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Research paper

Synthesis, *in vitro* and *in vivo* pharmacological evaluation of serotoninergic ligands containing an isonicotinic nucleus

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ABSTRACT

Isonicotinamide derivatives, linked to an arylpiperazine moiety, were prepared and their affinity to 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors were evaluated. The combination of structural elements (heterocyclic nucleus, alkyl chain and 4-substituted piperazine) known to play critical roles in affinity for serotoninergic receptors and the proper selection of substituents led to compounds with high specificity and affinity towards serotoninergic receptors. In binding studies, several molecules showed high affinity in nanomolar and subnanomolar range at 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors and moderate or no affinity for other relevant receptors (D_1, D_2, α_1 and α_2). *N*-(3-(4-(bis(4-fluorophenyl)methyl)piperazin-1-yl)propyl)isonicotinamide (**4s**) with Ki = 0.130 nM, was the most active and selective derivative for the 5-HT_{1A} receptor compared to other serotoninergic, dopaminergic and adrenergic receptors. Compound **4o**, instead, showed 5-HT_{2A} affinity values in subnamolar range. Moreover, the compounds having better affinity and selectivity binding profile towards 5-HT_{1A} and 5-HT_{2A} receptors were selected in order to be tested by *in vitro* and *in vivo* assays to determine their functional activity.

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1. Introduction

Serotonin (5-hydroxytryptamine, 5-HT) [1–4] is involved in several pivotal physiological and pathophysiological processes such as circadian rhythms, sleep and wake regulation, mood and emotions, sexual behaviors, aggression and anxiety [5]. Pharmacological manipulation of the 5-HT system is believed to have therapeutic potential, and therefore it is the subject of intense research [4].

Serotonin receptors are widely expressed throughout the brain and in many key structures responsible for cognition and basic brain functions. As a local hormone, serotonin is present in numerous other tissues including the gastrointestinal tract, the cardiovascular and immune systems.

Among the six classes of GPCR 5-HT receptors (5-HT_1, 5-HT_2, 5-HT_4, 5-HT_5, 5-HT_6 and 5-HT_7, each of them divided in additional subclasses, amounting to fifteen receptors), 5-HT_{1A} mediates effects on a wide range of psychiatric disorders, and it is involved in the proliferation of human tumor cells (PC3) and in human hormone refractory prostate cancer tissue [6]. The 5-HT_2 receptor family has three known subtypes, 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C}; activation of 5-HT_{2A} receptors stimulates the secretion of various hormones and influences neuronal plasticity; peripheral 5-HT_{2A} receptors mediate several processes such as vasoconstriction and platelet aggregation [4], it regulates also intestinal motility and secretions, by means of the conspicuous presence of serotonin-containing enterochromaffin cells; 5-HT causes diarrhea if present in excess, and constipation if any at fault [4,7–10]. The 5-HT_{2C} receptor is involved in physiological functions such as locomotor activity, anxiogenesis and neuroendocrine functions, and is

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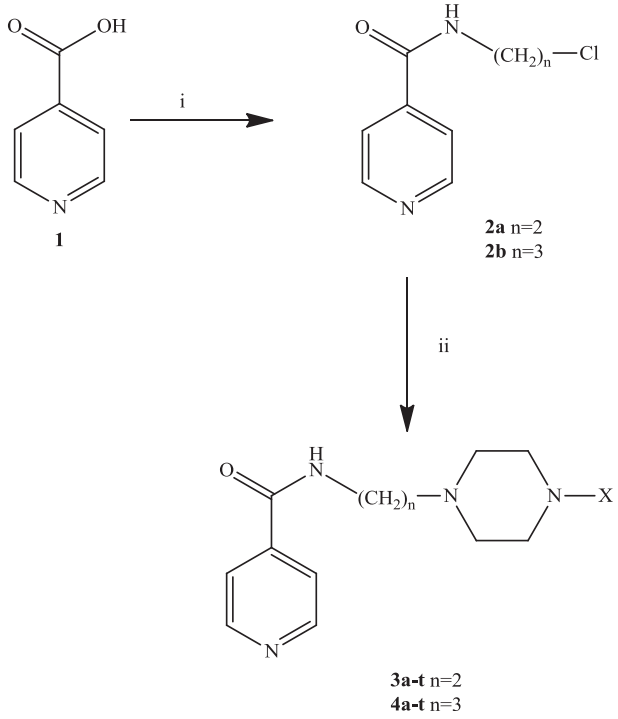
implicated in sexual dysfunction in males [7,8].

One of the most studied chemical classes, already known for their high affinity toward these receptors, is the long-chain N-1-substituted *N*-4-arylpiperazines (LCAPs) [8]. The significance of the respective parts of the LCAP structures on the 5-HT_{1A} receptor affinity, intrinsic activity, and selectivity has been the subject of many structure-activity relationship (SAR) studies. In particular, much effort has been devoted to understand both the role of the heterocyclic nucleus and of the substituent on the piperazine scaffold in the ligand-receptor interaction and, consequently, a great number of different fragments were used [11,12]. However, a limitation of many 5-HT_{1A} receptor ligands is their undesired high affinity for other receptor subtypes such as the dopaminergic D_2 receptor and α_1-adrenoceptor. In our laboratories, there has been an ongoing research work to develop more selective serotoninergic ligands [13–24] in order to have novel pharmacological tools that could improve our knowledge of the signal transduction mechanism leading to compounds with high affinity and selectivity.

