Novel Antagonists of 5-HT_3 Receptors. Synthesis and Biological Evaluation of Piperazinylquinoxaline Derivatives

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A series of piperazinylquinoxalines has been synthesized and studied as 5-HT_3 receptor antagonists in different preparations. Antagonism to 5-HT in the longitudinal muscle of the guinea pig ileum was particularly prominent in cyanoquinoxaline derivatives with an alkyl substitutuent on the piperazine moiety. The pA_2 of some selected compounds against the 5-HT_3 agonist 2-methyl-5HT in the guinea pig ileum was in the range of tropisetron or ondansetron, and one of them, **7e**, was more potent than these reference compounds by approximately 2 or 3 orders of magnitude. However, these compounds were markedly less potent than either tropisetron or ondansetron as displacers of ^{3}H-BRL 43694 binding to rat cortical membranes or as antagonists of the Bezold-Jarisch reflex in rats. Piperazinylcyanoquinoxalines represent a new class of 5-HT_3 antagonists with a selective effect on guinea pig peripheral receptors.

Introduction

Serotonin fulfills multiple physiological roles in both the central and peripheral nervous system by acting on a diversity of receptor subtypes[1] widely distributed in the organism.

Over the past few years, special attention has been paid to serotonin 5-HT_3 receptors. Some antagonists of this 5-HT receptor subtype (e.g., ondansetron or granisetron) are of high therapeutic interest in the prevention and treatment of vomiting associated with anticancer chemotherapy.[2] Another therapeutic use suggested for this class of compounds is the regulation of gastrointestinal motility, although typical prokinetic drugs such as metoclopramide or cisapride rather exert their effects through stimulation of serotonin 5-HT_4 receptors.[3-5] The psychiatric use of 5-HT_3 receptor antagonists in the treatment of anxiety disorders and schizophrenia is also the subject of intense investigation.[6,7]

The already developed 5-HT_3 receptor blockers do not seem to represent the ultimate answer to the problem of emesis. Among others, delayed emesis after chemotherapy continues to be a significant clinical problem.[8] Moreover, other therapeutic applications are envisaged for these compounds. Consequently, it is still of much interest to develop new 5-HT_3 antagonists. The probable heterogeneity of 5-HT_3 receptors in different animal species[9-11] perhaps suggests other hitherto unexpected uses for compounds selectively blocking some of the possible subtypes.

A previous publication described a series of piperazinylquinoxalines and their evaluation as serotonin agonists and neuronal serotonin reuptake inhibitors.[12] In this work, the authors discussed that the 6-CN derivatives appeared to have both serotonin mimetic and serotonin reuptake blocking properties. Other described arylpiperazines bind to 5-HT_3 serotonin receptors[13] but also to different 5-HT_1 receptor subtypes and to 5-HT_2 receptors.[14-16] For example, ^{3}H-quipazine, a piperazinylquinoline derivative, was initially reported to selectively label 5-HT_3 recognition sites in rat cerebral cortex;[17] however, this radioligand was later shown to also label 5-HT_{1B} and 5-HT_2 receptors.[14]

In the present study, we report the synthesis and initial pharmacological evaluation of several piperazinylquinoxaline derivatives as a novel class of 5-HT_3 receptor antagonists.

Chemistry

The general synthetic procedures used in this study are illustrated in Scheme I. By reaction of Beirut,[18,19] between benzofuroxan and malononitrile, according to the previously described method,[20] 3-amino-2-quinoxalinecarbonitrile 1,4-dioxide (**3**) was obtained. Treating this compound with sodium dithionite[21] allows for the obtainment of 3-amino-2-quinoxalinecarbonitrile (**4**), a product which by diazotization leads to the corresponding 3-chloro-2-quinoxalinecarbonitrile (**5**). Reaction of **5** with the corresponding arylpiperazines in an alcoholic medium leads to the 3-(arylpiperazinyl)-2-quinoxalinecarbonitriles **8**. By treating **5** with piperazine, **6** (R = H) is obtained, from which the corresponding alkylpiperazines **7**, R = alkyl, are generated.

3-Piperazinyl-2-quinoxalinecarbonitrile (**6**) is nitrated to the corresponding 6-nitroquinoxaline **9** which is reduced to the derived amine **10** through treatment with tin chloride.

With the aim of studying the importance of the cyano group in the quinoxaline system, 3-piperazinyl-2-quinoxalinecarbonitrile (**6**) was hydrolized to the corresponding derived amide **11**. The 2-ethoxycarbonyl derivatives (Scheme II) were prepared by reacting *o*-phenylenediamine (**12**) and diethyl ketomalonate (**13**) in order to obtain ethyl 3-hydroxy-2-quinoxalinecarboxylate (**14**). Treatment with $POCl_3$ leads to the substitution of the hydroxyl in position 3 by chloro in order to give **15**, which reacts with piperazine in order to give the substitution product, ethyl 2-piperazinyl-2-quinoxalinecarboxylate (**16**). Alkylation of **16** affords compounds of the general formula **17**.

All the compounds obtained were unequivocally identified by the corresponding elemental and spectroscopic analyses.

