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New 5-HT$_{1A}$ receptor ligands containing a *N*′-cyanoisonicotinamidine nucleus: Synthesis and in vitro pharmacological evaluation

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ABSTRACT

N′-Cyanoisonicotinamidine derivatives, linked to an arylpiperazine moiety, were prepared to identify highly selective and potent 5-HT$_{1A}$ ligands as potential pharmacological tools in studies of wide spread psychiatric disorders. The combination of structural elements (heterocyclic nucleus, alkyl chain and 4-substituted piperazine) known to be critical in order to have affinity on 5-HT$_{1A}$ receptor and the proper selection of substituents led to compounds with high specificity and affinity towards serotoninergic receptors. In binding studies, several molecules showed affinity in nanomolar and subnanomolar range at 5-HT$_{1A}$ and moderate to no affinity for other relevant receptors (5-HT$_{2A}$, 5-HT$_{2C}$, D$_1$, D$_2$, α_1 and α_2). *N*′-Cyano-*N*-(3-(4-(pyridin-2-yl)piperazin-1-yl)propyl)isonicotinamidine (**4o**) with K_i = 0.038 nM, was the most active and selective derivative for the 5-HT$_{1A}$ receptor with respect to other serotoninergic, dopaminergic and adrenergic receptors.

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The serotoninergic system is implicated in numerous physiological and pathophysiological processes.[1] Serotonin (5-hydroxytryptamine, 5-HT)[2–5] is found throughout the CNS in high levels and participates in modulating mood. According to the classic serotonin hypothesis, anxiety is usually associated with increased endogenous 5-HT, and anxiolytics tend to decrease endogenous 5-HT.[6] Discovery of ligands for 5-HT receptors (5-HTRs) is an area of intense research because of the potential for new therapeutic drugs. Among the 13 different serotonin receptors, belonging to the G protein coupled receptors superfamily,[5] 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors, are most frequently considered as important targets for neurobiological research and drug development. The activation of these receptors leads to a number of physiological changes that can be easily quantified.[7–9] Agonists and partial agonists have been proven to be effective in anxiety and depression.[10–13]

5-HT$_{1A}$ receptor, as a member of the group of G protein coupled receptors (GPCRs),[14] shows high similarity with other members of the family. A particular case is represented by the similarity between 5-HT$_{1A}$R and α_1-adrenoreceptor[15] that show a high degree of homology (45%) in their amino acid sequence.

Several structurally different compounds are already known for their high affinity toward these receptors and, from a chemical point of view, they can be subdivided into different classes. The most studied group is that of long-chain arylpiperazines (LCAPs).[9] Their general chemical structure contains an alkyl chain (2–4 methylene units) attached to the N-4 atom of the piperazine moiety on a terminal amide or imide fragment. 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 studies (SAR). In particular, much effort has been devoted to understand the role of the terminal part in the ligand–receptor interaction and, in consequence, a great number of many different fragments were used.[16,17] However, a limitation of many 5-HT$_{1A}$ receptor ligands for their potential use as drugs or pharmacological tools is their undesired high affinity for other receptor subtypes. The dopaminergic D$_2$ receptor and α_1-adrenoceptor are two examples of undesired binding sites to which several 5-HT$_{1A}$ ligands bind with high affinity.

In our laboratories, there has been an ongoing effort to develop more selective 5-HT$_{1A}$ ligands[18–26] in order to have novel pharmacological tools that could improve our knowledge of the signal transduction mechanism and lead to compounds with high affinity and selectivity. In continuation of our research program, we have analyzed a new set of derivatives where the piperazine-*N*-alkyl moiety has been linked to a novel *N*′-cyanoisonicotinamidine fragment as terminal part of LCAPs (Scheme 1). To gain insight into its influence on the serotoninergic activity, this original nucleus was linked via three methylene spacing units to piperazines substituted in position 4 with aliphatic and/or aromatic moieties. The choice of

