Journal of Medicinal Chemistry

Letter

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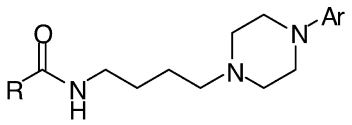
J. Med. Chem., **2005**, 48 (25), 7919-7922• DOI: 10.1021/jm050729o • Publication Date (Web): 18 November 2005



R= 9*H*-Fluoren-2-yl, 1-naphthalenyl, 2-naphthalenyl, 1,4-biphenyl, *trans*-4-phenyl-1-cyclohexyl, *cis*-4-phenyl-1-cyclohexyl, phenyl
Ar= 2,3-di-Cl-Ph, 2-CH_3O-Ph

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Journal of
Medicinal
Chemistry

Volume 48, Number 25 December 15, 2005

Letters

First Structure−Activity Relationship Study on Dopamine D_3 Receptor Agents with *N*-[4-(4-Arylpiperazin-1-yl)butyl]-arylcarboxamide Structure

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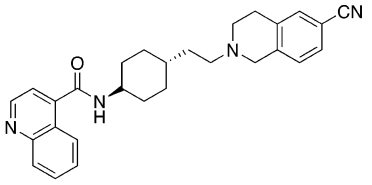
Received July 28, 2005

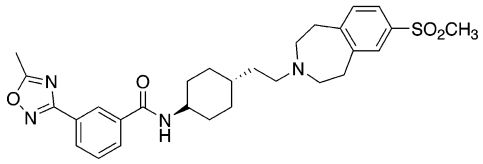
Abstract: Structure−*affinity* relationships of *N*-[4-(4-arylpiperazin-1-yl)butyl]arylcarboxamides as D_3 receptor ligands have been well characterized but not structure−*activity* relationships. In a first attempt to clarify this issue, seven 1-(2,3-dichlorophenyl)piperazine derivatives and their 2-methoxyphenyl counterparts were prepared by varying the arylcarboxamide moiety. They were tested for D_3 receptor binding affinities and in the Eu-GTP binding assay in order to evaluate their intrinsic activity. We have found that the intrinsic activity strongly depended on the nature of the arylcarboxamide moiety.

The dopamine D_3 receptor has received much attention because its potential involvement in the treatment of Parkinson's disease (PD) and schizophrenia and in substance abuse.[1] The therapeutic use of D_3 agents for PD treatment derived from the observation that pramipexole, a D_2/D_3 agent, is effective in early stages of PD and an effective adjunct therapy to levodopa in treating late PD. Moreover, it has been suggested that the D_3 receptor plays a pivotal role in the therapeutic action of levodopa.[2] The involvement of the D_3 receptor subtype in schizophrenia arose from D_3 receptor localization in limbic brain areas (islands of Calleja, ventral striatum/nucleus accumbens, dentate gyrus, and striate cortex), implicated in neuronal circuits believed to display defective functioning in schizophrenia.[3] Recently, the availability of selective D_3 agents has strengthened the likehood that D_3 receptor is significantly involved in mechanisms of drug dependence and abuse.[4] In fact, the D_3 selective antagonist SB-277011 (Chart 1) can reduce cocaine-, nicotine-, ethanol-, and heroin-seeking behaviors. Moreover, other selective D_3 receptor antagonists such as SB-414796 (Chart 1) and **1a** (NGB 2904) (Table 1) possess similar in vivo properties as SB-277011 and this further supports the hypothesis that central D_3 receptors play an important role in the rewarding and incentive motivating effects of cocaine. Moreover, a series of in vivo studies assessed the efficacy of the D_3 receptor partial agonist **2b** (BP 897) (Table 1) in animal models of drug addition.[5,6]

