# A New 5-$HT_3$ Receptor Ligand. II.[1] Structure–Activity Analysis of 5-$HT_3$ Receptor Agonist Action in the Gut

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**Several modified 2-piperazinyl benzoxazole derivatives, which exhibit an agonistic effect on gastrointestinal motility, were synthesized and their effects on the contraction of guinea-pig ileum were examined. The quaternary piperazinyl benzoxazole structure has a restricted conformation and stereostructure compared to those of the other 5-$HT_3$ receptor agonists, serotonin and *meta*-chlorophenylbiguanide. The mutual positions of the aromatic ring, nitrogen atom and terminal amine are considered to form the pharmacophore of the 5-$HT_3$ receptor agonist in the gut. In the serotonin-evoked reflex bradycardia [Bezold–Jarisch (B–J) reflex] inhibition test using rats the B–J reflex-inducing ratio was different for each synthesized compound. These results suggest that, in these 5-$HT_3$ receptor agonists, the substituents of the benzoxazole ring influence the B–J reflex-inducing activity in rats.**

**Key words** 1-allyl-1-methyl-4-(2-benzoxazolyl)piperazinium iodide (CP2289); 5-$HT_3$ receptor; agonist

Serotonin (5-HT, **2**) is an important molecule in medicinal chemistry,[2] and several 5-HT receptor subtypes have been found by molecular biological methods, as well as by using specific agonists or antagonists.[3] Particular attention has been focused on 5-$HT_3$ receptor-selective antagonists, which prevent emesis caused by cancer chemotherapy.[4] Structure–activity analysis of 5-$HT_3$ antagonists revealed that the pharmacophore of the 5-$HT_3$ receptor antagonist consists of an aromatic ring, a carbonyl function and a basic center.[5]

In contrast to the antagonist, there have been only a few reports on the 5-$HT_3$ receptor agonist and its pharmacophore.[6] We reported that 1-allyl-1-methyl-4-(2-benzoxazolyl)piperazinium iodide (CP2289, **1**), a new 5-$HT_3$ receptor ligand, had an agonistic effect on gastroenteric motility.[1] Piperazinyl benzoxazole **4a** also exhibited a characteristic agonist effect in an *in vitro* contraction test using isolated guinea-pig ileum, although the activity was weak.[1] A comparison of the structure of compound **1** with those of typical 5-$HT_3$ agonists such as 5-HT (**2**) and *meta*-chlorophenylbiguanide (**3**) indicated that the charged amine part in the piperazine ring corresponds to the terminal amines of compounds **2** and **3** and that the benzoxazole ring corresponds to the indole ring of **2** and the benzene ring of **3**. In this paper, we report the synthesis and agonistic profiles of some derivatives of **1** with a variety of alkyl substituents on the charged amine and a 5- and/or 6-substituent on the benzoxazole ring. The structural requirements of a 5-$HT_3$ receptor agonist are discussed.

## Chemistry and Pharmacology

The synthetic procedures are illustrated in Fig. 2. Compounds **4a**, **4d** and **5** were prepared by the reaction of piperazines with 2-chlorobenzoxazole **6**. Alkylations of **5** gave **4b** and **4c**. Methylation of **4a**—**4d** gave **7a**—**7d.** Compound **1** was obtained by the allylation of **4a**. The 5- or 6- substituted benzoxazole derivatives (**12a**—**12h**) were synthesized from the amino phenols **8a**—**8g**. 2-Mercaptobenzoxazoles **9a**—**9g** were prepared by the method of Dunner.[7] Treatment of **9a**—**9g** with phosphorus pentachloride gave the 2-chloro benzoxazoles **10a**—**10g**, which were allowed to react, without purification, with 1-methylpiperazine to give **11a**—**11g**. Allylation of **11a**—**11g** gave the quaternary ammonium salts **12a**—**12g**. The hydroxyl derivative **12h** was prepared by demethylation of **11g** under acidic conditions followed by allylation of the resulting phenol.

To determine the agonistic activity of the derivatives for the 5-$HT_3$ receptor in the gut, contraction tests were carried out using isolated guinea-pig ileum. Each $pD_2$ (the negative logarithm of the molar concentration which produced 50% of the maximum contraction generated by $10^{-5}$ M 5-HT) and intrinsic activity (*ia*, the ratio between the maximum response to a test compound and that to $10^{-5}$ M 5-HT) obtained are the mean ± SEM of five independent results. We also examined the affinity of the derivatives for the 5-$HT_3$ receptor at $10^{-7}$ M to avoid overlooking any unexpected antagonists. The results are summarized in Tables 1 and 2.

Introduction of a large alkyl or allyl substituent onto the nitrogen atom in the piperazine ring lowered the affinity for the receptor (Table 1, **7b**—**7d**). The simple dimethyl derivative **7a** ($pD_2 = 6.14$) exhibited an agonistic effect at a lower concentration than **1** ($pD_2 = 5.57$). The 5-$HT_3$ receptor agonist **3** showed a rather low affinity, but high intrinsic activity ($pD_2 = 4.83$, $ia = 0.86$). On the benzoxazole ring, a lipophilic substituent at the 5 position and a

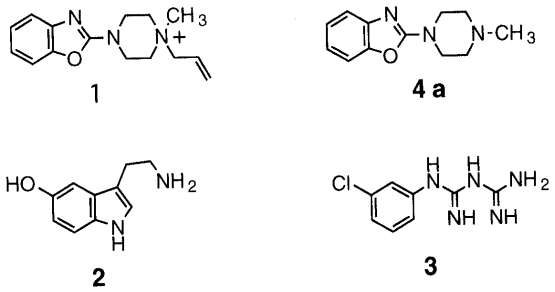


Fig. 1

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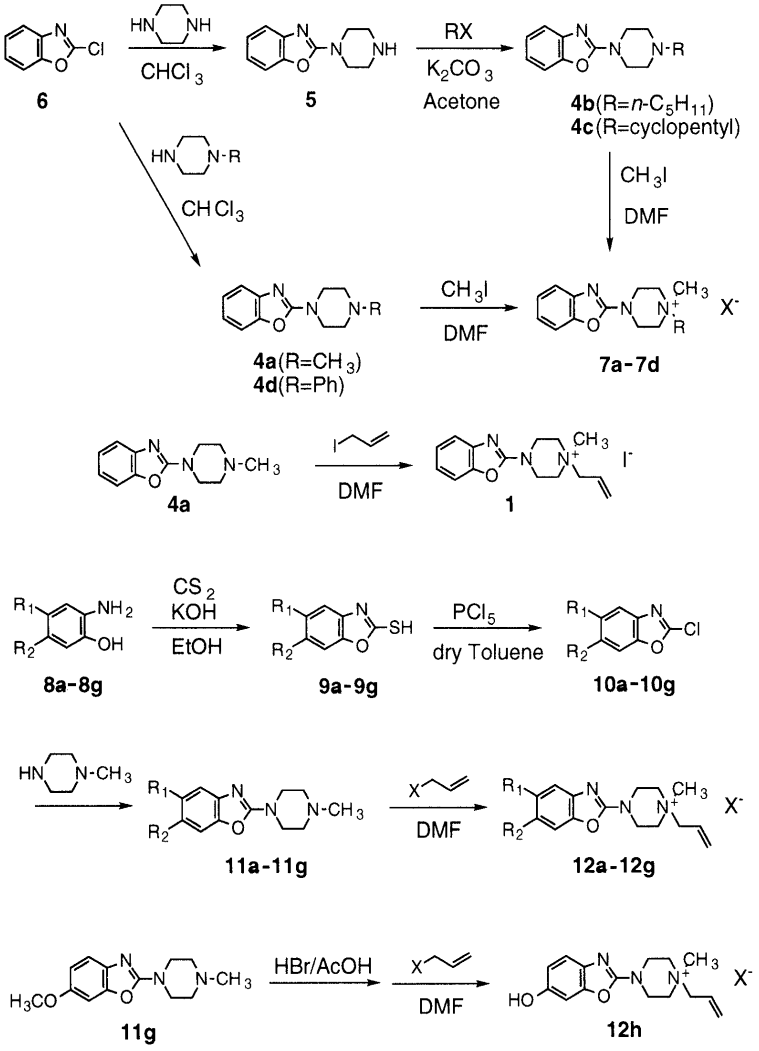



