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Kynurenic acid amides as novel NR2B selective NMDA receptor antagonists

István Borza,* Sándor Kolok, Kornél Galgóczy, Anikó Gere, Csilla Horváth, Sándor Farkas, István Greiner and György Domány

Gedeon Richter Ltd, Budapest 10, PO Box 27, H-1475, Hungary

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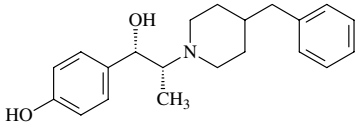
Abstract—A novel series of kynurenic acid amides, ring-enlarged derivatives of indole-2-carboxamides, was prepared and identified as in vivo active NR2B subtype selective NMDA receptor antagonists. The synthesis and SAR studies are discussed.

N-Methyl-D-aspartate (NMDA) receptors are ligand-gated cation-channels embedded in the cell membrane of neurones. Overactivation of NMDA receptors by glutamate, their natural ligand, can lead to calcium overload of the cells. Antagonists of the NMDA receptors may be used for treating many disorders that are accompanied with excess release of glutamate. The NMDA receptors are heteromeric assemblies built up mostly from NR1 and NR2 (NR2A-D) subunits. Particularly interesting of these is the NR2B subunit due to its restricted distribution (highest densities in the forebrain and substantia gelatinosa of the spinal cord). This subunit is the focus of increasing interest as therapeutic target in a wide range of CNS pathologies, including acute and chronic pain, stroke and head trauma, drug-induced dyskinesias, and dementias.[1] Ifenprodil (**1**) is the prototypical selective antagonist of these ionotropic receptors, a property that was discovered many years after its launch as a vasodilator.[2]



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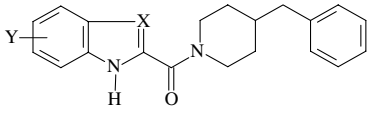
Modification of this structure with the aim of increasing affinity towards the NMDA receptors and at the same time eliminating its original cardiovascular effects resulted in a diverse set of compounds.[3–6]

Previously we reported indole-2-carboxamides **2**, **3**[7] and benzimidazole-2-carboxamide **4**,[8] as highly potent, selective and orally active NR2B antagonists.



2 X = CH, Y = 5-OH
3 X = CH, Y = 6-OH
4 X = N, Y = 5(6)-OH

As continuation of these studies a series of kynurenic acid amides, ring-enlarged derivatives of indole-2-carboxamides, were prepared and tested.[9]

Compounds **28**–**32** were synthesized as described in Scheme 1. The key step involves a standard coupling reaction[10] between an appropriately substituted kynurenic acid and a piperidine. The kynurenic ester intermediates (**15**–**18** and **20**) were prepared by modified Conrad–Limpach method, starting from the corresponding aniline derivatives.[11] Alkaline hydrolysis of esters readily produced the corresponding substituted kynurenic acids (**21**–**23**, **26**, and **27**). The acetylamino-substituted kynurenic ester (**19**) was prepared by catalytic reduction of the nitro precursor compound **18** followed by acetylation of the amine. The hydroxy substituted kynurenic

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* Corresponding author. Fax: +36 1 432 6002; e-mail: i.borza@richter.hu

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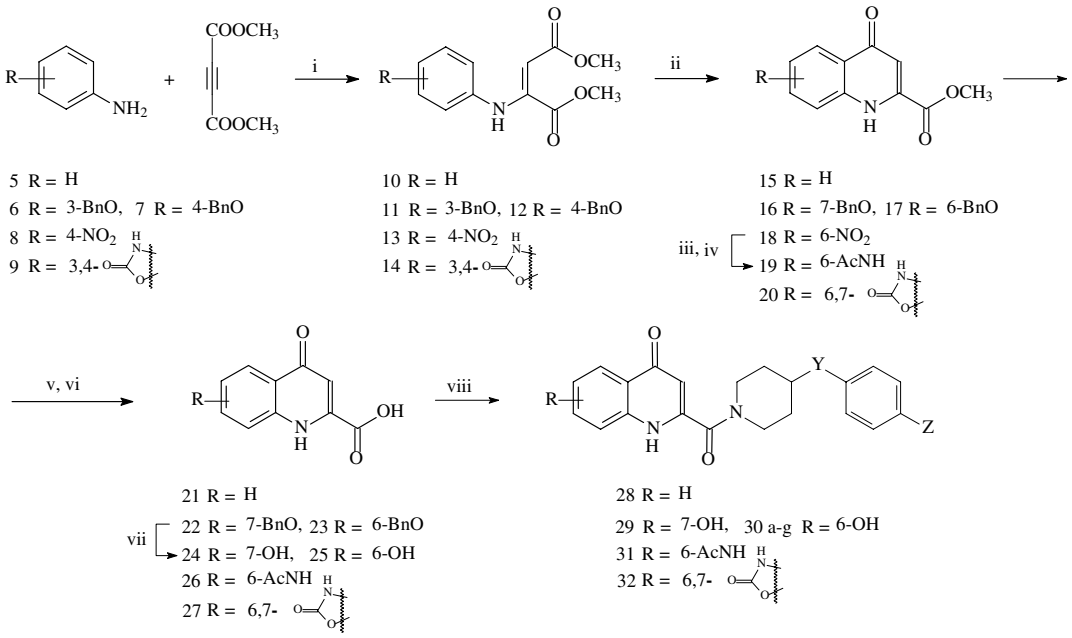


