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Novel N^1-(Benzyl)cinnamamidine Derived NR2B Subtype-Selective NMDA Receptor Antagonists

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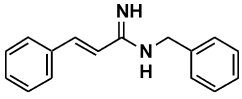
Abstract—Novel (*E*)-N^1-(benzyl)cinnamamidines were prepared and evaluated as NR2B subtype NMDA receptor ligands. Excellent affinity was achieved by appropriate substitution of either phenyl ring. The 2-methoxybenzyl compound **1h** had ~1000-fold lower IC_{50} in NR2B than NR2A-containing cells. Replacement of the styryl unit by 2-naphthyl was well tolerated.

Glutamate is the major excitatory neurotransmitter in the mammalian CNS. The *N*-methyl-D-aspartate (NMDA) receptor is one of the three families of ionotropic glutamate receptor and is a major therapeutic target for a wide range of clinical indications such as stroke, epilepsy, neuropathic pain and Parkinson's disease.[1] The NMDA receptor is a hetero-oligomeric cation channel complex comprising one or more NR1 subunits, of which there are eight isoforms (NR1a–h), and at least one of a family of NR2 subunits (NR2A–D), with different subunit combinations conferring diverse functional properties.[1] NR1 and NR2A are abundantly expressed throughout rat brain whereas localisation of NR2B subunits is more restricted, suggesting that agents acting selectively at this subtype may exhibit a reduced side-effect profile.[2,3] NR2B-selective NMDA antagonists have been developed,[4,5] such as the prototypic compound ifenprodil, Ro25-6981, CP-101,606 and CI-1041 (Fig. 1), and shown to be effective in preclinical pain models such as carrageenan-induced hyperalgesia and mechanical allodynia in nerve ligated rats.[3,6]

Screening for novel compounds with significant affinity for NR2B-containing receptors identified (*E*)-N^1-(benzyl)cinnamamidine (**1a**) as a promising candidate for initiation of a medicinal chemistry programme, particularly since it lacked the phenolic group and 4-benzylpiperidine moiety common to many NR2B antagonists.



1a K_i 9 nM

Amidines **1–4** were readily prepared by treatment of (*E*)-ethyl cinnamimidate hydrochloride with the appropriate amine and crystallizing the product hydrochloride (Scheme 1) or via Horner–Wadsworth–Emmons reaction of an amidine phosphonate intermediate (Scheme 2).

Benzamidines and 2-naphthamidines **6–8** were available from the corresponding nitrile via the imidate or directly using either a copper-mediated method[7] or a methylchloroaluminium amide derived from amine hydrochloride and trimethylaluminium[8] (Scheme 3).

The primary assay used to evaluate compounds was displacement of NMDA receptor-specific [^{3}H]ifenprodil

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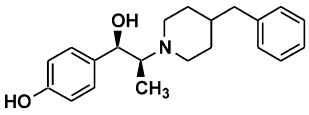
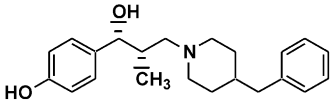
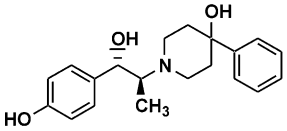


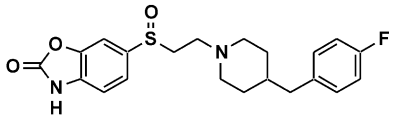
Ifenprodil



Ro25-6981

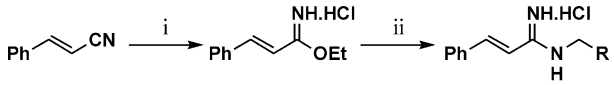


CP-101,606



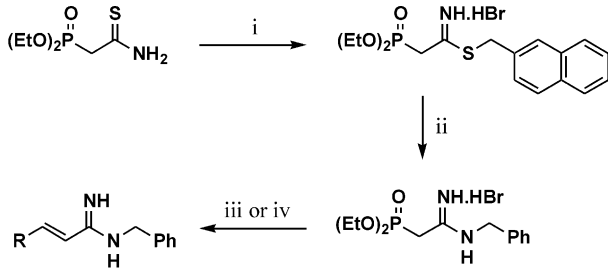
CI-1041

Figure 1. Structures of NR2B-selective NMDA receptor antagonists.



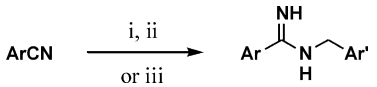
1a-n, 2, 3

Scheme 1. Reagents and conditions: (i) HCl, EtOH, Et_2O; (ii) RCH_2NH_2, MeOH.