Fig. 2. Synthetic Routes to Benzoxazole Derivatives

hydrophilic substituent at the 6 position were mostly examined (Table 2). The 5-chloro compound **12d** ($pD_2 = 6.41$) had the highest affinity followed by the 5-methyl compound **12a** ($pD_2 = 6.10$). Introduction of a large alkyl substituent at this position also significantly decreased the affinity (**12b**, **12c**). Among the 6-substituted compounds, the hydroxyl compound (**12h**) had approximately the same agonist effect as **1**, although the methyl, nitro and methoxy substituents lowered the affinity (**12e—g**).

With **12a**, **12d** and **12h** we performed the 5-HT-evoked reflex bradycardia [Bezold–Jarisch (B–J) reflex] inhibition test in rats (Table 3). In a similar result to that obtained with compound **1**, **12d** did not induce the B–J reflex at a concentration which inhibited the 5-HT-evoked bradicardia, although **12a** and **12h** showed B–J reflex-inducing activity.

## Discussion

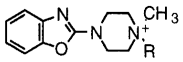
The stereostructure of compound **1** obtained by X-ray crystallographic analysis is given in Fig. 3. Compound **1** has a very rigid structure in which conformational variation is only possible due to the rotation of the C–N bond between the benzoxazole ring and piperazine ring. This was identical with the conformation obtained as the most stable one by molecular modeling. The relative positions of these groups are summarized in Fig. 4.

Superimposition of compounds **2** and **3** on the stable conformation of **1** revealed that the aromatic rings, nitrogen atoms neighboring the benzene rings, and terminal amines could occupy the same relative positions (Fig. 5). The agonistic activities of the synthesized compounds in contraction tests were in consistent with this correspondence. Terminal amines of some 5-$HT_3$ receptor ligands were quaternary-alkylated by methylation and the affinity for the 5-$HT_3$ receptor was maintained or increased, indicating that this terminal amine is protonated when it binds to the 5-$HT_3$ receptor.[8] The same finding was obtained in the cases of **4a** and **7a**. Furthermore, as with **3**,[9] the 5-Cl compound **12d** exhibited a higher affinity than the non-chlorinated compound. The relative spatial positions of the benzene ring, nitrogen atom and terminal amine in Fig. 4 are regarded as representing a phar-

macophore for the 5-$HT_3$ receptor agonist in the gut. As **1** and **12h** had similar agonistic activity, this suggests that the hydroxyl group is not essential for the agonistic effect in the gut. In contrast, the small lipophilic group on the 5 position of the benzoxazole ring (**12a**, **12d**) was important for increasing the affinity for the 5-$HT_3$ receptor.

In our previous paper, we noted that compound **1** behaved like a 5-$HT_3$ receptor antagonist in the 5-HT-evoked B–J reflex inhibition test.[1] Similarly, **12d** did not show reflex-inducing activity at a concentration that blocked the reflex caused by 5-HT. Administration of **12a** induced a weak B–J reflex (16%) and **12h** caused a 62% B–J reflex, which is consistent with its intrinsic activity (0.68) on isolated guinea-pig ileum. These results suggest that the substituents of the benzoxazole ring influence the 5-$HT_3$ receptor agonist activity for B–J reflex induction in rats. Several workers have commented on the diversity of 5-$HT_3$ receptors, based on differences in ligand potencies,[10] although it is not clear whether this di-

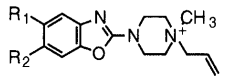
Table 1. *In Vitro* 5-$HT_3$ Agonist Activity and Binding Properties of the Derivatives



| Compd. | R | Contraction activity[a] | | 5-$HT_3$ receptor binding ($10^{-7}$ M, %)[c] |
|---|---|---|---|---|
| | | $pD_2 \pm SEM$[b] | $ia \pm SEM$[b] | |
| **4a** | — | 5.01±0.13 | 0.62±0.12 | 55 |
| **7a** | $-CH_3$ | 6.14±0.05 | 0.66±0.06 | 56 |
| **1** |  | 5.57±0.19 | 0.74±0.08 | 65 |
| **7b** |  | 5.0> | —[d] | 7 |
| **7c** |  | 5.0> | 0.19<[e] | 53 |
| **7d** |  | 5.0> | —[d] | 52 |
| **3** | (*m*-Cl-phenylbiguanide) | 4.83±0.05 | 0.86±0.08 | 30 |

*a*) See experimental section. *b*) Standard error of the mean ($n=5$); when SEM is not quoted, the values are the means of two experimental results. *c*) Compound was tested in duplicate at $10^{-7}$ M. Values are the means of two experimental results. *d*) No contraction was observed at $10^{-5}$ M. *e*) 19% contraction at $10^{-5}$ M.

Table 2. *In Vitro* 5-$HT_3$ Agonist Activity and Binding Properties of the Derivatives



| Compd. | $R_1$ | $R_2$ | Contraction activity[a] | | 5-$HT_3$ binding ($10^{-7}$ M, %)[c] |
|---|---|---|---|---|---|
| | | | $pD_2 \pm SEM$[b] | $ia \pm SEM$[b] | |
| **1** | H | H | 5.57±0.19 | 0.74±0.08 | 65 |
| **12a** | $CH_3$ | H | 6.10±0.19 | 0.82±0.08 | 86 |
| **12b** | *tert*-Bu | H | 5.0> | —[d] | −8 |
| **12c** | $-(CH_2)_4-$ | | 5.0> | —[d] | −14 |
| **12d** | Cl | H | 6.41±0.07 | 0.69±0.04 | 95 |
| **12e** | H | $CH_3$ | 5.0> | 0.44<[e] | 70 |
| **12f** | H | $NO_2$ | 5.0> | —[d] | −13 |
| **12g** | H | $OCH_3$ | 5.0> | —[d] | 19 |
| **12h** | H | OH | 5.52±0.08 | 0.68±0.05 | 85 |

*a*) See experimental section. *b*) Standard error of the mean ($n=5$); when SEM is not quoted, the values are the means of two experimental results. *c*) Compound was tested in duplicate at $10^{-7}$ M. Values are the means of two experimental results. *d*) No contraction was observed at $10^{-5}$ M. *e*) 44% contraction at $10^{-5}$ M.