Scheme 1. Reagents and conditions: (i) MeOH, reflux, 2 h; (ii) Dowtherm, 240 °C, 10 min; (iii) H_2, 10% Pd/C, MeOH, rt; (iv) Ac_2O, reflux; (v) NaOH, MeOH, H_2O, rt; (vi) HCl; (vii) H_2, 10% Pd/C, MeOH, rt; (viii) piperidine derivs., HBTU, Et_3N, DMF, rt.

acids (**24** and **25**), however, were prepared from their *O*-benzyl derivatives by catalytic hydrogenolysis. The synthesis of quinazoline derivative **38** started from commercially available 4-hydroxyanthranilic acid and proceeded according to Scheme 2. The anthranilamide **36** was prepared via standard procedures in six steps. The quinazoline ring was obtained by thermal condensation of the oxalic acid diamide **37** and catalytic hydrogenolysis of *O*-benzyl protecting group afforded the end-product. The IR, ^1H NMR, ^{13}C NMR and MS spectra for all intermediates and final compounds were consistent with the assigned structures. Moreover, the purity of the samples was checked by HPLC and HRMS analysis.

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 in 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.[12] Selectivity towards NR2A subunit containing NMDA receptors was tested by the same functional assay using cells expressing recombinant NR1/NR2A receptors. The results of these assays for selected compounds are summarized in Table 1. Although functional data were sufficient for lead optimisation the most active compound **30g** was also investigated in a binding assay on rat forebrain membrane using tritiated Ro-25,6981 as radioligand.[13–15] In vivo analgesic activity was tested in the mouse formalin test,[16,17] a model of persistent pain.

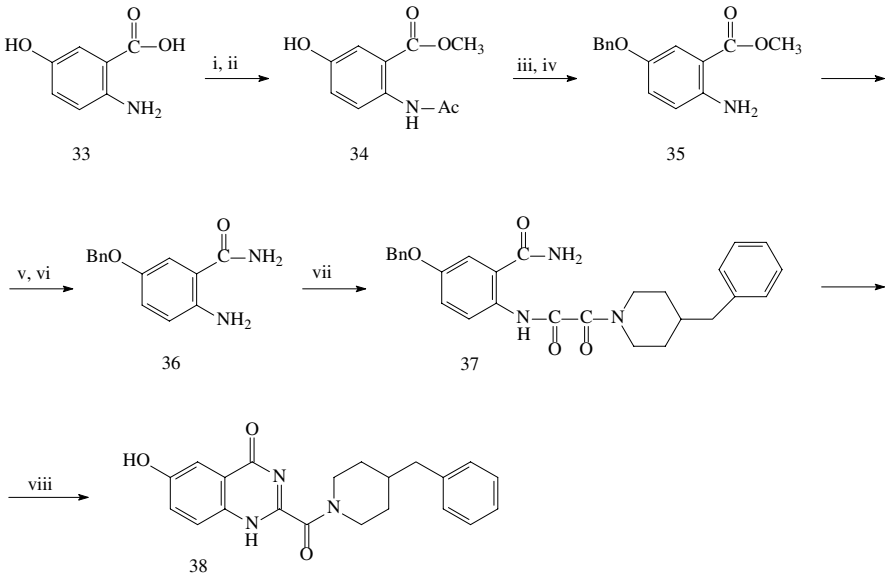
Similarly to many known NR2B antagonists the presence of an OH group, as a H-bond donor moiety, on the quinolinone ring-system was a prerequisite of high potency. The position of this OH, however, significantly influenced the activity of the compounds. In contrast to the analogous 5- and 6-hydroxyindole-2-carboxamides (**2** and **3**), which were almost equiactive, 7-hydroxykynurenic acid amid **29** was significantly less active than its 6-hydroxy analogue **30a**. Among the 6-hydroxykynurenic acid amides **30a–g** there were relatively slight variations in the potency. It seemed that substituents on the benzyl group or the replacement of the CH_2 of these benzyl groups for O did not affect the activity of the compounds. The only exception was **30e** which showed about 5-fold weaker potency compared to its congeners. In order to improve metabolic stability the H-bond donor OH was substituted for an NH on the left-hand side of the molecule. The acetamido analogue **31** lost its activity, while its heterotricyclic analogue **32**, bearing the NH at the same position, had slightly lower potency compared to 6-hydroxy derivatives.

