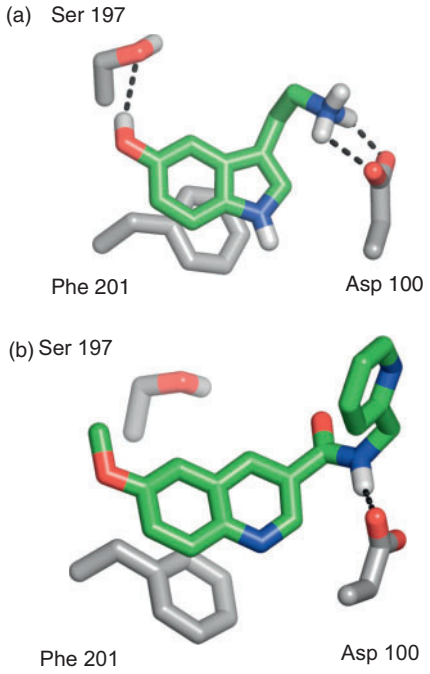


Fig. 2. (a) Compound **1** docked into the homology model of the 5-HT$_4$ receptor. (b) Compound **14** docked into the homology model of the 5-HT$_4$ receptor.

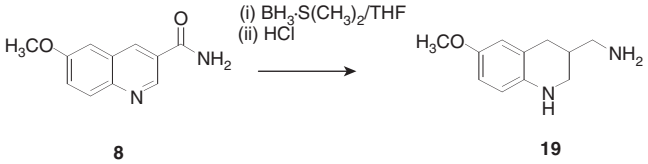
Table 1. Radioligand displacement values and G-scores
Displacement of [^{3}H]-GR113808 (± s.e.m.) at 1×10^{-6} M against the 5-HT$_{4b}$ receptor. G-score in Kcal mol^{-1}

Compound number	% Displacement	G-score
1	98 ± 2	−7.3
2	100 ± 3	−8.0
8	52 ± 3	−7.8
10	39 ± 5	−8.5
11	54 ± 4	−8.2
13	69 ± 3	−8.7
14	73 ± 4	−3.4
15	70 ± 6	−5.5
16	65 ± 3	−10.0
17	62 ± 4	−6.7
18	62 ± 5	−2.8
19	75 ± 3	−9.1 (*R* isomer)

and **18**), the best pose showed that the side chain of the analogue was placed adjacent to S197 and the amide made a hydrogen bond with D100. This suggests that the presence of an ionizable nitrogen is no doubt influential in orienting the compounds in the binding site.

Radioligand Binding

A series of radioligand binding assays were conducted using [^{3}H]-GR113808, which is a high affinity and highly selective 5-HT$_4$ receptor antagonist.[14] All molecules were screened against the 5-HT$_{4b}$ receptor, the most commonly expressed 5-HT$_4$ receptor splice variant.[5] Table 1 gives the displacement values for compounds **1**, **2**, **8**, **10**, **11**, **13**–**19**, in this series against the 5-HT$_{4b}$ receptor. The known agonists **1** and **2** showed



Scheme 3.

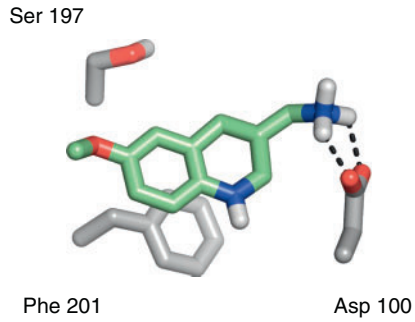


Fig. 3. Compound **19** (modelled as *R* isomer) docked into the homology model of the 5-HT$_4$ receptor.

displacements of 98 and 100% at 1×10^{-6} M, respectively, which were not significantly different. The highest displacement among the quinoline derivatives was observed with **14** (the pyridine analogue), followed by **13** and **15**.

