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DESIGN, SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS OF NOVEL STRYCHNINE-INSENSITIVE GLYCINE RECEPTOR LIGANDS

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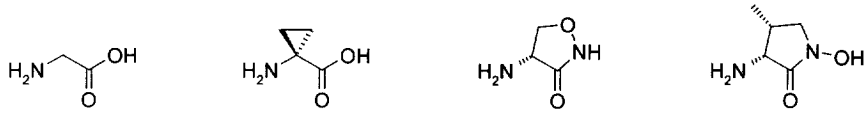
Abstract:. The *in vitro* activities of 3-hydroxy-imidazolidin-4-one derivatives demonstrated very restricted structure-activity relationships at the strychnine-insensitive glycine site of the NMDA receptor. The most active compound **(3a)** was completely unsubstituted and exhibited affinity and efficacy similar to that of D-cycloserine, the prototypical partial agonist at this site.

The discovery that glycine acts as a co-agonist at the NMDA-receptor gated ion channel[1] initiated intense research efforts aimed at the identification of antagonists and partial agonists for this site.[2] A diversity of disorders, such as cognitive deficits,[3] epilepsy,[4] schizophrenia,[5] pain,[6] depression[7] and stroke[8] have been evoked for such glycinergic ligands. Amongst the most representative compounds characterised as strychnine-insensitive glycine partial agonists are the amino acids, 1-aminocyclopropyl carboxylic acid (ACC),[9] D-cycloserine (DCS)[10] and L-687,414[11, 12] (figure 1).

Figure 1



Glycine | 1-Amino-cyclopropyl carboxylic acid (ACC) | D-Cycloserine (DCS) | L-687,414

In our search for novel ligands acting at this site, we elected DCS and L-687,414 as templates since these molecules have a rigid framework permitting the precise definition of the spatial orientation of acidic

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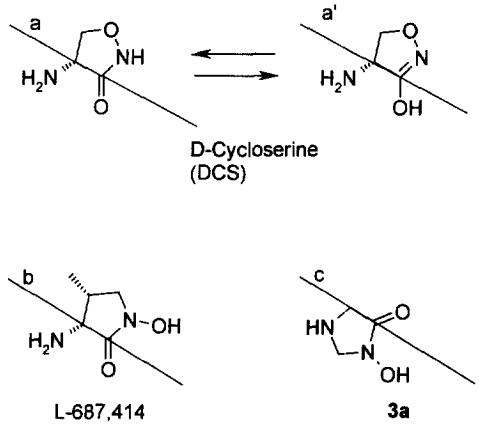
and basic pharmacophores. Indeed, these molecules are close to planar and, if a line is drawn between the two carbon atoms equivalent to the carbon atoms which are present in the glycine entity, the basic nitrogen and the acidic proton are located on opposite sides of this line (a & b, figure 2) in L-687,414 and in one of the tautomers of DCS. This observation indicates that this tautomer is maybe the active one. We attempted to test this hypothesis by designing compounds **3**, 3-hydroxy-imidazolidin-4-one derivatives where the basic nitrogen and the acidic proton are on the same side of the line c as in the supposedly less active tautomer of DCS (a'). We anticipated compounds **3** will be endowed with less affinity and lower efficacy than DCS for the strychnine-insensitive glycine site of the NMDA receptor.