Compared to the indole-2-carboxamides (**2** and **3**), the corresponding benzimidazole-2-carboxamide **4** had significantly higher activity. Analogously the addition of a further N to the kynurenic acid skeleton resulted in the quinazoline **38**. The activity of this compound, however, was much lower than that of the parent compound. In this case increasing the number of heteroatoms in the central ring-system decreased the potency.

All the kynurenic acid amides were found to be inactive on the NR2A subtype of the NMDA receptors.

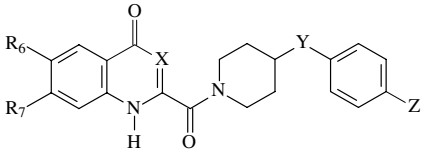
In the Ro-25,6981 binding assay compound **30g** was active, it showed high affinity (K_i: 4.2 nM ($n = 3$)) towards the ifenprodil binding site.

Compounds **30a–g** and **32** were tested in the mouse formalin test as well (Table 1). Several members of this series (**30b**, **30d**, **30e** and **30g**) showed good oral activity, however others with similarly good in vitro potency (**30a**, **30c**,



Scheme 2. Reagents and conditions: (i) $SOCl_2$, MeOH, −20 °C to rt; (ii) 1—Ac_2O, 50 °C; 2—aq Na_2CO_3, rt; (iii) benzylbromide, acetone, 4 h; (iv) HCl, MeOH, reflux, 1 h; (v) 1—10% NaOH, MeOH, reflux, 1 h; 2—HCl; (vi) 1—$SOCl_2$, $CHCl_3$, 1 drop DMF, reflux, 2 h; 2—NH_3, water, 0 °C; (vii) (4-benzylpiperidin-1-yl)(oxo)-acetic acid, HBTU, Et_3N, DMF; (viii) 1—250 °C, 1.5 h; 2—H_2, 10% Pd/C, THF, rt.

Table 1. Biological assay results for compounds **28**, **29**, **30a–g**, **31**, **32** and **38**



Compound	R^6	R^7	X	Y	Z	Mp (°C)	NMDA-evoked $\Delta[Ca^{2+}]_i$[a,b] Inhib (%)[c] IC_{50} (nM)	*n*	NR1/NR2A Inhib (%) in 15 μM	Formalin test po ED_{50} (mg/kg)
28	H	H	CH	CH_2	H	226–227	4.5%	1	1.1	
29	H	OH	CH	CH_2	H	174–175	27.9%	1	9.1	
30a	OH	H	CH	CH_2	H	126–127	9.1 ± 0. 8	5	8.9	Inactive[d]
30b	OH	H	CH	CH_2	F	125–126	8.7 ± 1.0	3	−0.9	9.5
30c	OH	H	CH	CH_2	Cl	190–192	9.1 ± 0.58	3	−5.1	Inactive[d]
30d	OH	H	CH	CH_2	CH_3	151–152	11.0 ± 0.8	3	−2.4	10.6
30e	OH	H	CH	O	H	268–270	49.6 ± 9.9	4	0.7	8.5
30f	OH	H	CH	O	Cl	191–193	7.1 ± 0. 8	3	5.1	Inactive[d]
30g	OH	H	CH	O	CH_3	258–260	6.2 ± 0.78	3	−3.5	8.2
31	AcNH	H	CH	CH_2	H	153–154	2.6%	3	12.4	
32	–HN–C(O)–O–		CH	CH_2	H	290–292	21.7 ± 2.8	3	−2.1	>20
38	OH	H	NH	CH_2	H	240–242	317 ± 8	6	13.9	
1							470 ± 51	9	−2.7	
2							25 ± 8	3	17.2	
3							18 ± 4	13	15.6	
4							2.2 ± 0.4	7	21.0	

[a] Values represent means ± SEM. The number of experiments (*n*) is indicated.
[b] NMDA-evoked changes of intracellular Ca^{2+}.
[c] Inhib (%) were obtained using 0.1 μM concentration of compound.
[d] No effect at 20 mg/kg.

30f and **32**) did not show activity at 20 mg/kg oral dose. One can assume that the differences in the ADME parameters (not measured) reflected in the in vivo activities.

In summary, we have developed a new class of NMDA subtype-selective antagonists designed from indole and benzimidazole derivatives.

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16. Peak effect of pain-related behaviour (licking/biting of paw) elicited by formalin injection in mice 20–25 min post-formalin was measured. Inhibition of this late phase response is considered as analgesic effect against chemically induced persistent pain. Fasted male NMRI mice (20–25 g, Charles River Hungary) were po treated with vehicle (5% Tween 80) or test drugs (in dose-range: **6a** = 0.5–8 mg/kg, **6c** = 2–32 mg/kg) 15 min pre-formalin. Then 20 ml of 1% formalin was sc injected into the right hindpaw. Animals were visually observed in an open glass cylinder. Percent decrease in licking time was determined for each dose in comparison with the daily vehicle-treated groups. Statistical significance was tested by Mann–Whitney *U*-test. ED_{50} values were calculated by Boltzmann's sigmoidal fitting.
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