Tetrahydroquinoline

Given the affinity of the quinoline analogues at 1×10^{-6} M together with their lack of an ionizable nitrogen in the side chain that could interact with D100, an additional scaffold was explored using the chemistry approaches described here. Compound **19** was generated by the reduction of **8** to the amine in anhydrous tetrahydrofuran, in the presence of borane–dimethyl sulfide at room temperature (Scheme 3) in 50% yield. The reduction of the pyridine ring formed an asymmetric centre at the 3-position of the piperidine ring, which could lead to the possibility of two enantiomers.

Molecular docking demonstrated that this molecule behaved in a similar manner to **2** showing an interaction with D100 (Fig. 3). Encouragingly this compound displayed good potency (Table 1) showing 75% displacement at 1×10^{-6} M. The compound itself has reduced flexibility relative to **2** and the ring nitrogen was estimated to be neutral at physiological pH (est. pK_a 4.7, ACD/Laboratories, Toronto, Canada). Molecular docking did not show any significant preference for the *R* or *S* isomers and the results presented here are given for the *R* isomer. We feel that compound **19** represents an important new scaffold for 5-HT$_4$ receptors and potentially for other serotinergic targets. As a lead compound it can be envisaged that substitution onto the basic side-chain nitrogen atom would enable the optimization of both potency and selectivity for the 5-HT$_4$ receptor. This compound will be the subject of future research in our laboratory.

Conclusions

The results presented here demonstrate that the 6-methoxyquinoline-3-carboxamide scaffold is a useful structure to generate potential ligands for the 5-HT$_4$ receptor. More importantly,

the 6-methoxy-tetrahydroquinoline compound (**19**) represents a novel and useful lead structure in the design of 5-HT$_4$ ligands. Synthetically, the procedure we outline is convenient and can be generally applied. The potency of several compounds was of great interest and will form the basis of future medicinal chemistry efforts. The combination of docking experiments and radioligand binding has given some insight into how these compounds may interact with the receptor. Of course, further screening is necessary at a range of concentrations to determine IC_{50} values as well as exploring additional 5-HT$_4$ splice variants for selectivity studies. Moreover, functional studies will be undertaken to determine whether these compounds act as agonists or antagonists. Although there was no correlation between the binding affinities and their G-score, it was useful to visualize each compound in the binding pocket to help influence future design efforts.

Experimental

Molecular Modelling

A homology model of the 5-HT$_4$ receptor developed by Mialet and coworkers[11–13] was used for the docking experiments. Details concerning the construction of this model can be found in the references above. Molecules were constructed using Sybyl (St Louis, MO, USA) employing Gasteiger Huckel charges. The Glide Package was used for the docking experiments employing the extra precision (XP) protocol without the specification of any constraints. The top five poses were examined and the highest scored pose was tabulated.

General

Diethyl (ethoxymethylene)malonate, diphenyl ether, and biphenyl were all obtained from Fluka. Borane–dimethyl sulfide complex and ρ-anisidine were obtained from Aldrich. Hydrochloric acid and phosphorus oxychloride as well as all other solvents were obtained from Merck. Anhydrous compressed ammonia gas was obtained from BOC.

All synthesized compounds* were assessed using electrospray ionization (ESI) mass spectrometry and ^{1}H NMR spectroscopy. NMR spectra were recorded at room temperature on a Bruker Avance 300 MHz NMR spectrometer. Chemical shifts are reported relative to tetramethylsilane at 0 ppm. Low-resolution mass spectrometry analyses were performed using a Micromass Platform II single quadropole mass spectrometer equipped with an atmospheric pressure (ESI/APCI) ion source. Sample management was facilitated by an Agilent 1100 series HPLC system and the instrument was controlled using MassLynx software version 3.5. High-resolution mass spectrometry analyses were collected on a Waters Micromass LCT Premier XE Time Of Flight mass spectrometer fitted with either an electrospray (ESI) or Ion Sabre (APCI) ion source and controlled with MassLynx software version 4.1. All compounds were named using Chemdraw Ultra 10.0 and predicted ^{1}H NMR shifts were also used for comparison with experimental data. It was observed that in (D$_6$)DMSO as NMR solvent the indole nitrogen proton was always observed at the same chemical shift, whereas in $CDCl_3$, it was consistently absent. In reporting spectroscopic data the following abbreviations have been used: DMSO, dimethyl sulfoxide; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, doublet of doublets; M, molecular ion.