Table 3. B–J Reflex Induction and 5-HT-Evoked B–J Reflex Inhibition Activity of the Compounds

| Compd. | B–J reflex induction%[a] (0.1 mg/kg) | B–J reflex inhibition%[a] (0.1 mg/kg) |
|---|---|---|
| **1** | Not detected | 72 |
| **12a** | 16[b] | 87 |
| **12d** | Not detected | 86 |
| **12h** | 62[b] | 70 |

*a*) Values are the means of two experiments. *b*) The ratio between the maximum response to a test compound and that to 10 μg/kg 5-HT.

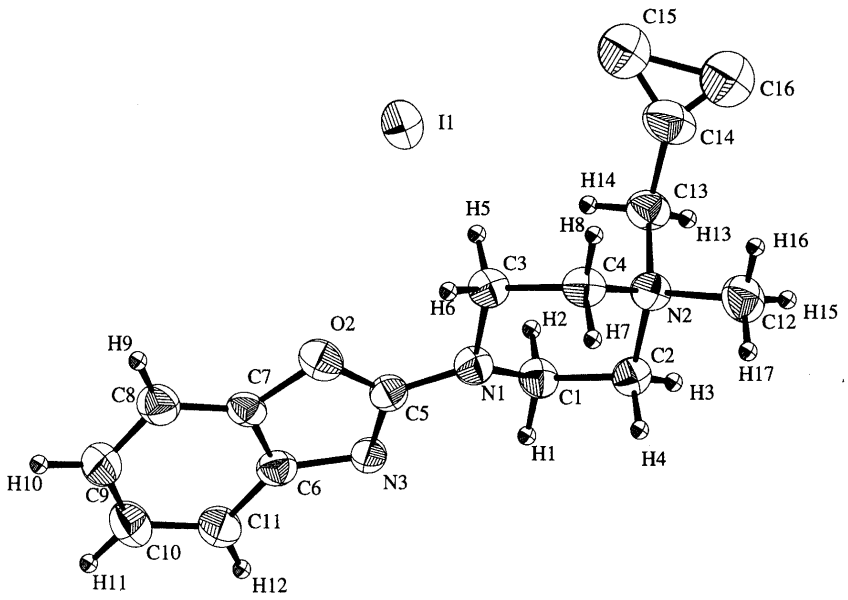



Fig. 3. X-Ray Crystallography Data for **1**

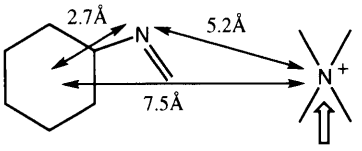
versity depends on the subtypes or species differences of 5-$HT_3$ receptors.[11] The selective agonistic activity of **1** and **12d** is of particular interest from the viewpoint of pharmacological and molecular-biological 5-$HT_3$ receptor studies.

## Experimental

**Chemistry** All melting points are uncorrected. IR spectra were recorded on Shimadzu FT-IR 8100 spectrometers. NMR spectra were obtained on JEOL GSX- or GX-400 FT-NMR spectrometers. The following abbreviations are used: s=singlet, d=doublet, t=triplet, q=quartet, quin=quintet, m=multiplet, and br=broad. MS were measured with Hitachi M-80B and JEOL JMS-700 instruments. 5-HT (**2**) and *meta*-chlorophenylbiguanide (**3**) were purchased from Sigma Co. All 2-SH-benzoxazoles (**9a**—**9g**) were prepared from *o*-aminophenols or *o*-nitrophenols by the method of Dunner.[7]

**1-Allyl-1-methyl-4-(2-benzoxazolyl)piperazinium Iodide (CP2289, 1)** Allyl iodide (1.7 g, 10 mmol) was added to a solution of 2-(4-methylpiperazinyl)benzoxazole (**4a**, 435 mg, 2 mmol) in *N*,*N*-dimethylformamide (DMF, 10 ml) at 0 °C. The reaction mixture was stirred at 20 °C for 2 h and concentrated *in vacuo*. The precipitates were collected by filtration and washed with acetone. The crude compound was recrystallized from methanol–acetone to give **1** (615 mg, 80%), mp 194 °C. NMR ($CD_3OD$) $\delta$: 3.24 (3H, s, $CH_3$–), 3.70—3.60 (4H, m, piperazine $-CH_2-\times 2$), 3.96—4.04 (2H, m, piperazine $-CH_2-$), 4.17—4.20 (4H, m, piperazine $-CH_2-$, allyl $-CH_2-$), 5.78—5.82 (2H, m, allyl $=CH_2$), 6.10—6.20 (1H, m, allyl $-CH=$), 7.14 (1H, t, $J$=7 Hz, benzoxazole 5-H), 7.23 (1H, t $J$=7 Hz, benzoxazole 6-H), 7.37 (1H, d, $J$=7 Hz, benzoxazole 7-H), 7.39 (1H, d, $J$=7 Hz, benzoxazole 4-H). Electrospray Ionization (ESI)-MS *m/z*: 259 ($M^+ + 1$). IR (KBr, $cm^{-1}$) 1630, 1574, 1455, 1406. *Anal.* (%) Calcd for $C_{15}H_{20}N_3OI$: C, 46.77; H, 5.23; N, 10.91. Found: C, 46.76; H, 5.10; N, 10.66.

**2-(4-Methyl-1-piperazinyl)benzoxazole (4a)** 2-Chlorobenzoxazole (**6**, 1.4 g, 9 mmol) was added to a solution of 1-methylpiperazine (1 g, 10 mmol) in chloroform (100 ml) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min and quenched in ice–water (150 ml). The resultant mixture was extracted with ethyl acetate (200 ml) and the extract was dried over $MgSO_4$. After concentration *in vacuo*, the residue was chromatographed on silica gel with chloroform–methanol (20 : 1) and recrystallized from water–acetone to give **4a** (1.8 g, 85%), mp 37—38 °C. NMR ($CDCl_3$) $\delta$: 2.37 (3H, s, $CH_3$–), 2.54 (4H, t, $J$=8 Hz, piperazine $-CH_2-\times 2$), 3.73 (4H, t, $J$=8 Hz, piperazine $-CH_2-\times 2$), 7.02 (1H, t, $J$=7 Hz, benzoxazole 5-H), 7.18 (1H, t, $J$=7 Hz, benzoxazole 6-H), 7.26 (1H, d, $J$=7 Hz, benzoxazole 7-H), 7.37 (1H, d, $J$=7 Hz, benzoxazole 4-H). EI-MS *m/z*: 217 ($M^+$). IR (KBr, $cm^{-1}$) 1630, 1575, 1460, 1397. *Anal.* (%) Calcd for $C_{12}H_{15}N_3O$: C, 66.34; H, 6.96; N, 19.34. Found: C, 66.46; H, 7.01; N, 19.08.




