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Synthesis and in vitro binding studies of substituted piperidine naphthamides. Part I: Influence of the substitution on the basic nitrogen and the position of the amide on the affinity for D_{2L}, $D_{4.2}$, and 5-HT_{2A} receptors

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Abstract—A series of 1- and 2-naphthamides has been prepared and tested for in vitro binding to D_{2L}, $D_{4.2}$, and 5-HT_{2A} receptors. Different compounds display selectivity for $D_{4.2}$ and 5-HT_{2A} receptors versus D_{2L} receptors. *N*-(1-Arylalkyl-piperidin-4-yl) carboxamides have higher affinities than the corresponding *N*-(4-arylalkylamino-piperidin-1-yl) carboxamide analogues. A benzyl moiety in position 1 of the piperidine in the 2-naphthamide series (**2**) appears to be the best choice for a favorable interaction with $D_{4.2}$ and 5-HT_{2A} receptors. Increasing the linker length between the phenyl ring and the basic nitrogen led to a decreased affinity for these receptors. In the 1-naphthamide series, the most potent $D_{4.2}$ ligand (**7**) possesses a phenylpropyl moiety while its affinity for 5-HT_{2A} receptors is strongly reduced. All compounds with significant affinity for $D_{4.2}$ and 5-HT_{2A} receptors were antagonists.

The discovery of new and effective chemical entities for the treatment of CNS pathologies is a huge challenge since the current understanding of etiological mechanisms of most mental illnesses remains unclear.[1] In continuation of our program dedicated to the development of atypical antipsychotics related to clozapine,[2–4] we intended to explore other approaches by developing new ligands which target both dopamine (DA) and serotonin (5-HT) receptors. Clozapine targets numerous receptors with higher or equal potency compared to D_2 receptor binding and thus the search for newer and improved agents is complex.[5] Because clozapine is a weak D_2 ligand, two receptors, D_4 and 5-HT_{2A}, have been proposed to explain the mechanism of action of this molecule[6,7] although others have questioned the role of D_4 receptors.[8] The hypothesis of an appropriate ratio of D_2 and 5-HT_{2A} affinity[9,10] has been the source of many research programs and the successful development of many effective atypical antipsychotic drugs (e.g., olanzapine, risperidone, quetiapine, ziprasidone). D_4 antagonism alone seems not to be sufficient for treating schizophrenia[11] but could be useful in the treatment of attention deficit hyperactivity disorder or mood disorder.[12] D_4 receptors in hippocampal neurons depress *N*-methyl-D-aspartate (NMDA) receptor activity through the activation of platelet-derived growth factor receptors[13] and inhibition of glutamatergic signaling in the frontal cortex.[14] These facts suggest a new and intriguing link between this receptor and the glutamate signaling system which has been clearly associated with cognition. A combination of D_4 and 5-HT_{2A} antagonism was proposed for treating schizophrenia but the clinical development of one such compound, fananserin,

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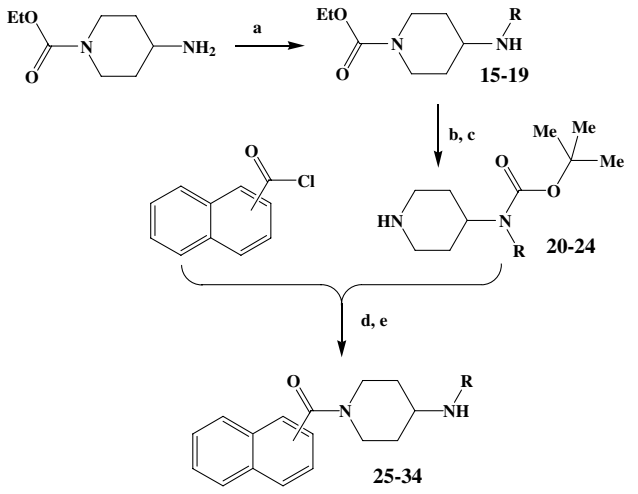
was stopped due to lack of efficacy.[15] Nevertheless, following chronic administration in rats, clozapine significantly influences the density of these receptors in various brain areas relevant to psychiatric disorders and displays a unique profile of mechanism in comparison with other antipsychotic drugs.[16,17] Therefore, the development of compounds sharing this mechanism is of considerable interest to better understand the corresponding pathological mechanisms or to treat other CNS disorders.