Chart 1



SB-277011



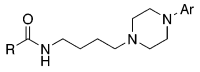
SB-414796

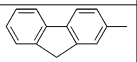
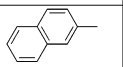
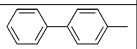
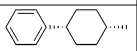
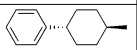
During the past decade, considerable research efforts have led to the identification of potent and selective D_3 receptor ligands.[7,8] We, as well as other research groups, have reported the synthesis and binding profile of a large number of D_3 receptor ligands with *N*-[4-(4-arylpiperazin-1-yl)butyl]arylcarboxamide structure.[9−14] These studies allowed the elucidation of structure−*affinity* relationships of this class of compounds as well as the identification of several potent and selective D_3 receptor ligands. By contrast, little is known on structure-*activity* relationships on *N*-[4-(4-arylpiperazin-1-yl)-butyl]arylcarboxamide derivatives. In fact, the intrinsic activities of several structurally unrelated ligands have been assessed. Moreover, the rationalization of the

* To whom correspondence should be addressed. Phone: +39-080-5442798. Fax: +39-080-5442231. E-mail: leopoldo@farmchim.uniba.it.

10.1021/jm050729o CCC: $30.25
Published on Web 11/18/2005

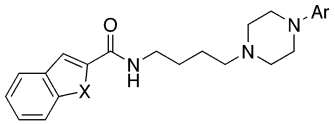
Table 1. D_3 Receptor Affinity and Intrinsic Activity of the Target Compounds



				Eu-GTP binding assay[a]		
compd	R	Ar	K_i ± S.E.M., nM[b]	% Max. activity	pEC_{50}	pA_2
1a		2,3-di-Cl-Ph	1.7 ± 0.30	0		8.70
1b		2-MeO-Ph	0.52 ± 0.03	100	7.31	
2a		2,3-di-Cl-Ph	7.9 ±	0		8.52
2b		2-MeO-Ph	4.6 ± 0.50	0		7.80
3a		2,3-di-Cl-Ph	1.98 ± 0.25	0		8.95
3b		2-MeO-Ph	0.30 ± 0.09	0		7.40
4a		2,3-di-Cl-Ph	11.9 ± 1.10	100	6.68	
4b		2-MeO-Ph	128 ± 15	54	6.81	
5a		2,3-di-Cl-Ph	6.9 ± 0.40	40	7.22	
5b		2-MeO-Ph	32.6 ± 2.50	0		7.14
cis-**6a**		2,3-di-Cl-Ph	10.6 ± 0.50	30	6.20	
cis-**6b**		2-MeO-Ph	20.6 ± 4.50	41	5.74	
trans-**6a**		2,3-di-Cl-Ph	22.8 ± 5.0	0		7.60
trans-**6b**		2-MeO-Ph	3.4 ± 0.25	0		8.10
quinpirole			0.41 ± 0.03	100	7.60	
haloperidol			16.0 ± 2.0	0		6.50

[a] All values represent the mean of three determinations, with each determination lying within 0.3 log unit of the mean. [b] Receptor and radioligand used in binding assay: human cloned D_3 receptors in CHO cells; [^{3}H]spiroperidol. All values represent the mean of three determinations. The Hill plot of listed compounds was between 0.9 and 1.2

Chart 2



X = O, S, Te

Ar = 2-CH_3O-Ph, 2,3-di-Cl-Ph

published intrinsic activity data is not possible, because different assay methods were used (i.e.: [^{35}S]GTPγS binding, stimulation/inhibition of mitogenesis). This aspect has been recently reviewed in depth by Newman and co-workers, who have pointed out the inconsistency of intrinsic activity data obtained from various labs.[15] Gmeiner and co-workers have reported the intrinsic activity of three 1-(2,3-dichlorophenyl)piperazine derivatives and of their 2-methoxyphenyl counterparts (Chart 2).[13] They demonstrated that the intrinsic activities were highly structure-dependent. However, structure−activity relationships were not drawn because of the limited number of compounds studied. On the basis of these observations, in a first attempt to shed light on structure−activity relationships of *N*-[4-(4-arylpiperazin-1-yl)butyl]arylcarboxamide derivatives, we have compared the intrinsic activities of the *N*-[4-(2,3-dichlorophenyl)piperazine]butyl]arylcarboxamides **1a**−**6a** with those of the 2-methoxyphenyl counterparts **1b**−**6b** (Table 1), including the D_3 receptor antagonist **1a** and the partial agonist **2b**. The arylcarboxamide moieties of the target compounds were structurally related to those of **1a** and **2b**.

