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Bioorganic & Medicinal Chemistry Letters 15 (2005) 5439–5441
Bioorganic & Medicinal Chemistry Letters

Indole-2-carboxamidines as novel NR2B selective NMDA receptor antagonists

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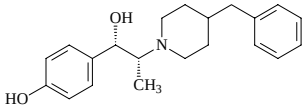
Received 27 July 2005; revised 30 August 2005; accepted 30 August 2005
Available online 5 October 2005

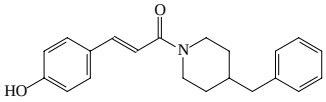
Abstract—A novel series of indole-2-carboxamidine derivatives was prepared and identified as NR2B selective NMDA receptor antagonists. The influence of the substituents on the indole skeleton as well as the substitution of the benzyl moiety on the biological activity of the compounds was studied. Compound **5a** was po active in the formalin test in mouse.

NR2B subtype-selective NMDA antagonists are an intensively studied family of compounds, the scientific and patent literature of which were reviewed from time to time during the past years.[1–3] The prototypical member of this family is ifenprodil (**1**) and its structure served as model for the design of a large number of much more selective analogues. The important features of these structures are two benzene rings connected by a spacer that contains a basic nitrogen, often as part of a piperidine ring. Even more important is a H-bond donor moiety, for example, a hydroxyl group, on one of the benzene rings generally in para position relative to the spacer.[4]



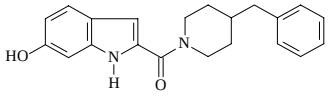
1

The establishment of SAR within this group of compounds revealed that the basic nitrogen in the spacer is not a prerequisite for the activity. Several carboxamides, among others compound **2**, were reported as potent and selective NR2B antagonists.[5]



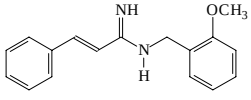
2

Further rigidification of this structure by inserting a NH group in the cinnamide part between the α-carbon of the side chain and one of the *o*-carbons of the phenol moiety resulted in compound **3**. The potency of this compound measured in a functional assay where the inhibition of NMDA-evoked increase of intracellular Ca^{2+} level was determined on rat cortical cell culture turned out to be about six times higher than that of **2**.[6]



3

Meanwhile, a new family of potent NR2B selective NMDA receptor antagonists, exemplified by **4**, was reported.[7] This group of compounds has a completely different pharmacophore. The most important difference between this pharmacophore and that described above is that a H-bond donor group on one of the terminal benzene rings is not a condition of good activity.



4

We assumed that the rigidification of the spacer in **4** may also result in active compounds as happened in the case of **2**. A series of indole-2-carboxamidines represented by **5a–o** was prepared and tested. In this paper, we describe the synthesis and structure–activity relationships developed in an effort to optimize this group of compounds.

Keywords: Indole-2-carboxamidine; NB2B; NMDA.
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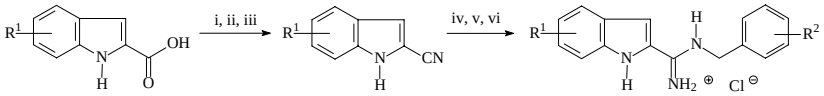
doi:10.1016/j.bmcl.2005.08.110

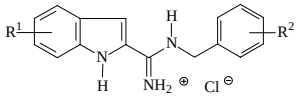


Scheme 1. Reagents and conditions: (i) $SOCl_2$, $CHCl_3$, reflux, 2 h; (ii) NH_4OH; (iii) $POCl_3$, $CHCl_3$, reflux 2 h; (iv) HCl, EtOH, rt, 2 h; (v) benzylamine derivative, rt, 6 h; (vi) HCl.

Solution-phase parallel synthesis was utilized to prepare a range of amidines (Scheme 1). Approximately 200 compounds were prepared from unsubstituted- or 5-substituted-indole-2-nitriles and 48 benzyl amines via the Pinner synthesis. The indol-2-carbonitriles can be synthesized by standard procedures from commercially available indol-2-carboxylic acids. The nitriles were transformed to the corresponding imidates by Pinner chemistry.[8] Amidines were prepared by treatment of indol-2-carboximidates with the appropriate benzyl amines, and purified by column chromatography. Their purity was determined by the HPLC–MS method. The potent compounds had been resynthesized and fully characterized (IR, ^1H and ^{13}C NMR, and high resolution MS).