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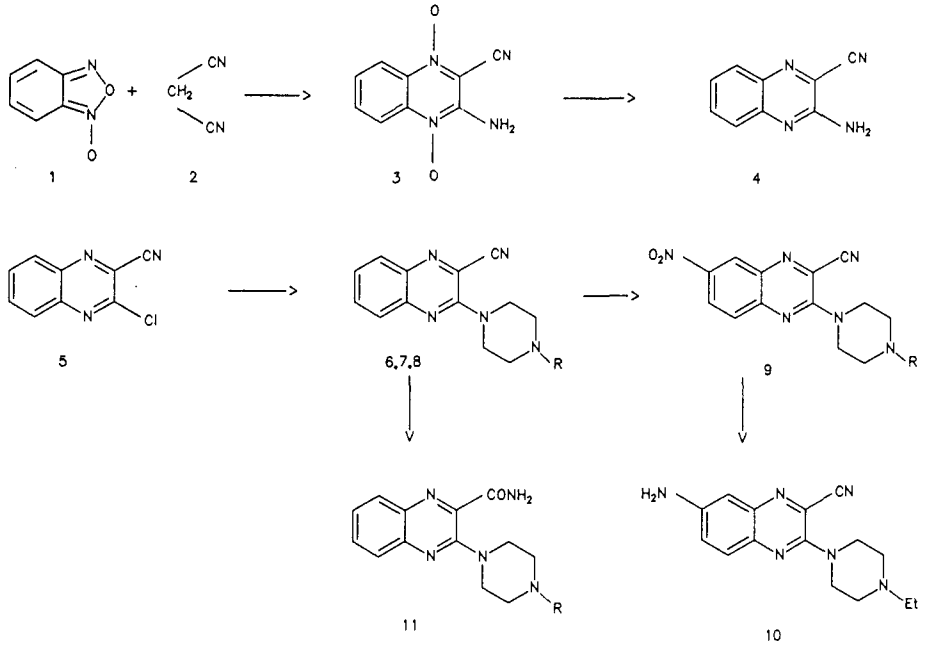
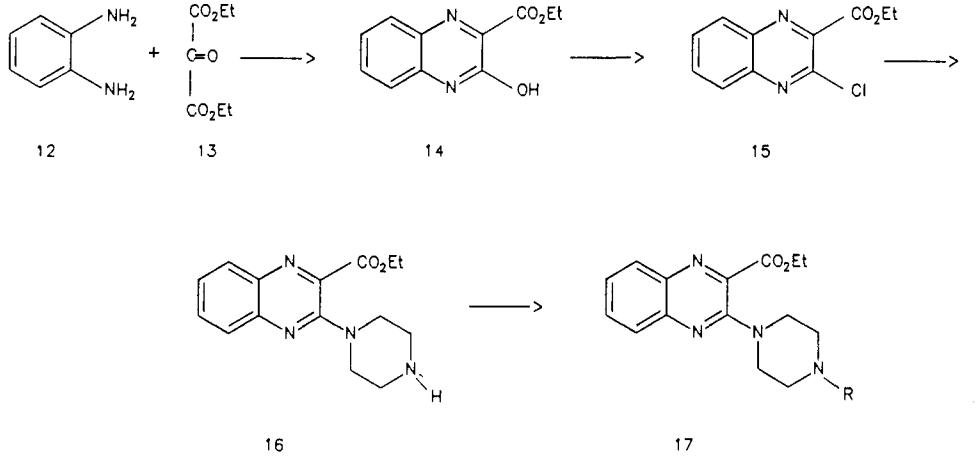
Scheme I



Scheme II



Biological Results and Discussion

The new quinoxaline derivaties were initially tested on the longitudinal muscle/myenteric plexus (LMMP) preparation of the guinea pig ileum, where two typical 5-HT_3 antagonists, ondansetron and tropisetron (10^{-5} M), inhibited the contraction induced by serotonin (10^{-5} M) by about 62 and 55%, respectively. Even though the contractile response to 5-HT in this preparation can be elicited by activation of other 5-HT receptors different from the 5-HT_3 subtype, this was considered to be a reliable initial screening test because 5-HT_3 receptors should be preferentially involved in the contractile response to this rather high 5-HT concentration.[22] Furthermore, all of the known 5-HT_3 antagonists clearly block this response. On the other hand, the correlation between binding studies to 5-HT_3 receptors from rat brain homogenates and the *in vivo* activity of these compounds is sometimes very poor.[23] Many of the new compounds were also tested for antagonism to 5-HT in the isolated rat thoracic aorta, a response presumably mediated by stimulation of 5-HT_2 receptors.

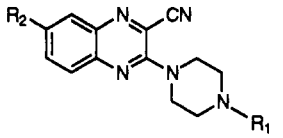
The results obtained with the new quinoxaline derivatives are depicted in Table I. Antagonism to 5-HT was particularly prominent in cyanoquinoxaline derivatives with an alkyl substituent on the piperazine moiety. Of special interest were the compounds **6**, **7a**, **7b**, and **7e**, which produced a marked inhibition on 5-HT-induced contractions in the LMMP as compared with the other new compounds. In contrast, when R1 was an aromatic radical (e.g., compounds **8e-8i**), the high antagonist activity was no longer observed. Substitution of the cyano group by carboxamide (compounds **11** in Scheme I) decreased the antagonist activity on the LMMP preparation. For example, the carboxamide analogs of compounds **6** and **7a** only inhibited the response to 5-HT by 10 and 42%, respectively.

Likewise, substitution of cyano by carboxyethyl (compounds **16** and **17** in Scheme II) also resulted in a lower antagonist effect in the same preparation. The corresponding carboxyethyl analogs of compounds **6** and **7a** reduced the 5-HT-reduced contraction by 24 and 34%, respectively.