Two pharmacophoric elements of clozapine are generally accepted to be responsible for the interaction with DA and 5-HT receptors: a basic distal nitrogen atom and a benzene ring at a distance of 7.72 Å. This differs from typical antipsychotic drugs where the corresponding distance is 5.97 .[18] Therefore, we decided to prepare a series of *N*-substituted naphthamides to mimic the aromatic ring with various 4-amino-piperidine side chains attached. A limited molecular modeling study indicated that the naphthyl moiety and the basic nitrogen would be in close vicinity of the corresponding elements in clozapine (data not shown).

The 4-amino-piperidine fragment was attached in the two possible flipped orientations to the 1- or 2-naphthoyl core and various substituents were added to the respective distal nitrogen. In vitro binding affinity for cloned human $D_{4.2}$ and D_{2L}, and native rat 5-HT_{2A} receptors was measured and intrinsic activity was tested for compounds having significant binding affinity.

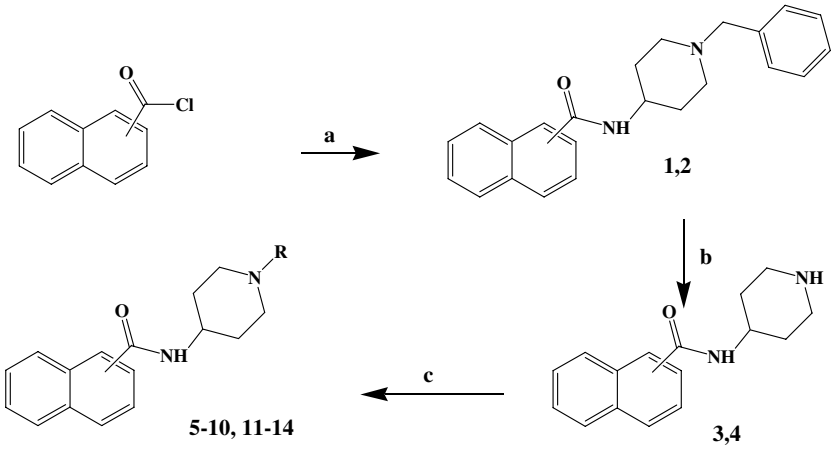
The compounds were prepared by two synthetic pathways (Schemes 1 and 2). The synthesis of *N*-(1-arylalkyl-piperidin-4-yl)naphthamides (**1**, **2**, **5**–**14**) was accomplished by the sequence of reactions outlined in Scheme 1. 1-Naphthoyl chloride or 2-naphthoyl chloride in ethyl acetate was reacted with 4-amino-1-benzyl-piperidine to afford *N*-(1-benzylpiperidin-4-yl)-1- naphthamide (**1**) or *N*-(1-benzylpiperidin-4-yl)-2-naphthamide (**2**). These compounds were used for preparing other



Scheme 2. Reagents and conditions: (a) R-X , K_2CO_3, DMF, rt; (b) Boc_2O, THF/H_2O, Δ*T*; (c) KOH, EtOH/H_2O, Δ*T*; (d) Et_3N, EtOAc, rt; (e) CF_3COOH, CH_2Cl_2, rt. R = benzyl (**15**, **20**, **25**, **30**), 2-phenylethyl (**16**, **21**, **26**, **31**), 3-phenylpropyl (**17**, **22**, **27**, **32**), (*S*)-3-hydroxy-3-phenylpropyl (**18**, **23**, **28**, **33**), (*R*)-3-hydroxy-3-phenylpropyl (**19**, **24**, **29**, **34**).

derivatives in the respective series. *N*-(1-Benzylpiperidin-4-yl)-1-naphthamide (**1**) was debenzylated with ammonium formate and Pd/C in methanol to give the corresponding *N*-(piperidin-4-yl)-1-naphthamide (**3**). *N*-(1-Benzylpiperidin-4-yl)-2-naphthamide (**2**) was debenzylated under 10 bar hydrogen in the presence of 10% Pd/C to give the corresponding *N*-(piperidin-4-yl)-2-naphthamide (**4**). Then these amines (**3**, **4**) were alkylated by using the appropriate arylalkyl halide under basic conditions to give compounds **5**–**14**.

