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Synthesis and pharmacological evaluation of benzannulated derivatives as potent and selective sigma-1 protein ligands

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ABSTRACT

The σ_1 proteins are considered to be a new class of target structures for several central nervous system disorders, including depression, anxiety, psychosis, and Parkinson's and Alzheimer's diseases. Recently, the involvement of these receptors in neuropathic pain and cancer has also been observed. So far, only a few ligands are in clinical trials. In a continuation of our previous studies on the development of σ_1 ligands, a new series of benzannulated heterocycles was designed and synthesised. In vitro competition binding assays showed that many of them possessed high σ_1 receptor affinity (K_i = 0.6–10.3 nM), and good σ_2/σ_1 subtype selectivity, without cytotoxic effects on SY5Y cells (human neuroblastoma cell line).

1. Introduction

Originally proposed as a subtype of opioid receptors [1], σ receptors are now recognised as a unique protein family. They have a characteristic distribution in the central nervous system, but they are also widely present in the peripheral organs and tissues such as lung, liver, kidney and heart [2–4]. Based on ligand selectivity assays, two subtypes have been identified and designated σ_1 and σ_2 [5,6]. These subtypes differ in size, anatomical distribution and ligand selectivity. While the human σ_1 receptor has been cloned from various tissues, and is well characterised at functional and structural level, the σ_2 receptor has not been yet cloned from any species, and is less well known [7].

The σ_1 receptors are integral membrane proteins consisting of 223 amino acids with two transmembrane domains [8]. They primarily reside in the specialised endoplasmic reticulum (ER) membrane directly apposing mitochondria, the so-called MAM (mitochondrial-associated endoplasmic reticulum membrane), and modulate Ca^{2+} efflux from ER by acting as molecular chaperones of inositol (1,4,5)-triphosphate receptors [9]. However, the localisation of σ_1 receptors is dynamic in nature. Indeed, they are also able to translocate from the MAM to the plasma membrane [10], where they regulate a variety of functional proteins, including ion channels, receptors and kinases. It has been shown that the σ_1 receptors are involved in the regulation of numerous neurotransmitter systems such as the cholinergic, dopaminergic and glutamatergic neurotransmission [11,12]. Although the signal transduction pathway after activation of σ_1 receptors is not completely understood, there is more and more evidence to suggest that they represent a potential therapeutic target in many diseases. Indeed, since their discovery, the σ_1 receptors have been implicated in various pathologies, including neurological and psychiatric disorders. Thus, ligands that bind to these receptors have been proposed to exhibit effects in several therapeutic areas such as mnesic disorders (Alzheimer's disease and amnesia) [13], drug addiction [14], depression, anxiety [15], epilepsy, multiple sclerosis [16], Parkinson's disease [17], stroke and pain. Recently, σ_1 receptors have been described as inter-organelle signalling modulators, thus being potentially involved in misfolded protein diseases

Abbreviations: DCM, dichloromethane; DMEM, Dulbecco's Modified Eagle Medium; DMSO, dimethylsulphoxide; P_{HPLC}, purity determined by HPLC; TLC, thin layer chromatography; TFA, trifluoroacetic acid; t_R, HPLC retention time.

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[18]. Furthermore, the σ_1 receptors are overexpressed in tumour cells, making them a possible target for cancer treatment [19]. Several compounds have undergone clinical trials, but no selective σ_1 receptor ligands have so far been marketed [20]. A phase 2 study to evaluate Anavex 2-73 in patients with Alzheimer's disease is ongoing [21]. Moreover, the effects of Igmesine on depressive patients have been studied in phase 3 clinical trials [22].

Biological and pharmacological investigations have pointed out several classes of structurally different compounds with high affinity for the σ_1 receptors. Based on optimisation studies, the pharmacophoric model of these ligands has been reported to consist of an amine binding site flanked on either side by hydrophobic pockets that display bulk tolerance [23]. A large diversity of hydrophobic moieties have already been described, including essentially heterocycles such as benzomorphanes, benzofurans [24], benzothiazolinones [25] and benzoxazolinones [26].

Work in our laboratory has focused on development of selective σ_1 receptor ligands with diverse therapeutic applications. In previous papers we presented the affinity of more constrained series containing tetrahydroisoquinoline-hydantoin (tic-hydantoin) structure [27–30]. Compounds **1** and **2** (Fig. 1) were identified as efficient σ_1 ligands [27–29] with nanomolar affinity (**1(S)**: $K_i = 4.5$ nM, **1(R)**: $K_i = 7.1$ nM, **2**: $K_i = 5.3$ nM), low affinity for σ_2 receptor ligands (**1(S)**: $K_i = 496$ nM, **1(R)**: $K_i = 1000$ nM, **2**: $K_i = 545$ nM), good σ_2/σ_1 selectivity (**1(S)**: $\sigma_2/\sigma_1 = 110$, **1(R)**: $\sigma_2/\sigma_1 = 141$, **2**: $\sigma_2/\sigma_1 = 103$) and very low cytotoxicity ($CC_{50} > 100$ μM), providing a high selectivity index ($CC_{50}/IC_{50} > 14{,}000$).

