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DESIGN AND SYNTHESIS OF A PIPERAZINE DERIVATIVE AS A HIGH AFFINITY 5-HT_{1A} RECEPTOR LIGAND

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ABSTRACT

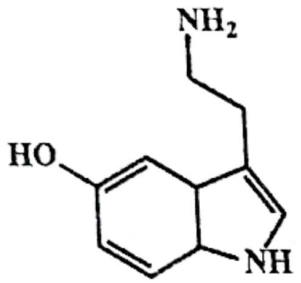
Serotonin (5-HT) 1A receptors have been shown to have physiological, biochemical pharmacological and clinical values. The piperazino derivative **1** was rationally designed as a bulky, lipophilic N4-substituted arylpiperazine. Radioligand binding assay of **1** at 5-HT_{1A} receptors indicates that it binds at these receptors with higher affinity than the famous 5-HT_{1A} ligand, buspirone (K_i = 15 nM). Buspirone is currently used to treat anxiety.

INTRODUCTION

The history of serotonin **2** began sixty years ago when it was discovered in the intestine and was then called enteramine[1]. In 1947, serotonin was characterized as a vasoconstrictor substance in the serum and was proven to be 5-hydroxytryptamine **2** (5-HT)[2]. About ten years later, Gaddum and Picarelli proposed two types of tryptamine receptors in guinea pig ileum[3]. The first type was blocked by dibenzyline (phenoxybenzamine) and called D receptors. The second type was antagonized by morphine and called M receptors[3]. In 1979, Peroutka and Snyder proposed two distinct types of serotonin receptors in the central nervous system[4]. Their classification was based on the radioligand binding technique. One type was called 5-HT_1 receptors and was labeled by [^{3}H]-5-HT. The second type was labeled by the D-2 neuroleptic, [^{3}H]-spiperone **3** and called 5-HT_2 receptors[4]. Additional studies revealed

that 3 showed biphasic effect on the 5-HT_1 receptors, i.e. showed higher affinity for a binding site and lower affinity for another site. The two binding sites were called 5-HT_{1A} and 5-HT_{1B}, respectively[5]. Recent studies suggested the existence of other kinds of 5-HT_1 receptors such as 5-HT_{1C}, 5-$HT_{1D\,(\alpha,\beta)}$, 5-HT_{1E}, 5-HT_{1S}, 5-HT_{1P} and 5-HT_{1R}[6].

In addition to 5-HT_1 and 5-HT_2 receptors, 5-HT_3 receptors were discovered. These receptors were suggested to be the Gaddum and Picarelli M receptors[7]. Additional classes of 5-HT receptors have been also suggested[6-8].



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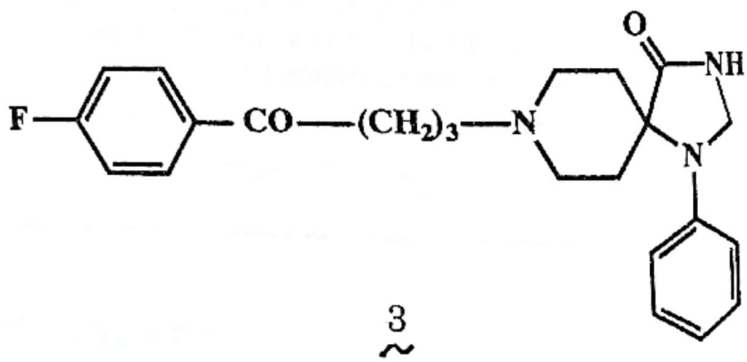

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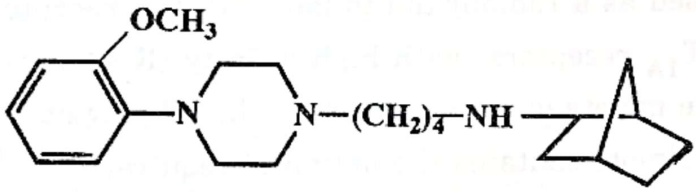
From the various kinds of 5-HT receptors, 5-HT_{1A} receptors have received the most attention. This kind plays an important role in the modulation of numerous biological functions such as cardiovascular functions, food intake, temperature, mood, sexual activity and hormonal activities. Various 5-HT_{1A} ligands have been shown to be useful in many disease states such as anxiety, depression, migraine and nociception[9-11].