Anhydrous sodium sulfate was used as the drying agent in organic solutions. Concentration and evaporation of organic solutions was performed using a Buchi rotary evaporator. All reactions requiring reflux were conducted under inert nitrogen atmosphere using oven-dried glassware (120°C). Analytical TLC was performed using 0.2 mm, aluminium backed Silica Gel 60 F_{254} sheets. Preparative TLC was performed on 2 mm glass backed Silica Gel 60 F_{254} sheets. Isolated compounds were analyzed by TLC to ensure only one spot was visible for high purity. In all cases purity was determined to be over 90%.

Ethyl 4-Hydroxy-6-methoxyquinoline-3-carboxylate[10] (***5***)

A mixture of ρ-anisidine (**3**) (2.46 g, 20.0 mmol) and diethyl (ethoxymethylene)malonate (**4**) (4.32 g, 20.0 mmol) was heated without solvent at 130°C for 4 h in a sand bath. The product was cooled in an ethanol/dry ice slurry until a solid black film developed on the glass flask. The solid was re-dissolved in 15 mL of diethyl ether, and again cooled in a dry ice slurry. White crystals formed upon cooling, and were filtered on a previously cooled Büchner funnel, and then quickly transferred to a beaker where they rapidly melted. Dowtherm A (25 mL, 26.5% biphenyl and 73.5% diphenyl ether) was added to the melted crystals and the dark coloured solution was heated at reflux (290°C) for 24 h. The reaction mixture was allowed to cool to room temperature, and 25 mL of petroleum ether (100–130°C fraction) was added to precipitate the product. The light brown coloured precipitate was filtered from the solution and washed twice with petroleum ether (10 mL). Ethyl 4-hydroxy-6-methoxyquinoline-3-carboxylate (**5**) (2.0 g, 41% yield) was obtained as the product; mp 280–283°C (lit.[10] 274–277°C). *m/z* 248 [M + H$^+$]. δ_H ((D$_6$)DMSO) 13.52 (1H, s, OH), 8.49 (1H, s, CH), 7.61 (1H, d, *J* 8.7, CH), 7.50 (1H, s, CH), 7.37 (1H, dd, *J* 9.0, CH), 4.23 (2H, q, *J* 7.2, CH_2), 3.87 (3H, s, OCH_3), 1.30 (3H, t, *J* 7.2, CH_3). δ_C ((D$_6$)DMSO) 165.7, 163.3, 156.1, 150.7, 143.0, 127.2, 123.5, 119.7, 107.6, 102.9, 62.3, 56.4, 15.7. (Found: [M + H$^+$] 248.0922. $C_{13}H_{13}NO_4$ requires [M + H$^+$] 248.0923.)

4-Hydroxy-6-methoxyquinoline-3-carboxylic Acid·HCl[10] (***6***)

Ethyl 4-hydroxy-6-methoxyquinoline-3-carboxylate (**5**) (2.0 g, 8.1 mmol) was heated to reflux in 20% hydrochloric acid for 3 h, and upon cooling, beige needle-like crystals precipitated from the solution. The product, 4-hydroxy-6-methoxyquinoline-3-carboxylic acid (**6**), was isolated in 95% yield (1.96 g, 7.7 mmol); mp 283–284°C (lit.[15] 278–279°C).

m/z (ESI) 220 [M + H$^+$]. δ_H ((D$_6$)DMSO) 17.75 (1H, s, OH), 13.47 (1H, s, OH), 8.77 (1H, s, CH), 7.76 (1H, d, *J* 9.3, CH), 7.61 (1H, s, CH), 7.51 (1H, dd, *J* 9.0, CH), 3.87 (3H, s, OCH_3). δ_C ((D$_6$)DMSO) 169.7, 161.7, 158.4, 147.3, 144.1, 129.2, 124.9, 117.1, 106.5, 101.7, 56.9. (Found: [M + H$^+$] 220.0609. $C_{11}H_9NO_4$ requires [M + H$^+$] 220.0610.)

