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DERIVATIVES OF 1-HYDROXY-3-AMINOPYRROLIDIN-2-ONE (HA-966). PARTIAL AGONISTS AT THE GLYCINE SITE OF THE NMDA RECEPTOR

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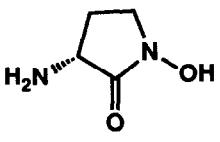
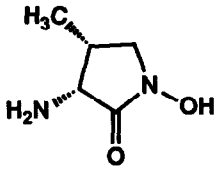
Abstract: The *in vitro* activities of 4-substituted and bicyclic analogues of the glycine-site NMDA partial agonist HA-966 (**1**) reveal strict structure-activity requirements reflecting subtle conformational and steric requirements for receptor binding. The most active compounds have *cis*-4-methyl or hydroxyl substituents and it is suggested that the *in vivo* anticonvulsant activity and good brain penetration of the optimal compound (+) **2** (L-687,414) result from the high fraction of (+) **2** which is not ionised at physiological pH.

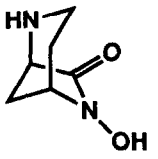
There have been intensive efforts to identify antagonists of the N-methyl-*D*-aspartate (NMDA) subtype of excitatory amino acid receptor for the treatment of cerebral ischaemia and epilepsy.[1] However currently available compounds, acting as blockers of the associated cation channel or as competitive antagonists at the neurotransmitter recognition site, are limited by side-effects[2] and poor brain penetration[3] respectively. Recently there has been considerable interest in developing antagonists acting at the glycine modulatory or "coagonist" site of the NMDA receptor. Amongst the available leads, we considered the α-amino hydroxamate *R*-(+)-HA-966 ((+) **1**)[4] to be particularly important, since its *in vivo* activity[5] suggests adequate CNS bioavailability. In contrast, alternative carboxyl-containing antagonists based on kynurenic acid[6] have poor systemic activity.[7]



(+) **1**

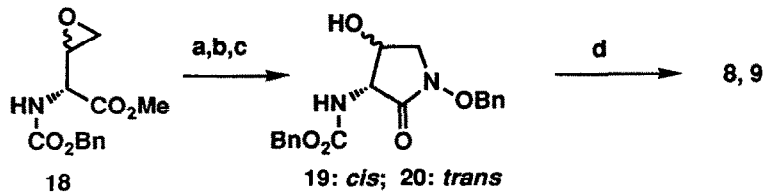


(+) **2**

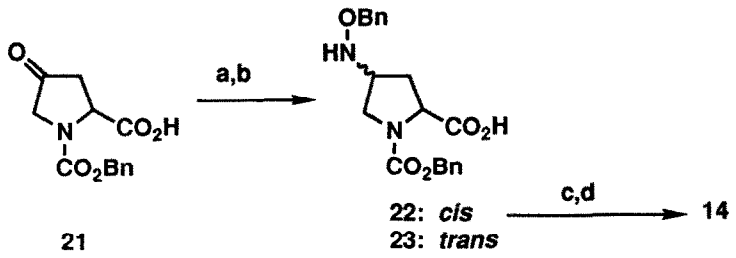


3

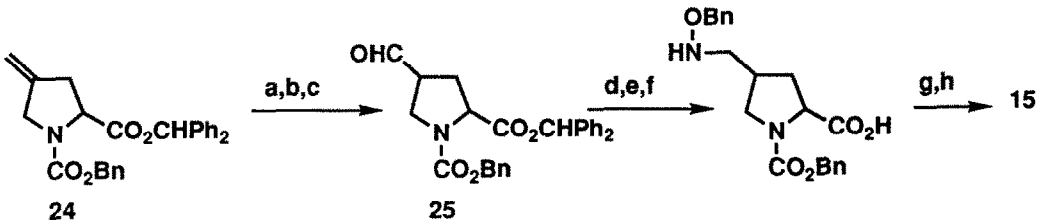
Derivatives of (+) **1**, including the more potent *cis*-4-methyl derivative L-687,414 ((+) **2**) and the racemic bicyclic analogue (**3**)[8] act as low-efficacy partial agonists at the glycine site.[9] Significantly, (+) **1**[5,10] and (+) **2**[11] have improved side-effect profiles relative to other classes of NMDA receptor antagonists, raising the possibility that *in vivo* advantages may be associated with partial agonist effects. We report here details of structure-activity relationships in a wider group of derivatives of **1** (Table 1) and **3** (Table 2).



