Bioorganic & Medicinal Chemistry Letters 23 (2013) 6083–6086

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Novel highly potent serotonin 5-HT$_7$ receptor ligands: Structural modifications to improve pharmacokinetic properties

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ARTICLE INFO

Article history:
Received 26 July 2013
Revised 5 September 2013
Accepted 9 September 2013
Available online 17 September 2013

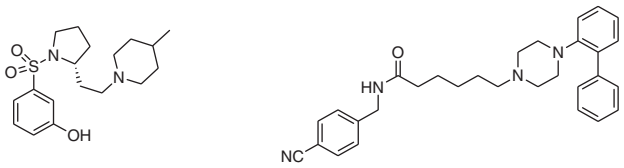
Keywords:
5-HT$_7$ receptors
Arylpiperazines
Serotonin
Microsomial S9 fraction

ABSTRACT

Here we report the synthesis, pharmacological and pharmacokinetic evaluation of a pilot set of compounds structurally related to the potent and selective 5-HT$_7$ ligand LP-211. Among the studied compounds, *N*-pyridin-3-ylmethyl-3-[4-[2-(4-methoxyphenyl)phenyl]piperazin-1-yl]ethoxy]propanamide (**4b**) showed high affinity for 5-HT$_7$ receptors (K_i = 23.8 nM), selectivity over 5-HT$_{1A}$ receptors (>50-fold), in vitro metabolic stability (82%) and weak interaction with P-glycoprotein (BA/AB = 3.3). Compound **4b** was injected ip in mice to preliminarily evaluate its distribution between blood and brain.

The 5-HT$_7$ subtype is the most recently discovered receptor in the serotonin (5-HT) receptor family and it has been cloned from several species including humans.[1] Since its identification, the 5-HT$_7$ receptor has been the subject of intense research efforts driven by its presence in functionally relevant regions of the brain. These receptors have been proposed as a therapeutic target for schizophrenia and mood disorders because several antipsychotics and antidepressants currently on the market bind to 5-HT$_7$ receptors with high affinity.[2–4] Several studies using selective 5-HT$_7$ receptor antagonists, such as **1** (SB-269970) (Fig. 1), or mice lacking the 5-HT$_7$ receptor have further supported a role for these receptors in depression.[5–7] Moreover, 5-HT$_7$ receptors have been implicated in other Central Nervous System (CNS) functions, including circadian rhythm, rapid eye movement sleep, thermoregulation, anxiety, epilepsy, learning and memory.[8]

Recent data suggest that the activation of 5-HT$_7$ receptors have a prominent role in regulating the neuronal cytoarchitecture of behaviorally relevant neuronal networks in an age-dependent manner suggesting new approaches in modulating CNS connectivity.[9,10] In fact, murine striatal and cortical neuronal cultures evidenced significant neurite outgrowth after treatment with 5-HT$_7$ agonists 8-OH-DPAT and **2** (LP-211).[11] Finally, recent studies have suggested that selective activation of 5-HT$_7$ receptors may represent a novel therapeutic strategy in the treatment of Fragile X syndrome, the most common form of inherited intellectual disability and autistic spectrum disorder.[12] On such basis, the identification of 5-HT$_7$-acting molecules with good brain penetrance is of key importance in view of proof-of-concept studies in animal models.



1 (SB-269970) 2 (LP-211)

Figure 1. Structure of selective 5-HT$_7$ ligands.

Our research group has been involved for several years in the study of structure–activity relationships (SARs) of 4-substituted-1-arylpiperazine derivatives, the so-called 'long-chain' arylpiperazines.[13–17] Our studies have led to the identification of **2** which showed high 5-HT$_7$ receptor affinity (K_i = 15.1 nM), good selectivity over 5-HT$_{1A}$ (K_i = 379 nM) and agonist properties in an ex vivo assay of 5-HT$_7$ receptor activation. Disposition studies in mice evidenced that **2** is brain penetrant and showed a half-life of 69 min.[18] Various studies have demonstrated that **2** is a valuable tool for studying the pharmacology of 5-HT$_7$ receptors in vitro[11,12]

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http://dx.doi.org/10.1016/j.bmcl.2013.09.025

and in vivo.[19] A drawback for in vivo use of **2** might arise from metabolic degradation since **2** undergoes N-dealkylation to the unsubstituted 1-(2-biphenyl)piperazine which is itself a 5-HT_7 receptor ligand.[20] This might be of relevance because N-unsubstituted 1-arylpiperazines can reach the brain and, thus, the final pharmacological effect of long-chain arylpiperazines might be the result of the interplay between the neurochemical actions of the parent drug and the active metabolite.[21]

In a first attempt to tackle the N-dealkylation, we prepared and studied a series of long-chain arylpiperazine derivatives with a short intermediate alkyl chain reasoning on the possibility that shortening the alkyl chain would increase the steric hindrance around the basic nitrogen and, consequently, would improve the stability of the compounds. This structural modification led to an improvement of metabolic stability for several compounds, but resulted in a loss of selectivity over 5-HT_{1A} receptor.[22] Therefore, we envisaged an alternative strategy and decided to modify the structure of **2** to improve pharmacokinetic properties by lowering lipophilicity of the compound. It is well known that lipophilicity is correlated with the metabolic liability of a molecule.[23] To this end, we inserted an oxygen atom in the intermediate alkyl chain of **2** with the two -fold aim of decreasing lipophilicity and limiting N-dealkylation.

