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Solid phase synthesis of sulphonamides: novel ligands of 5-HT$_6$ receptors

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Abstract

Using solid phase synthesis techniques, we have rapidly obtained a series of eight aryl sulphonamides derived from putrescine. These conjugates with various aryl groups were evaluated for their affinity towards 5-HT$_6$ receptors in man. This evaluation revealed the interest of two compounds which present the same activity level, in the submicromolar range, as two reference derivatives. The most potent will be considered as a new lead for further investigations.

Introduction

The neurotransmitter serotonin (5-HT) can activate seven types of receptors named 5-HT$_1$ to 5-HT$_7$. These 5-HT receptors are even subdivided into fourteen subtypes, among which the 5-HT$_6$ receptors are some of the more recently cloned (Ruat et al 1993). Their localisation – mainly in the CNS – associated with the high affinity of various anti-psychotic and anti-depressor drugs could suggest the involvement of these receptors in psychiatric diseases such as nervous breakdown or schizophrenia (Roth et al 1994). Recent studies have also shown that 5-HT$_6$ receptors are implicated in the modulation of central cholinergic transmission, selective 5-HT$_6$ receptor antagonists putatively being of interest in the field of anxiety and memory alterations (Bourson et al 1995; Sleight et al 1996).

However, a better knowledge of the physiological, pharmacological and patho-physiological roles of these receptors is dependent on the development of potent and selective ligands for the 5-HT$_6$ receptors. The first ligands described possess bisarylsulphonamide structures (Ro 04-6790, Ro 63-05635 (Sleight et al 1998), SB-271046 (Bromidge et al 1999), Figure 1) and basic moieties (amidine or amino groups). Their affinity range is of submicromolar to nanomolar concentrations.

Very recently, a functional organisation, named ATBI (Atelier de Binding) was established, that joins chemistry, molecular modelling and biology. Resulting from a collaboration between the CERMN (Centre d'Etudes et de Recherche sur le Médicament de Normandie) and the UMR (Unité Mixte de Recherche) CNRS 6551, this collaborative structure is dedicated to the identification of novel ligands of human serotonin 5-HT$_4$, 5-HT$_5$, 5-HT$_6$ and 5-HT$_7$ receptor subtypes.

In this context, we have synthesised a small library of sulphonamide derivatives from putrescine using solid phase chemistry. The effects of these compounds on human 5-HT$_6$ receptors were evaluated in-vitro.

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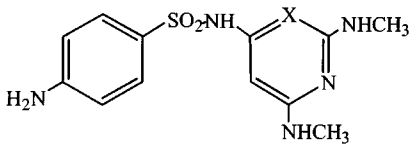
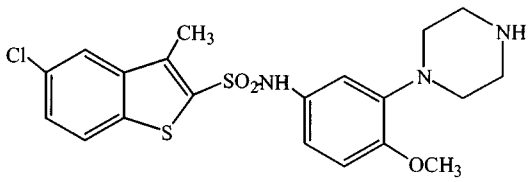


X = N : **Ro 04-6790**, pKi = 7.3
X = CH : **Ro 63-0563**, pKi = 7.9



SB-271046, pKi = 8.9

Figure 1 Chemical structures of 5-HT_6 receptor ligands.

Materials and Methods

Preparation of polymer-bound benzyl-4-nitrophenyl carbonate 1

A suspension of the hydroxylated polymer (Wang resin) (5g, 3.25 mmol OH) in anhydrous dichloromethane (15 mL) was treated successively by a solution of *N*-methylmorpholine (0.65 g, 6.50 mmol) then a solution of 4-nitrophenyl chloroformate (1.30 g, 6.50 mmol) in anhydrous dichloromethane. The suspension was allowed to stir for 18 h under nitrogen flush at room temperature. The polymer was then washed with dichloromethane (5×20 mL) to remove the excessive reagents and dried under reduced pressure. The procedure was repeated to lead the reaction to completeness.

