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SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF BENZOFURAN-ACETAMIDES AS "ANTINEOPHOBIC" MITOCHONDRIAL DBI RECEPTOR COMPLEX LIGANDS

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Abstract: A series of novel benzofuran analogues of *N,N*-di-*n*-hexyl-2-phenylindole-3-acetamide (**5**, FGIN-1-27), a potent and highly specific mitochondrial DBI receptor complex ligand, were synthesized by a modified Fischer method and found *in vitro* and *in vivo* to be equally potent and selective as FGIN-1-27.

In the central nervous system (CNS) of mammals, including man, there are two types of benzodiazepine recognition sites, each thought to be associated with a specific protein. The central benzodiazepine receptor (CBR) in the neuron is associated with the α-subunit of $GABA_A$ receptors and participates in the gating of chloride channels operated by GABA while the mitochondrial benzodiazepine receptor is located in the outer mitochondrial membrane of astroglial cells and linked to the production of neurosteroids such as pregnenolone sulfate, dihydroepiandrosterone sulfate, and others.[1,2] The mitochondrial receptor which binds benzodiazepine, non-benzodiazapines such as alpidem, and other ligands including the endogenous peptide DBI (diazepam binding inhibitor),[3,4] is a multimeric protein complex and was recently termed as mitochondrial DBI receptor complex[6] (mDRC; previously called PBR, the peripheral benzodiazepine receptors[5]). The mDRC when occupied by an agonist (e.g., 4'-Cl-diazepam[7] and alpidem[3]) facilitates the transport of cholesterol from the outer to the inner mitochondrial membrane, where a specific type of cytochrome P450scc catalyzes the side chain cleavage of cholesterol to form pregnenolone, the parent molecule of the endogenous steroids.[3] It has been assumed that mDCR ligands may indirectly modulate GABAergic and glutamatergic transmission by virtue of their effects on glial cell steroid biosynthesis.[6] Therefore, recent researches on mDRC ligands have been carried out in order to provide drugs that can be used to modify neurosteroid production and to rectify neuropsychiatric abnormalities (pathological anxiety, panic, depression, etc.) that have as an etiopathogenesis, an alternation in GABAergic function.

It is known that the potent mDRC ligands 4'-chlorodiazepam[7] and alpidem[3] produce some of their effects by binding to $GABA_A$ receptors directly, whereas the selective mDRC ligand PK11195 fails to show marked behavioral effects in rats, either because it may act as a partial agonist of the mDRC[8] or because it may have some additional action in the biosynthesis or release of glial cell neurosteroids.[6] Recently, Kozikowski et al. reported[6] the successful discovery of a class of 2-aryl-3-indoleacetamides (FGIN-1) as potent and highly specific mDRC ligands. A representative example is FGIN-1-27 (**5**) (K_i = 4.4 nM, as measured by the displacement of [^{3}H]4'-chlorodiazepam). A subset of these ligands was shown to stimulate pregnenolone formation in the mitochondria