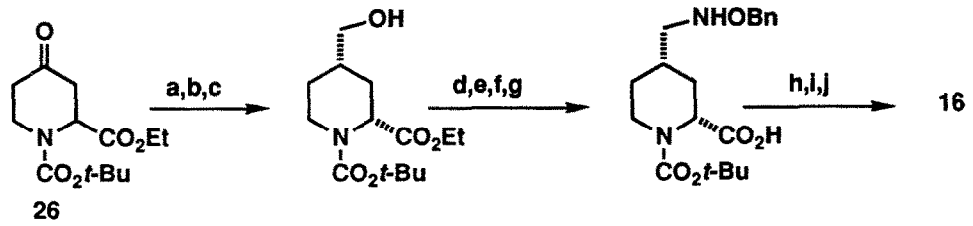
Scheme 1: a, H_2NOBn; b, NaOMe; c, separate (chromatography); d, H_2, Pd-C



Scheme 2: a, H_2NOBn, Et_3N; b, $NaCNBH_3$; c, dicyclohexylcarbodiimide; d, H_2, Pd-C



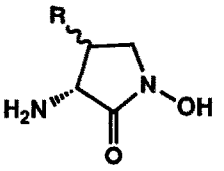
Scheme 3: a, disiamylborane; b, H_2O_2; c, DMSO, $(COCl)_2$, Et_3N; d, NH_2OBn; e, $NaCNBH_3$; f, NaOH; g, BOP-Cl, Et_3N; h, H_2, Pd-C



Scheme 4: a, $Ph_3P^+CH_3Br^-$, $NaNH_2$; b, disiamylborane; c, H_2O_2; d, DMSO, $(COCl)_2$, Et_3N; e, NH_2OBn; f, $NaCNBH_3$; g, NaOH; h, BOP-Cl, Et_3N; i, CF_3CO_2H; j, H_2, Pd-C

The syntheses of compounds **1**, (+) **1**, (-) **1**,[12] **2**, (+) **2**, (-) **2**, **3**, **4**, **12** and **13**[8] and **5**, **7**, **10**, **11** and **17**[13] have been reported. The propyl derivative (**6**) was made by the method described for **5**.[13] The 4-hydroxy derivatives (**8** and **9**) were obtained from the epoxide mixture (**18**)[14] (Scheme 1). Treatment with O-benzylhydroxylamine, followed by base-induced cyclization, gave a separable mixture of the diastereoisomers (**19** and **20**), which were individually hydrogenolysed to give **8** and **9**, isolated as their tosylate salts. The [2.2.1] bicyclic derivative (**14**) was synthesised from the 2-*R*-ketone (**21**)[15] (Scheme 2). Reduction of the oxime gave a separable mixture of the hydroxylamines (**22** and **23**). The *cis*-isomer was separated, cyclised and deprotected to give **14**, which as its tosylate salt proved to be unstable to hydrolysis, opening of the hydroxamate occurring in aqueous solution ($t_{1/2}$ approximately 30 minutes at pH 7.4). The stable higher homologue (**15**) was prepared from the racemic aldehyde (**25**, Scheme 3), which was made from olefin (**24**)[15] by successive hydroboration and oxidation. The [3.3.1] bicycle (**16**) was similarly prepared from the ketone (**26**)[8] (Scheme 4). Displacement of [^{3}H]-glycine binding to rat cortical membranes (IC_{50} values) and inhibition of glycine-stimulated NMDA currents in isolated cultured cortical neurones (K_i values) were determined *in vitro* for compounds **1** - **17** (Tables 1 and 2) using established procedures.[4,5]

Table 1. 4-Substituted derivatives.



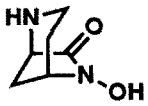
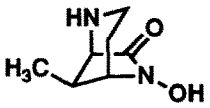
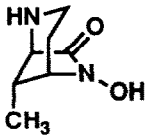
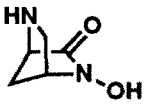
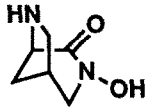
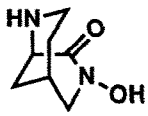
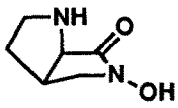
No[a]	R	IC_{50} (μM)[b] [^{3}H]-glycine	K_i (μM)[c] cortical neurone
1	H	27.2	6.3
(+) **1** (*R*)	H	12.5	2.5
(-) **1** (*S*)	H	339	
2	····CH_3	2.9	1.3
(+) **2** (*R,R*)	····CH_3	1.4	0.65
(-) **2** (*S,S*)	····CH_3	86	
4	◄CH_3	>100	
5	····CH_2CH_3	15	15.4
6	····$CH_2CH_2CH_3$	>100	39% inh. @ 300
7	····C_6H_5	>100	0% inh. @ 300
8	····OH	1.3	0.75
9	◄OH	313	30% inh. @ 300
10	····CH_2OH	>100	63% inh. @ 300
11	····CH_2CH_2OH	>100	59% inh. @ 300

a All compounds displayed spectral properties (^{1}H NMR and MS) consistent with their proposed structures.

b Concentration giving 50% inhibition of [^{3}H]-glycine binding to rat cortical membranes.[4,5]

c Inhibition of glycine (0.3 μM) potentiated NMDA (30 μM) responses.[4,5]

Table 2. Bicyclic Analogues.

