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Development of subnanomolar-affinity serotonin 5-HT₄ receptor ligands based on quinoline structures†

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Two small series of quinoline derivatives were designed starting from previously published quinoline derivatives **7a** and **b** in order to obtain information about their interaction with the 5-HT₄R binding site. Initially, the structure of **7a** and **b** was modified by replacing their basic moiety with that of partial agonist **4** (ML10302) or with that of reference ligand **6** (RS-67-333). Then, the aromatic moieties of 4-quinolinecarboxylates **7a**, **d-f**, and **h-k** or 4-quinolinecarboxamides **7b**, **c**, and **g** were modified into those of 2-quinolinecarboxamides **9a-e**. Very interestingly, this structure–affinity relationship study led to the discovery of **7h-j** as novel 5-HT₄R ligands showing *K_i* values in the subnanomolar range. The structures of all these compounds contain the *N*-butyl-4-piperidinylmethyl substituent, which appear to behave as an optimized basic moiety in the interaction of these 4-quinolinecarboxylates with the 5-HT₄R binding site. However, this basic moiety was ineffective in providing 5-HT₄R affinity in the corresponding 4-quinolinecarboxamide **7g**, but it did in 2-quinolinecarboxamide ligands **9c-e**. Thus, a subtle interrelationship of several structural parameters appeared to play a major role in the interaction of the ligands with the 5-HT₄R binding site. They include the kind of basic moiety, the position of the carbonyl linking group with respect to the aromatic moiety and its orientation, which could be affected by the presence of the intramolecular H-bond as in compounds **9c-e**.

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1. Introduction

The neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) is recognized to play pivotal roles in several different physiological functions that are mediated by seven receptor subtypes.^{1,2} Among them, the vast majority belongs to the G-protein coupled receptor (GPCR) super family, with the exception of the 5-HT₃ receptor, which is a ligand-gated ion channel.² The 5-HT₄ receptor (5-HT₄R) has received a great deal of attention as a target for drug discovery and development, and some 5-HT₄R agonists have entered the market as gastrointestinal drugs.^{3–5} Great efforts in the search of new drugs have produced a large number of 5-HT₄R ligands (see for example compounds **1–6** in Fig. 1).^{5,6}

Moreover, 5-HT₄R agonists have been proposed to play a role in the treatment of Alzheimer's disease (AD),^{7,8} potentially representing a new class of drugs for the treatment of the cognitive deficits associated with AD.⁷ For instance, the partial 5-HT₄R agonist PRX-03140 (**3**) by Epix Pharmaceuticals was reported to improve cognitive processes by increasing the release of acetylcholine and soluble amyloid precursor protein (sAPPα).⁷

In the context of our interest in developing new serotonin receptor ligands,^{9–16} the aromatic moieties (AM) of standard 5-HT₄R ligands **3** and **5** were replaced with the 2-methoxyquinoline system to obtain flexible compounds **7a** and **b** (Fig. 2), which were in turn transformed into the corresponding conformationally constrained derivatives **8a–g** in the aim of obtaining information on their interaction with the 5-HT₄R.¹⁶

Very interestingly, ester **7a** showed a 5-HT₄R affinity in the nanomolar range along with antagonist properties, and the structure–activity relationship analysis in the conformationally constrained derivatives **8a–g** suggested that the nature and the position of the substituents played a crucial role in determining the interaction with their receptor.¹⁶

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Fig. 1 Structures of some representative serotonin 5-HT₄R ligands.

Thus, the structure–affinity relationship studies in the class of quinoline derivatives **7a** and **b** were deepened by replacing their basic moiety (BM-1 in Fig. 3) with BM-2, typical of partial agonist **4**, or with BM-3 included in the structure of ligand **6**.

Moreover, the aromatic moiety of compounds **7a–k** (*i.e.* AM-2) was structurally modified into the quinoline AM-3 of compounds **9a–e**. The present paper reported on the synthesis and the structure–affinity relationship studies on quinoline 5-HT₄R ligands **7c–k** and **9a–e** and the discovery of some novel ligands showing K_i values in the subnanomolar range.

2. Results and discussion

Target quinoline derivatives **7h–k** were prepared starting from commercially available carboxylic acids **10a–d** by activation with 1,1-carbonyldiimidazole (CDI) and subsequent reaction with commercially available *N*-butyl-4-piperidinemethanol in the presence of DBU as the base (Method A, Scheme 1).

The synthesis of amide derivative **7g** was accomplished from acid **10a** by reaction with commercially available (1-butyl-4-piperidinyl)methanamine in the presence of BOP [(benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate, Castro's reagent]¹⁷ as the coupling reagent (Method B). Acid **10a** was used also in the preparation

Fig. 2 Structure of previously published 5-HT₄R ligands **7a** and **b** and **8a–g**.

Fig. 3 Design of quinoline ligands **7a–k** and **9a–e** (for the specific structures see Tables 1 and 2) from reference ligands **4**, **5**, and **6**.

of amide **7c** by using 1-(2-aminoethyl)piperidine as the amine and EDC hydrochloride as the activating agent (Method C). Acid **10a** was finally alkylated with 1,2-dibromoethane in the presence of DBU as the base to afford bromoderivative **11**, which was then reacted with the appropriate piperidine derivative to obtain the expected ester derivatives **7d–f**.