We have determined the intrinsic activity of the compounds **1a**,**b**−**6a**,**b** using a GTP binding assay because it provides a measurement of agonist efficacy targeting a mechanism that is directly associated to ligand−receptor interaction. We have used the recently introduced DELFIA (Dissociation Enhanced Lanthanide Fluoro Immuno Assay) Eu-GTP binding assay, that represents an alternative for use in filtration assays where [^{35}S]GTPγS is used.[16] By contrast, stimulation/inhibition of mitogenesis is an end-point event, downstream in different signaling pathways, and certainly encompasses other effects due to promiscuity of cellular signals and not only to dopamine D_3 receptor-mediated activity.[17]

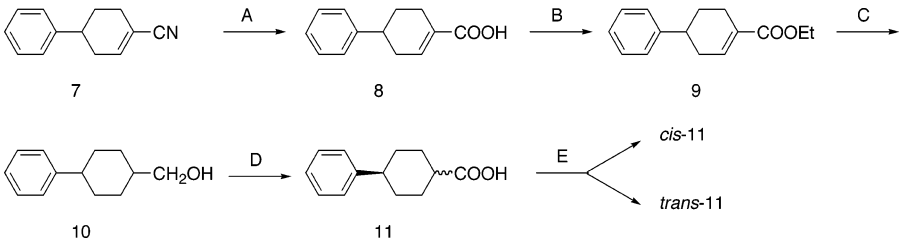
All carboxylic acids used to prepare the target compounds were obtained from commercial sources or prepared according to literature methods, except *cis*-4-phenyl-1-cyclohexanecarboxylic acid (*cis*-**11**). The synthetic procedures reported in the literature for the preparation of *cis*-**11** were not useful for preparative purpose (<6% yield).[18,19] We have used an alternative synthetic pathway which was characterized by low yield (36%) but fulfilled our requirements (Scheme 1). 4-Phenyl-1-cyano-1-cyclohexene (**7**)[20] was hydrolyzed with NaOH to afford the carboxylic acid **8**, which reacted with ethanol to afford ester **9**. This latter intermediate was reduced with borane methyl sulfide complex to give alcohol **10** as a mixture of cis and trans isomers. Oxidation of this mixture with Jones's reagent furnished 4-phenyl-1-cyclohexanecarboxylic acid **11** as a mixture of cis and trans isomers. These isomers were easily separated by column chromatography on silica gel. The target benzamides were prepared by condensing 4-(2,3-dichlorophenyl)-1-piperazinebutanamine (**12a**)[10] or 4-(2-methoxyphenyl)-1-piperazinebutanamine (**12b**)[21] with the appropriate carboxylic acid (Scheme 2).

All target compounds **1a**,**b**−**6a**,**b** display high affinity for D_3 receptor (Table 1), except the benzamide derivatives **4b**, which showed K_i values of 128 nM. Moreover, small differences in D_3 receptor affinity values can be noted between the 1-(2,3-dichlorophenyl)piperazine derivatives and their 2-methoxyphenyl counterparts, except for compound **4a** which is 10-fold more potent than **4b**. This trend was already observed in this class of compounds.[13]

Data of intrinsic activity of final compounds obtained by Eu-GTP binding assay are shown in Table 1. Considering the 2,3-dichlorophenyl derivatives, it can be observed that compounds **1a**, **2a**, **3a**, and *trans*-**6a** acted as D_3 receptor antagonists, **5a** and *cis*-**6a** behaved as partial agonists, and **4a** was found to be a full agonist. Therefore, the intrinsic activities of derivatives **1a**−**6a** seem to be dependent on the nature of the arylcarboxamide moiety. In particular, the orientation of the naphthalene system of derivatives **2a** and **5a** reflects on their intrinsic activity. Similarly, the geometric isomers *cis*-**6a** and *trans*-**6a** acted differently on D_3 receptor.