Incubation of the aorta rings with different compounds, tested at a fixed concentration of 10^{-5} M, resulted in practically no reduction of the contraction induced by stimulation of 5-HT_2 receptors (Table I).

Table II shows the effect of the selected compounds on the contractions induced by 2-methyl-5-HT in guinea pig LMMP, as well as the results of binding studies to 5-HT_3

Table I. Antagonism by Piperazinylquinoxaline Derivatives of the Response to 5-HT in the Isolated Guinea Pig Ileum (Longitudinal Muscle Myenteric Plexus Preparation) and in the Isolated Rate Thoracic Aorta[a]



compd	R_1	R_2	guinea pig ileum % inhibition (mean ± SE)	rat aorta % inhibition (mean ± SE)
6	H	H	63.7 ± 4.9	9.1 ± 5.8
7a	CH_3CH_2–	H	80.5 ± 1.8	9.1 ± 2.9
7b	$CH_3(CH_2)_2$–	H	63.3 ± 3.7	12.5 ± 6.7
7c	$CH_3(CH_2)_3$–	H	12.5 ± 8.6	
7d	$CH_3(CH_2)_4$–	H	18.0 ± 5.0	3.9 ± 2.8
7e	CH_2=$CHCH_2$–	H	63.5 ± 1.9	9.1 ± 3.8
7f	EtO_2CCH_2–	H	14.4 ± 5.5	5.1 ± 4.6
7g	$PhCH_2CH_2$–	H	29.2 ± 12.5	25.0 ± 6.5
8a	CH_3–	H	48.4 ± 7.4	
8b	$PhCH_2$–	H	12.4 ± 5.5	20.5 ± 8.4
8c	3,4-$OCH_2OC_6H_3CH_2$–	H	28.4 ± 7.2	
8d	Ph–	H	21.9 ± 10.3	8.4 ± 3.9
8e	2-$CH_3OC_6H_4$–	H	10.6 ± 6.1	
8f	3-$CH_3OC_6H_4$–	H	3.5 ± 3.5	4.6 ± 0.9
8g	4-$CH_3C_6H_4$–	H	15.8 ± 2.7	4.6 ± 4.1
8h	4-ClC_6H_4–	H	11.0 ± 2.8	7.0 ± 3.2
8i	4-$NO_2C_6H_4$–	H	18.0 ± 3.0	
8j	3-$CF_3C_6H_4$–	H	39.5 ± 10.7	18.3 ± 5.6
8k	4-$CH_3COC_6H_4$–	H	7.4 ± 10.4	
8l	4-FC_6H_4–	H	21.4 ± 5.4	
9a	H	NO_2	7.7 ± 2.1	
9b	CH_3CH_2–	NO_2	21.3 ± 6.9	
10	CH_3CH_2–	NH_2	43.5 ± 12.3	
ondansetron			62.3 ± 5.0	0.6 ± 4.9
tropisetron			55.6 ± 8.6	2.4 ± 4.7

[a] All compounds tested at the fixed concentration of 10–5 M. Results are means ± SE from four to six experiments.

and 5-HT$_1$ receptors from rat brain. The *in vivo* test considered was the Bezold–Jarisch reflex in anesthetized rats.

The pA_2 of the selected compounds against the 5-HT$_3$ agonist 2-methyl-5-HT was in the range of ondansetron and tropisetron or higher. When 5-HT was used as an agonist in preparations previously desensitized to 5-methoxytryptamine, the pA_2 values for compounds **6** and **7e** were 6.7 and 9.3, respectively. In all cases the antagonists produced a parallel shift to the right in the concentration–response curve to 2-methyl-5-HT (or to 5-HT). This was obviously indicative of a competitive antagonism by test compounds. Compound **7e** was almost 2 orders of magnitude more potent than tropisetron. Interestingly, this compound was a comparatively weaker displacer of binding of ^{3}H-BRL 43694 to rat cortical homogenates, its K_i being in the range of ondansetron or tropisetron. These new compounds caused little or no displacement of ^{3}H-5-HT binding to 5-HT$_1$ receptors (Table II). When the same compounds were tested *in vivo* for the antagonism of the bradycardia induced by 5-HT in anesthetized rats (von Bezold–Jarisch reflex), all the selected compounds were at least 1 order of magnitude less potent than tropisetron. Regardless of pharmacokinetic considerations, these contrasting findings are probably indicative of the heterogeneity of 5-HT$_3$ receptors in different animal species.[9-11] Preliminary results obtained in ferrets in which nausea and vomiting was induced by oral administration of 2-methyl-5-HT showed that all of the compounds included in Table II were able to prevent the emetic episodes at doses of 0.1–1 mg/kg.

In short, these cyanoquinoxaline derivatives represent a new class of 5-HT$_3$ antagonists with varying potency in different *in vitro* or *in vivo* assays. The different pharmacological profile of some of these new compounds merits a further exploration.

Experimental Section

Chemistry. Melting points were determined using a Mettler FP82+FP80 apparatus and are uncorrected. Elemental analyses were obtained from vacuum-dried samples (over phosphorus pentoxide at 3-4 mmHg, 24 h at about 60–70 °C). Infrared spectra were recorded on a Perkin-Elmer 681 apparatus, using potassium bromide tablets for solid products and sodium chloride plates for liquid products; the frequencies are expressed in cm^{-1}. The ^{1}H-NMR spectra were obtained on a Brucker AC-200E (200 MHz) instrument, with tetramethylsilane as the internal reference, at a concentration of about 0.1 g/mL and with dimethyl sulfoxide-d_6 as the solvent; the chemical shifts are reported in ppm of tetramethylsilane in δ units. The mass spectra were recorded on a Hewlett-Packard 5988-A instrument at 70 eV.