Preparation of Wang-carbonylputrescine polymer 2

To a suspension of the polymer **1** (5g, 3.25 mmol) in anhydrous dichloromethane (15 mL) was added, drop-wise, a solution of 1,4-diaminobutane (2.86 g, 32.5 mmol) in anhydrous dichloromethane (10 mL). The suspension was stirred for 18 h at room temperature then washed successively (general procedure for the washing) by dichloromethane (2×20 mL), tetrahydrofuran (2×20 mL), a mixture of water and tetrahydrofuran (2×20 mL), water (2×20 mL), tetrahydrofuran (2×20 mL), then diethyl ether (2×20 mL) and then dried under reduced pressure.

General procedure for Nω-(aryl)-1-sulphonyl-Nα-carbonyl-Wang-diamine polymers 3a–h

Polymer **2** (0.5 g, 0.325 mmol) was suspended in anhydrous dichloromethane. A solution of arylsulfonyl chloride (0.65 mmol) and *N*-methylmorpholine (0.065 g, 0.65 mmol) in anhydrous dichloromethane (5 mL) was added and the suspension was stirred for 3 h, and washed and dried according to the usual procedure. The procedure was repeated to lead the reaction to completeness.

General procedure for the cleavage

Polymer **3a–h** (0.5 g) was suspended in a mixture of trifluoroacetic acid (1 mL) and dichloromethane (1 mL). The suspension was stirred for 2 h at room temperature. The polymer was removed by filtration and washed with dichloromethane (5×2 mL). The filtrate was concentrated to dryness under reduced pressure, dissolved in the minimal amount of water (2 mL), alkalinised with aqueous sodium hydroxide (NaOH 10 %) then extracted with dichloromethane (3×5 mL). The organic layer was dried over potassium carbonate and the solvent removed under reduced pressure. A 1.5 M ethanolic solution of hydrochloric acid (1.2 equiv.) was added at 0°C to the crude residue dissolved in ethanol (2 mL). After a 30-min period of stirring, the ethanol was then evaporated and the residue was triturated in diethyl ether to yield **4a–h** (50 % average yield) as white precipitates which were collected and dried under reduced pressure.

Pharmacological characterisation of drugs on human 5-HT_6 receptors

Drugs were evaluated in terms of their ability to compete with the binding of [^{3}H]LSD on membranes of *sf9* cells transiently expressing the human 5-HT_6 receptor (CRM-044, NEN Life Sciences) according to Monsma et al (1993). In brief, 4 μg of proteins were incubated at 27°C for 90 min in duplicate in the absence or the presence of 10^{-6} or 10^{-8} M of each drug and 2 nM [^{3}H]LSD in 50 mM Tris-HCl buffer (pH 7.4) supplemented with 10 mM $MgSO_4$ and 0.5 mM EDTA. At the end of the incubation, the homogenates were filtered through Whatman GF/A filters and washed five times with ice-cold 50 mM Tris-HCl buffer. Non-specific binding was evaluated, in parallel, in the presence of 10^{-5} M clozapine. Radioactivity associated with proteins was then quantified

and expressed as the percentage of inhibition for each concentration of the drugs under study.

Results and Discussion

Polyamines synthesis is associated with two main problems: regio-selectivity (at least two amino groups whose differentiation is difficult) and high polarity (resulting in difficult purification). The use of protective groups is the first solution to these problems. Solid phase chemistry is the second approach: acting as an insoluble protective group, the polymer facilitates syntheses and highly simplifies the purification steps.

In our synthetic pathway (Tomasi et al 1998; Figure 2), the diamine is grafted on the support via a carbamate linker by nucleophilic displacement of the carbonate **1**. The primary amine of polymer **2** is then reacted by various arylsulphonyl chlorides in the presence of a tertiary amine. The cleavage in acidic medium provides the final compounds **4a**–**h**.