*4-Chloro-6-methoxyquinoline-3-carboxamide (**7**)*

4-Hydroxy-6-methoxyquinoline-3-carboxylic acid (**6**) (1.96 g, 8.9 mmol) was heated at reflux in phosphorus oxychloride for 5 h to afford the 4-chloro substituent of the quinoline and the formation of the acyl chloride in a one pot reaction. Conversion into the 3-amido derivative was achieved by addition of a

*Compounds **5**, **6**, **8**, and **11** have been reported previously but without detailed structure identification. As such, they have been described in the experimental section.

saturated ammonia solution in dichloromethane (50 mL) at 0°C for 30 min, and then slowly allowing the mixture to return to room temperature. The product, 4-chloro-6-methoxyquinoline-3-carboxamide (**7**), was isolated by filtration of inorganic salts, and washing with chloroform to obtain a 90% yield (1.9 g, 8.1 mmol); mp 261–263.5°C. *m/z* (ESI) 237 $[M+H^+]$. δ_H ((D_6)DMSO) 9.24 (1H, s, CH), 7.86 (1H, d, *J* 9.3, CH), 7.85 (2H, s, NH_2), 7.71 (1H, s, CH), 7.42 (1H, dd, *J* 9.0, CH), 3.83 (3H, s, OCH_3). δ_C ((D_6)DMSO) 168.7, 167.1, 158.1, 141.6, 139.3, 131.7, 129.7, 128.3, 119.4, 99.1, 57.3. (Found: $[M+H^+]$ 237.0421. $C_{11}H_9ClN_2O_2$ requires $[M+H^+]$ 237.0431.)

6-Methoxyquinoline-3-carboxamide[16] (***8***)

Sodium borohydride (304 mg, 8.1 mmol) was added portion wise to a stirred solution of 4-chloro-6-methoxyquinoline-3-carboxamide (**7**) (1.9 g, 8.1 mmol) and palladium chloride (1.41 g, 8.0 mmol) in dry methanol at room temperature over a period of 1 h.[17] The inorganic salts were filtered off, the methanol was removed under reduced pressure, and the reaction mixture was reconstituted in chloroform. The organic phase was washed with water and dried over anhydrous sodium sulfate. The dechlorinated product (**8**) was retained in 90% yield (1.45 g, 7.1 mmol); mp 230–232°C (lit.[16] 220°C). *m/z* (ESI) 203 $[M+H^+]$. δ_H ((D_6)DMSO) 9.18 (1H, s, CH), 8.73 (1H, s, CH), 7.71 (1H, d, *J* 9.3, CH), 7.91 (2H, s, NH_2), 7.43 (1H, dd, *J* 9.0, CH), 7.25 (1H, s, CH), 3.78 (3H, s, OCH_3). δ_C ((D_6)DMSO) 167.2, 157.3, 148.0, 143.9, 134.1, 132.2, 130.7, 127.8, 125.7, 108.4, 56.5. (Found: $[M+H^+]$ 203.0819. $C_{11}H_{10}N_2O_2$ requires $[M+H^+]$ 203.0821.)

Ethyl 6-Methoxyquinoline-3-carboxylate (***10***)

Ethyl 4-hydroxy-6-methoxyquinoline-3-carboxylate (**5**) (1.0 g, 4.1 mmol) was heated to reflux in phosphorus oxychloride for 3 h. Upon completion, excess phosphorus oxychloride was removed under reduced pressure. The reaction mixture was reconstituted in chloroform and filtered through silica gel, washed with water, and dried over anhydrous sodium sulfate. Ethyl 4-chloro-6-methoxyquinoline-3-carboxylate (**9**) was recovered in 90% yield (0.95 g, 3.6 mmol).