No[a]	Structure	IC_{50} (μM)[b] [^{3}H]-glycine	K_i (μM)[c] cortical neurone	HN-CH-C=O dihedral (°)[d]
3		19	3.5	84[f]
12		48	8.2	
13		>100	>80	
14			5-15[e]	109
15		>100	16% inh. @ 300	102
16		>100	28% inh. @ 300	85
17		>100	52% inh. @ 300	65

a,b,c See Table 1.

d Geometries determined using OPTIMOL (Molecular Systems Group, MSDRL, Rahway). Axial 1 and equatorial 1^{20} have dihedrals of 82^{o} and 44^{o} respectively.

e Approximation due to instability (see text).

f Geometry found in the X-ray crystal structure.

Synthesis and testing of each of the seven possible monomethyl derivatives of racemic **1** showed that only the *cis* 4-position allowed substitution. The results in Table 1 additionally suggest a strict tolerance to substituent size at the preferred *cis*-4-position. Thus whilst the ethyl derivative (**5**) is equipotent with **1**, further methylene homologation (**6**) or phenyl substitution (**7**) results in a large loss of activity. The hydroxyl derivatives **8** and **9** proved to have similar activities to the corresponding methyl derivatives (**2** and **4** respectively). However homologation of the *cis*-4-hydroxyl in the potent derivative **8** to hydroxymethyl (**10**) or hydroxyethyl (**11**) abolishes activity.

We have suggested previously that the axial amino conformation of the pyrrolidinone ring of **1** is required for recognition by the glycine site and the role of the *cis*-4-methyl substitutent in **2** is to enhance the population of the active conformer.[8] The [3.2.1] bicycle (**3**) mimics this conformation and is equiactive with **1** (Table 2). The relative affinities of the methyl stereoisomers (**12** and **13**) parallel the corresponding monocyclic compounds (**2** and **4**) but in contrast to **2**, the methyl in **12** does not enhance binding, a finding supporting the conformational hypothesis. Reducing the N - C5 ethylene bridge in **3** to methylene to give **14** retains activity, but the instability of **14** in aqueous solution did not allow determination of an accurate K_i or of an IC_{50} value. Comparisons of the geometries of **3** and **14** with the stable ring expanded homologues (**15** and **16**) show that all these compounds can act as mimics of the axial amino conformation of **1**, as shown by their comparable **NH-CH-C=O** dihedral angles (Table 2). However both **15** and **16** are one hundred fold less potent than **3** and **14**. The lack of activity of the bicycle **17**, which is substituted in the preferred *cis*-4-position, appears surprising, but conformational analysis suggests that the geometry of **17** is intermediate between the axial and equatorial conformers of **1**.

Overall, the results show subtle steric and conformational requirements for activity in this class of compounds. The evidence that the axial amino conformations of (+) **1** and (+) **2** are required for activity rests with compounds **3** and **12**, but the receptor clearly exhibits a high degree of steric congestion in the vicinity of the amino group and 4-substituent binding region. These findings contrast with glycine-site full antagonists, where recent studies suggest that considerably greater structural diversity is allowable.[16,17] The discovery that both *cis* 4-methyl and hydroxyl groups enhance activity equally is significant and although this may be a consequence of similar conformational effects of substitution, the hydroxyl group in **8** could act as a mimic of the hydroxyl present in the full agonist *R*-serine and form a hydrogen bond with the receptor.[18] In this respect, it is notable that **8** has higher efficacy than **1** in the cortical neurone test. The rank order of efficacies found was **8** > (+) **1** (10% of the maximal response induced by glycine[9]) > (+) **2** > **3**.

In vivo tests show that (+) **1** and (+) **2** have anticonvulsant and neuroprotective actions, with (+) **2** the most potent systemically effective glycine-site NMDA antagonist yet found, being active at i.p. and i.v. doses of 5-20 mg/kg.[9] The low octanol-water partition coefficients of **1** and **2** [log P (pH 7.4) -3.52 and -3.42 respectively] would suggest poor CNS penetration but following i.v. administration to rats, the concentration of (+) **2** in CSF reaches approximately 25% of blood levels.[19] The pKa values of **2** (8.0 and 6.1) and **1** and its O-benzyl derivative (8.1, 6.2 and 6.6 respectively) show that these molecules exist to the extent of approximately 30% as the non-ionised protomers at

physiological pH. This high fraction of the uncharged species probably accounts for the observed brain penetration and *in vivo* activity of (+) **2**.

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