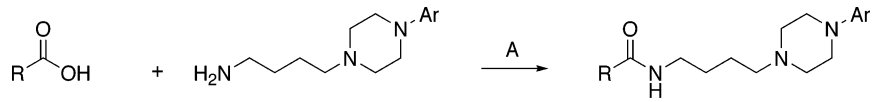
As far as the intrinsic activities of 2-methoxyphenyl derivatives are concerned, compounds **2b**, **3b**, **5b**, and *trans*-**6b** behaved as D_3 receptor antagonists, **4b** and *cis*-**6b** behaved as partial agonists, and **1b** acted as full

Scheme 1[a]



[a] Reagents: (A) NaOH; (B) ethanol, concd H_2SO_4; (C) borane methyl sulfide complex; (D) Jones's reagent; (E) silica gel column chromatography.

Scheme 2[a]



12a (Ar = 2,3-di-Cl-Ph)
12b (Ar = 2-CH_3O-Ph)

1a,b-6a,b

[a] Reagents: (A) 1,1′-carbonyldiimidazole or 1.2% NaOH or methyl chloroformate.

agonist. Also for this group of compounds, the intrinsic activities depended on the nature of the arylcarboxamide moiety. However, the structural features of the carboxamide moiety that are responsible of receptor activation are not clear. From the comparison of activity data, it could be hypothesized that compounds **1a**−**6a** interacted with dopamine D_3 receptor in a slightly different manner as did **1b**−**6b**.

The antagonists **1a**, **2a,b**, **3a,b**, **5b**, *trans*-**6a,b** inhibited quinpirole-stimulated Eu-GTP binding with potencies similar to those found in radioligand-binding experiments. On the other hand, the potencies of the agonists and partial agonists **1b**, **4a**,**b**, **5a**, *cis*-**6a**,**b** were slightly different from those found in radioligand-binding experiments. This might be due to the different [receptor]/[G-protein] ratio in the membrane preparations used for the experiments.[22]

The intrinsic activities of compounds **1a**, **2b**, and **3b** were assessed by other authors using a mitogenesis assay measuring the rate of [^{3}H]thymidine incorporation. Compound **1a**[10] was reported as an antagonist, and compounds **2b**[13] and **3b**[14] as partial agonists. As mentioned above, we have found that compounds **1a**, **2b**, and **3b** acted as antagonists in the Eu-GTP binding assay. Therefore, compounds **2b** and **3b** showed different behavior in these two assays. It is noteworthy that our intrinsic activity data of compound **2b** are in agreement with those published by Garcia-Ladona that tested such compound in the GTPγS binding assay.[17] The source of these differences is probably relates to the fact that mitogenesis measures a response that is distal to agonist-induced receptor/G-protein conformational changes.

The results presented herein show that structural modifications of the arylcarboxamide part of *N*-[4-(4-arylpiperazin-1-yl)butyl]arylcarboxamide framework influence the intrinsic activity at D_3 receptor in either 1-(2,3-dichlorophenyl) and 1-(2-methoxyphenyl)piperazine derivatives. A wide range of intrinsic activity can be observed by varying the arylcarboxamide moiety. The synthesis of a wider number of derivatives becomes necessary for the full elucidation of the role played by the arylcarboxamide moiety of this class of compounds on intrinsic activity.

Supporting Information Available: Physical and spectral data of all the synthesized compounds, experimental procedures for synthesis, and biological evaluation. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM050729O