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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 2769–2772

Synthesis and biological evaluation of a novel structural type of serotonin 5-HT$_3$ receptor antagonists

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Received 30 September 2005; revised 20 January 2006; accepted 2 February 2006
Available online 28 February 2006

Abstract—A series of novel 3-substituted quinoxalin-2-carboxamides were designed as per the pharmacophoric requirement for 5-HT$_3$ receptor antagonists and prepared by microwave irradiation and also by conventional method. The compounds were characterized by spectral data (IR, ^{1}H NMR, and MS) and the purity was ascertained by microanalysis. The synthesized compounds were evaluated for 5-HT$_3$ antagonisms in longitudinal muscle-myenteric plexus preparation from guinea pig ileum against 5-HT$_3$ agonist, 2-methyl-5-HT. Among the test compounds, *N*-{3-[(4-methylpiperazin-1-yl)methyl]-4-hydroxyphenyl}-3-methoxyquinoxalin-2-carboxamide **4e** showed most favorable 5-HT$_3$ receptor antagonism.

Serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter involved in various pharmacological effects in several peripheral and central nervous systems.[1] Fifteen 5-HT receptor subtypes belonging to 7 major classes (5-HT$_1$–5-HT$_7$) have been reported so far.[2] Recently, 5-HT$_3$ receptor subtype has gained much attention because of the clinical use of 5-HT$_3$ receptor antagonists (RAs) in the treatment of cancer chemotherapy-induced nausea and vomiting,[3] and also in postoperative nausea and vomiting.[4,5] Moreover, a number of preclinical studies suggest that 5-HT$_3$ RAs can be used in the treatment of various central nervous disorders such as anxiety, schizophrenia, drug abuse, withdrawal, and age-associated memory impairments.[6,7] Hibert et al.[8] proposed the pharmacophore of 5-HT$_3$ RAs, which consists of three components: an aromatic ring, a carbonyl-containing linking moiety, and a basic center in a specific spatial arrangement (Fig. 1). Based on this pharmacophore, a number of molecules have been reported so far.[9,10] In view of the various therapeutic implications of 5-HT$_3$ RAs and in continuation of our interest in developing novel 5-HT$_3$ RAs,[11–13] our efforts were focused on introducing different substituents in the 3rd position of the quinoxaline moiety of compound **1**[11]

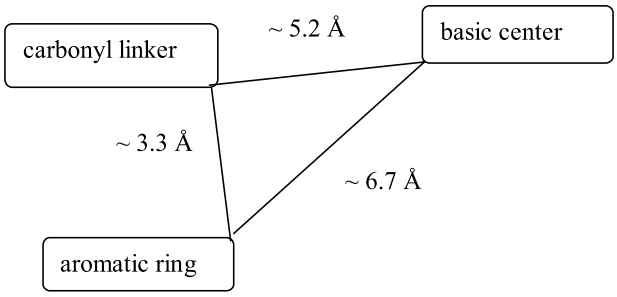

Figure 1. Pharmacophore of 5-HT$_3$ receptor antagonists.

(see Fig. 2). In the present paper, we describe the synthesis of a structurally novel series of 3-substituted quinoxalin-2-carboxamides and evaluated their 5-HT$_3$ antagonistic activities in the longitudinal muscle-myenteric plexus (LMMP) preparation of guinea pig ileum against 5-HT$_3$ agonist, 2-methyl-5-HT.

The title compounds, 3-substituted quinoxalin-2-carboxamides, were designed according to the pharmacophoric requirements (proposed by Hibert et al.[8]) for 5-HT$_3$ RAs. The pharmacophore consists of three components (Fig. 1): an aromatic/heteroaromatic ring, a carbonyl-containing linking moiety, and a basic center in a specific spatial arrangement. In the proposed series, the least energy conformation of the molecules was

Keywords: 5-HT3 receptor antagonist; Quinoxalin-2-carboxamides.
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doi:10.1016/j.bmcl.2006.02.006