We here report the synthesis, pharmacological and in vitro and in vivo pharmacokinetic evaluation of a pilot set of compounds structurally related to **2**.

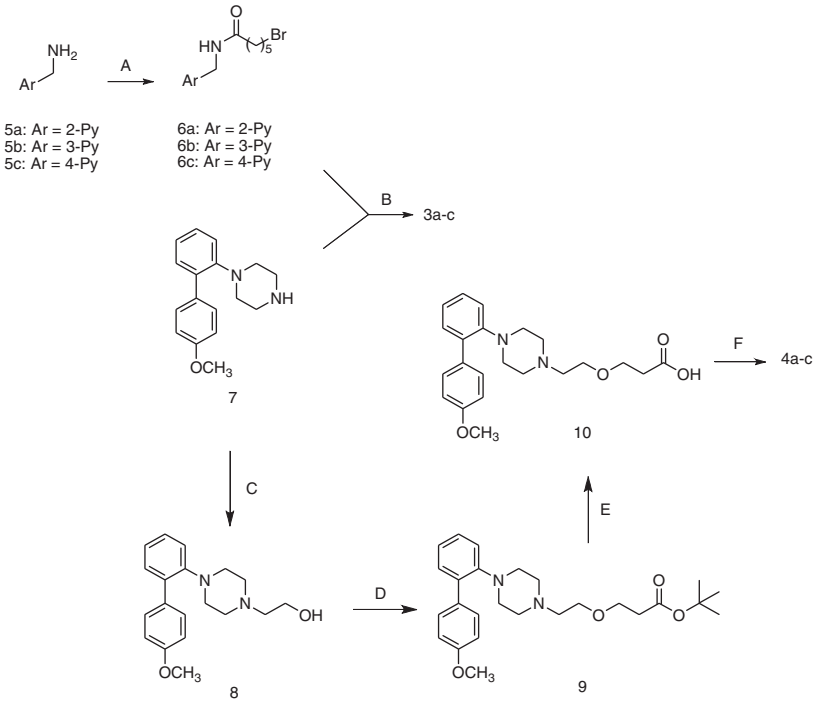
The synthesis of the target compounds **3a–c** having a pentamethylene spacer as compound **2** is reported in Scheme 1. The appropriate pyridinylmethylamine **5a–c** was reacted with 6-bromohexanoyl chloride to give the 6-bromohexanamides **6a–c**, that were used to alkylate 1-[2-(4-methoxyphenyl)phenyl]piperazine (**7**)[20] to afford the target compounds.

The target compounds having an ethoxyethyl spacer were synthesized following a different synthetic pathway (Scheme 1). The piperazine **7** was alkylated with 2-bromoethanol to give alcohol **8**. The latter underwent Michael addition to *t*-butylacrylate to give the ester **9**. Acidic hydrolysis of the ester afforded carboxylic acid **10** that was subsequently condensed with the appropriate pyridinylmethylamine to give the target compounds **4a–c**. For experimental details see Supplementary data.

All the compounds displayed the structural features of **2** that are responsible of 5-HT_7 receptor affinity, selectivity and agonistic properties. Our previous structure–activity relationship (SAR) studies on long-chain arylpiperazine derivatives indicated that (a) the biphenyl piperazine moiety is responsible for high affinity for 5-HT_7 receptor and selectivity over 5-HT_{1A} receptor; (b) the length of the spacer has influence on 5-HT_7/5-HT_{1A} selectivity; (c) marginal structural modifications on the terminal amidic fragment can be tolerated.[15–17,22] To modulate lipophilicity of **2** in order to obtain compounds with balanced lipophilicity for rapid brain penetration and low metabolic liability, we replaced the cyanophenyl ring of **2** with a pyridyl nucleus, because our previous SAR studies showed that this fragment was not detrimental for 5-HT_7 affinity. Also, the intermediate pentamethylene chain was replaced with the ethoxyethyl spacer and, to the best of our knowledge, this is the first time that such a modification is reported on the long chain arylpiperazine scaffold. Finally, we replaced the 1-(2-biphenyl)piperazine moiety with 1-[2-(4-methoxyphenyl) phenyl]piperazine, because this might lead to improve selectivity over 5-HT_{1A} and α_1 receptors as we have recently described.[20]

As shown in Table 1, all compounds displayed *c*log*P* values significantly lower than that of **2**.

The target compounds were evaluated for their affinity at human cloned 5-HT_7 receptor in radioligand binding assay and also for their affinity at 5-HT_{1A}. Moreover, they were also evaluated for adrenergic α_1 receptor affinity, because it has been reported that the 1-(2-biphenyl)piperazinyl scaffold might have affinity for such



Scheme 1. Reagents and conditions: (A) 6-bromohexanoyl chloride, 2% aqueous NaOH, CH_2Cl_2, 0 °C–rt, 53–87% yield; (B) Na_2CO_3, acetonitrile, reflux overnight, 38–48% yield; (C) 2-bromoethanol, K_2CO_3, KI, acetonitrile, reflux overnight, 65% yield; (D) NaH, *t*-butyl acrylate, anhydrous THF, 0 °C–rt, 20–22 h, 26% yield; (E) 3 N HCl, dioxane, rt, 48 h; (F) amine **5a–c**, 1,1′-carbonyldiimidazole, anhydrous THF, rt, overnight, 39–60% yield.