Sodium borohydride (137 mg, 3.6 mmol) was added portion wise to a stirred solution of ethyl 4-chloro-6-methoxyquinoline-3-carboxylate (**9**) (0.95 g, 3.6 mmol) and palladium chloride (634 mg, 3.6 mmol) in dry methanol at room temperature over a period of 1 h. The inorganic salts were filtered off, the methanol was removed under reduced pressure, and the reaction mixture was reconstituted in chloroform. The organic phase was washed with water and dried over anhydrous sodium sulfate. The dechlorinated product (**10**) was retained in 90% yield (0.74 g, 3.2 mmol); mp 203–205°C. *m/z* (ESI) 232 $[M+H^+]$. δ_H ((D_6)DMSO) 9.46 (1H, s, CH), 9.10 (1H, s, CH), 7.89 (1H, d, *J* 8.7, CH), 7.47 (1H, dd, *J* 9.0, CH), 7.35 (1H, s, CH), 4.33 (2H, q, *J* 7.2, CH_2), 3.83 (3H, s, OCH_3), 1.30 (3H, t, *J* 7.2, CH_3). δ_C ((D_6)DMSO) 166.2, 157.5, 145.9, 145.2, 138.6, 130.7, 127.5, 124.9, 121.5, 108.6, 61.2, 56.9, 15.9. (Found: $[M+H^+]$ 232.0969. $C_{13}H_{13}NO_3$ requires $[M+H^+]$ 232.0974.)

6-Methoxyquinoline-3-carboxylic Acid·HCl[18] (***11***)

Ethyl 6-methoxyquinoline-3-carboxylate (**10**) (0.74 g, 3.2 mmol) was heated to reflux in a solution of 20% HCl (10 mL) for 2 h. Upon cooling, the product precipitated out of solution as a black crystalline solid. This was filtered off and dried for use in derivatization steps. The product was isolated in 75% yield (0.58 g, 2.4 mmol); mp 210–211°C (lit.[18] 273°C for the free base). *m/z* (ESI) 204 $[M+H^+]$. δ_H ((D_6)DMSO) 12.95 (1H, s, OH), 9.51 (1H, s, CH), 8.75 (1H, s, CH), 7.96 (1H, d, *J* 9.3, CH), 7.31 (1H, dd, *J* 9.0, CH), 7.15 (1H, s, CH), 3.81 (3H, s, OCH_3). δ_C ((D_6)DMSO) 165.3, 157.6, 145.3, 144.0, 138.7, 130.2, 128.5, 124.3, 123.1, 108.5, 55.4. (Found: $[M+H^+]$ 204.0659. $C_{11}H_9NO_3$ requires $[M+H^+]$ 204.0661.)

N-*(4-Hydroxy-3-methoxybenzyl)-6-methoxyquinoline-3-carboxamide* (***13***)

The procedure for the preparation of compounds **13** to **18** employed compound **12** as an acyl chloride intermediate, which was generated by reflux of **11** in $POCl_3$ for 3 h followed by removal of excess solvent under reduced pressure. The acid chloride (**12**) was re-constituted in dichloromethane and reacted immediately with the appropriate amine to generate compounds **13** to **18**. During this procedure compound **12** was not isolated or characterized.

4-Hydroxy-3-methoxy benzylamine (75.0 mg, 0.5 mmol) was added to a stirred solution of 6-methoxyquinoline-3-carbonyl chloride (**12**) (100 mg, 0.5 mmol) and TEA (250 μL) in dichloromethane (5 mL) at room temperature. The solution was heated to reflux for 24 h, and monitored by analytical TLC. The reaction mixture was filtered through silica gel to facilitate removal of primary amines and inorganic salts. The filtered product was identified as *N*-(4-hydroxy-3-methoxybenzyl)-6-methoxyquinoline-3-carboxamide and was isolated as a yellow powder in 43% yield (71.0 mg). *m/z* (ESI) 339 $[M+H^+]$. δ_H ((D_6)DMSO) 9.83 (1H, s, OH), 9.18 (1H, s, CH), 8.93 (1H, d, *J* 9.3, CH), 8.76 (1H, s, NH), 7.89 (1H, d, *J* 9.3, CH), 7.39 (1H, dd, *J* 9.0, CH), 7.23 (1H, d, *J* 9.0, CH), 6.97 (1H, s, CH), 6.75 (1H, d, *J* 9.0, CH), 6.72 (1H, d, *J* 9.0, CH), 4.11 (2H, s, CH_2), 3.73 (3H, s, OCH_3). (Found: $[M+H^+]$ 339.1345. $C_{19}H_{18}N_2O_4$ requires $[M+H^+]$ 339.1345.)

