Pharmacophore Based Synthesis of 3-Chloroquinoxaline-2-carboxamides as Serotonin₃ (5-HT₃) Receptor Antagonist

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A series of 3-chloroquinoxaline-2-carboxamides were designed and prepared by the condensation of 3-chloro-2-quinoxaloylchloride with appropriate Mannich bases of the *p*-aminophenol in the microwave environment. The synthesized compounds were evaluated for serotonin₃ (5-HT₃) receptor antagonistic activities in longitudinal muscle-myenteric plexus (LMMP) preparation from guinea pig ileum against the 5-HT₃ agonist, 2-methyl-5-HT. Compound 3g exhibited comparable 5-HT₃ antagonistic activity (pA_2 6.4) to that of standard antagonist Ondansetron (pA_2 6.9), while the other compounds exhibited mild to moderate 5-HT₃ antagonistic activities.

Key words quinoxalinecarboxamide; Mannich base; serotonin₃ (5-HT₃) receptor antagonist

Chemotherapy regimens for the treatment of cancer are unfortunately well known for their toxicity rather than for their efficacy. Although some of the toxic effects may be life threatening, patients are often most fearful of the nausea and vomiting caused by cancer chemotherapy.[1] Prevention and control of nausea and vomiting are paramount in the treatment of cancer patients. Nausea and vomiting can result in serious metabolic derangements, nutritional depletion and anorexia, esophageal tears, wound dehiscence, deterioration of patient's physical and mental status which prompts him to discontinue the potentially useful and curative antineoplastic treatment.[2] The recent developments of serotonin₃ (5-HT₃) antagonists have dramatically improved the treatment of emesis induced by anticancer therapy. However, they are ineffective in 10—30% of the patients,[3] and also very few selective 5-HT₃ antagonists are available; since they possess asymmetric centers, the synthetic cost of these antagonists is high.[4] Several studies suggest a role of 5-HT₃ antagonists in the treatment of central nervous system disorders such as anxiety, schizophrenia, drug abuse and withdrawal, and age-associated memory impairments.[5] To overcome the above drawbacks and due to their etiology in several disorders, there is a need to develop more selective 5-HT₃ antagonists. The key pharmocophoric requirements of 5-HT₃ antagonists include an aromatic moiety, a linking acyl group and a basic amine of appropriate position and dimension.[6] Based on these pharmacophoric requirements, 3-chloroquinoxaline-2-carboxamides were designed using Tripos-Alchemy-2000 software and synthesized as shown in Fig. 1. The New Chemical Entities (NCEs) were evaluated for 5-HT₃ antagonism in longitudinal muscle-myenteric plexus (LMMP) preparation from guinea pig ileum against the 5-HT₃ agonist, 2-methyl-5-HT.

CHEMISTRY

Melting points were determined in open capillaries using Buchi 530 apparatus without correction. The purity of the compounds was checked by TLC using silica gel coated Al. plates (Merck) and the spots were visualized under ultra violet light at 254 and 366 nm. Microwave irradiations were carried out in domestic microwave oven (LG Electronics, model MG-605AP, 2450 MHz, 900 W). IR spectra were recorded in KBr pellets on an IR Prestige-21 FT IR spectrophotometer (cm⁻¹), ¹H-NMR spectra on a Bruker DRX300 spectrometer using tetramethylsilane as internal standard (chemical shifts in δ, ppm), mass spectra on a VG-70-S mass spectrometer and elemental analysis on a Perkin Elmer 2400 CHN ele-hyphen mental analyzer. The starting compound, 2-chloro-3-methylquinoxaline, was prepared as per the procedure described by Krishnan *et al.*,[7] and Mannich derivatives of *p*-aminophenol were prepared as per the method described by Mahesh *et al.*[8]

Synthesis of 3-Chloroquinoxaline-2-carboxylic Acid To a solution of sodium dichromate (8.9 g, 30.00 mmol) in H₂O (15 ml), 2-chloro-3-methylquinoxaline (3.5 g, 20.00 mmol) was added with stirring. To this H₂SO₄ (11.5 ml) was added drop by drop with occasional cooling. After the addition of the acid, the reaction mixture was heated on water bath for 30 min, followed by cooling and filtration. To the obtained residue 5% H₂SO₄ (12 ml) was added and digested for 5 min. After cooling, the product was filtered and dissolved in 5% NaOH solution and refiltered to remove any chromium hydroxide. The filtrate was acidified with 5% H₂SO₄ and the resultant product was filtered, washed with H₂O, dried and recrystallized from ethanol–acetone mixture to give the desired compound 2.7 g in 65% yield as a white colour solid. mp >300 °C. ¹H-NMR (DMSO-d_6) δ: 7.58 (2H, d, J=12.0 Hz, H₆, H₇ quinoxaline), 8.06 (2H, d, J=12.0 Hz, H₅, H₈ quinoxaline), 12.78 (1H, s, CO₂H). IR (KBr) cm⁻¹: 3269, 1688, 1521. MS *m/z*: 208 (M⁺). *Anal.* Calcd for C₉H₅ClN₂O₂: C, 51.92; H, 2.40; N, 13.46. Found: C, 51.70; H, 2.31; N, 13.72.