N-(4-Arylalkylamino-piperidin-1-yl)-naphthamide analogues (**25**–**34**) were synthesized by using 1- or 2-naphthoyl chloride and the appropriate Boc-protected 4-aminopiperidine derivatives (**20**–**24**) which were prepared in two steps as outlined in Scheme 2. First, ethyl-4-amino-1-piperidine carboxylate was alkylated



Scheme 1. Reagents and conditions: (a) 4-amino-1-benzyl-piperidine, Et_3N, EtOAc, rt; (b) ammonium formate, 10% Pd/C, MeOH, Δ*T* for **3** or 10 bar H_2, 10% Pd/C, MeOH, rt for **4**; (c) R-Br, K_2CO_3, DMF, Δ*T*. R = benzyl (**1**, **2**), 2-phenethyl (**5**, **11**), 1-phenethyl (**6**, **12**), 3-phenylpropyl (**7**, **13**), (*S*)-3-(1-hydroxy-1-phenyl)propyl (**8**), (*R*)-3-(1-hydroxy-1-phenyl)propyl (**9**), 4-phenylbutyl (**10**, **14**).

with the appropriate benzyl or arylalkyl halide in DMF in the presence of potassium carbonate. Then, the substituted 4-amino-piperidine analogues (**15**–**19**) were Boc-protected and subsequently treated under basic conditions to remove the carbamate protecting group. In the second step, piperidine intermediates (**20**–**24**) were reacted with the appropriate naphthoyl chloride and the Boc group was cleaved by treatment with trifluoroacetic acid to provide the appropriate naphthamides (**25**–**34**). All compounds were analyzed by ^{1}H NMR, FTIR, and elemental analysis.

The affinity of the compounds for cloned human $D_{4.2}$ and D_{2L}, and native rat 5-HT_{2A} receptors was evaluated in in vitro binding assays using the radioligands [^{3}H]nemonapride, [^{3}H]spiperone, and [^{3}H]ketanserin, respectively, according to previously described procedures.[19] Binding affinities were determined for compounds showing significant activity in initial screens (>50% inhibition) conducted at 1 μM. K_i values were calculated according to the Cheng–Prusoff equation[20] and the in vitro receptor binding data are reported in Tables 1 and 2.

The aminopiperidine template was previously used in tricyclic series and the corresponding molecules showed significant affinity for D_{2L}, $D_{4.2}$, and 5-HT_{2A} receptors.[19] The current naphthamides bound preferentially to $D_{4.2}$ and 5-HT_{2A} receptors but were devoid of D_{2L} affinity. The 1-naphthamide and the 2-naphthamide series showed clearly distinct binding profiles. The 2-naphthamides interacted more potently with $D_{4.2}$ and 5-HT_{2A} receptors than the corresponding 1-naphthamides. The latter had some $D_{4.2}$ affinity but generally lower 5-HT_{2A} affinity. The orientation of the 4-amino-piperidine side chain had a strong influence on $D_{4.2}$ and 5-HT_{2A} affinity.

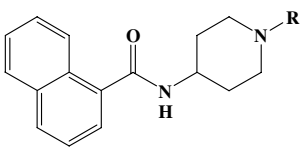
In the *N*-(piperidin-4-yl)-2-naphthamide series, the $D_{4.2}$ receptor affinity was higher in the presence of a benzyl group on the piperidine moiety (**2**). When the spacer between the distal aromatic ring and the piperidine increased from 2 to 4 carbons (compounds **11**–**14**), affinity decreased. The affinity for 5-HT_{2A} receptors only decreased significantly when the linker became a butyl group (**14**). The 1-phenethyl moiety (**12**) was also less favorable.