*6-Methoxy-*N*-(pyridin-2-ylmethyl)quinoline-3-carboxamide* (***14***)

2-(Aminomethyl)pyridine (50 μL, 0.5 mmol) was added to a stirred solution of 6-methoxyquinoline-3-carbonyl chloride (**12**) (100 mg, 0.5 mmol) and TEA (250 μL) in dichloromethane (5 mL) at room temperature. The solution was heated to reflux for 4 h, and monitored by analytical TLC. The reaction mixture was filtered through silica gel to facilitate removal of primary amines and inorganic salts. The filtered product was identified as 6-methoxy-*N*-(pyridin-2-ylmethyl)quinoline-3-carboxamide and obtained as a white solid in 73% yield (105 mg); mp 113–114°C. *m/z* (ESI) 294 $[M+H^+]$.

δ_H ((D_6)DMSO) 9.28 (1H, s, CH), 8.84 (1H, d, *J* 9.3, CH), 8.76 (1H, s, NH), 8.46 (1H, d, *J* 9.3, CH), 7.79 (1H, d, *J* 9.3, CH), 7.73 (1H, d, *J* 9.3, CH), 7.31 (1H, dd, *J* 9.0, CH), 7.31 (1H, dd, *J* 9.0, CH), 7.17 (1H, d, *J* 9.0, CH), 4.37 (2H, s, CH_2), 3.83 (3H, s, OCH_3). (Found: $[M+H^+]$ 294.1241. $C_{17}H_{15}N_3O_2$ requires $[M+H^+]$ 294.1242.)

*6-Methoxy-*N*-(4-sulfamoylphenethyl)quinoline-3-carboxamide* (***15***)

4-(2-Aminoethyl)benzenesulfonamide (98.0 mg, 0.5 mmol) was added to a stirred solution of 6-methoxyquinoline-3-carbonyl chloride (**12**) (100 mg, 0.5 mmol) and TEA (250 μL) in dichloromethane (5 mL) at room temperature. The solution was heated to reflux for 24 h, and monitored by analytical TLC. The reaction mixture was filtered through silica

gel to facilitate removal of primary amines and inorganic salts. The filtered product was identified as 6-methoxy-*N*-(4-sulfamoylphenethyl)quinoline-3-carboxamide and obtained as a beige powder in 29% yield (55 mg). *m/z* (ESI) 386 [M + H$^+$]. δ_H ((D$_6$)DMSO) 9.15 (1H, s, CH), 8.78 (1H, d, *J* 9.3, CH), 8.56 (1H, s, NH), 7.81 (2H, dd, *J* 9.3, CH, CH), 7.70 (1H, d, *J* 9.3, CH), 7.45 (2H, dd, *J* 9.3, CH, CH), 7.39 (2H, s, NH$_2$), 7.25 (1H, dd, *J* 9.0, CH), 7.19 (1H, d, *J* 9.0, CH), 3.76 (3H, s, OCH$_3$), 3.33 (2H, m, CH$_2$), 2.53 (2H, m, CH$_2$). (Found: [M + H$^+$] 386.1175. $C_{19}H_{19}N_3O_4S$ requires [M + H$^+$] 386.1172.)

