Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 4638–4640

Benzimidazole-2-carboxamides as novel NR2B selective NMDA receptor antagonists

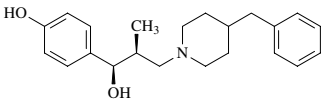
István Borza,[a,*] Sándor Kolok,[a] Anikó Gere,[a] József Nagy,[a] László Fodor,[a] Kornél Galgóczy,[a] József Fetter,[b] Ferenc Bertha,[b] Béla Ágai,[b] Csilla Horváth,[a] Sándor Farkas[a] and György Domány[a]

[a]*Gedeon Richter Ltd., Budapest, 10. POB. 27, H-1475, Hungary*
[b]*Budapest Univ. of Techn. and Econ., Budapest, POB. 91., H-1521, Hungary*

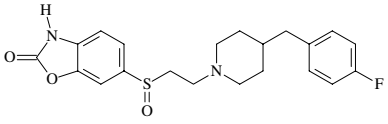
Received 11 April 2006; revised 1 June 2006; accepted 1 June 2006
Available online 16 June 2006

Abstract—A novel series of benzimidazole-2-carboxamide derivatives was prepared and identified as NR2B selective NMDA receptor antagonists. The influence of some structural elements, like H-bond donor groups placed on the benzimidazole skeleton and the substitution pattern of the piperidine ring, on the biological activity was studied. Compound **6a** showed excellent analgetic activity in the mouse formalin test following po administration.

The NR2B subunit containing NMDA receptors became thoroughly studied targets in a wide range of CNS pathologies including chronic pain states.[1] Numerous structurally diverse antagonists of these receptors have been reported and have recently been reviewed as well.[2–4] An important class of the NR2B subtype selective NMDA receptor antagonists can be characterised as an H-bond donor moiety containing benzene ring connected to the nitrogen of a 4-benzylpiperidine via a three-atom long spacer, such as Ro 25-6981[5] and CI-1041.[6]

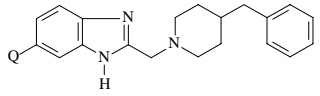


Ro 25-6981



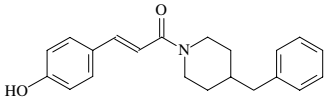
CI-1041

Researchers at Merck have recently reported a series of benzimidazole derivatives. The screening of their compound library led to the identification of the benzimidazole derivative **1** with modest NR2B activity. On the basis of this lead, **2** and **3** were designed by adding the 5(6)-MsNH and 5(6)-OH hydrogen-bond-donating substituents, that showed excellent in vitro and in vivo activity.[7]

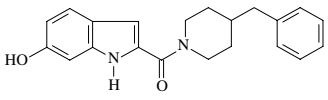


1 Q = H; **2** Q = MsNH; **3** Q = OH

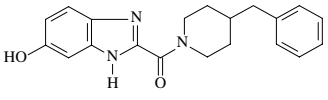
Their publication prompted us to report on our own results achieved in the field of NR2B selective NMDA antagonist benzimidazole-2-carboxamides.



4



5



6

Cinnamide derivatives were the first NR2B subtype-selective NMDA receptor antagonists which did not con-

Keywords: NMDA; NR2B; Benzimidazole.
* Corresponding author. Fax: +36 1 432 6002; e-mail: i.borza@richter.hu

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.06.002

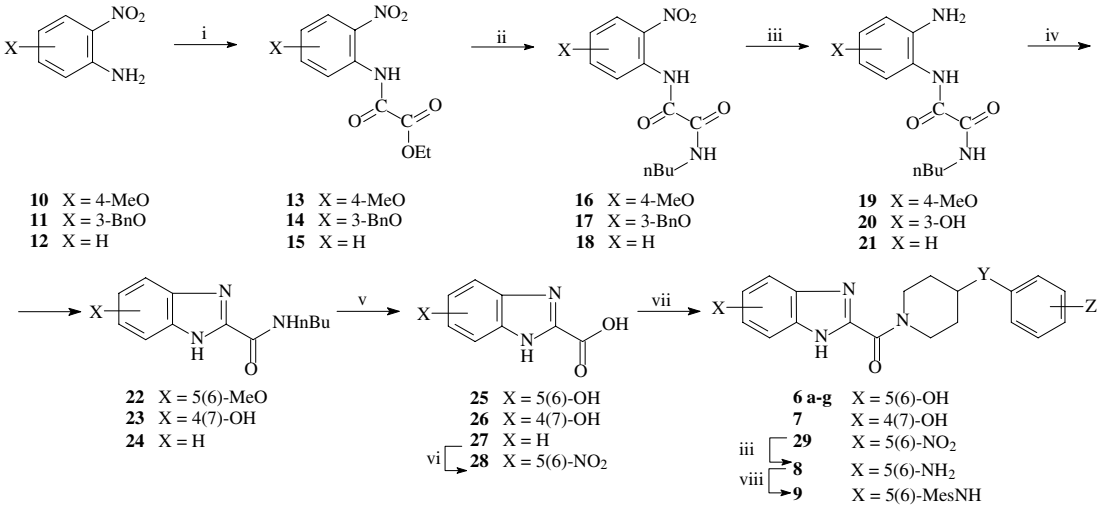
tain basic N.[8] Piperidine-based cinnamide **4** was selected from this family as starting point of our research. In an earlier publication we verified our hypothesis that potency may be enhanced by increasing the rigidity of cinnamide moiety. Incorporation of an NH group between the α carbon atom and the benzene ring of the cinnamic acid part led to the 6-hydroxy-indole-2-carboxamide (**5**), with more than 6-fold higher potency.[9] Encouraged by this result, we decided to explore other heterobicyclic systems. Replacing of the indole skeleton in compound **5** with the potentially bioequivalent benzimidazole ring-system resulted in a more active compound (**6a**), so we decided to prepare a series of benzimidazole-2-carboxamides.

