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New benzoyl urea derivatives as novel NR2B selective NMDA receptor antagonists

Dedicated to the memory of Professor Dr. Kálmán Harsányi

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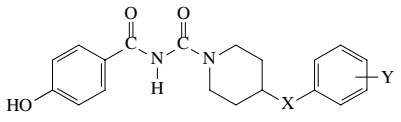
A novel series of benzoyl urea derivatives was prepared and identified as NR2B selective NMDA receptor antagonists. The influence of the substitution of the piperidine ring on the biological activity of the compounds was studied. Compound **9** was active in the formalin test in mice.

During recent years much attention has been devoted to development of NR2B-selective NMDA receptor antagonists as potentially attractive drugs for the treatment of several human diseases, among others of neuropathic pain (Chazot 2004). Typical antagonists of competitor companies contain basic nitrogen, in most cases as N of a 1,4-diaralkyl piperidine for example, in Ro 25-6981 (Fischer et al. 1997) or in CI-1041 (Wright et al. 2000). We have previously described indole-2-carboxamide (Borza et al. 2003) and oxamide (Barta-Szalai et al. 2004) derivatives demonstrating that the basic N is not a condition for activity. Herein we report on a novel class of NR2B-selective antagonists which do not contain basic N. A novel series of benzoyl urea derivatives was prepared and tested in our laboratories as potent NR2B selective NMDA receptor antagonists.

Because of the rapidity, solid phase parallel synthesis was used to prepare a range of benzoyl ureas. Approximately 50 compounds were prepared in a straightfoward manner (Scheme). As a first step of the solid phase synthesis, the Wang resin was reacted with 4-hydroxy-benzamide under Mitsunobu conditions. The benzamide **2** was transformed to benzoyl isocyanate **3** by treatment with oxalyl chloride (Holland 1979). Benzoyl urea derivatives were prepared by addition of benzoyl isocyanate (**3**) to the appropriate piperidines. Finally the 4-hydroxybenzoyl urea derivatives were released from the resin by treatment with 10% TFA/dichloromethane, purified by column chromatography and their purity was determined by HPLC-MS method. The potent compounds were resynthesised and fully characterized (IR, ^{1}H NMR, high resolution MS). The resynthesis was started from 4-benzyloxy-benzamide, and performed analogously to the above solid phase procedure. The end-products (**4**–**12**) were prepared from the corresponding benzyloxy derivatives with catalytic hydrogenolysis.

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 cells expressing recombinant NR1/NR2B receptors (Nagy et al. 2003). Baseline and NMDA-evoked changes of intracellular Ca^{2+} were monitored by fluorimetry using a Ca^{2+}-selective fluorescent dye (Fluo-4/AM) and a plate reader fluorimeter (Nagy et al. 2003). Selectivity towards NR2A subunit containing NMDA receptors was tested by the same functional assay using cells expressing recombinant NR1–3/NR2A receptors and none of the compounds exhibited significant activity up to 15 μM concentration. Potencies for inhibition of NR1A/2B and NR1–3/2A are listed in the Table. *In vivo* analgesic activity was tested in the mouse formalin test, a model of persistent pain (Hunskaar et al. 1985; Richards et al. 1997).

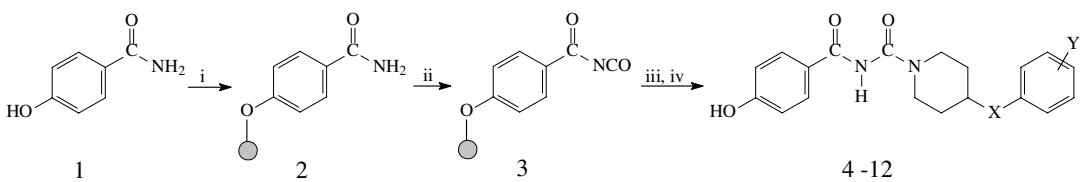
Table: NMDA antagonist activity of compounds measured by fluorimetric method on cells expressing NR1a/NR2B or NR1-3/NR2A subunits



