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NGB 2904 AND NGB 2849: TWO HIGHLY SELECTIVE DOPAMINE D_3 RECEPTOR ANTAGONISTS.

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Abstract: N-(4-[4-{2, 3-dichlorophenyl}-1-piperazinyl]butyl)-3-fluorenylcarboxamide and N-(4-[4-{2, 3-dichlorophenyl}-1-piperazinyl]butyl)-2-biphenylenylcarboxamide were prepared in several steps from 2,3-dichloroaniline. These compounds were identified as highly selective dopamine D_3 receptor antagonists.

In 1990, Sokoloff reported the identification of a new dopamine receptor. This receptor, termed D_3, was cloned from a rat complimentary DNA library using probes derived from the D_2 receptor.[1] This new receptor displayed 75% homology with the rat D_2 receptor in the transmembrane domains. The human version of the D_3 receptor was subsequently identified by Giros.[2]

The high degree of sequence homology as well as related pharmacology and gene organization allowed the D_3 receptor to be classified as D_2-like. The presence of introns within the D_3 receptor gene further associated it with the D_2 receptor. The genes encoding the D_1 and related D_5 receptor lack such introns.[3]

Although D_3 has been associated with both D_2 and the subsequently identified D_4 receptor for the reasons previously described, certain characteristics of the receptor appear to make it unique among the D_2-like family. The D_2 and D_4 receptors couple negatively to adenylate cyclase in that stimulation of the receptors leads to decreased levels of cAMP in the brain. In contrast, the binding of agonists at the D_3 receptor does not appear to be diminished in the presence of guanyl nucleotides (G shift). Although somewhat controversial, the absence of this effect suggests that D_3 is not functionally coupled to G-proteins and may use alternate signal transduction pathways.[4]

Recently it was found that D_3 receptors trigger C-fos expression in the selected cell lines.[5] This observation contrasts with the opposite effects found in the case of D_2 receptors and suggests that D_2 and D_3 receptors might mediate opposite behavioral effects.

Dopamine D_3 receptor messenger RNA and protein were found to be selectively expressed in brain limbic structures. Autoradiographic visualization shows localization of D_3 receptors in tha limbic areas of the nucleus accumbens and islands of Callega.[6] In addition, D_3 receptors were found to be blocked by a number of antipsychotic agents.[1] These findings, taken as a whole, support the idea that D_3 receptors may play a role in the etiology or behavioral expression of schizophrenia. Any confirmation of the role of D_3 receptors in animal behavior or human neuropsychiatric disorders would require sufficiently selective D_3

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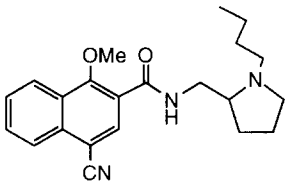
ligands. A number of selective D_3 receptor antagonists have been disclosed. Sokoloff et al reported that the methoxynaphthalenamide nafadotride (**1**) to be a D_3 selective antagonist with a D_2/D_3 K_i selectivity of 10.[7] Glaxo disclosed a series of arylpiperazines with high affinity and selectivity for the D_3 receptor.[8] These compounds, a representative of which is the diphenylamide **2**, are reportedly 100-fold selective for D_3 over D_2 receptors but also have appreciable affinity for the $\alpha 1$ and $5HT_{1A}$ receptors. Researchers at Warner-Lambert identified the halogenated phenylpiperazinonaphthamide **3** as a D_3 receptor partial agonist with high D_3/D_2 selectivity.[9] Compound **3** also has moderate affinity for the D_4 receptor (K_i = 65 ± 8 nM).



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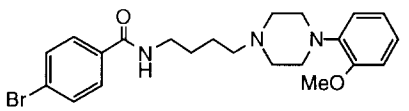

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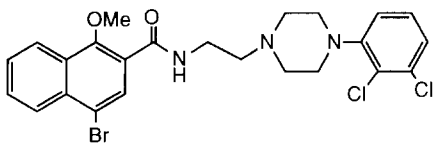

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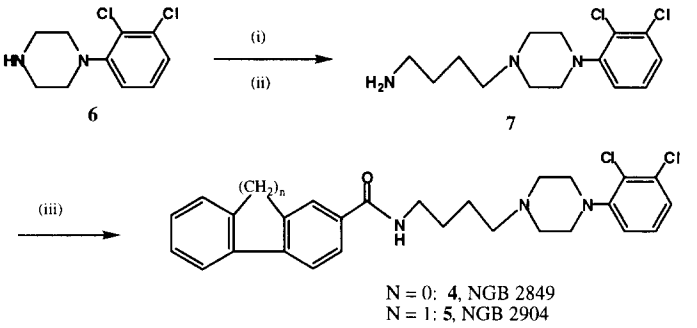
Examination of the structures of **2** and **3** reveals certain common and divergent structural features. Compounds **2** and **3** have the common structural elements of a 4-phenylpiperazine connected via an alkyl chain to a napthalamide (**3**) or pseudonaphthalamide (**2**). The compounds differ chiefly in the aromatic substitution pattern on the 4-phenylpiperazine and in the length of the connecting alkyl chain. As part of a program to identify novel D_3 ligands, we sought to combine selected structural features of compounds **2** and **3**. These efforts culminated in the identification of (**4**, NGB 2849) and (**5**, NGB 2904) as D_3 antagonists having improved selectivity over the parent compounds.