Several classes of ligands were tested for 5-HT_{1A} receptors of these classes, arylpiperazines have been shown to possess affinity for 5-HT_{1A} receptors[12-14]. There was a previous hypothesis that arylpiperazines

bind at 5-HT_{1B} receptors more than at 5-HT_{1A} receptors. Later investigations showed that substitution on the N_4 of the piperazine ring provide compounds with modest affinity and selectivity for 5-HT_{1A} receptors[13]. Recent studies indicated that increasing the carbon chain (four carbons are optimum length) results in increasing the affinity for 5-HT_{1A} receptor sites. Various amido derivatives of 2-methoxyphenylpiperazines bind at these receptors with K_i in the nanomolar range. The highest affinity ligands were the amides containing branched, bulky and hydrophobic substituents[14,15].



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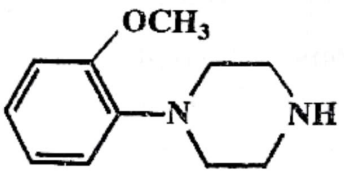

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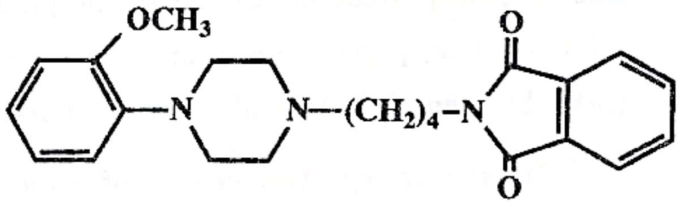

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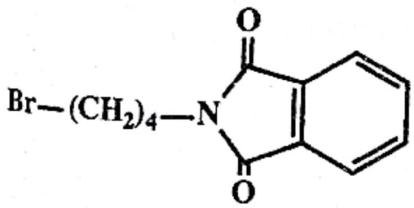

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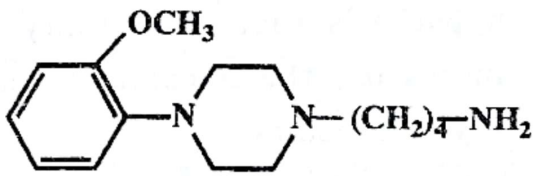

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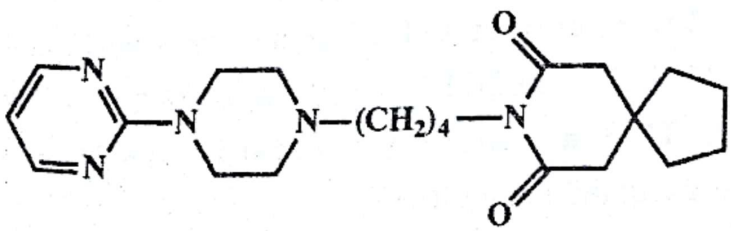

Buspirone

To further investigate structure affinity relationship (SAFIR), the target ligand **1** was rationally designed and synthesized. It contains a branched, lipophilic and bulky group (the norbornyl moiety) directly attached to an NH not to an amide.

RESULTS AND DISCUSSION

Radioligand binding studies[16] were performed by using Sprague-Dawley rats. [^{3}H]-8-hydroxy-n-dipropylaminotetralin (8-OH-DPAT) was used as a radioligand to label 5-HT$_{1A}$ receptors. Compound **1** binds at 5-HT$_{1A}$ receptors with high affinity (K_i=4 nm). This indicates that the amide moiety is not essential for the affinity at 5-HT$_{1A}$ receptors. Compound **1** almost contains the optimum requirements[16-18] for 5-HT$_{1A}$ receptor ligands which are : (i) methoxy group at the 2 position of the phenyl group attached to N-1 of the piperazine ring (ii), the basic nitrogen (N-4 of the piperazine moiety), (iii) four carbons attached to N-4 and (iv) a branched, bulky-alkyl group, norbornyl).

In summary, the result of this study is in agreement with the previous SAFIR and molecular modeling studies. It supports the hypothesis that the affinity for 5-HT$_{1A}$ receptors enhances with increasing the lipophilicity and the bulkiness of the N4-substituted arylpiperazines.

EXPERIMENTAL

Melting points were determined by using a Thomas-Hoover melting point apparatus and were uncorrected. Microanalysis were conducted by Atlantic Microlab, USA. Proton NMR spectra were performed on GE-300 spectrometer with TMS as internal standard. Spectral data were consistent with the assigned structures.