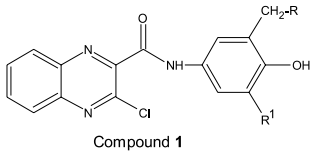
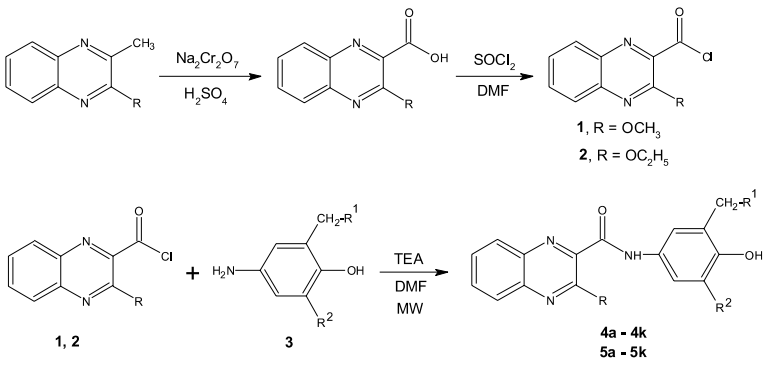


Figure 2.

generated by Tripos-Alchemy 2000 software (Tripos Associated Inc., St. Louis, USA) and the pharmacophoric distances were measured from the heteroaromatic ring to carbonyl oxygen, carbonyl oxygen to basic nitrogen of Mannich derivatives, and heteroaromatic ring to basic nitrogen. The molecules identified for synthesis comply with the pharmacophoric model.[8]

The synthetic procedures are illustrated in Scheme 1. The starting material, 2-methoxy-3-methylquinoxaline, was prepared as per the procedure described by Cheeseman,[14] and 2-ethoxy-3-methylquinoxaline was prepared as per the procedure described by Newbold and Spring[15] The intermediates, Mannich derivatives of *p*-aminophenol, were prepared as per our previously reported method.[16] 2-Methoxy-3-methylquinoxaline, and 2-ethoxy-3-methylquinoxaline on oxidation with a mixture of sodium dichromate and sulfuric acid, as per our previously reported method,[13] afforded 3-methoxyquinoxalin-2-carboxylic acid,[17] and 3-ethoxyquinoxalin-2-carboxylic acid,[18] respectively. The obtained product was refluxed with excess thionyl chloride and few drops of dimethylformamide for about 1 h. Excess thionyl chloride was removed under reduced pressure to obtain the crude acid chloride. To the solution of acid chloride in DMF, a mixture of appropriate hydrochloride salt of Mannich derivatives of *p*-aminophenol[16] in DMF and triethylamine was added. This reaction mexture was then irradiated with microwaves for 2 min at 720 W. DMF was removed using rotary flask evaporator and to the residue, water was added. The separated product was filtered, washed with water, dried, and recrystallized from ethanol–acetone mixture to give **4** and **5**, respectively. When this reaction was carried out by conventional heating (oil bath), the reaction was completed (monitored by TLC) in 6–8 h. The product obtained by both methods was identical in all aspects (mp, mixed mp, co-TLC, and superimposable IR). Almost similar yields were obtained by both the methods. It was observed that the reaction was accelerated greatly when carried out in microwave environment. All the synthesized compounds were characterized by spectral (IR, ^{1}H NMR, and MS) and elemental analysis data. IR spectral analysis of the final compounds (**4a**–**4k** and **5a**–**5k**) showed absorption bands at ~3400, ~3200, and ~1670 cm^{-1} due to OH, NH, and C=O functions, respectively. In ^{1}H NMR spectra, OH showed singlet at $\delta \sim 11.5$, NH showed singlet at $\delta \sim 9.8$, and aromatic protons showed multiplet in the range of δ 7.1–8.4. Elemental analysis indicated that the calculated and observed values were within the acceptable limits (±0.4%). Physical data of the final compounds are given in Table 1.