Thin layer chromatography (TLC) was carried out on silica gel (DSF-5, Cammaga 0.3-mm thickness) with toluene/dioxane/acetic acid (90:25:4) as the solvent, and the plates were scanned under ultraviolet light = 254 and 366 nm. Column chromatography was carried out with silica gel 60 Merck (70–230 mesh ASTM) and indicated solvents.

Solvents were usually removed under vacuum or in a rotovapory evaporator when stated.

3-Amino-2-quinoxalinecarbonitrile 1,4-Dioxide (3). Benzofuroxan (1.36 g, 10 mmol) and malononitrile (0.7 g, 10.6 mmol) were dissolved in *N,N*-dimethylformamide (4 mL). The solution was cooled in an ice bath. A dissolution of triethylamine (0.2 mL) in *N,N*-dimethylformamide (3 mL) was added. The temperature of the mixture was kept below 25 °C. The mixture was maintained under these conditions for 1.5 h, after which it was filtered and washed with ethyl ether, obtaining **3** (1.51 g, 75% yield): mp = 190 °C (dioxane); ^{1}H-NMR (DMSO-d_6, 200 MHz) δ 7.62–8.31 (m, 4H, H_5, H_8, H_7, H_8), 8.06 (s, 2H, NH_2); IR 3350 (w), 3251 (w, NH_2), 2236 (w, CN), 1350 (s, NO), 766 (s), 608 (m, 2,3-quinoxaline) cm^{-1}. Anal. ($C_9H_6N_4O_2$) C, H, N.

3-Amino-2-quinoxalinecarbonitrile (4). 3-Amino-2-quinoxalinecarbonitrile 1,4-dioxide (**3**) (2 g, 9.9 mmol) was suspended in methanol (18 mL). The mixture was heated to 50 °C with stirring. A freshly prepared solution of $Na_2S_2O_4$ (4.8 g, 27.58 mmol) in distilled water (18 mL) was added in drops. Once the addition was complete, the mixture was stirred at said temperature for 3 more h, obtaining **4** (1.47 g, 87% yield): mp = 201 °C (DMF/H_2O) (1:1); ^{1}H-NMR (DMSO-d_6, 200 MHz) δ 7.32–7.75 (m, 4H, H_5, H_8, H_7, H_8), 7.33 (s, 2H, NH_2); IR 3412 (s), 3322 (m, NH_2), 2220 (m, CN), 755 (s), 612 (m, 2,3-quinoxaline) cm^{-1}. Anal. ($C_9H_6N_4$) C, H, N.

3-Chloro-2-quinoxalinecarbonitrile (5). 3-Amino-2-quinoxaline carbonitrile (**4**) (2.4 g, 14.11 mmol) was mixed with glacial acetic acid (40 mL) and HCl (35%, 50 mL). The mixture was magnetically stirred in a bath with ice and salt. When the temperature was below 4 °C, a freshly prepared solution of $NaNO_2$ (3 g, 43 mmol) in distilled water (25 mL) was added. This solution was added in drops to the former mixture. This new mixture was maintained in the same conditions for 1 h after the addition of $NaNO_2$ was complete. The obtained precipitate **5** was filtered, washed with water, and dried (1.47 g, 55% yield): mp = 200 °C (methanol); ^{1}H-NMR (DMSO-d_6, 200 MHz) δ 7.30–8.30 (m, 4H, H_5, H_8, H_7, H_8); IR 3039 (w, C-H), 2234 (w, CN), 779 (s), 612 (m, 2,3-quinoxaline) cm^{-1}. Anal. ($C_9H_4N_3Cl$) C, H, N.

3-(1-Piperazinyl)-2-quinoxalinecarbonitrile (6). 3-Chloro-2-quinoxalinecarbonitrile (**5**) (0.95 g, 5 mmol) and sodium bicarbonate (0.53 g, 6.5 mmol) were added to piperazine (20 mL) and ethanol (20 mL). The reaction was maintained with stirring at 100 °C for 6 h. The mixture was concentrated to 5 mL, and water (20 mL) was added. The precipitate which formed was filtered, obtaining a yellow solid **6** (0.60 g, 50% yield): ^{1}H-NMR (DMSO-d_6, 200 MHz) δ 7.91 (d, 1H, H_5 quinoxaline), 7.82–7.72 (m, 2H, H_8 and H_8 quinoxaline), 7.68–7.60 (m, 1H, H_7 quinoxaline), 3.62 (bs, 5H, piperazine and NH), 2.93 (bs, 4H, piperazine); IR 3500–3400 (s, NH), 2980–2820 (m, CH, saturated), 2200 (w, CN),

Table II. Compared Antagonist Effects of Piperazinilquinoxaline Derivatives on 5-HT3 Receptors[a]

compd	antagonism to 2-methyl-5HT in guinea pig ileum: pA_2	displacement of binding of ^{3}H-BRL 43694 to rat cerebral cortex: K_i, nM	displacement of binding of H-5HT to rat cerebral cortex: K_i, nM	Bezold-Jarisch reflex in rats ID50, μg/kg iv (confidence interval)
6	5.8	90	345	0.027 (0.01–0.06)
7a	7.2	100	>1000	0.015 (0.004–0.051)
7e	10.2	8	>1000	0.02 (0.004–0.096)
7b	6.0	40	>1000	0.38 (0.11–1.2)
ondansetron	6.9	6.5	>1000	0.0004 (0.00003–0.007)
tropisetron	8.4	3.8	>1000	0.002

[a] Experiments were performed as described in the Experimental Section. Values are the means from three to six separate experiments. SE for pA_2 and K_i values were less than 10% of the mean. When a discrete value was not generated, K_i is reported as >1000 nM.