1-2(-Methoxyphenyl)-4-[4-(2-phthalimido)butyl] piperazine 6 :

This compound was prepared as previously reported[15] with slight modification. N-(4-Bromobutyl)phthalimide **5** (6 g, 0.021 mol) in CH_3CN (20 mL) was added to a mixture of 1-(2-methoxyphenyl)piperazine HCl (4.87 g, 0.021 mol) , K_2CO_3 (6 g, 0.034 mol), and KI (10 mg, 0.047 mol) in CH_3CN (20 mL). The reaction mixture was heated under reflux for 24 h and filtered while hot. The filtrate was evaporated under reduced pressure. The residue was recrystallized from absolute EtOH to afford 7.12 g (85%) of **6**, mp 80-81 °C as in literature[15].

4-(4-Aminobutyl)-1-(2-methoxyphenyl)piperazine 7 :

This compound was prepared from **6** by Gabriel synthesis[16].

1-(2-methoxyphenyl)-4-[4-(norbornylamino) butyl] piperazine fumarate 1:

The amine **7** (200 mg, 0.76 mmol) was dissolved in absolute EtOH (25 ml) and mixed with norcamphor (210 mg, 1.91 mmol) and 10% Pd/C (70 mg). The mixture was hydrogenated at 45 psi for 18 h then filtered over celite pad. The solvent was evaporated under reduced pressure. The resulting residue was dissolved in anhydrous Et_2O (10 mL). The fumarate salt was formed by the slow addition of ethereal fumaric acid. The resulting solid was collected by filtration and recrystallized from a mixture of absolute EtOH and anhydrous Et_2O to afford 79 mg (58%) of the title compound as white crystals, mp: 154-156°C. Anal. ($C_{22}H_{35}N_3O$. Fumarate monohydrate) C, 63.52; H, 8.41; N, 8.55. Found C, 63.39; H, 8.02; N, 8.33: ^{1}HNMR (DMSO-d_6) δ : 1.01-1.7 (m, 8 H, CH_2), 2.2-2.49 (m, 8 H, CH_2), 3.75 (S, 3 H, OCH_3), 6.83-6.95 (m, 4 H, ArH).

Radioligand binding studies[17]:

The radioligand binding assay was performed in the laboratory of professor Milt Teitler, Department of Pharmacology, Albany Medical College, USA. Sprague-Dawley rats (≈ 220 g) were used. Their brains were isolated after decapitation and placed in ice-cold saline. The tissues were homogenized in 30 volumes of ice-cold buffer containing 50 mM tris-HCl, 0.5 mM Na_2EDTA, and 10 mM $MgSO_4$. The homogenized tissues were centrifuged at 30,000 g for 15 min. The formed pellet was washed and resuspended. The $5\text{-}HT_{1A}$ receptors were labeled with 0.1 nM [^{3}H]-8-OH-DPAT. The nonspecific binding was determined by using 8-OH-DPAT. Various concentrations of the compound to be tested were used and incubated with the tissues and [^{3}H)-8-OH-DPAT for 20 min at 37°C. Samples were filtered over glass filters. The individual filters were poured into vials and equilibrated with 5 mL of scintillation fluid then counted at 45% efficiency in Beckman 3801 counter. Computer program EBDA was used to analyze the data and to determine K_i .

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تصميم وتخليق مشتق بيبرازين ذو فاعلية عالية لمستقبلات سيروتونين - ١أ (5-HT_{1A})

محمد عبد الشافى البرماوى

قسم الكيمياء العضوية – كلية الصيدلة – جامعة الزقازيق – مصر

ثبت أن مستقبلات السبروتونين 5-HT_{1A} لها أهمية فسيولوجية وكيموحيوية وفارماكولوجية وإكلينيكية . ولذلك فى هذا البحث تم تصميم أحد مشتقات البيبرارين الذى يحتوى على مجموعة ضخمة ولها قابلية لليبيدات وهذه المجموعة متصلة بذرة النيتروجين رقم ٤ لحلقة البيبرازين . وبفحص هذا المشتق بطريقة الترابط الاشعاعى . ثبت أنه يتماسك مع مستقبلات 5-HT_{1A} بقابلية عالية أكثر من مستقبل 5-HT_{1A} المعروف "بيسبيرون ، الذى يتماسك بمستقبلات 5-HT_{1A} بدرجة عالية ($K_i = 15$ nm) والذى يستخدم حاليا لعلاج الاضطرابات العصبية.