Target 2-quinolinecarboxamide derivatives **9a–d** were prepared according to the reaction sequence described in Scheme 2.

Acid **12** (ref. 18) was activated with thionyl chloride and then reacted with the suitable piperidine derivatives to afford amide derivatives **7a–c** and **13**. The reaction of **13** with sodium methoxide in methanol gave methoxyderivative **14**, which was deprotected with HCl in ethanol to give intermediate **15**. Finally, ligand **7d** was obtained by alkylation of **15** with *n*-butyl iodide in the presence of sodium carbonate as the base.

The synthesis of monovalent ligand **9e** is described in Scheme 3.

Commercially available carboxylic acid **16** was converted into amide derivative **9e** by reaction with (1-butyl-4-piperidinyl)methanamine in the presence of BOP, as the

Scheme 1 Synthesis of target quinoline derivatives **7c–k**. Reagents: (Method A, for **7h–k**) BM-OH, CDI, DBU, DMF; (Method B, for **7g**) BM-NH₂, BOP, TEA, DMF; (Method C, for **7c**) BM-NH₂, EDC hydrochloride, DMAP, CH₂Cl₂; (i) BrCH₂CH₂Br, DBU, THF; (ii) suitable piperidine derivative, DIPEA, CH₃CN (for **7d–f**).

Scheme 2 Synthesis of target 2-quinolinecarboxamide derivatives **9a–d**. Reagents: (i) SOCl_2 , DMF; (ii) BM- NH_2 , (TEA), CH_2Cl_2 ; (iii) 1-Boc-4-(aminomethyl)piperidine, CH_2Cl_2 ; (iv) NaH, CH_3OH ; (v) HCl, $\text{C}_2\text{H}_5\text{OH}$; (vi) $\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{I}$, Na_2CO_3 , $\text{C}_2\text{H}_5\text{OH}$.

coupling agent. The structure of target compounds **9a** and **e** was confirmed by crystallographic studies (Fig. 4 and 5).

The binding affinity values of target quinoline derivatives **7c–k** and **9a–e** were measured as the activity in inhibiting the specific binding of $[^3\text{H}]\text{GR113808}$ ([1-[2-(methylsulfonylamino)ethyl]-4-piperidinyl]methyl 1-methyl-1H-indole-3-carboxylate) to the 5-HT₄R in guinea pig striatum membranes¹⁹ in comparison with reference ligands **1** and **4–6**, and the results of the binding studies are summarized in Tables 1 and 2.

In agreement with the results obtained with the previously published quinoline derivatives **7a** and **b**,¹⁶ amide **7c**, was found to be virtually inactive, whereas the corresponding ester **7d** showed an inhibition constant value in the nanomolar range. Thus, the replacement of BM-1 of ester **7a** with BM-2 as in ester **7d** produced a negligible effect on the binding affinity, which could be further modulated by the presence of the substituents in position 4 of the piperidine moiety as in ester derivatives **7e** and **f**. In particular, the introduction of a $-\text{CH}_2\text{OH}$ group (as in **7e**) was well tolerated by the receptor binding site, whereas the presence of Boc-amino as in **7f** produced a significant decrease in the affinity.

Also in the small subseries of quinoline derivatives **7g–k** bearing BM-3 as the basic moiety, the amide member **7g** was found to be virtually inactive, while the corresponding ester **7h** showed an outstanding binding potency, thus BM-3 appeared to be the optimized basic moiety capable of maxi-

Scheme 3 Synthesis of target 2-quinolinecarboxamide derivative **9e**. Reagents: (i) (1-butylpiperidin-4-yl)methanamine, BOP, TEA, DMF.

Fig. 4 Structure of target compound **9a** found by crystallography. Ellipsoids enclose 50% probability.

mizing the productive interaction of these quinoline ligands with the 5-HT₄R binding site. Thus, the subnanomolar affinity shown by ester **7h** led us to explore further the structure–affinity relationships by changing the substituent in position 2 of the quinoline nucleus. In particular, the replacement of the methoxy group of **7h** with a chlorine atom as in compound **7i** produced a negligible effect on the 5-HT₄R binding affinity, but in the small subseries of ester derivatives **7h–k**, the highest binding potency was shown by the cyclopropyl derivative **7j** ($K_i = 0.44$ nM), while the unsubstituted derivative **7k** showed an inhibition constant value in the nanomolar range ($K_i = 1.5$ nM).

The analysis of the binding data obtained with quinoline derivatives **9a–e** (Table 2) showed not only interesting analogies but also some apparent discrepancies with the structure–affinity relationship trends discussed above. First, in agreement with the results obtained with amide derivatives **7b** and **c**, the corresponding amide derivatives **9a** and **b** were found to be virtually inactive. By contrast, BM-3 based amide **9c** showed an inhibition constant value in the nanomolar range. This apparent discrepancy seemed to suggest that the stereoelectronic features of AM-3 caused differences in the interaction of this compound with the 5-HT₄R binding site with respect to that of subnanomolar ligands **7h–j**. In particular, X-ray diffraction data obtained with single crystals of compounds **9a** and **e** suggested the presence of an intramolecular H-bond between the amide NH group and quinoline nitrogen. This interaction rigidified the structure of compounds **9a–e** so that the amide carbonyl was coplanar with the aromatic moiety and the chlorine atom could play a significant role in the interaction with the receptor.