Synthesis of targeted compounds **6a**–**9** is shown in Scheme 1. Acylation of the corresponding *o*-nitro-anilines **10**–**12** with ethyl chlorooxoacetate under basic conditions (Et_3N/CH_2Cl_2), followed by amidation with *n*-butylamine, afforded the oxalic acid diamides **16**–**18**. Reduction of their nitro groups and debenzylation of **17** at the same time gave the phenylenediamines **19**–**21**. The benzimidazole ring was obtained by thermal condensation of **19**–**21**. Hydrolysis of amides **22**–**24**, simultaneous demethylation of **22** and nitration of **27** readily produced the corresponding substituted benzimidazole-2-carboxylic acids **25**–**28**, which were coupled with various piperidine derivatives to give the desired compounds **6a**–**7**, **29**. Nitrobenzimidazole-carboxamide **29** was transformed via the amine derivative **8** to methanesulfonamide **9** with standard procedures. For the preparation of 4-(substituted-benzyl)piperidines a recently published procedure was used.[10] The IR, ^1H NMR, ^{13}C NMR and MS spectra for all intermediates and final compounds were consistent with the assigned structures. Purity of the samples was checked by HPLC and HRMS analysis.

Biological activity of the prepared compounds was measured in a binding assay on rat forebrain membrane using tritiated Ro 25-6981 as radioligand[5,11,12] and 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 by fluorimetric method using a Ca^{2+}-selective fluorescent dye (Fluo-4/AM) and a plate reader fluorimeter.[13] The results of the functional assay for selected compounds are summarized in Table 1. Selectivity towards 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. **6a** was selected for further examination of selectivity towards other NR2 subunit containing NMDA receptors. **6a** up to 10 μM concentration did not inhibit (inh. < 20%) NMDA evoked current in NR1/NR2A and NR1/NR2D transfected cells measured by standard patch clamp technique.[14] In vivo analgesic activity was tested in the mouse formalin test,[15,16] a model of persistent pain.

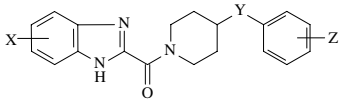
Majority of 5(6)-OH-benzimidazole-2-carboxamide derivatives showed excellent activities. Surprisingly modifications of Y and Z did not influence significantly the potency of compounds. The meta (**6d**–**6f**) and para (**6b**, **6c**) position of Z substituents resulted in nearly equipotent compounds, even the ortho (**6g**) position was well tolerated. A slightly reduced potency could be observed in case of para Me (**6c**) and meta F (**6d**) derivatives. The phenoxy-piperidines (**6h**–**6j**) were the most effective compounds of the series. Replacement of OH group by NH_2 (**8**) led to loss of activity, whereas the corresponding mesylamino derivative (**9**) showed high activity. IC_{50} value of **9** was 6-fold lower than that of **2** while having the same affinity towards NR2B receptors. The 4(7)-OH isomer of **6a** (**7**) was practically equipotent with it.

Compound **6a** had the best oral efficacy in formalin test (ED_{50}: 1.6 mg/kg po).



Scheme 1. Reagents and conditions: (i) ClCOCOOEt, Et_3N, CH_2Cl_2, 0 °C–rt; (ii) *n*-$BuNH_2$, toluene, rt, 10 h; (iii) H_2, 10% Pd/C, MeOH, rt; (iv) 240 °C, 10 min; (v) 48% HBr, reflux, 48 h; (vi) H_2SO_4–HNO_3; (vii) 4-substituted piperidine, HBTU, Et_3N, DMF; (viii) MesCl, pyridine, CH_2Cl_2, 0 °C–rt.