Compd.	X	Y	NR1a/NR2B[a] IC_{50} (nM)	NR1-3/NR2A[a] Inhib % at 15 μM
4	CH_2	4-Cl	5.3	1.8
5	CH_2	4-CH_3	6.2	8.7
6	CH_2	4-OCH_3	7.6	−5.7
7	CH_2	4-CF_3	8.3	19.7
8	CH_2	4-F	19.3	4.9
9	CH_2	H	28.0	2.6
10	CH_2	3-OCH_3	58.9	−0.9
11	CH_2CH_2	4-CH_3	14.3	11.4
12	CH_2S	H	59.1	−2.9
Ro 25–6981			57.0	1.0
CI-1041			8.0	21.0

[a] Values represent the means ± S.E.M. The number of experiments is 2–4 for NR1a/NR2B and 1 for NR1-3/NR2A measurements

Scheme: Reagents and conditions: (i) Wang resin, Ph_3P, DEAD, THF, 0 °C – r.t, 24 h; (ii) ClCOCOCl, 1,2-dichloroethane, 75 °C, 0.5 h; (iii) piperidine derivs., 1,2-dichloroethane, DIEA, r.t, 1 h; (iv) 10% TFA-DCM, 2 h, r.t.



1 2 3 4 -12

Substitution on the 4-position of the phenyl ring was well tolerated, both electron-donating and -withdrawing groups retained excellent potency and selectivity. Phenyl (**9**) and 4-fluoro derivatives (**8**) showed a modest 3-4-fold drop in NR1A/2B potency. The order of potency suggest that a hydrophobic interaction was responsible for the improved affinity rather than electronic factors. Moving the methoxy substituent to the meta position (**10**) produced a significant decrease in potency. Extending the X spacer in **6** with an additional methylene group (**11**) resulted in only a modest drop in potency. The lengthening of this methylene group in **9** by a sulphur atom (**12**) halved the activity, too.
All compounds showed high subtype selectivity (Table). The unsubstituted analogue **9** had excellent oral efficacy in the formalin test (ED_{50} 1.6 mg/kg p.o.), demonstrating that not only their in vitro activity and selectivity were comparable with the reference compounds but some representatives of this class possess outstanding *in vivo* activity too.

Experimental

1. 4-Hydroxybenzamide anchored onto resin (**2**)

A mixture of 7.86 g (6.3 mmol) of Wang resin (Novabiochem; capacity: 0.8 mM/g; size: 100–200 mesh), 200 ml of tetrahydrofuran, 2.9 g (21.1 mmol) of 4-hydroxybenzamide (Aldrich), 6.3 g (24.0 mmol) of triphenylphosphine was stirred at 0 °C for 20 min, then 3.8 ml (24.1 mmol) of diethyl azodicarboxylate was added. The reaction mixture was stirred at 20 °C for 24 h, then the product was filtered off, washed with DMF and THF, and dried at room temperature to yield 8.8 g of **2**.

2. 4-Benzyl-piperidine-1-carboxylic acid 4-hydroxy-benzoylamide anchored onto resin

To a mixture of 0.2 g (0.14 mmol) of **2** in 4 ml of 1,2-dichloroethane 40 µl (0.46 mmol) of oxalyl chloride was added. The reaction mixture was shaken at 75 °C for 0.5 h, cooled to 20 °C and 150 µl (0.86 mmol) of *N*,*N*-diisopropylethylamine, 2 ml of 1,2-dichloroethane, 73 mg (0.41 mmol) of 4-benzyl-piperidine were added. The reaction mixture was shaken for 1 h. Then the resin was filtered off and washed several times with dichloromethane and methanol.