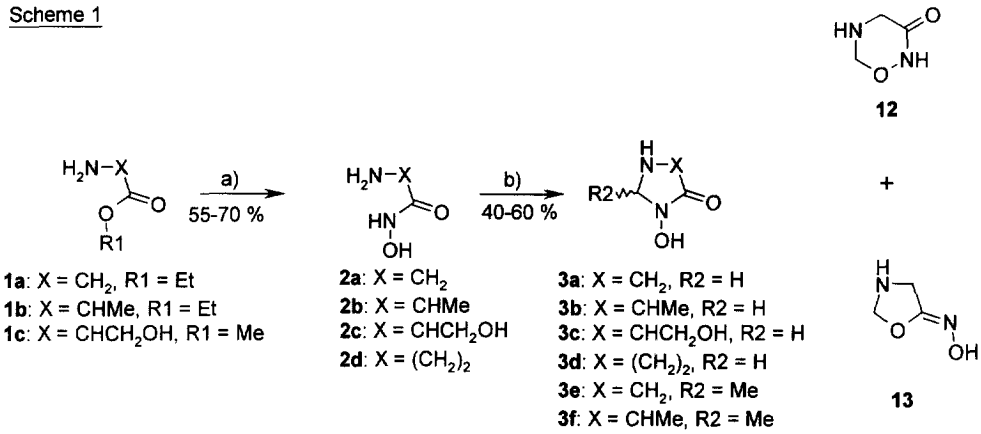
Figure 2



A first series of molecules (**3a-f**) was synthesised according to scheme 1 starting from the amino esters (**1a-c**) which were transformed into the corresponding amino hydroxamate by reaction with hydroxylamine in a mixture of methanol and water. These amino hydroxamates were smoothly cyclized by interaction with aldehydes. One of these compounds has already been described : **3b**.[13] Because the last step in the synthesis can lead depending of the heteroatom which was involved in the cyclization, to three isomeric structures 3-hydroxy-imidazolidin-4-one **3a**, [1,2,5]oxadiazinan-3-one **12** and oxazolidin-5-one oxime **13**, which are difficult to distinguish by routine spectroscopic methods (IR & NMR), the structure of **3a** was confirmed by single crystal X ray diffraction performed by Mrs C. Pascard at Gif sur Yvette.[14] Inasmuch as the D-

enantiomers of the amino acid ligands are systematically more active than L-enantiomers at the strychnine-insensitive glycine site of the NMDA receptor, racemic amino acids were used as starting material throughout this study.

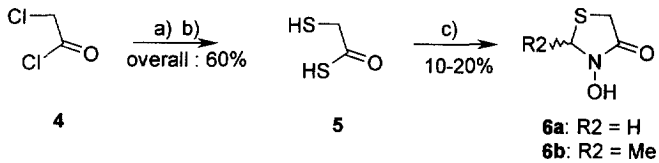
Scheme 1



Reagents and conditions : a) NH_2OH, KOH, MeOH, H_2O, T = -5°C & t = 12h ; b) R2CHO, EtOH, reflux 1h, then 0°C & 12h.

The synthesis of a further set of molecules in which the basic nitrogen was replaced by a sulfur atom, is described in scheme 2. Treatment of 2-chloroacetyl chloride (**4**) by hydrogen sulfide in the presence of a Lewis acid, such as aluminium trichloride, leads to 2-chlorothioacetic acid which reacts with hydrogen sulfide under basic conditions to yield 2-mercaptothioacetic acid (**5**). Reaction of this intermediate with formaldoxime and acetaldoxime under basic conditions, produced the 3-hydroxy-thiazolidin-4-one **6a** and **6b**[15], respectively.

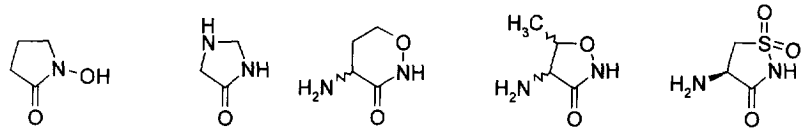
Scheme 2



Reagents and conditions : a) H_2S, $AlCl_3$, T = 0°C, t = 9h, then T = 20°C & t = 10h ; b) H_2S, KOH, EtOH, T = -10°C & t = 3h, then T = 20°C & t = 2h ; c) R2CH=NOH.HCl, Et_3N, EtOH, T = 20°C & t = 2h.

With the aim of further defining the structure-activity relationships at the strychnine-insensitive glycine site of the NMDA receptor, various compounds related to a lesser or greater degree to structure **3a** (**7**,[16] **8**[17]) and DCS (**9**,[18] **10**,[19] **11**[20]) were synthesised according to literature procedures and are presented in figure 3.

Figure 3



7 **8** **9** **10** **11**

The affinity of all compounds for the strychnine-insensitive glycine site was defined by measuring the inhibition of [^{3}H]-glycine binding at a concentration of 10^{-4} M. Dose-response curves were determined only for compounds which inhibited binding by more than 50%. Efficacy of the key ligand **3a** was determined by the modulation of [^{3}H]-MK 801 binding[21] : the higher the maximal effect (ME), the higher the efficacy.