1550 (m, aromatic), 1260 (s, CN) cm^{-1}; ^{13}C-NMR (DMSO-d_6) δ 153.13 (C, bonded to piperazine), 140.21 (1C, quinoxaline), 135.52 (1C, quinoxaline, 132.60 (1C, quinoxaline), 127.76 (1C, quinoxaline), 126.32 (1C, quinoxaline), 125.77 (1C, quinoxaline), 121.51 (1C, bound to CN), 115.43 (CN), 47.92 (2C, piperazine), 43.98 (2C, piperazine); MS (*m/e*, parent) 239 (M$^+$, 6.07), 209 (5.00), 197 (78.11), 184 (44.36), 183 (10.29), 171 (12.83), 156 (20.13), 155 (12.49), 154 (7.34), 149 (8.64), 129 (16.53), 103 (13.41), 102 (28.07), 71 (17.96), 69 (100), 57 (39.16), 56 (40.38). Anal. ($C_{13}H_{13}N_5$) C, H, N.

3-[1-(4-Methylpiperazinyl)]-2-quinoxalinecarbonitrile (7). 2-Chloro-3-quinoxalinecarbonitrile (**5**) (0.95 g, 5 mmol) and *N*-methylpiperazine (10 mL) were heated at 100 °C for 1 h. The reaction mixture was poured over H_2O–ice (100 mL) and stirred for 30 min. The obtained yellow precipitate was filtered and recrystallized with hot water and 2-propanol. A yellow cottony solid (**7**) was obtained (0.80 g, 63% yield): ^{1}H-NMR (DMSO-d_6) δ 7.95 (d, 1H, H_5 quinoxaline), 7.84–7.77 (m, 2H, H_8 and H_8 quinoxaline), 7.73–7.64 (m, 1H, H_7 quinoxaline), 3.69 (bs, 4H, piperazine), 2.53 (bs, 4H, piperazine), 2.26 (s, 3H, methyl); IR 2950–2810 (m, CH saturated), 2240 (w, CN), 1550 (m, aromatic), 1160 (s, CN) cm^{-1}. Anal. ($C_{14}H_{15}N_5$) C, H, N.

3-[1-(4-Phenylpiperazinyl)]-2-quinoxalinecarbonitrile (8). A mixture of 3-chloro-2-quinoxalinecarbonitrile (**5**) (0.95 g, 5 mmol), *N*-phenylpiperazine hydrochloride (5.67 g, 0.03 mL), and sodium bicarbonate (2.55 g, 0.03 mol) was stirred in ethanol (70 mL) and H_2O (10 mL) for 10 h, maintaining the temperature at 70 °C. Once the reaction had finalized, H_2O (50 mL) was added. The mixture was concentrated in the rotavapory evaporator until a total volume of 50 mL was obtained. The mixture was treated with ethyl acetate (3 × 100 mL). The organic extracts were dried and concentrated over silica gel. The obtained reddish oil was crystallized in 2-propanol and identified as **8** (1.40 g, 89% yield); ^{1}H-NMR (DMSO-d_6) δ 7.97 (d, 1H, H_5 quinoxaline), 7.85–7.70 (m, 2H, H_6 and H_8 quinoxaline), 7.65 (m, 1H, H_7 quinoxaline), 7.23 (m, 2H, H_3 and H_5 phenyl), 7.06 (d, 2H, H_2 and H_8 phenyl), 6.85 (m, 1H, H_4 phenyl), 3.86 (bs, 4H, H_{eq} piperazine), 3.40 (bs, 4H, H_{ax} piperazine); IR 2970–2820 (m, CH, saturated), 2220 (w, CN), 1610 (m, aromatics), 1560 (m, aromatics), 950 (m, ortho-substituted), 770 (s, ortho-substituted), 705 (m, ortho-substituted) cm^{-1}; ^{13}C-NMR (DMSO-d_6) δ 153.74 (C, bounded to piperazine), 150.55 (C_1, phenyl), 141.04 (C, quinoxaline), 136.58 (C, quinoxaline), 133.55 (C, quinoxaline), 128.85 (2C, C_3, phenyl), 128.69 (C, quinoxaline), 127.39 (C, quinoxaline), 126.70 (C, quinoxaline), 122.37 (C, bonded to CN), 119.08 (C_4, phenyl), 116.33 (CN), 115.46 (2C, C_2, phenyl), 47.75 (2C, piperazine), 47.66 (2C, piperazine); MS (*m/e*, parent) 315 (M$^+$, 8.35), 155 (9.25), 154 (6.54), 144 (5.77), 133 (9.71), 132 (100), 105 (31.32), 105 (58.25), 103 (13.92), 102 (21.62), 91 (8.72), 77 (30.13). Anal. ($C_{19}H_{17}N_5$) C, H, N.