Table 1. Receptor binding and functional assay results for compounds **6a**–**9**



Compound	X	Y	Z	Mp (°C)	NMDA-evoked $\Delta[Ca^{2+}]_i$[a,b] IC_{50} (nM)	*n*	[^{3}H]Ro 25-6981 binding[a] IC_{50} (nM)	*n*
6a	5(6)-OH	CH_2	H	90–92	2.2 ± 0.4	4	4	2
6b	5(6)-OH	CH_2	4-F	99–100	1.2	5	4	2
6c	5(6)-OH	CH_2	4-Me	158–160	8.7 ± 1.0	5	4.4 ± 1.2	3
6d	5(6)-OH	CH_2	3-F	92–94	6.0 ± 1.1	3	5.2 ± 1.9	3
6e	5(6)-OH	CH_2	3-Me	95–96	2.4 ± 0.5	4	5	2
6f	5(6)-OH	CH_2	3-MeO	86–87	2.5 ± 0.6	3	3.2 ± 1.1	3
6g	5(6)-OH	CH_2	2-Me	74–76	12.0 ± 2.8	10	8.4	2
6h	5(6)-OH	O	4-Me	115–117	1.1 ± 0.2	4	3	2
6i	5(6)-OH	O	4-Cl	194–195	1.7 ± 0.4	6	14	2
6j	5(6)-OH	O	4-F	147–151	2.9 ± 0.6	3	6	2
7	4(7)-OH	CH_2	H	165-166	3.0 ± 0.4	4	10 ± 4	3
8	5(6)-NH_2	CH_2	H	74–75	40 ± 0.4	3	143	2
9	5(6)-MsNH	CH_2	H	222–223	1.3 ± 0.2	4	3	2
2					8.6 ± 1.7	5	3.5 ± 1.1	3
4					191 ± 10	2	196 ± 43	3
5					18 ± 4	2	12 ± 2	3
Ro 25-6981					159 ± 29	3	6 ± 1	3
CI-1041					6.6 ± 1.2	3	4 ± 1	3

[a] Values represent means ± SEM. The number of experiments (*n*) is indicated.
[b] NMDA-evoked changes of intracellular Ca^{2+}.

In summary, a series of new benzimidazole-2-carboxamide derivatives were prepared and found to be potent and selective antagonists of the NR2B subtype of NMDA receptors. Modifications of Y and Z (**6a**–**6j**), transfer of the OH group from the 5(6) position to the 4(7) position and replacement of phenolic OH with mesylamino group did not influence significantly the activity of the compounds. Results of further SAR studies and detailed biological data will be published under separate cover.

References and notes

1. Chazot, P. L. *Curr. Med. Chem.* **2004**, *11*, 389–396.
2. Chenard, B. L.; Menniti, F. S. *Curr. Pharm. Des.* **1999**, *5*, 381–404.
3. Nikam, S. S.; Meltzer, L. T. *Curr. Pharm. Des.* **2002**, *8*, 845–855.
4. McCauley, J. A. *Expert Opin. Ther. Patents* **2005**, *15*, 389–407.
5. Fischer, G.; Mutel, V.; Trube, G.; Malherbe, P.; Kew, J. N.; Mohacsi, E.; Heitz, M. P.; Kemp, J. A. *J. Pharmacol. Exp. Ther.* **1997**, *283*, 1285–1292.
6. Wright, J. L.; Kesten, S. R.; Upasani, R. B.; Lan, N. C. PCT Int. Appl. WO2000000197-A1. 2000; *Chem. Abstr.* **2000**, *132*, 64255.
7. McCauley, J. A.; Theberge, C. R.; Romano, J. J.; Billings, S. B.; Anderson, K. D.; Claremon, D. A.; Freidinger, R. M.; Bednar, R. A.; Mosser, S. D.; Gaul, S. L.; Connolly, T. M.; Condra, C. L.; Xia, M.; Cunningham, M. E.; Bednar, B.; Stump, G. L.; Lynch, J. J.; Macaulay, A.; Wafford, K. A.; Koblan, K. S.; Liverton, N. J. *J. Med. Chem.* **2004**, *47*, 2089–2096.
8. Tamiz, A. P.; Cai, S. X.; Zhou, Z. L.; Yuen, P. W.; Schelkun, R. M.; Whittemore, E. R.; Weber, E.; Woodward, R. M.; Keana, J. F. *J. Med. Chem.* **1999**, *42*, 3412–3420.
9. 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. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3859–3861.
10. Ágai, B.; Proszenyák, Á.; Tárkányi, G.; Vida, L.; Faigl, F. *Eur. J. Org. Chem.* **2004**, *2004*, 3623–3632.
11. Mutel, V.; Buchy, D.; Klingelschmidt, A.; Messer, J.; Bleuel, Z.; Kemp, J. A.; Richards, J. G. *J. Neurochem.* **1998**, *70*, 2147–2155.
12. Richards, J. G.; Messer, J.; Buchy, D.; Klingel-schmidt, A.; Mutel, V. *Br. J. Pharmacol* **1997**, *120*, Proc. Suppl., 39P.
13. Nagy, J.; Boros, A.; Dezső, P.; Kolok, S.; Fodor, L. *Neurochem. Int.* **2003**, *43*, 19–29.
14. Kurko, D.; Dezső, P.; Boros, A.; Kolok, S.; Fodor, L.; Nagy, J.; Szombathelyi, Zs. *Neurochem. Int.* **2005**, *46*, 369–379.
15. 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 s.c. 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.
16. Hunskaar, S.; Fasmer, O. B.; Hole, K. *J. Neurosci. Meth.* **1985**, *14*, 69–76.