A previously described study focused on the synthesis and pharmacological evaluation of a set of arylpiperazine derivatives containing a *N*′-cyanoisonicotinamidine nucleus; the binding data reported in this study identified this original scaffold as an optimal structural element to enhance 5-HT_{1A} receptor affinity [22].

In continuation of our research program, we designed a new set of derivatives where the piperazine-*N*-alkyl moiety has been linked to an isonicotinic fragment as terminal part of LCAPs (Scheme 1); this choice was made considering that it represents a molecular simplification of derivatives previously synthesized, characterized by *N*′-cyanoisonicotinamidine nucleus as an optimal structural element to enhance 5-HT_{1A} receptor binding [22]. Consequently, we decided to investigate how the substitution of a cyanoamidine group with a simple amide bond may influence the binding affinity/selectivity profile. The isonicotinic scaffold was linked via two and three methylene spacing units to the *N*-4-aryl-substituted piperazines and this choice was done in order to obtain a complete and comparative structure-affinity and structure-selectivity relationship study. All the new compounds were tested for their affinity to 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors and the multireceptor profiles of promising derivatives were also evaluated in terms of binding affinities for dopaminergic (D_1, D_2) and adrenergic (α_1, α_2) receptors. Moreover, the compounds showing better affinity and selectivity binding profile towards 5-HT_{2A} receptors have been tested by *in vitro* assay to evaluate their agonist or antagonist activity towards serotonin evoked contractions. Finally, compounds with a better affinity/selectivity profile towards 5-HT_{1A} have been evaluated by *in vivo* assay (e.i. behavioural tests), to determine their functional activity.



Scheme 1. Reagents and conditions: (i) $Cl(CH_2)_nNH_2 \cdot HCl$, DCC, HOBt, TEA, CH_3CN, r.t., 24h; (ii) 4-X-substituted-piperazine, K_2CO_3, NaI, CH_3CN, 70 °C, 24 h.

2. Results and discussion

The synthetic strategy used for the preparation of the isonicotinamide derivatives is summarized in Scheme 1. Isonicotinic acid (**1**) reacted with 2-chloroethanamine hydrochloride or 3-chloropropan-1-amine hydrochloride in acetonitrile, in presence of *N*,*N*′-dicyclohexylcarbodiimide (DCC), hydroxybenzotriazole (HOBt) and triethylamine (TEA) to give the corresponding chloroalkylisonicotinamides **2a** and **2b**. Subsequent condensation of intermediates **2a** and **2b** with the appropriate 4-X-substituted-piperazine, performed in CH_3CN with K_2CO_3 and NaI, under reflux, provided the final compounds **3a**–**t** and **4a**–**t**. Each final product was purified by chromatography on silica gel column and further crystallized from the appropriate solvent. All the compounds were characterized by ^1H NMR, ^{13}C NMR and triple quadrupole mass spectrometry (API 2000 Applied Biosystem) and the obtained data were consistent with the proposed structures.

Several of the synthesized derivatives showed affinities in the nanomolar range towards 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors and moderate to no affinity for other relevant receptors (D_1, D_2 and α_1, α_2) (Tables 1 and 2). Besides the outstanding 5-HT_{2A} receptor affinity and selectivity of compound **4o** (K_i = 0.00985 nM), other interesting K_i values were those of compounds **3s** (0.00956 nM), **3l** (0.0126 nM), **3m** (0.046 nM) and **4l** (0.0390 nM). Moreover compounds **3n**, **4p** and **4s**, showed the most interesting affinity/selectivity profile towards 5-HT_{1A} receptors with K_i values of 1.08, 0.0113 and 0.130 nM respectively, whereas compounds **3b** (that was already described) [25,26], **3h** and **3i** presented a mixed 5-HT_{1A}/5-HT_{2C} activity with K_i values of 0.36/0.933 nM, 2.85/6.79 nM and 0.82/0.156 nM, respectively. The two series, **3a**–**t** and **4a**–**t**, differ in the length of the connecting chain between the isonicotinic fragment and the piperazine ring. Unlike the previously reported series of arylpiperazines [13–24], where three units alkyl chain compounds showed the best affinity/selectivity profile towards 5-HT_{1A} receptors, in this new series, the alkyl chain length, as well as a simple amide bond instead of cyanoamidine group characterizing *N*′-cyanoisonicotinamidine derivatives [22], doesn't seem to be decisive in determining a general trend towards 5-HT_{1A} receptors but the affinity/selectivity profile is more influenced by the particular substituent on the piperazine moiety. The bis(4-fluorophenyl)methyl group associated to a longer chain spacer (n = 3, **4s**), conferred the highest affinity and selectivity values for the 5-HT_{1A} receptor. The presence of a piperonyl group (**4p**), a 2,3-dimethylphenyl group (**4g**) or a pyridine-2-yl group associated to a shorter chain spacer (n = 2, **3n**) on the N-4 of the piperazine moiety, led also to compounds which exhibited high affinity for 5-HT_{1A} receptor (**3n** K_i = 1.08 nM; **4g** K_i = 8.42 nM and **4p** K_i = 0.011 nM). Instead, the pyrimidinyl and 2-fluorophenyl moieties (**4o**, K_i = 0.00985 nM and **4l**, K_i 0.0390 nM, respectively) associated to a propyl chain spacer and the 2-fluorophenyl (**3l** K_i = 0.0126 nM), 4-fluorophenyl (**3m** K_i = 0.046 nM) as well as the bis(4-fluorophenyl)methyl (**3s** K_i = 0.00956 nM), associated to an

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