The Institutional Animal Ethics Committee of the Birla Institute of Technology & Science, Pilani, India, approved the experimentation on animals (Protocol No. IAEC/RES/6, dated 21.04.03). Male Dunkin Hartley guinea pigs (250–300 g; Hissar Agricultural University, Hissar, Haryana, India) were sacrificed by cervical dislocation. The abdomen was cut open and a length of ileum was excised about 2 cm from the ileo-caecal junction. The longitudinal muscle-myenteric plexus (LMMP), 3–4 cm in length, was prepared and mounted as described by the literature method.[19] The tissue was equilibrated for 30 min. under a resting tension of 500 mg and constant aeration in a 40 ml organ bath containing Tyrode solution maintained at ca. 37 °C. Noncumulative concentrations of 2-methyl-5-HT (Tocris, UK) were added with a 15 min dosing cycle (to prevent desensitization) and left in contact with the tissue until the maximal contraction had developed. To study the antagonist effect of the test compounds on the response evoked by 2-methyl-5-HT, the compounds were added to the organ bath and left in contact with the tissue for at least 10 min prior to the addition of 2-methyl-5-HT. The contractions were recorded using a T-305 Force transducer coupled to a Student's physiograph (Bio Devices, Ambala, India). Antagonism was expressed in the form of pA_2 values, which were graphically determined.[20] The pA_2 values of the test compounds were compared with the standard antagonist Ondansetron (Natco Pharma, Hyderabad, India). The observed pharmacological data are represented in Table 1.



Scheme 1.

Table 1. Physical and pharmacological data of compounds **4a–4k** and **5a–5k**

Compound	R	R^1	R^2	Yield (%)	Mp (°C)	Molecular formula[a]	Molecular weight[b]	Antagonism to 2-Me-5-HT pA_2[c]
4a	OCH_3	Pyrrolidinyl	H	61	156–158	$C_{21}H_{22}N_4O_3$	378	3.9
4b	OCH_3	Piperidinyl	H	65	170–171	$C_{22}H_{24}N_4O_3$	392	3.5
4c	OCH_3	Morpholinyl	H	62	124–126	$C_{21}H_{22}N_4O_4$	394	3.0
4d	OCH_3	Piperazinyl	H	67	161–163	$C_{21}H_{23}N_5O_3$	393	4.5
4e	OCH_3	*N*-Methyl-piperazinyl	H	69	149–150	$C_{22}H_{25}N_5O_3$	407	5.7
4f	OCH_3	*N*-Ethyl-piperazinyl	H	65	137–138	$C_{23}H_{27}N_5O_3$	412	4.9
4g	OCH_3	Pyrrolidinyl	Pyrrolidinyl	56	177–178	$C_{26}H_{31}N_5O_3$	461	<3.0
4h	OCH_3	Morpholinyl	Morpholinyl	55	143–145	$C_{26}H_{31}N_5O_5$	493	<3.0
4i	OCH_3	Piperazinyl	Piperazinyl	59	181–183	$C_{26}H_{31}N_7O_3$	492	4.0
4j	OCH_3	*N*-Methyl-piperazinyl	*N*-Methyl-piperazinyl	60	170–172	$C_{28}H_{37}N_7O_3$	520	4.8
4k	OCH_3	*N*-Ethyl-piperazinyl	*N*-Ethyl-piperazinyl	58	155–156	$C_{30}H_{41}N_7O_3$	548	4.1
5a	OC_2H_5	Pyrrolidinyl	H	63	194–196	$C_{22}H_{24}N_4O_3$	392	3.5
5b	OC_2H_5	Piperidinyl	H	65	207–208	$C_{23}H_{26}N_4O_3$	406	3.1
5c	OC_2H_5	Morpholinyl	H	63	159–160	$C_{22}H_{24}N_4O_4$	408	<3.0
5d	OC_2H_5	Piperazinyl	H	66	196–197	$C_{22}H_{25}N_5O_3$	407	3.4
5e	OC_2H_5	*N*-Methyl-piperazinyl	H	67	182–183	$C_{23}H_{27}N_5O_3$	421	4.6
5f	OC_2H_5	*N*-Ethyl-piperazinyl	H	65	169–170	$C_{24}H_{29}N_5O_3$	435	3.9
5g	OC_2H_5	Pyrrolidinyl	Pyrrolidinyl	55	219–220	$C_{27}H_{33}N_5O_3$	475	<3.0
5h	OC_2H_5	Morpholinyl	Morpholinyl	56	177–179	$C_{27}H_{33}N_5O_5$	507	<3.0
5i	OC_2H_5	Piperazinyl	Piperazinyl	58	211–212	$C_{27}H_{33}N_7O_3$	506	<3.0
5j	OC_2H_5	*N*-Methyl-piperazinyl	*N*-Methyl-piperazinyl	54	202–203	$C_{29}H_{39}N_7O_3$	534	<3.0
5k	OC_2H_5	*N*-Ethyl-piperazinyl	*N*-Ethyl-piperazinyl	57	189–190	$C_{31}H_{43}N_7O_3$	562	<3.0

[a] Elemental (C, H, and N) analysis indicated that the calculated and observed values were within the acceptable limits (±0.4%).
[b] Molecular weight determination by mass spectral analysis.
[c] Results are means from three separate experiments except for those pA_2 values <3.0, which are means of two separate experiments. SE was less than 10% of the mean.