2-(1-Piperazinyl)-6-nitro-3-quinoxalinecarbonitrile (9a). 3-(1-Piperazinyl)-2-quinoxalinecarbonitrile (0.96 g, 4 mmol) in sulfuric acid (15 mL) was placed in an ice bath, maintaining the temperature below 5 °C and adding nitric acid (4 mL) in drops. The reaction was maintained with stirring at 0–5 °C for 30 min. The mixture was poured over ice (200 g), and the precipitate that formed was filtered and washed with abundant water that was saturated with potassium carbonate. The solid was recrystallized from ethanol, and a red solid (**9a**) was obtained (0.81 g, 71% yield): ^{1}H-NMR (DMSO-d_6, 200 MHz) 8.52 (s, 1H, H_5 quinoxaline), 8.30 (d, 1H, H_7 quinoxaline), 7.63 (d, 1H, H_8 quinoxaline), 3.78 (bs, 4H, H_{eq} piperazine), 2.88 (bs, 4H, H_{ax} piperazine), 2.72 (bs, 1H, NH); IR 3205 (m, NH), 2922 (m, CH aliphatics), 2223 (w, CN), 1615 (m, aromatics), 1548 (s, NO_2), 1510 (s, NO_2), 1427 (m, aromatics), 1272 (s, CN) cm^{-1}. Anal. ($C_{13}H_{12}N_6O_2$) C, H, N.

2-[1-(4-Ethylpiperazinyl)]-6-nitro-3-quinoxalinecarbonitrile (9b). 2-[1-Piperazinyl]-6-nitro-3-quinoxalinecarbonitrile (**9a**) (12 g, 42 mmol) and iodoethane (8.56 g, 0.055 mol) were refluxed in ethanol (200 mL) for 5 h. The solvent was removed, and the residue was treated with water saturated with sodium carbonate (200 mL) and chloroform (200 mL). The organic extracts were dried, concentrated, and chromatographed with silica gel, eluting with a mixture of chloroform/ethanol (95:5). An orange solid (**9b**) was obtained (6.5 g, 50% yield): ^{1}H-NMR (DMSO, 200 MHz) δ 8.79 (s, 1H, H_5 quinoxaline), 8.52 (d, 1H, H_7 quinoxaline), 7.88 (d, 1H, H_8 quinoxaline), 3.97 (bs, 4H, H_{eq} piperazine), 2.66 (bs, 4H, H_{ax} piperazine), 2.49 (q, 2H, CH_2), 1.14 (t, 3H, CH_3); IR 3450–3200 (m, NH associated), 2970 (m, CH aliphatics), 2815 (m, CH aliphatics), 1612 (m, aromatics), 1549 (s, NO_2), 1455 (m, aromatics), 1260 (s, CN) cm^{-1}. Anal. ($C_{15}H_{16}N_6O_2$) C, H, N.

2-[1-(4-Ethylpiperazinyl)]-6-amino-3-quinoxalinecarbonitrile (10). 2-[1-(4-Ethylpiperazinyl)]-6-nitro-2-quinoxalinecarbonitrile (5.6 g, 1.8 mmol) and tin(II) chloride dihydrate (22.5 g, 0.1 mol) were refluxed in ethanol (200 mL) for 2 h. The solvent was removed, and the residue was treated with water saturated with sodium carbonate (200 mL) and ethyl acetate (200 mL). The organic extracts were dried, concentrated, and chromatographed with silica gel, eluting with a mixture of dichloromethane/ethanol (9:1). A red solid (**10**) was obtained (1.4 g, 27% yield): ^{1}H-NMR (DMSO-d_6, 200 MHz) δ 7.53 (d, 1H, H_6 quinoxaline), 7.30 (d, 1H, H_7 quinoxaline), 6.85 (s, 1H, H_5 quinoxaline), 5.96 (s, 2H, NH_2), 3.40 (bs, 4H, H_{eq} piperazine), 2.51 (bs, 4H, H_{ax} piperazine), 2.34 (q, 2H, CH_2), 0.99 (t, 3H, CH_3); IR 3450 (m, NH associated), 3360 (m, NH), 2980 (m, CH aliphatics), 2800 (m, CH aliphatics), 2230 (w, CN), 1620 (m, aromatics), 1540 (m, aromatics), 1450 (m, aromatics), 1265 (s, CN) cm^{-1}. Anal. ($C_{15}H_{16}N_8$) C, H, N.

3-(1-Piperazinyl)-2-quinoxalinecarboxamide (11a). 3-(1-Piperazinyl)-2-quinoxalinecarbonitrile (0.96 g, 4 mmol) was added to a dissolution of hydrogen peroxide (30%, 5 mL) and sodium hydroxide (6 N, 5 mL) in acetone (50 mL). The mixture was stirred at 25 °C for 24 h. The acetone was eliminated in the rotavapory evaporator. Distilled water (50 mL) was added. The mixture was then treated with ethyl acetate (3 × 50 mL). The extracts were dried, concentrated, and chromatographed with silica gel, eluting the extract with a ternary mixture of dichloromethane/methanol/triethylamine (70:30:1), thereby obtaining a yellow solid (**11a**) (0.25 g, 24% yield): ^{1}H-NMR (DMSO-d_6, 200 MHz) δ 8.74 (s, 1H, amide), 7.87 (d, 1H, H_5 quinoxaline), 7.67 (bs, 2H, H_8 and H_8 quinoxaline), 7.51–7.43 (m, 1H, H_7 quinoxaline), 3.53 (bs, 6H, 4H piperazine, 1H NH piperazine, 1H amide), 2.82 (bs, 4H, piperazine); IR 3500–3280 (w, bs NH and NH_2), 1670 ($CONH_2$), 1603 (w, aromatics), 1545 (m, aromatics), 1275 (s, CN) cm^{-1}. Anal. ($C_{13}H_{15}N_5O$) C, H, N: calcd 27.22; found 26.45.