1o-s, 4

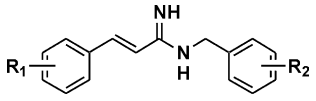
Scheme 2. Reagents and conditions: (i) 2-bromomethylnaphthalene, $CHCl_3$, reflux; (ii) $PhCH_2NH_2$, EtOH; (iii) RCHO, K_2CO_3, THF, reflux; (iv) RCHO, CH_2Cl_2, aq NaOH, *n*-Bu_4NBr, rt.



6, 7, 8a-d

Scheme 3. Reagents and conditions: (i) HCl, EtOH; (ii) $Ar'CH_2NH_2$, MeOH; (iii) $Ar'CH_2NH_2$, CuCl, EtOH, reflux; (iv) $Ar'CH_2NHAlClCH_3$, toluene, 80 °C.

Table 1. [^{3}H]Ifenprodil binding affinities for cinnamamidines **1a–s**



Compd	R_1	R_2	[^{3}H]Ifenprodil K_i (nM)[a]
1a	H	H	9.0
1b	H	2-Cl	1.0
1c	H	3-Cl	0.8
1d	H	4-Cl	77
1e	H	2-CF_3	1.4
1f	H	3-CF_3	31
1g	H	4-CF_3	1400
1h	H	2-OCH_3	0.7
1i	H	3-OCH_3	1.9
1j	H	2-OCH_2CH_3	0.5
1k	H	2-OCF_3	0.6
1l	H	2,3-di-Cl	3.6
1m	H	2,5-di-Cl	2.7
1n	H	3,5-di-Cl	1.4
1o	2-Cl	H	93
1p	3-Cl	H	12
1q	4-Cl	H	52
1r	4-F	H	5.7
1s	3,4-di-F	H	1.7

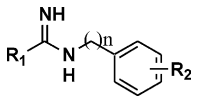
[a]Displacement of [^{3}H]ifenprodil binding to recombinant human NR1a/NR2B receptors stably expressed in L(tk-) cells (ref 9). Values represent the geometric mean of 3 to 5 determinations.

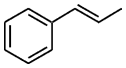
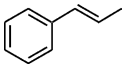
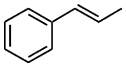
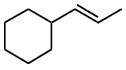
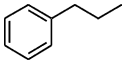
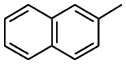
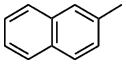
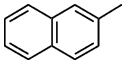
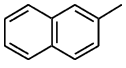
binding to recombinant human NR1a/NR2B receptors stably expressed in L(tk-) cells.[9] Thus, in the [^{3}H]ifenprodil binding assay amidine **1a** had K_i 9 nM, which was comparable to that determined in-house[9] for (±)-Ro25-6981 (K_i 10 nM) and (±)-CP-101,606 (K_i 11 nM). In patch-clamp electrophysiology studies **1a** demonstrated selectivity for inhibition of the response to glutamate/glycine in NR1a/NR2B expressing cells (IC_{50} 70±6 nM, $n=4$) over that observed in NR1a/NR2A cells (no effect up to 3 μM, $n=4$).[10] In the discussion, NR1a/NR2B has been abbreviated to NR2B for the sake of brevity.

Initial investigation of the lead amidine **1a** focussed on substitution of either phenyl ring (Table 1). Substitution of the benzyl moiety (R_2 substituent) showed that 4-substitution was poorly tolerated; for example 4-chloro (**1d**) gave an order of magnitude reduction in NR2B affinity and 4-trifluoromethyl (**1g**) a 150-fold reduction. A 3-chloro group (**1c**) gave a 10-fold improvement, yet the larger 3-trifluoromethyl (**1f**) substituent was less well tolerated. A variety of 2-substituents showed improved affinity over the parent to provide subnanomolar compounds, for example, 2-chloro (**1b**) or 2-alkoxy (**1h**, **1j**), suggesting that a hydrophobic interaction was responsible for the improved affinity over **1a** rather than electronic factors. Dichloro substitution (**1l–n**) offered no particular advantage over the 2- or 3- monochloro analogues.