Chemistry

Compounds **4** and **5** were prepared as outlined in Scheme 1. The dichloropiperazine **6** was prepared from 2,3-dichloroaniline via a dehydrative cyclization using diethanolamine.[10] Alkylation of **6** with N-(4-bromobutyl)phthalimide and subsequent de-phthaloylation provided the 4-aminobutylpiperazine **7**. Condensation of **7** with 3-carboxyfluorene or 2-carboxybiphenylene provided **4** and **5**, respectively. [11]

Scheme 1.



(i) N-(4-bromobutyl)phthalimide, Na_2CO_3 (ii) H_2NNH_2, EtOH (iii) 2-biphenylene carboxylic acid or 3-fluorenylcarboxylic acid, CDI, THF.

Pharmacology

The receptor binding profiles of compounds **4** and **5** as summarized in Table 1 were determined by in vitro binding studies using membranes from CHO cells transfected with individual human, primate or rat receptor subtype cDNAs. Both compounds displayed high affinity for the D_3 receptor with greater than 150-fold selectivity over all other dopamine receptor subtypes. Similar selectivity was observed over $\alpha 1$ receptors. In the case of **4**, moderate affinity (125 nM) and selectivity (100-fold) was found for the serotonin 5-HT_2. A global receptor screen (Panlabs) for binding sites not listed in Table 1 indicated no inhibition of greater than 50% at 1 mM.

Table 1. Affinity of NGB 2849 and 2904 for selected cloned receptor subtypes (K_i, nM)

Assay	D_1 hum	D_2 prim	D_3 hum	D_4 hum	D_5 hum	$\alpha 1$ rat	$5HT_2$ rat
NGB 2849	>10000	262 ± 21	0.9 ± 0.3	>5000	>10000	547 ± 62	125 ± 8
NGB 2904	>10000	217 ± 12	1.4 ± 0.6	>5000	>10000	642 ± 41	223 ± 3

In order to determine the functional action of **4** and **5** at the D_3 receptor, the effect on mitogenesis in D_3-transfected CHO cells was measured.[12] Agonist activation of D_3 receptors stimulates [3H]thymidine uptake in CHO cells. The D_3 receptor agonists dopamine and quinpirole both stimulate [3H]thymidine incorporation into CHO.hD3 cells in a dose dependent manner, while the D_3 antagonist haloperidol, and both NGB 2849 and NGB 2904 are receptor antagonists. Haloperidol, NGB 2849 and NGB 2904 antagonize 100 nM quinpirole stimulated mitogenesis with an IC_{50} values of 8.8, 6.8 and 5.0 nM, respectively.

The concentration of D_3 receptors in limbic areas suggest it as a promising target for antipsychotic agents. Although high affinity for the D_3 receptor is observed in a number of commonly used antipsychotic mediciations, some questions remain concerning the contribution of thioos receptor to the overall profile of these agents in the clinical setting. On a cellular level, the lack of an identifiable second messenger system for this dopamine receptor is puzzling. The examination of highly selective ligands for this receptor should provide insight into these questions.

References and Notes

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10. A mixture of phosphorus pentoxide (140 g) and triethylamine hydroichloride (67 g) was melted at 200 °C under a nitrogen atmosphere with mechanical stirring. To this was added 20 g of 2,3-dichloroaniline and diethanolamine (13 g) and heating continued for an additional 3 h. After cooling to 100 °C the mixture was poured into water and the pH adjusted to 7 with the addition of NaOH. The solid material was filtered, suspended in 10% HCl and washed with hexane to remove unreacted aniline. The aqueous was basified with 10 N NaOH solution and extracted with chloroform. The organic layer was dried, concentrated and purified by chromatography to provide 2,3-dichlorophenylpiperazine (5.9 g, 21%). The fumarate salt was prepared in methanol and crystallized from ethanol, mp 213-215 °C.

11. Compound **4** fumarate: mp 191-194 °C; ^{1}H NMR (DMSO- d_6) 7.33 (dd, J = 7, 1 Hz, 1H), 7.27-7.30 (m, 2H), 7.15 (m, 2H), 6.78-6.86 (m, 5H), 6.59 (s, 2H, fumarate), 3.22 (m, 2H), 2.98 (m, 4H), 2.57 (m, 2H), 2.40 (m, 2H), 1.52 (m, 2H). Anal. C, H, N calc. 62.42, 5.24, 7.04; found 62.13, 5.08, 7.10. Compound **5** fumarate: mp 164-166 °C; ^{1}H NMR (DMSO- d_6) hydrobromide 8.60 (m, 1H), 8.08 (s, 1H), 7.95 (dd, *J* = 7, 2 Hz, 2H), 7.95 (m, 1H), 7.90 (d, *J* = 8 Hz, 1H), 7.60 (d, *J* = 7 Hz, 1H), 7.32- 7.42 (m, 3H), 7.19 (dd, *J* = 7, 2 Hz, 2H), 4.0 (s, 2H), 3.58 (m, 2H), 3.3 (m, 2H), 3.2 (m, 6H), 1.8 (m, 2H), 1.6 (m, 2H). Anal. C, H, N calc. 62.95, 5.45, 6.88; found 63.19, 5.43, 6.69.

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