3-[1-(4-Ethylpiperazinyl)]-2-quinoxalinecarboxamide (11b). 3-[1-(4-Ethylpiperazinyl)]-2-quinoxalinecarbonitrile (1.07 g, 4 mmol) was added to a dissolution of hydrogen peroxide (30%, 5 mL) and sodium hydroxide (6 N, 5 mL) in acetone (50 mL). The mixture was stirred at 25 °C for 24 h. The acetone was eliminated in the rotavapory evaporator. Distilled water (50 mL) was added. The mixture was then treated with ethyl acetate (3 × 50 mL). The extracts were dried, concentrated, and chromatographed over silica gel, eluting with a mixture of dichlo-

romethane/ethanol (1:1), thereby obtaining a yellow solid (**11b**) (0.60 g, 52% yield): ^{1}H-NMR (DMSO-d_6, 200 MHz) δ 8.40–7.24 (m, 4H, quinoxaline), 4.21–3.50 (bs, 2H, NH_2), 3.67 (bs, 4H, piperazine), 2.56 (bs, 4H, piperazine), 2.42 (q, 2H, methylene), 1.09 (t, 3H, methyl); IR 3500–3200 (w, bs NH_2), 2899 (m, CH, st aliphatics), 2812 (m, CH aliphatics), 1652 ($CONH_2$), 1548 (m, aromatics), 1435 (m, aromatics), 1268 (s, CN) cm^{-1}. Anal. ($C_{15}H_{21}N_5O$) C, H, N.

Ethyl 3-Hydroxy-2-quinoxalinecarboxylate (14). This product is obtained according to the procedure described in the literature:[24] IR 2835 (m, CH), 1735 (vs, C=O ester), 1660 (vs, C=O, lactam) cm^{-1}.

Ethyl 3-Chloro-2-quinoxalinecarboxylate (15). This product is obtained according to the earlier described procedure:[20] IR 2973 (m, CH, aliphatics), 2928 (m, CH, aliphatics), 1725 (vs, C=O, ester), 1606 (s, aromatic), 1536 (m, aromatic), 1249 (m, CN), 773 (m, CCl) cm^{-1}.

Ethyl 3-(1-Piperazinyl)-2-quinoxalinecarboxylate, Hydrochloride (16). A mixture of ethyl 3-chloro-2-quinoxalinecarboxylate (7.10 g, 30 mmol) and piperazine (12.90 g, 0.15 mol) in ethanol (200 mL) was refluxed with stirring for 3 h. Once this time had elapsed, the mixture was vacuum dried, and the resulting residue was recrystallized from water, thereby obtaining a yellow solid **16** (6.78 g, 70%): ^{1}H-NMR (DMSO-d_6, 200 MHz) δ 8.00 (d, 1H, H_5 quinoxaline), 7.79 (bs, 2H, H_6 and H_8 quinoxaline), 7.66–7.60 (m, 1H, H_7 quinoxaline), 6.97 (bs, 2H, NH and HCl), 4.49 (q, 2H, CH_2), 3.73 (bs, 4H, piperazine), 3.20 (bs, 4H, piperazine), 1.41 (t, 3H, CH_3); IR 3850–3450 (w, bs, NH), 2952 (m, CH aliphatics), 2847 (m, CH aliphatics), 2813 (m, CH aliphatics), 1728 (CO_2Et), 1547 (m, aromatics), 1428 (m, aromatics), 1275 (s, CN) cm^{-1}. Anal. ($C_{15}H_{19}N_4O_2Cl$) C, H, N.

Ethyl 3-(1-Ethylpiperazinyl)-2-quinoxalinecarboxylate (17). Ethyl 3-(1-piperazinyl)-2-quinoxalinecarboxylate (5.72, 20 mmol) and iodoethane (3.90 g, 25 mmol) were added to ethanol (50 mL). The mixture was refluxed with stirring for 6 h. The mixture was then vacuum dried and treated with water saturated with potassium carbonate (50 mL) and dichloromethane (50 mL). The aqueous phase was washed with dichloromethane (2 × 50 mL). The organic extracts were dried, concentrated, and chromatographed over silica gel, eluting with a mixture of dichloromethane/methanol (95:5). A yellow oil (**17**) was obtained (4.40 g, 70%): ^{1}H-NMR (DMSO-d_6, 200 MHz) δ 7.89 (d, 1H, H_5 quinoxaline), 7.68 (bs, 2H, H_6 and H_8 quinoxaline), 7.49 (bs, 1H, H_7 quinoxaline), 4.41 (q, 2H, OCH_2), 3.45 (bs, 4H, piperazine), 2.43 (bs, 4H, piperazine), 2.29 (q, 2H, NCH_2), 1.34 (t, 3H, CH_3), 0.96 (t, 3H, CH_3); IR 2972 (m, CH aliphatics), 2813 (m, CH aliphatics), 1738 (CO_2Et), 1557 (m, aromatics), 1436 (m, aromatics), 1265 (s, CN) cm^{-1}. Anal. ($C_{17}H_{22}N_4O_2$) C, H, N.