Biological activity of the prepared compounds was measured in a functional assay where the inhibition of NMDA-evoked increase of intracellular Ca^{2+} level was determined on rat cortical cell culture. Baseline and NMDA-evoked changes of intracellular Ca^{2+} were monitored with fluorimetry using a Ca^{2+}-selective fluorescent dye (Fluo-4/AM) and a plate reader fluorimeter.[9] The results of the functional assay for selected compounds are summarized in Table 1.

Table 1. Functional assay results for compounds **5a**–**o**



5a-5o

Compound	R^1	R^2	NMDA-evoked $\Delta[Ca^{2+}]_i$[a,b] Inhib (%)[c] IC_{50} (nM)	*n*
5a[d]	H	2-OCH_3	35 ± 5	5
5b[d]	5-MeO	2-OCH_3	19 ± 3	3
5c[d]	5-F	2-OCH_3	33 ± 6	3
5d[d]	5-Cl	2-OCH_3	64 ± 12	3
5e[d]	H	2,6-$(OCH_3)_2$	5.4 ± 0.8	3
5f[d]	5-MeO	2,6-$(OCH_3)_2$	26 ± 5	5
5g[d]	5-F	2,6-$(OCH_3)_2$	17 ± 3	3
5h	5-C1	2,6-$(OCH_3)_2$	69 ± 13	4
5i	H	2,6-Di-F	143 ± 27	4
5j	H	3,5-Di-Cl	159 ± 17	3
5k	H	2-CH_3	170 ± 34	4
5l	H	2-F	232 ± 36	3
5m	H	3-OCH_3	57.0%	2
5n	H	4-OCH_3	10.5%	1
5o	H	H	32.8%	1
1			470 ± 51	9
2			131 ± 10	2
3			18 ± 4	13
4			6.6 ± 1.1	3

[a] Values represent mean ± SEM. The number of experiments (*n*) is indicated.

[b] NMDA-evoked changes of intracellular Ca^{2+}.

[c] Inhib (%) were obtained using 1 μM concentration of compound.

[d] The salt form was proved by potentiometric titration. Melting points for the compounds are as follows: **5a**, mp 186–187 °C; **5b**, mp 195–196 °C; **5c**, mp 164–165 °C; **5d**, mp 190–191 °C; **5e**, mp 243–244 °C; **5f**, mp 242–244 °C; **5g**, mp 248–250 °C.

Selectivity toward NR2A subunit containing NMDA receptors was tested by the same functional assay using cells expressing recombinant NR1/NR2A receptors and none of the compounds exhibited significant activity up to 15 μM concentration. In vivo analgesic activity was tested in the mouse formalin test,[10,11] a model of persistent pain.

Initial investigation of the indole-2-carboxamidines focussed on substitution of some of the phenyl rings. Substitution of the benzyl moiety (R^2) showed that substitution at either 3 or 4-position was poorly tolerated; for example, 4-methoxy (**5n**), and 3-methoxy (**5m**) analogues were practically inactive in the functional test. On the other hand, the 2-methoxy substituent (**5a**) showed high activity, while other groups at this position (**5k** or **5l**) were less active. Disubstitution by MeO groups at the 2 and 6 positions, as in **5e**–**h**, gave enhanced activity. Other disubstitution seemed to give inferior compounds (**5i** and **j**). A possible reason why ortho substitution, and to an even greater extent 2,6-disubstitution, leads to potent compounds is that the ortho substituents force the aromatic B-ring out of planarity with the indolecarboxamide moiety. Holding 2-methoxy R^2 group as constant, significant effects on affinity were observed by the substitution of the indol ring. The activity depended on the electron-donating ability of R^1 substituents, increased from 5-Cl toward 5-F and 5-MeO.

The most potent compound in this series is **5e** which is a 2,6-dimethoxybenzyl derivative and its indole portion is unsubstituted. Compound **5e** showed good subtype selectivity too ($NR1_A/2A$ inhibition at 15 μM was 32.1%). The unsubstituted analogue (**5o**) was almost inactive, indicating that the common scaffold in itself was not enough for the activity.

Compound **5a** had the best oral efficacy in formalin test (ED_{50} 15 mg/kg po).

In summary, a series of new indole-2-carboxamidine derivatives were prepared and found to be potent and selective antagonists of the NR2B subtype of NMDA receptors. The conclusion of this study was that the activity of this type of compounds unusually strongly depended on the character and position of the substituents on the aromatic rings. Results of detailed biological investigations will be published under separate cover.

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