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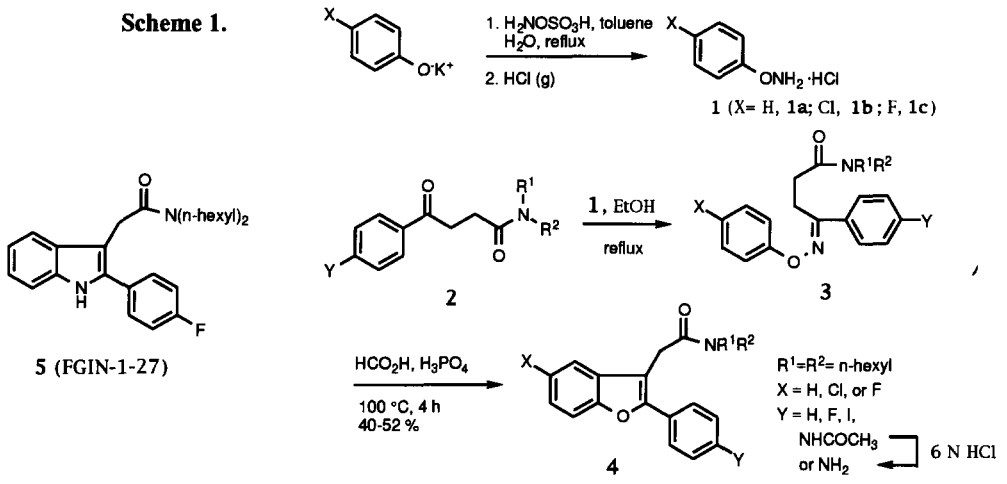
of C6-2B glioma cells with an EC_{50} of about 3 nM.[9] In animal experiments, some selected ligands were found to exhibit antineophobic effects, in spite of the fact that they exhibit no direct action on $GABA_A$ receptors, and to reduce anxiety without causing sedation, a major problem with many of the benzodiazepines.

In continuation of our study of the structure-activity relationship (SAR) of these potent and selective mDRC ligands, we present here the synthesis and binding assays of a series of benzofuranacetamide derivatives which are about equally potent and selective antineophobic mDRC ligands as the corresponding indoleacetamide.

Chemical Synthesis

The compounds presented here are a series of 2-aryl substituted benzofuran-3-acetamide derivatives which we synthesized successfully by a modified Fischer methodology[10] as shown in Scheme 1. *O*-arylhydroxylamine hydrochlorides (**1a-c**) were prepared from potassium aryloxide and hydroxylamine-*O*-sulphonic acid (**1a** is also commercially available from Fluka co.).[11] The ketoamides **2** were prepared according to ref. 6. The reactions of **1** with **2** were carried out in refluxing ethanol to form intermediates **3** which were converted to the desired benzofuran derivatives **4** by heating in a mixture of 96% formic acid and 85% phosphoric acid (10:1). All products were purified by flash chromatography (SiO_2, 9:1 n-hexane/ethyl acetate as eluent) and characterized by spectroscopy (IR, NMR, MS) and elemental analysis.

Scheme 1.



Binding Studies

Preparations of mitochondria and crude synaptic membranes were conducted according to the methods described previously.[9] Binding studies were carried out using primary cultures of glial cells prepared from the cerebella of 8-day-old rat pups.[12] The mitochondrial DBI receptors were specially labeled by [^{3}H]4'-chlorodiazepam (4'CD) and the binding data were measured to represent the ability of our compounds to displace [^{3}H]4'CD binding from the mDRC (Table 1). The data are expressed as K_i values (nM) which were calculated from the corresponding IC_{50} values according to the method of Bylund et al.[13]

No significant binding affinities (Ki > 1000 nM) were found for the binding of our compounds to other neurotransmitter receptors, including GABA (A and B), glycine, glutamate (NMDA and non-NMDA), 5-HT, DA, opiate, cholecystokinin, cannabinoid, and α- and β-adrenergic receptors. For details of the binding protocols, see ref 9 and the articles cited therein.

Antineophobic Activity

In preliminary animal experiments, the action of some of the synthesized benzofuranacetamide derivatives against neophobia in rats were measured using the elevated plus maze test following per os administration.[9] This test is responsive to several classes of neuroactive drugs including serotonin and cholecystokinin antagonists and GABAa receptor modulators.[14] Animals were tested 45 min after receiving the drugs. The data were recorded as the number of entries made by the animal into the open arms of the maze together with the percentage of time spent in the open arm. Data were also presented in Table 1.