Surprisingly, compound **3a** was as potent a ligand for the strychnine-insensitive glycine site of the NMDA receptor as DCS (table 1). Additionally, it was somewhat more efficacious than DCS in the [^{3}H] MK 801 binding assay. Modification of this skeleton by addition of a methyl (**3b**) or a hydroxymethyl (**3c**) group in position 4 induced a complete loss of activity or a ten fold increase in IC_{50} (78 μM) respectively. Expanding the ring size to 6 (**3d**) also led to an inactive compound. Addition of a methyl group in position 2 (**3e**) provoked a ten fold increase in IC_{50} (83 μM), while combining two methyl groups in positions 2 and 4, led to an inactive molecule (**3f**). Replacement of the basic nitrogen by a sulfur atom (**6a** & **b**) also eliminated activity. The same result was obtained upon deletion of the basic nitrogen (**7**) or of the N-hydroxyl moiety (**8**). DCS analogues with the ring expanded (**9**), a 5-methyl substitution (**10**) or replacement of the ring oxygen by a sulfone function (**11**), were also inactive.

Table 1 : *In Vitro* Activity[22] of Selected Compounds

Compounds	[^{3}H]-glycine binding : IC_{50} (μM)	[^{3}H]-MK 801 binding	
		EC_{50} (μM)	ME (%)
3a	6.8 ± 0.5	4.33 ± 1.73	146 ± 6
Glycine	0.25 ± 0.05	0.068 ± 0.013	159 ± 6
D-serine	0.67 ± 0.2	0.16 ± 0.08	169 ± 20
DCS	7.37 ± 1.75	2.95 ± 0.68	122 ± 9
L 687,414	1.87 ± 0.63	9.5 (1)	18 (1)

IC_{50} : Concentration of compound inhibiting radioligand binding by 50% ; EC_{50} : Concentration of compound eliciting half maximal effect on [^{3}H]-MK 801 binding as defined by its own maximal response ; ME : Maximal effect of the compound on [^{3}H]-MK 801 binding as compared to a basal value of 100%; Values are the means ± s.e.m. of 2-5 independent determinations performed in triplicate. All the drugs were tested up to a maximal concentration of 100 μM.

In conclusion, we have discovered an original ligand (**3a**) for the strychnine-insensitive glycine site of the NMDA receptor. This compound possesses moderate affinity and exhibits efficacy slightly lower than the full agonist, glycine, but higher than that of the partial agonist, DCS. This compound should prove a useful tool in the exploration of the therapeutic potential of ligands interacting with the modulatory glycine site of the NMDA receptor.

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[22] Methods were essentially as described previously (Yoneda Y.; Ogita K.; Suzuki T. *J. Neurochem.* **1990**, *55*, 237-244) : Buffers were made with sterile, deionated and filtered (nitrocellulose, 0.45 mM) Milli-Q water (Millipore) to avoid glycine contamination. For preparation of membranes, rat brains (without cerebellum) from Wistar males (240-260 gr.) were placed in ice cold buffer A (Tris-Acetate 1mM, EGTA 1mM, sucrose 320 mM, pH 7) and homogenised using an Ultra-Turrax TP-1810 (Lucerne, Switzerland) homogeniser. The suspension was centrifuged 10 min at 1000g, the supernatant was then centrifuged 20 min at 35000g. The pellet was resuspended in buffer B (Tris-Acetate 1mM, EGTA 1mM, pH 8) and incubated 15 min at 4°C and centrifuged 20 min at 35000g. The pellet was resuspended in buffer C (Tris-acetate 50 mM pH 7.4, Triton 0.08%) and further incubated 30 min at 4°C. After another two successive centrifugations (20 min at 35000g), the pellet was resuspended in buffer C and aliquots stocked at –80°C for use within 3 weeks. On the day of experiment, membranes were washed twice with buffer C. Binding experiments were performed in buffer C. For glycine sites, [^{3}H]-glycine was employed at 10 nM and non-specific binding defined using glycine (10 mM). Incubation was performed for 20 min at 4°C. For NMDA sites, [^{3}H]-MK 801 was employed at 1 nM, non-specific binding defined with PCP (10 μM), and incubations performed for 120 min at room temperature. Assays were terminated by filtration over 0.1% polyethylenimine-pretreated GF/B glass fiber filters using a Brandel harvester (Gaithersburg, MD) and rapidly washed with ice cold buffer C (containing $MgSO_4$ 10 mM for [^{3}H]-glycine experiments). Data were analysed by nonlinear regression using the program PRISM (Graphpad Software Inc., San Diego, CA, U.S.A.) to yield IC_{50} (Inhibitory Concentration)$_{50}$ values for competition experiments and EC_{50} (Effective concentration)$_{50}$ values for stimulation of [^{3}H]-MK 801 binding.