In the corresponding 1-naphthamide series (**1**, **5**–**10**), the $D_{4.2}$ affinity decreased similarly from the benzyl (**1**) to the phenylbutyl (**10**) with the exception of the phenylpropyl analogue (**7**) which had a higher $D_{4.2}$ affinity than the benzyl one (**1**). In this series, 5-HT_{2A} affinity was strongly reduced, particularly for the benzyl analogue (**1**). The presence of a polar substituent on the side chain was unfavorable since the corresponding hydroxylated compounds (**8**, **9**) had weak or no affinity for the tested receptors. This side chain was previously introduced in other series[21] where both isomers had a high affinity for $D_{4.2}$ receptors and also a clear difference in affinity between both stereoisomers.

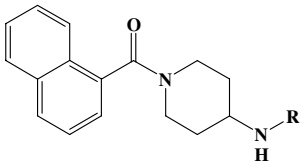
In the series with the opposite orientation of the 4-aminopiperidine where the piperidine nitrogen is incorporated into the amide (**25**–**34**), only the *N*-phenethyl (**31**), the *N*-phenylpropyl (**32**), and especially the *N*-benzyl (**30**) analogues had a weak affinity for $D_{4.2}$ receptors.

Compounds with significant affinity for $D_{4.2}$ and 5-HT_{2A} receptors were tested for in vitro agonism

Table 1. In vitro binding affinities of substituted *N*-(piperidin-4-yl)-1-naphthamides and *N*-(4-aminopiperidin-1-yl)-1-naphthamides for D_{2L}, $D_{4.2}$, and 5-HT_{2A} receptors



1, 5-10

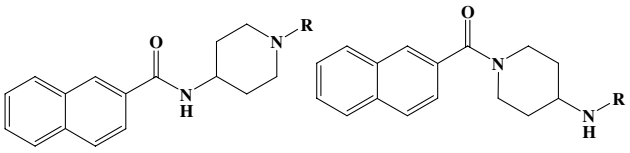


25-29

Compound	R	$D_{4.2}$[a]	5-HT_{2A}[a]	D_{2L}[a]
1	Benzyl	162 ± 48[b]	>1000[b]	>1000
5	2-Phenylethyl	230 ± 162[b]	543 (1)[b]	3%
6	1-Phenylethyl	21%	248 ± 83	11%
7	3-Phenylpropyl	71 ± 14[b]	228 ± 62[b]	9%
8	(*S*)-3-(1-hydroxy-1-phenyl)propyl	45%	390 ± 65	0%
9	(*R*)-3-(1-hydroxy-1-phenyl)propyl	15%	26%	0%
10	4-Phenylbutyl	362 ± 33[b]	487 (1)[b]	18%
25	Benzyl	1%	10%	0%
26	2-Phenylethyl	16%	211 ± 49	0%
27	3-Phenylpropyl	52%	35%	0%
28	(*S*)-3-(1-hydroxy-1-phenyl)propyl	13%	28%	0%
29	(*R*)-3-(1-hydroxy-1-phenyl)propyl	13%	20%	0%

[a] K_i (in nM; mean ± SD; $n \geqslant 2$ if unspecified) or percentage of inhibition at 1 μM.
[b] The compound had no agonistic activity in functional assays and blocked the effect of 100 nM serotonin (5-HT_{2A} receptors) or 500 nM dopamine ($D_{4.2}$ receptors).

Table 2. In vitro binding affinities of substituted *N*-(piperidin-4-yl)-2-naphthamides and *N*-(4-aminopiperidin-1-yl)-2-naphthamides for D_{2L}, $D_{4.2}$, and 5-HT_{2A} receptors