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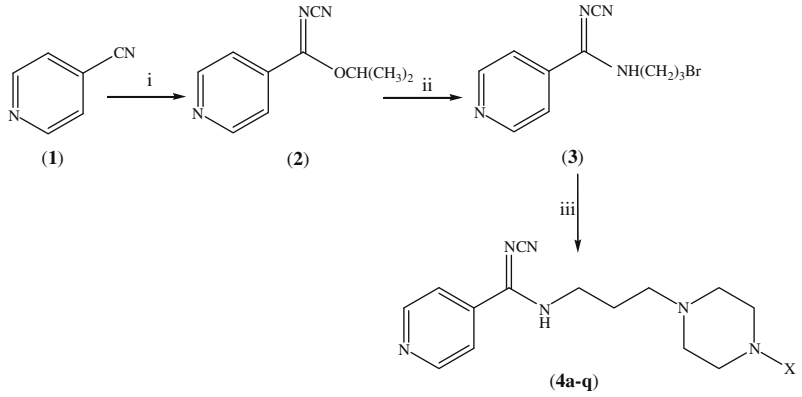


Scheme 1. Reagents and conditions: (i) CH_3ONa, H_2NCN, *i*PrOH; (ii) $Br(CH_2)_3NH_2{\cdot}HBr$, CH_3ONa, anhydrous MeOH; (iii) 4-X-substituted-piperazine, K_2CO_3, NaI, CH_3CN, 70 °C, 4 h.

the alkyl chain length (three units), was performed on the basis of our previous investigations, where, as a general trend, compounds with piperazinylpropyl chain linked to different nuclei showed good and preferential affinity for the 5-HT_{1A}R, with respect to compounds in which the spacer is one atom shorter. Instead the choice of aliphatic substituents on the N-4 of the piperazine moiety, that could appear in contrast with the statement that protonable nitrogen and aromatic system are necessary for binding of aminergic ligand,[32,35] was done in order to obtain a complete structure–affinity and structure–selectivity relationship study. All the new compounds were tested for their affinity for 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors. Moreover, the multireceptor profiles of promising derivatives were also evaluated in terms of binding affinities for dopaminergic (D_1 and D_2) and adrenergic (α_1 and α_2) receptors.

The synthetic strategy employed for the preparation of the target compounds (Table 1) is summarized in Scheme 1. The general procedure is as follows: isopropyl-*N*-cyano-4-pyridinecarboximidate (**2**) was prepared starting from 4-cyanopyridine (**1**) by base-promoted reaction with isopropanol followed by treatment with cyanamide in aqueous phosphate buffer ($NaH_2PO_4{\cdot}2H_2O/Na_2HPO_4$ = 4:1). Compound (**2**) was converted to *N*-(3-bromopropyl)-*N*′-cyanoisonicotinamidine (**3**) by reaction with 3-bromopropylamine···HBr and NaOMe in methanol. Subsequent condensation of compound (**3**) with the desired 4-X-substituted-piperazine, performed in CH_3CN in the presence of K_2CO_3 and NaI, under reflux, provided the final compounds **4a–q**. Purification of each final product was obtained by chromatography on silica gel column and further by crystallization from the appropriate solvent. All new compounds gave satisfactory elemental analyses and were characterized by ^{1}H NMR and mass spectrometry (API 2000 Applied Biosystem). ^{1}H NMR and MS data for all final compounds were consistent with the proposed structures. As already reported in the literature, due to tautomeric equilibrium about sp^2 carbon, cyanoamidine derivatives may exist as a mixture of two tautomers (I and II in Fig. 1). The NMR spectra of the final compounds **4a–q**, confirmed that all final compounds exist predominantly as the cyanoimino form (I). Moreover, about the configurational determination of geometrical isomers (*E*/*Z*) on the amidine bond C=N, the *N*-cyanoamidine derivatives **4a–q** exhibited only one set of relevant ^{1}H NMR signals, implying the existence of either a single geometric form or, more probably, a fast equilibrium of two isomeric forms as already described in the literature.[27]