Fig. 5 Structure of target compound **9e** monohydrate found by crystallography. Ellipsoids enclose 50% probability.

Table 1 Affinity of quinoline ligands **7a–k** for the 5-HT₄R

Compd	X	R	BM	K_i (nM) $\pm$ SEM ^a
7a^b	O	OCH ₃		52
7b^b	NH	OCH ₃		>1000
7c	NH	OCH ₃		>1000
7d	O	OCH ₃		15 $\pm$ 2.3
7e	O	OCH ₃		8.0 $\pm$ 1.5
7f	O	OCH ₃		304 $\pm$ 62
7g	NH	OCH ₃		>1000
7h	O	OCH ₃		0.62 $\pm$ 0.05
7i	O	Cl		0.86 $\pm$ 0.15
7j	O			0.44 $\pm$ 0.11
7k	O	H		1.5 $\pm$ 0.27

^a Each value is the mean $\pm$ SEM of 3 independent determinations performed in duplicate and represents the apparent affinity constant assessed in the [³H]GR113808 (final concentration 0.2 nM) specific binding assay to guinea pig striatum membranes. ^b See ref. 16.

In fact, the replacement of the chlorine atom of **9c** with a methoxy group as in compound **9d** produced a significant decrease in the receptor affinity, confirming the importance of the chlorine atom. However, its replacement with a hydrogen atom and the simultaneous introduction of a hydroxy group in position 8 of the quinoline nucleus as in compound **9e** was tolerated by the receptor, suggesting that closely related compounds may adopt different binding modalities in the interaction at the 5-HT₄R.

3. Conclusions

The structure–affinity relationship studies in the class of previously published quinoline derivatives **7a** and **b** were deepened by replacing their basic moiety with that of partial agonist **4** or with that of reference ligand **6**. Furthermore, the aromatic moieties of 4-quinolinecarboxylates **7a**, **d–f**, and **h–k** or 4-quinolinecarboxamides **7b**, **c**, and **g** were modified into those of 2-quinolinecarboxamides **9a–e**. Very interestingly, this study led to the discovery of some novel 5-HT₄R ligands

showing K_i values in the subnanomolar range. In fact, in the subseries of 4-quinolinecarboxylates **7d–f** and **h–k**, cyclopropyl derivative **7j** showed an inhibition constant of 0.44 nM that was slightly lower than that of the corresponding methoxy-derivative **7h** (K_i = 0.62 nM) or the one shown by chloro-derivative **7i** (K_i = 0.86 nM). All these compounds show the *N*-butyl-4-piperidinylmethyl BM-3 substituent in their structure. Therefore, BM-3 emerged as an optimized basic moiety for the interaction of these 4-quinolinecarboxylates with the 5-HT₄R binding site. Curiously, BM-3 was ineffective in providing 5-HT₄R affinity in the corresponding 4-quinolinecarboxamide **7g**, but it did in 2-quinolinecarboxamide ligands **9c–e**. Thus, the interaction with the 5-HT₄R binding site appeared to be regulated by the subtle interrelationship of structural parameters such as the kind of basic moiety, the position of the carbonyl linking group with respect to the aromatic moiety and its orientation, which could be affected by the presence of the intramolecular H-bond as in compounds **9c–e**.

Experimental procedures were in compliance with national and international laws and policies on the protection

Table 2 Affinity of 2-quinolinecarboxamide ligands **9a–e** for the 5-HT₄R

Compd	R ₁	R ₂	BM	K _i (nM) ± SEM ^a
9a	Cl	H		>1000
9b	Cl	H		>1000
9c	Cl	H		39 ± 5.5
9d	OCH ₃	H		148 ± 55
9e	H	OH		76 ± 29
1				20 ± 5.5
4				6.0 ± 1.5
5				19 ± 3.5
6				3.9 ± 0.55

^a Each value is the mean ± SEM of 3 independent determinations performed in duplicate and represents the apparent affinity constant assessed in the [³H]GR113808 (final concentration 0.2 nM) specific binding assay to guinea pig striatum membranes.

of animals used for scientific purposes (Italian Legislative Decree 26/2014 that transposed the EU Directive 2010/63/EU; Guide for the Care and Use of Laboratory Animals, U.S. National Research Council, 2011), approved by the Rottapharm Biotech Review Board, and authorized by the Italian Minister of Health.

Author contributions

Synthesis and preliminary characterization: F. C., M. P., G. G., C. M. F., A. R., G. G., and M. A.; crystallographic studies: G. G.; binding assays: L. M., C. S., M. L., and G. C.; design of experiments, analysis of results, supervision, and writing of the paper: A. C.

Conflicts of interest

The authors declare no conflict of interest.

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