3. 4-Benzyl-piperidine-1-carboxylic acid 4-hydroxy-benzoylamide (**9**)

A mixture of 4-benzyl-piperidine-1-carboxylic acid 4-hydroxy-benzoylamide anchored onto resin and 3 ml of 10% TFA/dichloromethane was shaken for 2 h. Then the resin was filtered off and concentrated. The residue was purified by column chromatography using Kieselgel 60 as adsorbent (Merck) and toluene : methanol = 4 : 1 as eluent to yield 14 mg of 9. The so obtained 9 was dissolved in DMSO and characterised by HPLC-MS method. The resynthesised compound **9**, starting from 4-benzyloxy-benzoylamide was fully characterised: m.p. 176 °C; ^{1}H NMR (300 MHz, DMSO-d_6, 30 °C) δ 9.87 (vbrs, 2 H), 7.83–7.74 (m, 2 H), 7.33–7.23 (m, 2 H), 7.22–7.13 (m, 3 H), 6.88–6.80 (m, 2 H), 4.30–3.60 (vbrm, 2 H), 2.92–2.67 (m, 2 H), 2.52 (d, J = 7.1 Hz, 2 H), 1.86–1.65 (m, 1 H), 1.65–1.49 (m, 2 H), 1.27–1.03 (m, 2 H) ppm. MS[10]: $[M + H]^+ = 339$ ($C_{20}H_{22}N_2O_3$, M = 338) can be detected using LSIMS (Cs ion, glycerine matrix, 20 kV, Finnigan MAT95 SQ), using EI (70 eV, 220 °C source temperature, Finnigan MAT95 XP) only m/z 121, 163 and 175 fragment ions can be detected. Accurate mass measurements (perfluorotributyl amine was used as a reference compound) were performed for m/z 163 and 175 ion peaks (which correspond to two parts of the molecule): calcd 163.0264 (for $C_8H_5O_3N$), found 163.027, delta: 3.8 ppm; calcd 175.1356 (for $C_{12}H_{17}N$), found 175.1354, delta: −0.8 ppm.
Compounds **4**–**8**, **10**–**12** were prepared and determined similarly.

References

Barta-Szalai G, Borza I, Bozó É, Kiss Cs, Ágai B, Proszenyák Á, Keserű GyM, Gere A, Kolok S, Galgóczy K, Horváth Cs, Farkas S, Domány Gy (2004) Oxamides as novel NR2B selective NMDA receptor antagonists. Bioorg Med Chem Lett 14: 3953–3956.

Borza I, Kolok S, Gere A, Ágai-Csongor É, Ágai B, Tárkányi G, Horváth Cs, Barta-Szalai G, Bozó É, Kiss Cs, Bielik A, Nagy J, Farkas S, Domány Gy (2003) Indole-2-carboxamides as novel NR2B selective NMDA receptor antagonists. Bioorg Med Chem Lett 13: 3859–3861.

Chazot PL (2004) The NMDA Receptor NR2B Subunit: A valid therapeutic target for multiple CNS pathologies. Curr Med Chem 11: 389–396.

Fischer G, Mutel V, Traube G, Malherbe P, Kew JNC, Mohacsi E, Heitz MP, Kemp JA (1997) Ro-25,6981, a highly potent and selective blocker of *N*-methyl-D-aspartate receptors containing the NR2B subunit. Characterization *in vitro*. J Pharmacol Exp Ther 283: 1285–1292.

Holland GF (1979) Heterocyclylcarbonyl derivatives of urea, agents for dissolution of gallstones. US 4, 163 784.

Hunskaar S, Fasmer OB, Hole K (1985) Formalin test in mice, a useful technique for evaluating mild analgesics. J Neurosci Meth 14: 69–76.

Nagy J, Boros A, Dezső P, Kolok S, Fodor L (2003) Inducible expression and pharmacology of recombinant NMDA receptors, composed of NR1a/NR2B subunits. Neurochemistry International 43: 19–29.

Richards JG, Messer J, Buchy D, Klingelschimdt A, Mutel V (1997) *In vitro* binding characteristics of a selective NMDA receptor 2B subtype antagonist [^{3}H] Ro 25-6981 in rat brain. Br J Pharmacol 120: 39.

Wright JL, Kesten SR, Upasani RB, Lan NC (2000) 4-Benzyl piperidine alkylsulfoxide heterocycles and their use as subtype-selective NMDA receptor antagonists. WO2000000197-A1; C.A. (2000) 132: 64255.