Pharmacology. 1. Isolated Longitudinal Muscle-Myenteric Plexus Preparation from Guinea Pig Ileum. Guinea pigs of either sex weighing 300–400 g were stunned by a blow to the head and bled. The ileum was excised, approximately 10 cm from the ileo–caecal junction, and longitudinal muscle strips with the myenteric plexus attached (LMMP) were prepared as previously described.[25] LMMP strips were suspended in a 10-mL organ bath containing Tyrode's solution (composition in mM: NaCl, 136; KCl, 2.7; $CaCl_2$ 1.8; $MgCl_2$, 1.05; NaH_2PO_4, 0.42; $NaHCO_3$, 11.9; glucose, 5.5), aereated with 95% O_2/5% CO_2 and maintained at 37 °C. Contractile responses were isometrically recorded with a resting tension of 0.5 g. Following a 30-min equilibration period, tissues were stimulated with increasing concentrations of 5-HT, from 10^{-8} to 10^{-4} M. A fixed 5-HT concentration (10^{-5}), approximately the ED_{80}, was used for the subsequent antagonism studies since this rather high 5-HT concentration should preferentially stimulate 5-HT$_3$ receptors.[22] The response to 10 μM 5-HT was expressed as 100%. After 30 min of incubation with the test compounds, serotonin was added into the bath, and the subsequent response was measured. The antagonist effect of the compounds was expressed as a percentage of the previous response to 5-HT. The pA_2 values were calculated against 2-methyl-5HT by means of the method described by Furchgott[26] in which a single concentration of antagonist is used, according to the formula: pA_2 = log [antagonist concentration]/(agonist dose ratio-1). When pA_2 values against 5-HT were determined, the preparations were preincubated with 5-methoxytryptamine (10 μM) for 30 min.[27]

2. Isolated Rat Thoracic Aorta. Male Wistar rats (200-250 g) were sacrificed by decapitation and exsanguinated. The descending thoracic aorta was removed and cut into rings of approximately 2–3 mm width. These rings were suspended in 10 mL organ baths containing a Krebs–Henseleit solution (composition in mM: NaCl, 118.4; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; $NaHCO_3$, 25 and glucose, 11). The rings were prepared as previously described.[28] The solutions were kept at 37 °C and gassed continuously with 95% O_2/5% CO_2. The tissues were stretched to an initial tension of 1.5 g and equilibrated for 60–90 min. After the initial equilibration period, the aortic rings were contracted with a submaximal concentration of 5-HT (3×10^{-5} M). Control contractile responses to 5-HT were obtained and considered as 100%.

After 30 min of incubation with the new compounds, 5-HT was added into the bath and the subsequent response was measured. The antagonist effect was expressed as the percentage of the previous response to 5-HT.

3. Binding to Several Neurotransmitter Receptors. Binding of [^{3}H]BRL-43694 to 5-HT$_3$ receptors from rat cerebral cortex homogenates was performed according to a previously described method.[29] Whole cerebral cortex tissue was obtained from male Wistar rats (150-200 g) and homogenized in 10 volumes of ice-cold HEPES buffer (50 mM, pH 7.5). The homogenate was washed three times by centrifugation (50 000 g/10 min), and the final pellet was suspended in 10 volumes of HEPES buffer and stored on ice until required. Displacement studies were performed with 1 nM [^{3}H]BRL-43694 (final concentration) and eight different concentrations of new compounds. The incubation (23 °C for 30 min) was terminated by rapidly filtering through GB/B filters using a Brandel Cell Harvester. The filters were rinsed immediately and measured by liquid scintillation counting.

Binding to [^{3}H]-5HT-labeled 5-HT$_1$ receptors of rat cortical membranes was performed using a previously described procedure.[30] The tissue was homogenized in 20 volumes of 50 mM Tris–HCl and centrifuged at 45000*g* for 10 min. The supernatant was discarded, and the pellet was rehomogenized in the same buffer. The tissue homogenate was then incubated at 37 °C for 10 min before being subjected to a second 10-min centrifugation. The final pellet was resuspended in 100 volumes of buffer containing 0.1% ascorbic acid and 10 μM pargyline. The final mixture consisted of 0.1 mL of 0.3 nM ^{3}H-5HT (30 Ci/mmol, NEN), 0.1 mL of buffer or 5-HT (final concentration 10 μM), and 0.8 mL of tissue suspension.

Following incubation at 37 °C for 10 min, the tubes were rapidly filtered and washed twice with 5 mL of buffer each time. Radioactivity was determined by liquid scintillation counting.

4. Von Benzold–Jarisch Reflex. The antagonism to the bradycardic effect of 5-HT in anesthetized rats was evaluated as described.[31] Male Sprague–Dawley rats (500–550 g) were anesthetized with urethane (1 g/kg ip). The carotid artery was cannulated and connected to a TRA-021 Letica pressure transducer. Heart rate was derived from the arterial blood pressure signal using a cardiotachometer (CAR-306 Letica). A jugular vein was exposed, cannulated and used for iv administration of compounds. Bolus intravenous injection of 4 μg/kg of serotonin repeated every 15 min reproducibly elicited the von Bezold–Jarisch reflex. Antagonists were injected iv 2 min before serotonin, and their effect was expressed as percent inhibition of the serotonin response. ID_{50}'s were calculated by the method of Litchfield and Wilcoxon.[32]

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