N-*(4-Hydroxyphenethyl)-6-methoxyquinoline-3-carboxamide (**16**)*

Tyramine (67.0 mg, 0.5 mmol) was added to a stirred solution of 6-methoxyquinoline-3-carbonyl chloride (**12**) (100 mg, 0.5 mmol) and TEA (250 μL) in dichloromethane (5 mL) at room temperature. The solution was heated to reflux for 24 h, and monitored by analytical TLC. The reaction mixture was filtered through silica gel to facilitate removal of primary amines and inorganic salts. The filtered product was identified as *N*-(4-hydroxyphenethyl)-6-methoxyquinoline-3-carboxamide and isolated as a yellow powder in 24% yield (39 mg). *m/z* (ESI) 323 [M + H$^+$]. δ_H ((D$_6$)DMSO) 9.43 (1H, s, OH), 9.31 (1H, s, CH), 8.79 (1H, d, *J* 9.3, CH), 8.56 (1H, s, NH), 7.83 (1H, d, *J* 9.3, CH), 7.35 (1H, dd, *J* 9.0, CH), 7.27 (1H, d, *J* 9.0, CH), 7.16 (2H, d, *J* 9.0, CH, CH), 6.76 (2H, d, *J* 9.0, CH, CH), 3.86 (3H, s, OCH$_3$), 3.27 (2H, m, CH$_2$), 2.72 (2H, m, CH$_2$). (Found: [M + H$^+$] 323.1390. $C_{19}H_{18}N_2O_3$ requires [M + H$^+$] 323.1396.)

*6-Methoxy-*N*-morpholinoquinoline-3-carboxamide (**17**)*

4-Aminomorpholine (47 μL, 0.5 mmol) was added to a stirred solution of 6-methoxyquinoline-3-carbonyl chloride (**12**) (100 mg, 0.5 mmol) and TEA (250 μL) in dichloromethane (5 mL) at room temperature. The solution was heated to reflux for 4 h, and monitored by analytical TLC. The reaction mixture was filtered through silica gel to facilitate removal of primary amines and inorganic salts. The filtered product was identified as 6-methoxy-*N*-morpholinoquinoline-3-carboxamide and isolated as a brown solid in 31% yield (43 mg); mp 107–108°C. *m/z* (ESI) 288 [M + H$^+$]. δ_H ((D$_6$)DMSO) 9.19 (1H, s, CH), 8.71 (1H, d, *J* 9.3, CH), (1H, s, NH), 7.65 (1H, d, *J* 9.3, CH), 7.45 (1H, dd, *J* 9.0, CH), 7.28 (1H, d, *J* 9.0, CH), 3.75 (3H, s, OCH$_3$), 3.51 (4H, m, CH$_2$, CH$_2$), 2.90 (4H, m, CH$_2$, CH$_2$). (Found: [M + H$^+$] 288.1346. $C_{15}H_{17}N_3O_3$ requires [M + H$^+$] 288.1348.)

N-*(3-(1*H*-Imidazol-1-yl)propyl)-6-methoxyquinoline-3-carboxamide (**18**)*

1-(3-Aminopropyl)imidazole (58 μL, 0.5 mmol) was added to a stirred solution of 6-methoxyquinoline-3-carbonyl chloride (**12**) (100 mg, 0.5 mmol) and TEA (250 μL) in dichloromethane (5 mL) at room temperature. The solution was heated to reflux for 4 h, and monitored by analytical TLC. The reaction mixture was filtered through silica gel to facilitate removal of primary amines and inorganic salts. The filtered product was identified as *N*-(3-(1*H*-imidazol-1-yl)propyl)-6-methoxyquinoline-3-carboxamide and obtained as a yellow oil in 34% yield (52 mg). *m/z* (ESI) 311 [M + H$^+$]. δ_H ((D$_6$)DMSO) 9.21 (1H, s, CH), 8.79 (1H, d, *J* 9.3, CH), 8.47 (1H, s, NH), 7.98 (1H, s, CH), 7.72 (1H, d, *J* 9.3, CH), 7.36 (1H, dd, *J* 9.0, CH), 7.27 (1H, d, *J* 9.0, CH), 7.23 (1H, d, *J* 9.0, CH), 6.69 (1H, d, *J* 9.0, CH), 4.07 (2H, m, CH$_2$), 3.71 (3H, s, OCH$_3$), 3.12 (2H, m, CH$_2$), 2.54 (2H, m, CH$_2$). (Found: [M + H$^+$] 311.1510. $C_{17}H_{18}N_4O_2$ requires [M + H$^+$] 311.1508.)