These σ_1 ligands were evaluated in different pharmacological models. Compound **1(R)** was identified as a potent anti-cocaine agent which is able to increase cocaine-induced locomotor stimulation and sensitisation [31]. The S-enantiomer of compound **1** brought about a 57% decrease of infarct volume in an ischaemia model [32]. Finally, when evaluated as neuroprotective agents, these compounds showed strong anti-inflammatory and neuroprotective effects in an experimental autoimmune encephalomyelitis model [submitted for publication]. Although high potency and efficacy in *in vivo* experiments is a prerequisite for a candidate drug, the ADME profile is also essential in the perspective of drug development. Tic-hydantoin derivative **1** showed all properties compatible with development except metabolic stability. Indeed, despite chemical stability in neutral and acidic media, they demonstrated a low metabolic stability [31]. The major part of metabolites resulted from tic-hydantoin instability. However, among all the metabolites of compound **1**, demethylated and debenzylated compounds have been identified (respectively 5% and 9% of all metabolites, unpublished data). An isoindoline moiety was thus introduced to avoid this metabolic instability.

In this paper, we report our efforts to replace the tic-hydantoin core with different heterocycles. Many benzannulated moieties have already proved their interest in the design of novel σ_1 ligands. Based on the results obtained with benzoxazolinone derivatives [26], we decided to focus our work on the evaluation of related heterocycles. In the light of previous results with tic-hydantoin series, both side chains of lead compounds **1** and **2** (*i.e.* propylbenzylmethylamine and propylisoindoline) were selected for the study. Moreover, the presence of chlorine in many known σ_1 ligands, such as haloperidol, guided our work on the introduction of halogens on the heterocyclic moieties.

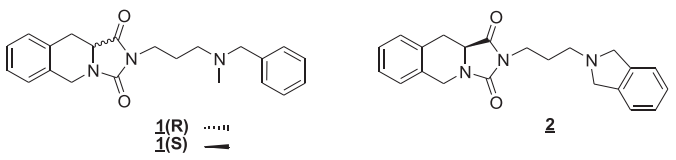

Fig. 1. Structure of compounds **1**and **2**.

2. Chemistry

A series of benzannulated derivatives were synthesised and investigated for their affinity towards σ_1 receptors. The heterocycles used were either commercially available, such as 2-methyl-1*H*-benzimidazole and 1-methyl-1,3-dihydro-2*H*-benzimidazol-2-one, or synthesised. 2-benzoxazolinone and 5-bromo-2-benzoxazolinone were synthesised from the corresponding 2-aminophenol derivatives according to the method previously reported by Nachman et al. [33]. 2-Benzothiazolinone and 5-bromo-2-benzothiazolinone were prepared from the corresponding 2-nitrobenzenethiol derivatives and triphosgene [34]. An aromatic bromination reaction of 2-benzoxazolinone or 2-benzothiazolinone was performed in chloroform with bromine to obtain 6-bromo derivatives [35,36]. Ring closure of 4-bromo-2-aminophenol with ethyl bromoacetate in the presence of potassium ethoxide gave rise to 6-bromo-1,4-benzoxazinone [37]. Finally, 6-bromo-1,4-benzothiazinone was synthesised from 1,4-dibromo-2-nitrobenzene according to a multi-step reaction previously described by Badger et al. [38].

According to the general procedure shown in Scheme 1, a library of 18 novel benzannulated derivatives was synthesised. Synthesis started from 3-bromo-1-chloropropane, and reaction with the appropriate amines yielded amino side chains **4**–**5**. The preparation of compounds **6**–**7** involved direct nucleophilic substitution using various heterocycles and potassium carbonate in DMF to obtain our desired derivatives. Heterocycles **a**–**l** were all introduced on *N*-benzyl-3-chloro-*N*-methylpropan-1-amine **4**. For derivatives **7**, only heterocycles **a**, **c**, **e**, **g**, **i** and **k** were used. The structures of all final compounds were determined by ^{1}H NMR, ^{13}C NMR and LC-MS analyses. Purity was evaluated under HPLC conditions and exceeded 96%. Results are presented in the experimental section. For the pharmacological evaluation, all final products were converted into their water soluble hydrochloride salts.

3. Results and discussion

3.1. Biological evaluations and SAR

Receptor affinities were investigated in competition experiments with radioligands according to the methods of Ganapathy et al. [39]. In the σ_1 assay, the selective ligand [^{3}H] (+)-pentazocine was employed as the radioligand. Since a σ_2 selective radioligand was not commercially available, the non selective ligand [^{3}H]-DTG was employed for σ_2 assay, in the presence of an excess of non-tritiated (+)-pentazocine, which selectively occupies σ_1 receptors. In both assays, Jurkat cell membranes were used as a source of receptors.

The K_i values for σ_1 receptors were determined from the corresponding IC_{50} values for each compound **6**–**7**. For compounds showing high σ_1 affinity, the K_i values for σ_2 receptors and the σ_2/σ_1 selectivity ratios were also calculated (Table 1).

The tic-hydantoin moiety was replaced with various benzannulated heterocycles such as benzoxazolinone, benzothiazolinone, benzoxazinone, benzothiazinone [40], 2-methyl-1*H*-benzimidazole or 1-methyl-1,3-dihydro-2*H*-benzimidazol-2-one, substituted with halogen atoms (Cl, Br) or unsubstituted. From the results obtained, it appears that the replacement of the tic-hydantoin core with different heterocycles can strongly affect the σ_1 affinity. Indeed, although a variety of modulations leads to a dramatic loss of affinity compared to the lead compounds **1** and **2**, high affinity for σ_1