In the present study, we have demonstrated the synthesis and 5-HT_3 receptor antagonistic activity of novel 3-substituted quinoxalin-2-carboxamides in the isolated guinea pig ileum. Ethoxy group (**5a–5k**) in the 3rd position of the quinoxaline moiety showed lesser 5-HT_3 antagonism than the methoxy group (**4a–4k**), which is lesser than the chloro group (compound **1**[13]). Piperazines attached with methylene group of *p*-aminophenol (**4d–4f**, **4i–4k**, and **5d–5f**) exhibit higher 5-HT_3 antagonism than other cyclic secondary amines (**4a–4c**, **4g**, **4h**, and **5a–5c**). These results clearly indicate that N^4 piperazine is essential for activity. Compounds **4d**, **5d** (with no substitution at N^4 piperazine) showed pA_2 4.5 and 3.4, respectively; with increased lipophilicity (i.e., methyl group, **4e**, **5e**) activity increased (pA_2 5.7 and 4.6, respectively). Further increase in lipophilicity (i.e., ethyl group, **4f**, **5f**) decreased the activity (pA_2 4.9 and 3.9, respectively). It has been observed that when the side chain of the 3-substituted quinoxalin-2-carboxamides is attached with bis Mannich derivatives of *p*-aminophenol (**4g–4k** and **5g–5k**) the antagonism decreased when compared to mono Mannich derivatives of *p*-aminophenol (**4a–4f** and **5a–5f**). Most of the reported 5-HT_3 RAs contain aromatic/heteroaromatic esters or amides having bridged bicyclic amines like azabicyclo[2.2.2]octane,[21] azabicyclo[3.2.1]octane[22] and azabicyclo[3.3.1]nonane[21,22] for the basic nitrogen in the pharmacophore and moreover the bicyclic amine is directly attached to the amide/ester moiety. In contrary, we have developed novel molecules which contain heteroaromatic amide having monocyclic amine viz., pyrrolidine, piperidine, morpholine, piperazine, and 4-substituted piperazines for the basic nitrogen and also the monocyclic amines are attached to the amide moiety through a benzyl group.

Acknowledgments

This research was supported by grants from the University Grants Commission, New Delhi, India. We are thankful to the CSIR, New Delhi, India, for awarding SRF to one of the authors (R.V.P.). We are grateful to the Birla Institute of Technology & Science, Pilani, for providing necessary facilities to carry out this research and the RSIC, Punjab University, Chandigarh, India, for providing spectral and analytical data.

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17. Yield 76%; mp 101–102 °C. IR (KBr) (cm^{-1}): 3303, 1701, 1594. ^1H NMR (300 MHz) ($CDCl_3$) (δ) ppm: 3.71 (s, 3H, OCH_3), 7.71 (d, 2H, H_6 and H_7 quinoxaline), 8.17 (d, 2H, H_5 & H_8 quinoxaline), 12.60 (s, 1H, CO_2H). Anal. Calcd for $C_{10}H_8N_2O_3$: C, 55.04; H, 3.95; N, 13.72. Found. C, 55.31; H, 4.17; N, 13.51.
18. Yield 73%; mp 120–121 °C. IR (KBr) (cm^{-1}): 3310, 1706, 1585. ^1H NMR (300 MHz) ($CDCl_3$) (δ) ppm: 1.31 (t, 3H, CH_3), 3.97 (q, 2H, CH_2), 7.67 (d, 2H, H_6 and H_7 quinoxaline), 8.10 (d, 2H, H_5 and H_8 quinoxaline), 12.67 (s, 1H, CO_2H). Anal. Calcd for $C_{11}H_{10}N_2O_3$: C, 60.55; H, 4.62; N, 12.84. Found. C, 60.31; H, 4.40; N, 13.02.
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