Height above the plane defined by the benzoxazole ring : 0.8Å

Fig. 4. Intramolecular Distances in the Stable Conformation of **1**

**2-(1-Piperazinyl)benzoxazole (5)** 2-Chlorobenzoxazole (**6**, 5 g, 32.6 mmol) was added to a solution of anhydrous piperazine (5.6 g, 65.2 mmol) in dichloromethane (100 ml)–triethylamine (4.5 ml) solution at 0 °C. The reaction mixture was stirred at 0 °C for 30 min and quenched in ice-water (150 ml). The resultant mixture was extracted with ethyl acetate (200 ml) and the extract was dried over $MgSO_4$. After concentration *in vacuo*, the residue was chromatographed on silica gel with dichloromethane–methanol (5 : 1) to give **5** (4.8 g, 72%), mp 68—70 °C. NMR ($CDCl_3$) $\delta$: 2.99 (4H, t, $J$=5 Hz), 3.48 (1H, s), 3.68 (4H, t, $J$=5 Hz), 7.02 (1H, dt, $J$=1, 7 Hz), 7.16 (1H, dt, $J$=1, 7 Hz), 7.25 (1H, dd, $J$=1, 7 Hz), 7.36 (1H, dd, $J$=1, 7 Hz). ESI-MS *m/z*: 204 ($M^+ + 1$). IR (KBr, $cm^{-1}$) 3213, 1630, 1578, 1460, 1271, 1240. *Anal.* (%) Calcd for $C_{11}H_{13}N_3O \cdot 1/3H_2O$: C, 63.14; H, 6.58; N, 20.08. Found: C, 62.98; H, 6.42; N, 19.99.

**1,1-Dimethyl-4-(2-benzoxazolyl)piperazinium Iodide (7a)** Methyl iodide (1.4 g, 10 mmol) was added to a solution of 2-(4-methylpiperazinyl)benzoxazole (**4a**, 435 mg, 2 mmol) in DMF (10 ml) at 0 °C. The reaction mixture was stirred at 20 °C for 2 h and concentrated *in vacuo*. The precipitates were collected by filtration and washed with acetone. The crude compound was recrystallized from methanol–acetone to give **7a** (488 mg, 85%), mp 269.5—270 °C. NMR ($CD_3OD$) $\delta$: 3.23 (6H, s), 3.58 (4H, t, $J$=6 Hz), 3.99 (4H, t, $J$=6 Hz), 7.10 (1H, t, $J$=8 Hz), 7.21 (1H, t, $J$=8 Hz), 7.38 (1H, d, $J$=8 Hz), 7.48 (1H, d, $J$=8 Hz). ESI-MS *m/z*: 233 ($M^+ + 1$). IR (KBr, $cm^{-1}$) 1630, 1574, 1464, 1452, 1368. *Anal.* (%) Calcd for $C_{13}H_{18}N_3OI$: C, 43.47; H, 5.05; N, 11.70. Found: C, 43.66; H, 4.89; N, 11.40.

**2-(4-*n*-Pentyl-1-piperazinyl)benzoxazole (4b)** 2-(1-Piperazinyl)benzoxazole (**5**, 100 mg, 0.49 mmol) was added to a stirred mixture of *n*-pentyl chloride (0.16 ml, 1.36 mmol), sodium iodide (204 mg, 1.36 mmol) and $K_2CO_3$ (188 mg, 1.36 mmol) in acetone (5 ml). The reaction mixture was refluxed for 3 d and quenched with water. The resultant mixture was extracted with ethyl acetate, washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on silica gel with *n*-hexane–ethyl acetate (1 : 1) to give **4b** (50.5 mg, 38%), mp 66—68 °C. NMR ($CDCl_3$) $\delta$: 0.91 (3H, t, $J$=8 Hz), 1.20—1.40 (4H, m), 1.52 (2H, quin, $J$=8 Hz), 2.38 (2H, t, $J$=8 Hz), 2.55 (4H, t, $J$=5 Hz), 3.72 (4H, t, $J$=5 Hz), 7.01 (1H, dt, $J$=1, 8 Hz), 7.16 (1H, dt, $J$=1, 8 Hz), 7.25 (1H, d, $J$= 8Hz), 7.35 (1H, d, $J$=8 Hz). EI-MS *m/z*: 273 ($M^+$). IR (KBr, $cm^{-1}$) 1635, 1578, 1452, 1358. *Anal.* (%) Calcd for $C_{16}H_{23}N_3O \cdot 1/6H_2O$: C, 69.53; H, 8.51; N, 15.20. Found: C, 69.68; H, 8.36; N, 14.99.

**1-Pentyl-1-methyl-4-(2-benzoxazolyl)piperazinium Iodide (7b)** 2-(4-*n*-Pentylpiperazinyl)benzoxazole (**4b**, 23 mg, 0.08 mmol) was allowed to react with methyl iodide (0.1 ml, 1.6 mmol) as described for the preparation of **7a** to afford **7b** (9 mg, 26%) as an oil. NMR ($CD_3OD$) $\delta$: 1.03 (3H, t, $J$=7 Hz), 1.40—1.60 (4H, m), 1.85—1.95 (2H, m), 3.30 (3H, s), 3.57 (2H, t, $J$=5 Hz), 3.71 (4H, t, $J$=5 Hz), 4.00—4.15 (2H, m), 4.15—4.25 (2H, m), 7.18 (1H, t, $J$=8 Hz), 7.28 (1H, t, $J$=8 Hz), 7.41 (1H, d, $J$=8 Hz), 7.44 (1H, d, $J$=8 Hz). FAB-MS *m/z*: 288 ($M^+$). IR

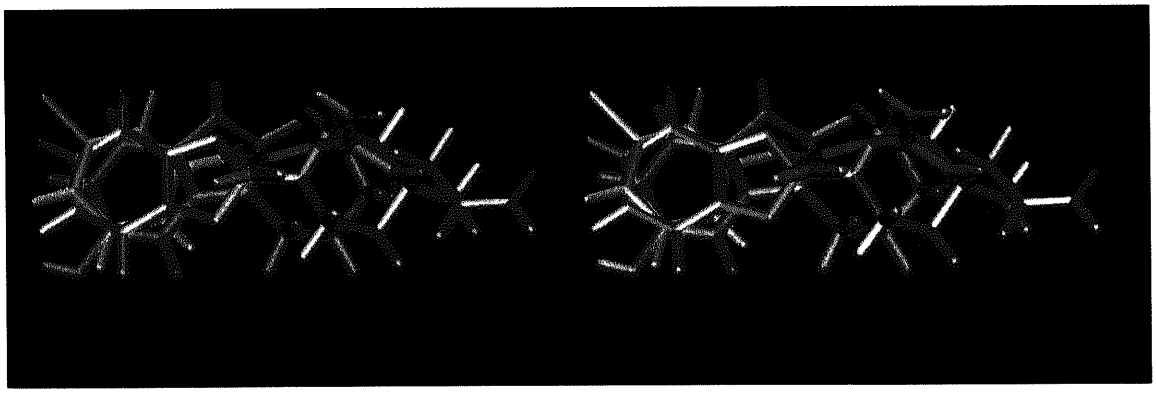


Fig. 5. Superimposition of **2** (Red) and **3** (Blue) on **1** (Green).

Parameters (energy difference between the superposed conformation and the global minimum conformation, $\Delta E$; root mean square index measured for three superposed points, RMS) for each compound were as follows: **2**; $\Delta E$=9.6 kcal/mol, RMS=0.72. **3**; $\Delta E$=3.5 kcal/mol, RMS=0.62.