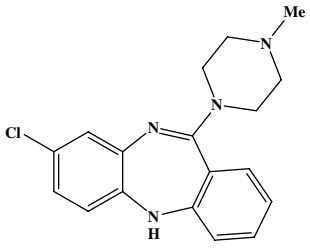
2, 11-14 **30-34**

Compound	R	$D_{4.2}$[a]	5-HT_{2A}[a]	D_{2L}[a]
2	Benzyl	11 ± 1[b]	44 ± 5[b]	>1000
11	2-Phenylethyl	139 ± 33[b]	42 ± 11[b]	7%
12	1-Phenylethyl	29 ± 2[b]	110 ± 47[b]	—
13	3-Phenylpropyl	63 ± 13[b]	50 ± 12[b]	13%
14	4-Phenylbutyl	281 ± 15[b]	213 ± 83[b]	22%
30	Benzyl	167 ± 15[b]	8%	0%
31	2-Phenylethyl	210 ± 12[b]	50%	0%
32	3-Phenylpropyl	222 ± 13	41%	0%
33	(*S*)-3-(1-hydroxy-1-phenyl)propyl	49%	—	—
34	(*R*)-3-(1-hydroxy-1-phenyl)propyl	39%	44%	—

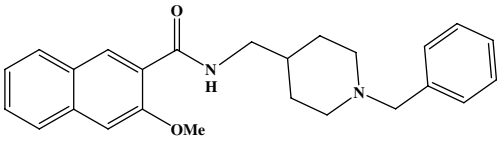
[a] K_i (in nM; mean ± SD; $n \geqslant 2$ if unspecified) or percentage of inhibition at 1 μM.
[b] The compound had no agonistic activity in functional assays and blocked the effect of 100 nM serotonin (5-HT_{2A} receptors) or 500 nM dopamine ($D_{4.2}$ receptors).

and antagonism. For D_4 receptors a GTP-$\gamma^{32}S$ functional assay was used.[22] For 5-HT_{2A} receptors, intracellular calcium flux assays were performed using a FLIPR Calcium Assay Kit and a FLEX Station II fluorescence plate reader (both from Molecular Devices) essentially as described previously.[23] The compounds were tested at a concentration of 5 μM. Selected compounds were also tested in dose–response experiments using the same assays. In both procedures, all tested compounds (see Tables 1 and 2) showed no agonist activity and antagonized the effect of 100 nM serotonin or 500 nM dopamine depending on their binding affinity.

Regarding the absence of D_{2L} affinity, a homologue of compound **2** with a 3-methoxy substituent (**35**) was previously described[24] and showed affinity of 20 and 126 nM for D_4 and D_2 receptors, respectively. The presence of significant D_2 affinity is most probably due to the presence of a pseudocycle formed between the NH amide group and the oxygen of the *ortho*-methoxy group through an intramolecular hydrogen bond. This fact was described as a key feature in a series of benzamide D_2 antagonists.[25] The omission of this structural element in the present study leads to a decrease of binding to D_{2L} sites and thereby to an increased selectivity for $D_{4.2}$ receptors. Several 2-thienylbenzamide derivatives of YM-43611, a non-selective dopamine receptor ligand with an orthopramide structure, have recently been described as D_4 and 5-HT_{2A} ligands with low D_2 affinity.[26] In that series the absence of an *ortho*-methoxy group probably accounted for the reduction in D_2 affinity, while other structural features permitted to maintain a significant D_2 affinity and a decreased 5-HT_{2A} affinity.



CLOZAPINE



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Compound **2** is a mixed and medium potency $D_{4.2}$ and 5-HT_{2A} ligand. High affinity does not always appear to be one of the most important criteria for clinical efficacy of a drug.[5,9] In vivo occupancy and reversibility of the interaction as found with clozapine,[27] quetiapine,[28] or more recently with *N*-methyl-laudanosine[29] are also important parameters and need to be determined in a follow-up study.

The effect of different benzyl substituents of *N*-(piperidin-4-yl)naphthamides on the affinity for $D_{4.2}$, D_{2L}, and 5-HT_{2A} receptors will be reported separately.

In these series depending on the amide linkage, we have obtained different compounds with either $D_{4.2}$ selectivity or with a mixed $D_{4.2}$ and 5-HT_{2A} profile.

The compounds were antagonists at both sites. Their affinities are in a similar range compared to clozapine, however, D_{2L} affinity is strongly reduced. Compound **2** appears to be promising for further biological evaluation.

Acknowledgments

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