Synthesis of 3-Chloro-*N*-{3-[(dimethylamino)methyl]-4-hydroxyphenyl}quinoxaline-2-carboxamide (3a; R= N(CH₃)₂, R′=H) A mixture of 3-chloroquinoxaline-2-carboxylic acid (1.04 g, 5.00 mmol), thionyl chloride (10 ml) and 4 drops of dimethyl formamide (DMF) was refluxed for 1 h. Excess thionyl chloride was removed under reduced pressure to obtain the crude acid chloride. To the acid chloride DMF (2 ml) was added and this solution was added slowly to a solution of hydrochloride salt of 4-amino-2-[(dimethylamino)methyl]phenol[8] (1.01 g, 5.00 mmol) in DMF (2 ml) and triethylamine (1.5 ml, 10.00 mmol). This reaction

mixture was then irradiated with microwaves for 2 min at 720 Watt. DMF was removed using rotary flash evaporator and to the residue H$_2$O (10 ml) was added. The obtained product was collected by filtration and dried. The crude product was recrystallized from ethanol to give the desired compound 1.02 g in 57% yield as a yellow solid. mp 225—226 °C. ^{1}H-NMR (CDCl$_3$) δ: 2.28 (6H, s, N(CH$_3$)$_2$), 3.74 (2H, s, CH$_2$–N(CH$_3$)$_2$), 7.12—8.34 (7H, m, ArH), 9.86 (1H, br s, CONH), 11.47 (1H, br s, OH). IR (KBr) (cm^{-1}): 3406, 3217, 1670, 1545. MS m/z: 356 (M+). *Anal.* Calcd for C$_{18}$H$_{17}$ClN$_4$O$_2$: C, 60.67; H, 4.77; N, 15.73. Found: C, 60.93; H, 4.56; N, 16.03. Compounds (**3b**—**n**) were prepared by the same methodology. Physical data of compounds (**3a**—**n**) are listed in Table 1. To make the compounds soluble in water (for pharmacological evaluation), the free bases were converted into the corresponding hydrochloride salts as described by Blackburn *et al.*[9]

Fig. 1.   Synthesis of 3-Chloroquinoxaline-2-carboxamides

Fig. 2.   Pharmacophore of 5-HT$_3$ Receptor Antagonists

PHARMACOLOGY

Evaluation of 5-HT$_3$ Antagonism in the LMMP of Guinea Pig Ileum[10]   Experimentation on animals was approved by the Institutional Animal Ethics Committee of the Birla Institute of Technology & Science, Pilani, India. (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 LMMP, 3—4 cm in length was prepared and mounted as described by Paton and Zar.[11] 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. Non-cumulative concentrations of 2-methyl-5-HT (Tocris, U.K.) 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-methy-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.[12] The pA_2 values of the test compounds were compared with the standard antagonist Ondansetron (Natco Pharma, Hyderabad, India).

RESULTS AND DISCUSSION

The title compounds, 3-chloroquinoxaline-2-carboxamides, were designed according to the pharmacophoric requirements (proposed by Hibert *et al.*[6]) for 5-HT$_3$ receptor antagonists. The pharmacophore consists of three components (Fig. 2): 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 were generated by Tripos-Alchemy 2000 software (Tripos Associates Inc., St. Louis, U.S.A.) and the pharmacophoric distances were measured from the heteroaromatic ring to carbonyl oxygen, carbonyl
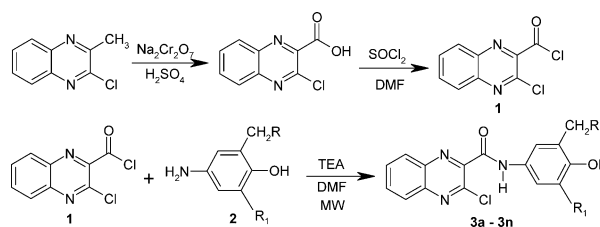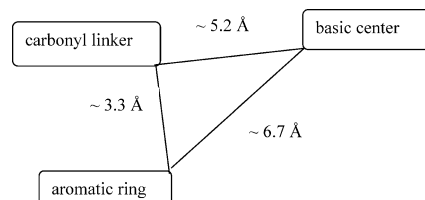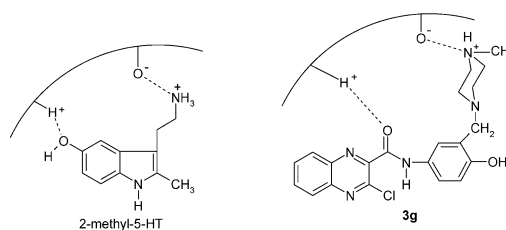