Preliminary investigation of substitution on the styryl phenyl ring of **1a** (R_1 substituent) suggested less scope for improving NR2B affinity than with changes made to the benzyl moiety (Table 1). 3-Chloro was tolerated (**1p**), but 4- and 2-chloro had 5- and 10-fold lower affinity than **1a**, respectively. 4-Fluoro (**1r**) was incorporated to

Table 2. Variation of chain length and replacement of the styryl group



Compd	R_1	n	R_2	[^{3}H]Ifenprodil K_i (nM)[a]
1a		1	H	9.0
2		2	H	97
3		3	H	120
4		1	H	19
5		1	H	530
6		1	H	10,000
7		2	H	55
8a		1	H	32
8b		1	2-OCH_3	1.3
8c		1	2-Cl	7.3
8d		1	3-Cl	1.6

[a]See corresponding footnote in Table 1.

block a potential site of metabolism, and was found to be well tolerated. Indeed, 3,4-difluoro subsitution (**1s**) gave 5-fold higher affinity than the unsubstituted parent structure **1a**.

NMDA subtype selectivity for this series of cinnamamidines was further illustrated by patch-clamp electrophysiology. Thus, **1h** had IC_{50} 28.6±2.6 nM ($n=5$) for inhibition of the response to glutamate/glycine in NR1a/NR2B expressing cells, compared with approximately 26 μM ($n=6$) in NR1a/NR2A cells.[10]

Having identified several high affinity substituted N^1-(benzyl)cinnamamidine NR2B antagonists of potential use as biological tools, notably **1h**,[11] we turned our attention to variation of the amidine group, the overall chain length and the styryl unit. Attempts to replace the

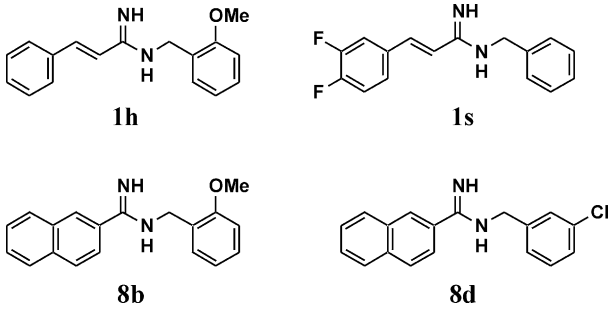


Figure 2. Representative substituted N^1-(benzyl)cinnamamidine and N^1-(benzyl)naphthamidine compounds displaying low nanomolar NR2B receptor affinity.

amidine functional group by amine, amide[12] or imidazole significantly attenuated activity (data not shown). Extension of the benzyl moiety of **1a** by one or two carbon atoms to give the corresponding phenethyl and phenylpropyl compounds, **2** and **3**, respectively, reduced NR2B affinity 10-fold (Table 2). Replacement of the phenyl ring of the styryl unit by cyclohexyl (**4**) retained equivalent activity.

In order to obviate the potential Michael-acceptor reactivity of **1a**, the double bond was hydrogenated to afford **5**. Unfortunately, this was accompanied by a 50-fold drop in binding affinity. Instead, the styryl group was truncated to phenyl by deleting the double bond. However, *N*-(benzyl)benzamidine (**6**) was essentially devoid of NR2B affinity. Interestingly, significant activity could be restored by homologation of the benzyl group to give phenethyl compound **7**, suggesting some tolerance for the position of the amidine functionality along the chain linking the two aromatic groups.

Replacement of the styryl group of **1a** with 2-naphthyl (**8a**) gave a modest reduction in affinity. However, the activity could be improved by benzyl substitution found to be beneficial in the cinnamamidine series. Hence, 2-methoxy and 3-chlorobenzyl naphthamidines, **8b** and **8d** respectively, had low nanomolar activity without the potential Michael-acceptor liability.

In conclusion, investigation of the lead N^1-(benzyl)cinnamamidine **1a** (K_i 9 nM) demonstrated that NR2B affinity could be improved by aromatic substitution of either phenyl ring, as exemplified by the 2-methoxybenzyl compound **1h** (K_i 0.7 nM) and 3,4-difluorocinnamamidine **1s** (K_i 1.7 nM; Fig. 2). The styryl group could be replaced by 2-naphthyl as in **8b** (K_i 1.3 nM) and **8d** (K_i 1.6 nM). Selectivity over NR2A was good, where determined; three orders of magnitude in the case of **1h**. Tritiation of **1h** has provided an NR2B-selective radioligand and further elaboration of the compounds identified in this study has led to the discovery of orally efficacious NR2B-selective NMDA receptor antagonists.[11]

Acknowledgements

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12. The amide analogue of **1a** (C=NH replaced by C=O) had NR2B K_i 3.2 μM. *N*-(Phenylalkyl)cinnamide NR2B subtype-selective antagonists have been reported; *J. Med. Chem*. **1999**, *42*, 3412.