The compounds dissolved in ethanol or in 5% DMSO were tested for in vitro affinity for 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors by radioligand binding assays. The compounds showing the highest affinity towards serotonin receptors have been selected and evaluated for their affinity for dopaminergic (D_1 and D_2) and adrenergic (α_1 and α_2) receptors. All The following specific radioligands and tissue sources were used: (a) serotonin 5-HT_{1A} receptor, [^{3}H]-8-OH-DPAT, rat brain cortex[36]; (b) serotonin 5-HT_{2A} receptor, [^{3}H]ketanserin, rat brain cortex[37]; (c) serotonin 5-HT_{2C} receptor, [^{3}H]mesulergine, rat brain cortex[37]; (d) dopamine D_1 receptor, [^{3}H]SCH-23390, rat striatum[38]; (e) dopamine D_2 receptor, [^{3}H]spiperone, rat striatum[39]; (f) α_1 adrenergic receptor, [^{3}H]prazosin, rat brain cortex[40]; (g) α_2 adrenergic receptor, [^{3}H]yohimbine, rat brain cortex.[41]

Non-specific binding was determined as described in the Supplementary data, and specific binding as the difference between total and non-specific binding. Blank experiments were carried out to determine the effect of 5% DMSO on the binding and no effects were observed. Competition experiments were analyzed by the 'EASYFIT' program[28] to obtain the concentration of unlabeled drug that caused 50% inhibition of ligand binding (IC_{50}), with six concentrations of test compounds, each performed in triplicate. The IC_{50} values obtained were used to calculate apparent inhibition constants (K_i) by the method of Cheng and Prusoff,[29] from the following equation: $K_i = IC_{50}/(1 + S/K_D)$ where S represents the concentration of the hot ligand used and K_D its receptor dissociation constant (K_D values, obtained by Scatchard analysis,[30] were calculated for each labeled ligand).

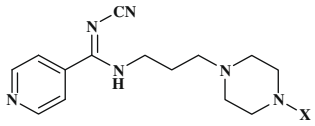
Some of the reported derivatives were potent 5-HT_{1A} receptor ligands, in fact, they showed nanomolar or even subnanomolar 5-HT_{1A} receptor affinities (Table 1). Besides the outstanding 5-HT_{1A} receptor affinity of compound **4o** (K_i = 0.038 nM), other interesting K_i values were those of compounds **4d** (1.47 nM), **4l** (6.17 nM) and **4f** (21.3 nM) while compounds **4h, 4i, 4m** and **4n** where less active with K_i values of 101, 166, 141 and 172 nM, respectively. Other derivatives showed K_i values of above 10^4 nM or no affinity.

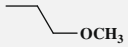
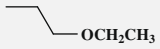
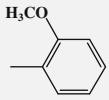
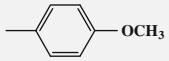
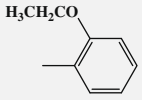
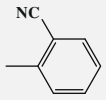
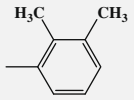
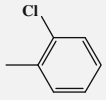
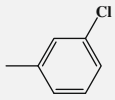
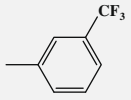
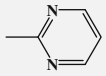
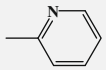
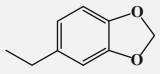
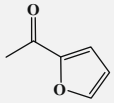
The derivatives **4a–q** differ in the substituents on the N-4 of the piperazine moiety that represents a critical feature in determining 5-HT_{1A} receptor affinity and selectivity. These results confirm those obtained with previously described compounds[25,26] in fact, the introduction of non aromatic or larger substituents on the piperazine nitrogen, even in this series of derivatives, causes a dramatic decrease of the receptor affinity. These results were also in accordance with recently reported structure affinity relationships of new model of arylpiperazines.[31]

Concerning the influence of the N-4 substituent of the piperazine moiety, the pyridin-2-yl group, (**4o**), and the 2-methoxyphenyl group (**4d**), conferred the highest affinity and selectivity for the 5-HT_{1A} receptor. The presence of a 3-chlorophenyl group and 2-ethoxyphenyl group on the N-4 of the piperazine moiety, led also to compounds which exhibited high affinity for 5-HT_{1A} receptor (**4l** K_i = 6.17 nM; **4f** K_i = 21.3 nM). Non aromatic moiety as well as pyrimidinic and piperonyl groups seem especially unfavorable determining a high decrease in binding affinity compared to the other N-4 substituents at the piperazine ring.