Fig. 3.   Possible Interaction of 2-Methyl-5-HT and **3g** with the 5-HT$_3$ Receptor

oxygen to basic nitrogen of mannich derivatives and heteroaromatic ring to basic nitrogen. The molecules identified for synthesis complies with the pharmacophoric model.[6]

The 3-chloroquinoxaline-2-carboxamides were prepared by the condensation of 3-chloro-2-quinoxaloylchloride (**1**) with Mannich derivatives of *p*-aminophenol (**2**) in microwave environment (Fig. 1). This method was optimized on the basis of the conventional method in which the title compounds were prepared by refluxing the mixture of 3-chloro-2-quinoxaloylchloride, appropriate Mannich derivates of *p*-aminophenol, DMF and triethylamine for about 6 h. 3-Chloro-2-quinoxaloylchloride was prepared by the oxidation of 2-chloro-3-methylquinoxaline by sodium dichromate and sulfuric acid mixture,[13] followed by chlorination with thionyl chloride. 2-Chloro-3-methylquinoxaline was prepared according to the method of Krishnan *et al.*[7] by the reaction of *o*-phenylene diamine and pyruvic acid to yield 3-methylquinoxalin-2-ol, followed by chlorination with POCl$_3$. Mannich derivates of *p*-aminophenol were prepared[8] by the reaction of paracetamol, dialkylamine and formaldehyde followed by acid hydrolysis, which is a modified method of Burckhalter *et al.*[14] and Stout *et al.*[15] These compounds were evaluated for their 5-HT$_3$ antagonistic activity in the LMMP preparation from guinea pig ileum. The 5-HT$_3$ antagonism of the 3-chloroquinoxaline-2-carboxamides is represented as pA_2 and is shown in Table 1. From the fourteen compounds tested, compound **3g** showed higher antagonism (pA_2 6.4) than other compounds but lesser than standard antagonist Ondansetron (pA_2 6.9). Other compounds (**3a**—**f**, **3h**—**n**) exhibited mild to moderate antagonist activity. It has

Table 1.   Physical and Pharmacological Data of Compounds **3a**—**n**
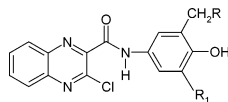
Compd.	R	R_1	Yield (%)	mp (°C)	Molecular formula[a]	Molecular weight[b]	Antagonism to 2-methyl-5-HT pA_2[c]
3a	Dimethylamino	H	57	225—226	$C_{18}H_{17}ClN_4O_2$	356	2.0
3b	Diethylamino	H	63	210—211	$C_{20}H_{21}ClN_4O_2$	384	2.7
3c	Pyrrolidinyl	H	56	218—219	$C_{20}H_{19}ClN_4O_2$	382	4.8
3d	Piperidinyl	H	56	236—239	$C_{21}H_{21}ClN_4O_2$	396	4.6
3e	Morpholinyl	H	64	188—190	$C_{20}H_{19}ClN_4O_3$	398	4.0
3f	Piperazinyl	H	66	221—223	$C_{20}H_{20}ClN_5O_2$	397	5.4
3g	*N*-Methylpiperazinyl	H	65	211—213	$C_{21}H_{22}ClN_5O_2$	411	6.4
3h	*N*-Ethylpiperazinyl	H	59	202—205	$C_{22}H_{24}ClN_5O_2$	425	5.9
3i	Diethylamino	–CH$_2$-diethylamino	60	222—224	$C_{25}H_{32}ClN_5O_2$	470	2.2
3j	Pyrrolidinyl	–CH$_2$-pyrrolidinyl	55	229—231	$C_{25}H_{28}ClN_5O_2$	465	4.0
3k	Morpholinyl	–CH$_2$-morpholinyl	62	205—208	$C_{25}H_{28}ClN_5O_4$	497	3.3
3l	Piperazinyl	–CH$_2$-piperazinyl	65	240—243	$C_{25}H_{30}ClN_7O_2$	496	5.0
3m	*N*-Methylpiperazinyl	–CH$_2$-*N*-methylpiperazinyl	63	232—234	$C_{27}H_{34}ClN_7O_2$	524	6.0
3n	*N*-Ethylpiperazinyl	–CH$_2$-*N*-ethylpiperazinyl	59	218—220	$C_{29}H_{38}ClN_7O_2$	552	5.6
Ondansetron							6.9

a) Elemental (C, H, N) analysis indicated that the calculated and observed values were within the acceptable limits ($\pm 0.4\%$).   *b*) Molecular weight determination by mass spectral analysis.   *c*) Values are the means from three separate experiments. S.E. was less than 10% of the mean.

been hypothesized that two electrostatic interactions[16] are possible with the 5-HT$_3$ receptors, one with the oxygen atom and another with the basic nitrogen, as shown in Fig. 3. We observed that when the side chain of the 3-quinoxaline-2-carboxamides is attached with bis Mannich derivaties of *p*-aminophenol the antagonism decreased when compared to mono Mannich derivatives of *p*-aminophenol.

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