Table 1. K_i Values (nM) and Antineophobic Activity of Benzofurans **4** and FGIN-1-27 (**5**)

Compound	X	Y	K_i (nM) [^{3}H]4'CD	Dose μmol/kg po	no. of open arm entries	% of time in open arms
5	H	F	4.4 ± 0.1	57	2.1 ± 0.4*	4.5 ± 0.8*
4a	H	H	14 ± 1.1	59	2.6 ± 0.3*	4.5 ± 2.3*
4b	H	F	8 ± 0.5	57	2.1 ± 0.4*	5.1 ± 2.5*
4c	Cl	H	32 ± 2.8	70	2.1 ± 0.8*	4.7 ± 2.7
4d	Cl	F	32 ± 2.8	53	2.3 ± 0.5*	4.7 ± 2.0*
4e	H	I	80 ± 6	-	NT	NT
4f	H	NH_2	35 ± 2.5	-	NT	NT
Saline					0.3 ± 0.26*	1.02 ± 0.8

Note: * $p < 0.01$ versus saline, + - test (analysis of variance, Dunnett's test)

Results and Discussion

The synthesized benzofuran acetamide derivatives (**4a-f**) have been evaluated in binding assays for displacement of [^{3}H]4'CD in brain mDRCs. The binding affinities were found to be in a range from as high as 8 nM for **4b** to 80 nM for **4e**. All of these compounds are highly selective for mDRC receptors because of the facts that they failed to bind with significant affinities (K_i > 1000 nM) to major neurotransmitter receptors in crude synaptic membranes of the rat brain. Selected compounds **4a-d** were further tested to induce antineophobic effects *in vivo* in the elevated-plus maze, an action which is mimicked by the neurosteroid THDOC,[9] and demonstrated having good antineophobic activity by increasing the number of entries made by the animals into the open arms as well as the percentage of time spent in the open arms. From the SAR study of the previously reported indole series as mDRC ligands, the optimal structure appeared to be a 2-aryl-indole-3-acetamide whose amide nitrogen bears two *n*-hexyl groups. Additionally, the presence of one or more halogen substituents preferably located at either the 5-position of the indole ring or the para position of the 2-aryl ring would further increase binding affinities (FGIN-1-27 as representative).[6] The present evaluation of the selected compounds **4a-f** further demonstrates that the replacement of the indole moiety by a benzofuran still retains most of its potency

and selectivity. Thus, the acetamide side chain appears to be more important for mDRC ligands than the nature of the ring heteroatom.

In conclusion, the presented new ligands modulate the mitochondrial DBI receptor, and their anxiolytic action can be related exclusively to the binding of mDRC since they fail to bind to the benzodiazepine receptor site located on $GABA_A$ receptors or to the binding sites for other known putative neurotransmitters. These compounds make an interesting addition to the family of potent and highly selective mDBI receptor complex ligands with antineophobic properties, that could be useful in treating neurological and psychiatric disorders.

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15. Selected data for **4b**: mp below 40 °C; yield 52%; IR (film) 1645 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.74 (d, 1 H, J = 5.4 Hz), 7.71 (d, 1 H, J = 5.4 Hz), 7.62 (d, 1 H, J = 7.3 Hz), 7.47 (d, 1 H, J = 7.6 Hz), 7.29 - 7.14 (m, 4 H), 3.88 (s, 2 H), 3.31 (t, 2 H, J = 7.6 Hz), 3.15 (t, 2 H, J = 7.9 Hz), 1.47 - 1.44 (m, 4 H), 1.23 - 1.00 (m, 8 H), 0.88 - 0.82 (m, 6 H); ^{13}C NMR ($CDCl_3$) δ 169.0, 163.2 (d, J = 189 Hz), 154.0, 151.4, 129.8, 129.4 (d, J = 6.3 Hz), 126.9, 124.5, 122.8, 120.2, 115.8 (d, J = 23.4 Hz), 111.0, 110.1, 48.3, 46.2, 31.5, 31.4, 30.5, 29.1, 27.6, 26.2, 26.4, 22.5, 14.0, 13.9 ppm; MS (EI) *m/z* 437 (M^+). Anal. ($C_{28}H_{36}NO_2F$) C, H, N (within ±0.3% of the theoretical value).