The 5-HT_{2A} and 5-HT_{2C} receptor affinities of the tested compounds were always lower than those observed for 5-HT_{1A} receptors except for compounds **4m** and **4f** that show higher affinities

Table 1
Affinities of compounds **4a**–**q** for 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors



Compd	Receptor affinity K_i ± SD (nM)			
	X	5-HT_{1A} [^{3}H]8OH-DPAT	5-HT_{2A} [^{3}H]Ketanserin	5-HT_{2C} [^{3}H]Mesulergine
4a		No affinity	No affinity	No affinity
4b		No affinity	>10^4	No affinity
4c		>10^4	No affinity	No affinity
4d		1.47 ± 0.05	>10^4	No affinity
4e		>10^4	>10^4	No affinity
4f		21.3 ± 0.8	1900 ± 100	11.5 ± 3.0
4g		No affinity	>10^4	No affinity
4h		101 ± 38	>10^4	No affinity
4i		166 ± 8	>10^4	No affinity
4l		6.17 ± 0.14	>10^4	No affinity
4m		141 ± 9	32.1 ± 0.5	No affinity
4n		172 ± 24	No affinity	No affinity
4o		0.038 ± 0.001	No affinity	No affinity
4p		>10^4	>10^4	No affinity
4q		>10^4	>10^4	No affinity

For purpose of comparison, 8-OH-DPAT, Ketanserine and Mesulergine binds 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors with values of 0.80, 0.85 and 1.90 nM, respectively, under these assay conditions.

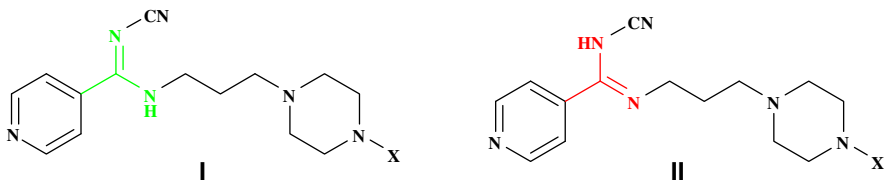


Figure 1. Tautomeric equilibrium about sp^{2}carbon of the cyanoamidine derivatives.

Table 2
Affinities of compounds **4d**, **4f**, **4l**, and **4o** for D_1, D_2, α_1 and α_2 receptors

Compd	Receptor affinity $K_i \pm$ SD (nM)			
	D_1 [^{3}H]SCH-23390	D_2 [^{3}H]spiperone	α_1 [^{3}H]prazosin	α_2 [^{3}H]yohimbine
4d	$>10^4$	$>10^4$	$>10^4$	2570 ± 258
4f	$>10^4$	$>10^4$	190 ± 58	516 ± 64
4l	1630 ± 60	$>10^4$	912 ± 11	$>10^4$
4o	$>10^4$	$>10^4$	$>10^4$	$>10^4$

towards 5-HT_{2A}R (K_i = 32.1 nM) and 5-HT_{2C} receptor (K_i = 11.5 nM), respectively. *N*′-Cyano-*N*-(3-(4-(pyridin-2-yl)piperazin-1-yl)propyl)isonicotinamidine (**4o**) (K_i = 0.037 nM), showed not only the highest affinity but also the highest selectivity with respect to other serotoninergic receptors studied.

Additionally, the affinity of the most active compounds (**4d, 4f, 4l** and **4o**) on several other receptors (α_1 and α_2 adrenergic and D_1 and D_2 dopaminergic receptors) was examined in order to verify the selectivity of these compounds. Results are summarized in Table 2. All the compounds proved highly selective against dopaminergic receptors with K_i values of above 10^4 nM except for compound **4l**, which exhibited K_i value of 1630 nM on D_1 receptor. Regarding α_1 and α_2 adrenergic receptors, only compound **4f** showed quite moderate affinity (190 nM and 516 nM, respectively), while compounds **4d** and **4o** showed K_i values higher than 10^4 nM; these data are very interesting considering the high degree of homology existing between these two receptors and demonstrates that these compounds possess a very good binding profile, preferring 5-$HT_{1A}R_s$ over all other evaluated receptors.

These results emphasize the high selectivity afforded by the discussed *N*′-cyanoisonicotinamidine scaffold and confirm that the propyl alkyl chain together with the aromatic ring substitution plays a critical role in determining 5-HT_{1A} receptor affinity and selectivity.