(neat, $cm^{-1}$) 1628, 1578, 1458, 1401. HR-MS *m/z*: Calcd for $C_{17}H_{26}N_3O$: 288.2076 Found: 288.2078.

**2-(4-Cyclopentyl-1-piperazinyl)benzoxazole (4c)** 2-(1-Piperazinyl)-benzoxazole (**5**, 100 mg, 0.49 mmol) was refluxed for 3 d with cyclopentyl bromide (73 μl, 0.68 mmol), sodium iodide (102 mg, 0.68 mmol) and $K_2CO_3$ (94 mg, 0.68 mmol) in acetone. The product was purified as described for the preparation of **4b** to afford **4c** (56.5 mg, 43%), mp 162—163 °C ($CH_2Cl_2$–ether). NMR ($CDCl_3$) δ: 1.40—1.50 (2H, m), 1.50—1.65 (2H, m), 1.65—1.80 (2H, m), 1.80—2.00 (2H, m), 2.57 (1H, quin, *J*=8 Hz), 2.62 (4H, t, *J*=5 Hz), 3.73 (4H, t, *J*=5 Hz), 7.02 (1H, dt, *J*=1, 7 Hz), 7.16 (1H, dt, *J*=1, 7 Hz), 7.25 (1H, d, *J*=7 Hz), 7.36 (1H, d, *J*=7 Hz). EI-MS *m/z*: 271 ($M^+$). IR (KBr, $cm^{-1}$) 1632, 1578, 1509, 1460. *Anal.* (%) Calcd for $C_{16}H_{21}N_3O$: C, 70.82; H, 7.80; N, 15.48. Found: C, 70.52; H, 7.55; N, 15.64.

**1-Cyclopentyl-1-methyl-4-(2-benzoxazolyl)piperazinium Iodide (7c)** 2-(4-Cyclopentylpiperazinyl)benzoxazole (**4c**, 27 mg, 0.1 mmol) was allowed to react with methyl iodide (0.16 ml, 2.6 mmol) as described for the preparation of **7a** to afford **7c** (25.2 mg, 61%) as an oil. NMR ($CD_3OD$) δ: 1.70—1.85 (2H, m), 1.85—2.00 (2H, m), 2.00—2.10 (2H, m), 2.10—2.20 (2H, m), 3.23 (3H, s), 3.72 (4H, t, *J*=5 Hz), 4.00 (1H, quin, *J*=8 Hz), 4.24 (4H, t, *J*=5 Hz), 7.18 (1H, dt, *J*=1, 8 Hz), 7.28 (1H, dt, *J*=1, 8 Hz), 7.41 (1H, d, *J*=8 Hz), 7.44 (1H, d, *J*=8 Hz). GC-MS *m/z*: 286 ($M^+$). IR (neat, $cm^{-1}$) 1669, 1634, 1576, 1509, 1458. HR-MS *m/z*: Calcd for $C_{17}H_{24}N_3O$: 286.1919 Found: 286.1907.

**2-(4-Phenyl-1-piperazinyl)benzoxazole (4d)** 2-Chlorobenzoxazole (**6**, 0.1 ml, 0.9 mmol) was allowed to react with 4-phenylpiperazine (0.14 ml, 0.9 mmol) as described for the preparation of **4a** to afford **4d** (132 mg, 53%), mp 150—151 °C (MeOH–ether). NMR ($CDCl_3$) δ: 3.31 (4H, t, *J*=5 Hz), 3.87 (4H, t, *J*=5 Hz), 6.93 (2H, t, *J*=7 Hz), 6.98 (1H, d, *J*=7 Hz), 7.04 (1H, dt, *J*=1, 7 Hz), 7.18 (1H, dt, *J*=1, 7 Hz), 7.27 (1H, d, *J*=7 Hz), 7.30 (2H, d, *J*=7 Hz), 7.38 (1H, d, *J*=7 Hz). EI-MS *m/z*: 279 ($M^+$). IR (KBr, $cm^{-1}$) 1640, 1597, 1582, 1505, 1458. *Anal.* (%) Calcd for $C_{17}H_{17}N_3O \cdot 1/3H_2O$: C, 71.56; H, 6.24; N, 14.73. Found: C, 71.51; H, 6.12; N, 14.86.

**1-Methyl-1-phenyl-4-(2-benzoxazolyl)piperazinium Iodide (7d)** 2-(4-Phenylpiperazinyl)benzoxazole (**4d**, 28 mg, 0.1 mmol) was allowed to react with methyl iodide (0.16 ml, 2.6 mmol) at 40 °C as described for the preparation of **7a** to afford **7d** (29.1 mg, 69%) as an oil. NMR ($CD_3OD$) δ: 3.69 (3H, s), 3.83—3.95 (2H, m), 4.25—4.42 (4H, m), 4.70—4.80 (2H, m), 7.23 (1H, t, *J*=8 Hz), 7.31 (1H, t, *J*=8 Hz), 7.42 (1H, d, *J*=8 Hz), 7.47 (1H, dd, *J*=1, 8 Hz), 7.72 (1H, t, *J*=8 Hz), 7.79 (2H, t, *J*=8 Hz), 8.03 (2H, d, *J*=8 Hz). FAB-MS *m/z*: 294 ($M^+$). IR (neat, $cm^{-1}$) 1636, 1578, 1458, 1246. HR-MS *m/z*: Calcd for $C_{18}H_{20}N_3O$: 294.1606 Found: 294.1599.

**5-Methyl-2-(4-methyl-1-piperazinyl)benzoxazole (11a)** Phosphorus pentachloride (0.5 g, 2.4 mmol) was added to a solution of 5-methyl-2-mercaptobenzoxazole (**9a**, 435 mg, 2 mmol) in dry toluene (10 ml) at 20 °C. The reaction mixture was stirred at 120 °C for 1 h and cooled in an ice-bath. 1-Methylpiperazine (2 g, 20 mmol) was added dropwise to the mixture and the whole was stirred for 30 min at 0 °C. The reaction mixture was diluted with chloroform and washed with water. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on silica gel with chloroform–methanol (20 : 1) to give **11a** (1.8 g, 85%), mp 63—64 °C (MeOH–ether). NMR ($CDCl_3$) δ: 2.35 (3H, s), 2.39 (3H, s), 2.52 (4H, t, *J*=5 Hz), 3.71 (4H, t, *J*=5 Hz), 6.82 (1H, d, *J*=8 Hz), 7.11 (1H, d, *J*=8 Hz), 7.15 (1H, s). EI-MS *m/z*: 231 ($M^+$). IR (KBr, $cm^{-1}$) 1638, 1586, 1451, 1356. *Anal.* (%) Calcd for $C_{13}H_{17}N_3O \cdot 1/8H_2O$: C, 66.86; H, 7.44; N, 17.99. Found: C, 66.77; H, 7.40; N, 18.00.