The main advantage of this method, when compared with solution phase chemistry, is its rapidity. After the common steps (anchorage of the diamine), the resin **2** is divided into amounts of 0.5 g in syringes for the parallel synthesis of sulphonamides. After cleavage, trifluoroacetate salts are obtained. Their treatment with sodium hydroxide allows their extraction by dichloromethane and salification by hydrogen chloride. The compounds **4a**–**h** were then obtained with an average yield of 50% and purity was evaluated at 95% from their TLC (eluent: CH_3OH–NH_4OH, 90:10) and ^{1}H NMR studies.

The compounds **4a**–**h** are displayed in Table 1, as well

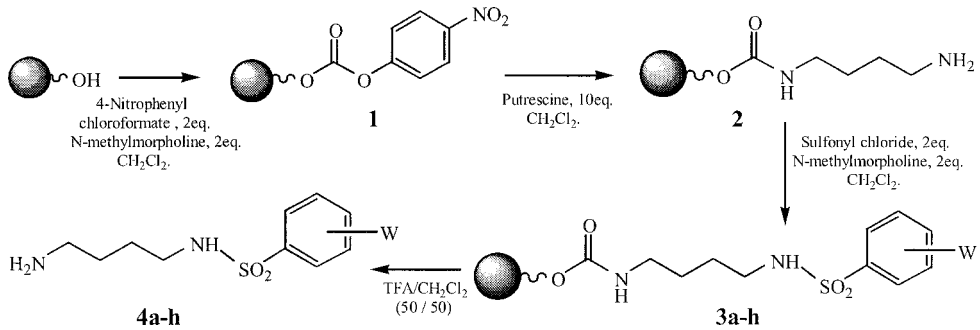
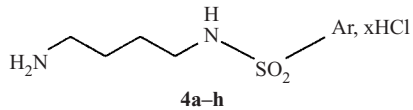
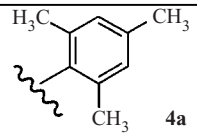
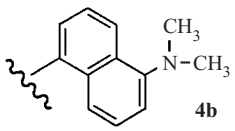
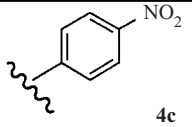
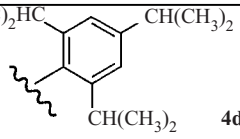


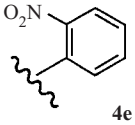
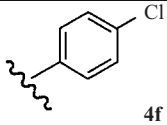
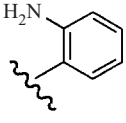
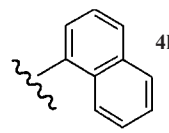
Figure 2 Synthetic pathway to compounds **4a**–**h**.

Table 1 Inhibition (%) of [^{3}H]LSD binding on human 5-HT$_6$ receptor by compounds **4a**–**h**.



4a–h

Ar =	 **4a**	 **4b**	 **4c**	 **4d**
10^{-6} M	53	30	0	97
10^{-8} M	0	4	2	10

Ar =	 **4e**	 **4f**	 **4g**	 **4h**
10^{-6} M	16	13	28	3
10^{-8} M	5	6	0	1

as the percentage of inhibition of [^{3}H]LSD binding on human 5-HT_6 receptors measured for two concentrations.

Despite moderate affinities towards 5-HT_6 receptors for most of the compounds tested, this study first demonstrates the high affinity of compound **4d** for these receptors (apparent IC50 (concentration of drug that inhibits 50% of the ligand binding) and pK_i ($-\log K_i$, K_i: inhibition constant) of 30 nM and 7.4, respectively; similar to the affinities published (Sleight et al 1996) for Ro 04-6790 and Ro 63-0563).

A structure–activity relationship has to be studied to complete these first results. Three parameters must be investigated: the importance of the sterical hindrance in the *ortho* position of the sulphonamide group; the length of the polyamine chain; and the functional modifications on the amino group. To improve the affinity and selectivity of compounds towards the 5-HT_6 receptor, a modulation could thus be carried from our first structure **4d**.

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