As already observed in a previously described series of norbornene derivatives,[24] the exceptional high affinity of **4o**, supporting a pyridin-2-yl moiety, could be explained in terms of solvent accessibility and hydrophobic interaction with the receptor that are decisive compared to all other compounds, whereas the lower affinity of **4n** appear to agree data already reported in the literature,[32,33] where as general trend the pyrimidin-2yl derivatives result poorer ligands with respect to 2-methoxyphenyl and pyridin-2-yl moiety. In fact as already described[33] this aspect could be due to a unfavorable conformation of the pyrimidinylpiperazine, where the pyrimidin-2yl ring is parallel to that of the piperazine due to delocalization of sp^2/sp^3 nitrogen into the aromatic system. Instead the high affinity of **4d** is most probably explained by an additional hydrogen bond between the methoxy substituent in *ortho* position and the side chain of Asn-386.

Moreover, results obtained with compound **4f** (K_i value of 21.3 nM) supporting an *o*-ethoxyphenylpiperazine moiety, compared with *o*-methoxyphenylpiperazine derivative **4d** (K_i value of 1.47 nM), could be discussed in order to better explain the influence of different substituents in *ortho* position on the phenylpiperazine moiety. In fact, the simple change from $-OCH_3$ to $-OCH_2CH_3$ produces a reduction of affinity and a loss of selectivity. This result could be, probably, attributed to unfavorable steric interaction of the ethoxy group (**4f**) with the surrounding receptor residues and loss of the hydrogen bond to Asn-386, that molecular modeling studies[24] suggested as important for *o*-methoxy analogues. In fact compound **4f** presented a mixed 5-HT_{1A}/5-HT_{2C} affinity with K_i values of 21.3/11.5 nM, respectively. Additionally **4m**, characterized by a very hydrophobic trifluoromethyl group, exhibits a mixed 5-HT_{1A}/5-HT_{2A} affinity with K_i values of 141/32.1 nM, respectively. This data is of particular interest and outlines a potential atypical antipsychotic profile for this derivative. In fact second-generation antipsychotics combine a concomitant activity at serotoninergic receptors (5-HT_{1A} and 5-HT_{2A}) with D_2 receptor occupancy, to provide drug therapies for resistant schizophrenic patients, with prompter therapeutic benefits and the improvement of cognitive symptoms.[34]

Finally the bad affinity/selectivity profile of the derivatives characterized with the presence of an aliphatic group (**4a**–**4c**) on the N-4 position has clarified the role of N-4 position of the piperazine moiety on the 5-HT_{1A} receptor affinity and selectivity. Zlatović et al.[35] have recently reported that some arylpiperazines can interact directly with the hydrophobic part of the 5-HT_{1A} receptor binding site. In particular the hydrophobic part of the binding site in the 5-HT_{1A} receptor, formed by Trp-358, Phe-361 and Tyr-390, is significant for the stabilization of the ligand–receptor complex. Therefore, these aspects can be useful to explain the low affinity that these new piperazine derivatives have shown; in these compounds, in fact, a non aromatic moiety is less favorable to the hydrophobic interaction with the receptor, determining the formation of weaker complexes with the receptor.

In conclusion, in this Letter, we have described the synthesis of a new series of arylpiperazines as 5-HT_{1A} ligands (**4a–q**), containing a novel heterocyclic fragment. Some of the described compounds showed high in vitro affinity and selectivity towards 5-HT_{1A} receptors. Compound **4o** was the most potent (K_i = 0.038 nM) and selective derivative for the 5-HT_{1A} receptor with respect to the other serotonin, dopaminergic and adrenergic receptors, besides appear interesting also the affinity/selectivity profile of compound **4d** (K_i = 1.47 nM) supporting on the N-4 of the piperazine moiety the 2-methoxyphenyl group. The binding data presented in this study identified the *N*′-cyanoisonicotinamidine nucleus as an optimal structural element to enhance 5-HT_{1A} receptor binding, although piperazinylpropyl chain and the nature of the substituent on the N-4 piperazine ring play an important role in determining affinity and selectivity.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.02.106.

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