**1-Allyl-1-methyl-4-(5-methylbenzoxazol-2-yl)piperazinium Iodide (12a)** 5-Methyl-2-(4-methylpiperazinyl)benzoxazole (**11a**, 250 mg, 1.1 mmol) was reacted with allyl bromide (650 mg, 5.5 mmol) as described for the preparation of **7a** to afford **12a** (285 mg, 75%) as an oil. NMR ($CD_3OD$) δ: 2.43 (3H, s), 3.28 (3H, s), 3.60—3.80 (4H, m), 3.98—4.10 (2H, m), 4.14—4.30 (4H, m), 5.80—5.90 (2H, m), 6.12—6.25 (1H, m), 6.99 (1H, d, *J*=8 Hz), 7.21 (1H, s), 7.30 (1H, d, *J*=8 Hz). ESI-MS *m/z*: 272 ($M^+$). IR (KBr, $cm^{-1}$) 1646, 1590, 1486, 1406. HR-MS *m/z*: Calcd for $C_{16}H_{22}N_3O$: 272.1763 Found: 272.1767.

**5-*tert*-Butyl-2-(4-methyl-1-piperazinyl)benzoxazole (11b)** 5-*tert*-Butyl-2-mercaptobenzoxazole (**9b**, 1 g, 4.8 mmol) was treated with phosphorus pentachloride (1.2 g, 5.8 mmol) and the resultant 2-chloro-5-*tert*-butylbenzoxazole (**10b**) was allowed to react with 1-methylpiperazine (5.4 ml, 48 mmol) as described for the preparation of **11a** to afford **11b** (820 mg, 62%), mp 73.5—75 °C (MeOH–$H_2O$). NMR ($CDCl_3$) δ: 1.39 (9H, s), 2.42 (3H, s), 2.6 (4H, t, *J*=4 Hz), 3.81 (4H, t, *J*=4 Hz), 7.15 (1H, dd, *J*=8, 2 Hz), 7.24 (1H, d, *J*=8 Hz), 7.50 (1H, d, *J*=2 Hz). ESI-MS *m/z*: 274 ($M^+ + 1$). IR (KBr, $cm^{-1}$) 1636, 1578, 1269, 1227. *Anal.* (%) Calcd for $C_{16}H_{23}N_3O \cdot 1/3H_2O$: C, 68.79; H, 8.54; N, 15.04. Found: C, 68.81; H, 8.67; N, 15.13.

**1-Allyl-1-methyl-4-(5-*tert*-butylbenzoxazol-2-yl)piperazinium Bromide (12b)** 5-*tert*-Butyl-2-(4-methylpiperazinyl)benzoxazole (**11b**, 100 mg, 0.36 mmol) was allowed to react with allyl bromide (443 mg, 3.6 mmol) as described for the preparation of **7a** to afford **12b** (130 mg, 90%), mp 198—199 °C (MeOH–ethyl acetate). NMR ($CD_3OD$) δ: 1.35 (9H, s), 3.22 (3H, s), 3.50—3.70 (4H, m), 3.90—4.05 (2H, m), 4.10—4.20 (4H, m), 5.70—5.80 (2H, m), 6.05—6.20 (1H, m), 7.18 (1H, d, *J*=7 Hz), 7.26 (1H, d, *J*=7 Hz), 7.39 (1H, s). ESI-MS *m/z*: 315 ($M^+ + 1$). IR (KBr, $cm^{-1}$) 1638, 1582, 1480, 1429. *Anal.* (%) Calcd for $C_{19}H_{28}N_3OBr \cdot 2/3H_2O$: C, 56.16; H, 7.28; N, 10.19. Found: C, 56.19; H, 7.18; N, 9.91.

**2-(4-Methyl-1-piperazinyl)-5,6,7,8-tetrahydronaphtho[2,3-*d*]oxazole (11c)** 2-Mercapto-5,6,7,8-tetrahydronaphtho[2,3-*d*]oxazole (**9c**, 100 mg, 0.5 mmol) was treated with phosphorus pentachloride (122 mg, 0.6 mmol) and the resultant 2-chloro-5,6,7,8-tetrahydronaphtho[2,3-*d*]-oxazole was allowed to react with 1-methylpiperazine (976 mg, 10 mmol) as described for the preparation of **11a** to afford **11c** (69 mg, 52%), mp 198—199 °C (MeOH–ethyl acetate). NMR ($CDCl_3$) δ: 1.79—1.82 (4H, m), 2.35 (3H, s), 2.56 (4H, t, *J*=5 Hz), 2.80 (4H, br s), 3.66 (4H, t, *J*=5 Hz), 6.95 (1H, s), 6.99 (1H, s). EI-MS *m/z*: 271 ($M^+$). IR (KBr, $cm^{-1}$) 1634, 1574, 1466, 1445, 1300. *Anal.* (%) Calcd for $C_{16}H_{21}N_3O \cdot 1/6H_2O$: C, 70.04; H, 7.84; N, 15.32. Found: C, 70.18; H, 7.93; N, 15.41.

**1-Allyl-1-methyl-4-(5,6,7,8-tetrahydronaphtho[2,3-*d*]oxazol-2-yl)piperazinium Iodide (12c)** 2-(4-Methylpiperazinyl)-5,6,7,8-tetrahydronaphtho-[2,3-*d*]oxazole (**11c**, 30 mg, 0.1 mmol) was allowed to react with allyl iodide (170 mg, 1 mmol) as described for the preparation of **7a** to afford **12c** (42 mg, 88%) as an oil. NMR ($CD_3OD$) δ: 1.79—1.82 (4H, m), 2.81 (4H, br s), 3.23 (3H, s), 3.62—3.66 (4H, m), 3.93—4.01 (2H, m), 4.11—4.20 (4H, m), 5.77—5.81 (2H, m), 6.09—6.20 (1H, m), 7.02 (1H, s), 7.06 (1H, s). ESI-MS *m/z*: 312 ($M^+$) IR (neat, $cm^{-1}$) 1624, 1574, 1468, 1269. HR-MS *m/z*: Calcd for $C_{19}H_{26}N_3O$: 312.2077 Found 312.2022.

**5-Chloro-2-mercaptobenzoxazole (9d)** 2-Amino-4-chlorophenol (**8d**, 10 g, 70 mmol) was refluxed for 8 h with potassium hydroxide (4.7 g, 84 mmol) and carbon disulfide (100 ml) in ethanol (150 ml). The reaction mixture was concentrated *in vacuo*. A 1 N aqueous hydrochloric acid solution (100 ml) and ethyl acetate (200 ml) were added to the residue. The organic layer was washed with water (100 ml), dried over $MgSO_4$ and concentrated *in vacuo*, to afford 11.5 g (89%) of crude **9d** as a yellow powder. This was used in the next reaction without further purification.

**5-Chloro-2-(4-methyl-1-piperazinyl)benzoxazole (11d)** 5-Chloro-2-mercaptobenzoxazole (**9d**, 1 g, 5.4 mmol) was treated with phosphorus pentachloride (1.35 g, 6.5 mmol) and the resultant 2,5-dichlorobenzoxazole (**10d**) was allowed to react with 1-methylpiperazine (5.4 g, 54 mmol) as described for the preparation of **11a** to afford **11d** (1 g, 74%), mp 118—119 °C (acetone–*n*-hexane). NMR ($CD_3OD$) δ: 2.34 (3H, s), 2.57 (4H, t, *J*=5 Hz), 3.71 (4H, t, *J*=5 Hz), 7.03 (1H, d, *J*=7 Hz), 7.22 (1H, s), 7.26 (1H, d, *J*=7 Hz). EI-MS *m/z*: 251 ($M^+$). IR (KBr, $cm^{-1}$) 1634, 1574, 1449, 1398, 1368, 1356. *Anal.* (%) Calcd for $C_{12}H_{14}ClN_3O$: C, 57.26; H, 5.61; N, 16.70. Found: C, 56.98; H, 5.56; N, 16.50.