*(±)(6-Methoxy-1,2,3,4-tetrahydroquinolin-3-yl)methanamine (**19**)*

Borane dimethyl sulfide (1.33 mL, 14 mmol) was added dropwise to a stirred solution of 6-methoxyquinoline-3-carboxamide (**8**) (1.45 g, 7.2 mmol) in dry THF (10 mL) at room temperature over a period of 30 min.[19] Bubbling was observed as sulfide gas and hydrogen were evolved. The solution was stirred for a further hour upon completion of the addition and then brought to reflux. Hydrochloric acid (6 M, 10 mL) was slowly added to the refluxing solution to quench the remaining hydride. The reaction was cooled to 0°C in an ice bath and neutralized with NaOH pellets. The amine product was extracted with 3 × 15 mL quantities of diethyl ether. Analytical HPLC produced a single clean peak, and the corresponding TLC showed only 1 spot. The product was identified as (6-methoxy-1,2,3,4-tetrahydroquinolin-3-yl)methanamine (**19**) in 50% yield (0.670 g). *m/z* (ESI) 193 [M + H$^+$]. δ_H ((D$_6$)DMSO) 6.85 (1H, s, CH), 6.59 (1H, s, CH), 6.44 (1H, d, *J* 9.0, CH), 4.25 (1H, s, NH), 3.83 (1H, s, OCH$_3$), 3.15–2.90 (2H, m, *J* 9.0, CH$_2$), 2.73–2.34 (5H, m, CH$_2$, CH$_2$, CH), 2.30 (2H, s, NH$_2$). δ_C ((D$_6$)DMSO) 148.1, 138.3, 124.7, 113.6, 112.3, 111.9, 55.8, 55.6, 45.8, 42.7, 31.1. (Found: [M + H$^+$] 193.1341. $C_{11}H_{16}N_2O$ requires [M + H$^+$] 193.1338.)

Biology

The standard compounds **1** and **2** were screened along with intermediates **8**, **10**, and **11**, and derivatives **13**–**19** against the 5-HT$_{4b}$ receptor. Each compound was screened at 100 × 10^{-6} M (data not shown) and 1 × 10^{-6} M in duplicate, which was repeated three times to give $n = 3$. All assays were carried out in 96-well plates using the Filtermate harvester (Packard, CT, USA) to harvest the membrane-ligand complex onto a GF/B Unifilter (Perkin–Elmer, Waltham, MA, USA). GF/B Unifilter plates were prepared for the binding study by soaking in 0.5% polyethyleneimine overnight. The potent and selective 5-HT$_4$ receptor antagonist [^{3}H]-GR113808 (GE Healthcare) was used as the radioligand at a final concentration of 0.25 × 10^{-9} M per well (300 μL final well volume). Test compounds, dissolved in phosphate buffered saline (PBS) and DMSO (max. conc. 1% v/v) were then added, along with 20 μg of membrane preparation (prepared from transient transfection of COS-7 cells which expressed the 5-HT$_{4b}$ splice variant). The binding reaction occurred at 25–30°C for 60 min. The membrane complexes were harvested onto the GF/B Unifilter plates using a vacuum pump and then washed three times with PBS. The filter was then dried at 37°C for 4–6 h before adding 50 μL of scintillation fluid (Microscint 40). Binding was measured using a TopCount Microplate Scintillation Counter (Packard). The GraphPad Prism 5 package (La Jolla, CA, USA) was used for data analysis. The mean displacement values of the compounds in the screening exercise were determined. The standard error of the mean was also calculated and the differences between means were determined by one-way ANOVA followed by the Tukey–Kramer post-hoc test. The level of significance was taken as $P < 0.01$.

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