**1-Allyl-1-methyl-4-(5-chlorobenzoxazol-2-yl)piperazinium Iodide (12d)** 5-Chloro-2-(4-methylpiperazinyl)benzoxazole (**11d**, 630 mg, 2.5 mmol) was allowed to react with allyl iodide (2.1 g, 12 mmol) as described for the preparation of **7a** to afford **12d** (957 mg, 92%), mp 198 °C (EtOH–*n*-hexane). NMR ($CD_3OD$) δ: 3.26 (3H, s), 3.95—4.10 (2H, m), 4.10—4.30 (4H, m), 5.75—5.90 (2H, m), 6.05—6.95 (1H, m), 7.10 (1H, dd, *J*=2, 8 Hz), 7.32 (1H, d, *J*=2 Hz), 7.37 (1H, d , *J*=8 Hz). ESI-MS *m/z*: 292 ($M^+$). IR (KBr, $cm^{-1}$) 1628, 1568, 1455, 1395, 1287. *Anal.* (%) Calcd for $C_{15}H_{19}ClN_3OI$: C, 42.93; H, 4.56; N, 10.01. Found: C, 42.78; H, 4.39; N, 9.71.

**6-Methyl-2-(4-methyl-1-piperazinyl)benzoxazole (11e)** 6-Methyl-2-mercaptobenzoxazole (**9e**, 200 mg, 1.21 mmol) was treated with phosphorus pentachloride (302 mg, 1.45 mmol) and the resultant 2-chloro-6-methylbenzoxazole (**10e**) was allowed to react with 1-methylpiperazine (1.34 ml, 12.1 mmol) as described for the preparation of **11a** to afford **11e** (166 mg, 59%), mp 62—63 °C (MeOH–ether). NMR ($CDCl_3$) δ: 2.35 (3H, s), 2.40 (3H, s), 2.52 (4H, t, *J*=5 Hz), 3.70 (4H, t, *J*=5 Hz), 6.97 (1H, d, *J*=8 Hz), 7.07 (1H, s), 7.23 (1H, d, *J*=8 Hz). EI-MS *m/z*: 231 ($M^+$). IR (KBr, $cm^{-1}$) 1650, 1578, 1489, 1397. *Anal.* (%)

Calcd for $C_{13}H_{17}N_3O$: C, 67.51; H, 7.41; N, 18.17. Found: C, 67.21; H, 7.30; N, 17.87.

**1-Allyl-1-methyl-4-(6-methylbenzoxazol-2-yl)piperazinium Bromide (12e)** 6-Methyl-2-(4-methylpiperazinyl)benzoxazole (**11e**, 15 mg, 0.06 mmol) was allowed to react with allyl bromide (51 $\mu$l, 0.6 mmol) as described for the preparation of **7a** to afford **12e** (15 mg, 66%) as an oil. NMR ($CD_3OD$) $\delta$: 2.45 (3H, s), 3.27 (3H, s), 3.60—3.80 (4H, m), 3.95—4.10 (2H, m), 4.10—4.30 (4H, m), 5.80—5.90 (2H, m), 6.10—6.25 (1H, m), 7.10 (1H, d, $J$=8 Hz), 7.26 (1H, s), 7.27 (1H, d, $J$=8 Hz). ESI-MS $m/z$: 272 ($M^+$). IR (KBr, $cm^{-1}$) 1636, 1578, 1509, 1487, 1431. HR-MS $m/z$: Calcd for $C_{16}H_{22}N_3O$: 272.1763 Found 272.1758.

**6-Nitro-2-(4-methyl-1-piperazinyl)benzoxazole (11f)** 6-Nitro-2-mercaptobenzoxazole (**9f**, 600 mg, 3.06 mmol) was treated with phosphorus pentachloride (764 mg, 3.67 mmol) and the resultant 2-chloro-5-nitrobenzoxazole (**10f**) was allowed to react with 1-methylpiperazine (3.4 ml, 30.6 mmol) as described for the preparation of **11a** to afford **11f** (669 mg, 83%), mp 109—110 °C (MeOH–ether). NMR ($CDCl_3$) $\delta$: 2.37 (3H, s), 2.56 (4H, t, $J$=5 Hz), 3.81 (4H, t, $J$=5 Hz), 7.32 (1H, d, $J$=9 Hz), 8.14 (1H, d, $J$=2 Hz), 8.19 (1H, dd, $J$=2, 9 Hz). EI-MS $m/z$: 262 ($M^+$). IR (KBr, $cm^{-1}$) 1655, 1593, 1505, 1472, 1397, 1374. *Anal.* (%) Calcd for $C_{12}H_{14}N_4O_3$: C, 54.96; H, 5.38; N, 21.36. Found: C, 55.26; H, 5.37; N, 21.13.

**1-Allyl-1-methyl-4-(6-nitrobenzoxazol-2-yl)piperazinium Bromide (12f)** 6-Nitro-2-(4-methylpiperazinyl)benzoxazole (**11f**, 18 mg, 0.07 mmol) was allowed to react with allyl bromide (59 $\mu$l, 0.7 mmol) as described for the preparation of **7a** to afford **12f** (18.1 mg, 70%), mp 254—255 °C (MeOH–ether). NMR ($CD_3OD$) $\delta$: 3.30 (3H, s), 4.10—4.20 (2H, m), 4.23—4.35 (4H, m), 5.80—5.90 (2H, m), 6.15—6.30 (1H, m), 7.49 (1H, d, $J$=9 Hz), 8.27 (1H, dd, $J$=2, 9 Hz), 8.34 (1H, d, $J$=2 Hz). FAB-MS $m/z$: 303 ($M^+$). IR (KBr, $cm^{-1}$) 1647, 1590, 1507, 1439, 1397, 1366. *Anal.* (%) Calcd for $C_{15}H_{19}N_4O_3Br$: C, 47.01; H, 5.00; N, 14.62. Found: C, 46.73; H, 4.92; N, 14.52.

**6-Methoxy-2-(4-methyl-1-piperazinyl)benzoxazole (11g)** 6-Methoxy-2-mercaptobenzoxazole (**9g**, 600 mg, 3.31 mmol) was treated with phosphorus pentachloride (827 mg, 3.97 mmol) and the resultant 2-chloro-6-methoxybenzoxazole (**10g**) was allowed to react with 1-methylpiperazine (3.67 ml, 33.1 mmol) as described for the preparation of **11a** to afford **11g** (369 mg, 45%), mp 38—39 °C (MeOH–$H_2O$). NMR ($CD_3OD$) $\delta$: 2.32 (3H, s), 3.56 (4H, t, $J$=5 Hz), 3.59 (3H, s), 3.68 (4H, t, $J$=5 Hz), 7.36 (1H, d, $J$=8 Hz), 7.74 (1H, dd, $J$=2, 8 Hz), 7.83 (1H, d, $J$=2 Hz). EI-MS $m/z$: 247 ($M^+$). IR (KBr, $cm^{-1}$) 1647, 1622, 1585, 1489, 1445. *Anal.* (%) Calcd for $C_{13}H_{17}N_3O_2 \cdot 3/4H_2O$: C, 59.87; H, 7.15; N, 16.11. Found: C, 59.81; H, 6.92; N, 15.86.

**1-Allyl-1-methyl-4-(6-methoxybenzoxazol-2-yl)piperazinium Bromide (12g)** 6-Methoxy-2-(4-methylpiperazinyl)benzoxazole (**11g**, 200 mg, 0.81 mmol) was allowed to react with allyl bromide (978 mg, 8.1 mmol) as described for the preparation of **7a** to afford **12g** (229 mg, 77%), mp 144 °C (MeOH–ethyl acetate). NMR ($CD_3OD$) $\delta$: 3.24 (3H, s), 3.61—3.70 (4H, m), 3.81 (3H, s), 3.90—4.20 (2H, m), 4.11—4.15 (2H, m), 4.19 (2H, d, $J$=6 Hz), 5.78—5.82 (2H, m), 6.10—6.18 (1H, m), 6.84 (1H, dd, $J$=2, 8 Hz), 7.05 (1H, d, $J$=2 Hz), 7.24 (1H, d, $J$=8 Hz). ESI-MS $m/z$: 288 ($M^+$). IR (KBr, $cm^{-1}$) 1624, 1581, 1489, 1396. *Anal.* (%) Calcd for $C_{16}H_{22}N_3O_2I \cdot 3/4H_2O$: C, 44.82; H, 5.17; N, 9.60. Found: C, 45.06; H, 5.16; N, 9.45.

**6-Hydroxy-2-(4-methyl-1-piperazinyl)benzoxazole (11h)** 6-Methoxy-2-(4-methylpiperazinyl)benzoxazole (**11g**, 130 mg, 0.5 mmol) was treated with HBr–acetic acid (12 ml) and the mixture was heated at 100 °C for 40 h in a sealed tube. The reaction mixture was concentrated under reduced pressure and the residue was dissolved with chloroform. The organic layer was washed with 1 N aqueous NaOH and saturated aqueous NaCl, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on silica gel with chloroform–methanol (20 : 1) and recrystallized from ethyl acetate–*n*-hexane to give **11h** (81 mg, 65%), mp 213 °C. NMR ($CD_3OD$) $\delta$: 2.31 (3H, s), 2.52 (4H, t, $J$=5 Hz), 3.60 (4H, t, $J$=5 Hz), 6.59 (1H, dd, $J$=3, 8 Hz), 6.73 (1H, d, $J$=3 Hz), 7.02 (1H, d, $J$=8 Hz). EI-MS $m/z$: 233 ($M^+$). IR (KBr, $cm^{-1}$) 1647, 1617, 1589, 1495, 1450. *Anal.* (%) Calcd for $C_{12}H_{15}N_3O_2$: C, 61.79; H, 6.48; N, 18.02. Found: C, 61.91; H, 6.25; N, 17.80.

**1-Allyl-1-methyl-4-(6-hydroxybenzoxazol-2-yl)piperazinium Iodide (12h)** 6-Hydroxy-2-(4-methylpiperazinyl)benzoxazole (**11h**, 100 mg, 0.43 mmol) was allowed to react with allyl iodide (730 mg, 4.3 mmol) as described for the preparation of **7a** to afford **12h** (125 mg, 72%), mp 222—224 °C (dec., MeOH–ethyl acetate). NMR ($CD_3OD$) $\delta$: 3.23 (3H, s), 3.57—3.75 (4H, m), 3.90—4.00 (2H, m), 4.09—4.16 (2H, m), 4.19 (2H, d, $J$=6 Hz), 5.78—5.83 (2H, m), 6.11—6.19 (1H, m), 6.71 (1H, dd, $J$=2, 7 Hz), 7.05 (1H, d, $J$=2 Hz), 7.24 (1H, d, $J$=7 Hz). ESI-MS $m/z$: 274 ($M^+$). IR (KBr, $cm^{-1}$) 1635, 1590, 1462, 1448. *Anal.* (%) Calcd for $C_{15}H_{20}N_3O_2I \cdot 3/2H_2O$: C, 42.00; H, 5.31; N, 9.81. Found: C, 42.29; H, 5.01; N, 9.54.

**X-Ray Structure Determination of Compound 1** The X-ray structure determination was performed with a Rigaku AFC5R diffractometer and the teXsan software on a Silicon Graphics workstation. The crystal was prismatic with cell parameters of $a$=6.553(3) Å; $b$=19.440(4) Å; $c$=12.893(3) Å, space group $P2_1/c$ (#14) and $Z$=4. The $R$ factor was 4.0.

**Molecular Modeling** Molecular modeling was performed using the QUANTA/CHARMm molecular modeling software, running on a Silicon Graphics workstation.

**Contraction Test** Male Hartley guinea pigs weighing 500—800 g were killed by bleeding from the neck and the ileum was excised. Pieces (about 20 mm) of ileal longitudinal muscles were placed in a 5 ml organ bath containing Krebs solution aerated with 95% $O_2$ and 5% $CO_2$ at 37 °C. The composition of the solution was as follows (mM): NaCl 118, KCl 4.7, $KH_2PO_4$ 1.19, $MgSO_4$ 1.2, $CaCl_2$ 2.54, $NaHCO_3$ 25 and glucose 11. The solution also contained ritanserin ($10^{-7}$ M) to inhibit the 5-$HT_2$ receptor. The preparations were allowed to equilibrate for at least 30 min under 0.5 g tension. After equilibration, the preparations were repeatedly exposed to $3 \times 10^{-7}$ M of 5-HT to desensitize the 5-$HT_4$ receptor. Compounds were added to the bath and contractions were recorded isometrically. The sensitivities of agonists were expressed as $pD_2$ value, *i.e.*, the negative logarithm of the molar concentration which produced 50% of the maximum contraction obtained from individual concentration–response curves. The *ia* of partial agonists were expressed as the ratio between the maximum response to a test compound and that to 5-HT ($10^{-5}$ M).

**5-$HT_3$ Receptor Binding Assay** The assay was performed according to the method of Kilpatrick *et al.*[12] Brain cortices were isolated from male Wistar rats (250—300 g) and a membrane fraction was prepared by standard techniques. The membrane fraction (0.04 mg) was incubated with 0.2 nM [$^3$H]GR656630 for 60 min at 22 °C. Membranes were collected by filtration and washed 3 times. The radioactivity on the filters was counted to determine [$^3$H]GR656630 specifically bound. Non-specific binding was estimated in the presence of 1 mM ICS205-930.

**Effect on the B–J Reflex in Rats** The assay was conducted according to the procedure of Fozard and Host.[13] Male Wistar rats (250—300 g) were anesthetized with urethane (1.25 g/kg i.p.) and blood pressure and heart rate were recorded. A submaximal dose of 5-HT (10 $\mu$g/kg i.v.) was given repeatedly, and changes in heart rate were observed. Test compounds were given intravenously 5 min prior to administration of 5-HT, and their effect was expressed as percent induction and inhibition of the 5-HT response.

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## References

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