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Il Farmaco 56 (2001) 609–613

IL FARMACO

Synthesis and preliminary biological evaluation at the glycine$_B$ site of (+)- and (−)-3-oxetanylglycine, novel non-proteinogenic amino acids

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Received 31 October 2000; accepted 5 February 2001

Abstract

Two novel non-proteinogenic amino acids, (+)- and (−)-3-oxetanylglycine were synthesized and evaluated for their ability to diplace [^{3}H]-glycine from the glycine site of the NMDA receptor complex. The lack of activity of these compounds at concentrations up to 100 μM may help in understanding the topological requirements of the glycine site of the NMDA receptor complex

Keywords: Novel non-proteinogenic amino acids; Glycine site; NMDA receptor complex

1. Introduction

The simplest natural amino acid glycine (**1**, Chart 1) plays a fundamental role in the mammalian central nervous system, interacting with either inhibitory (strychnine-sensitive) or excitatory (strychnine-insensitive) receptors. More than a decade has elapsed since the first connection between the strychnine-insensitive glycine sites and the NMDA receptor complex [1], and glycine (**1**) is now recognized to be essential for NMDA activation and is considered a co-agonist of glutamate.

The considerable interest in the modulation of the glycine site of the NMDA receptor complex (glycine$_B$ site, according to the most recent nomenclature) is motivated by the intriguing possibility of achieving pharmacological control of the fundamental functions mediated by this class of ionotropic receptors [2].

Central to the development of glycine$_B$ site modulators is the notion that a partial blockage of the glycine$_B$ site is insufficient to completely switch-off the NMDA receptor functions, thus avoiding many of the side-effects associated with competitive NMDA antagonists.

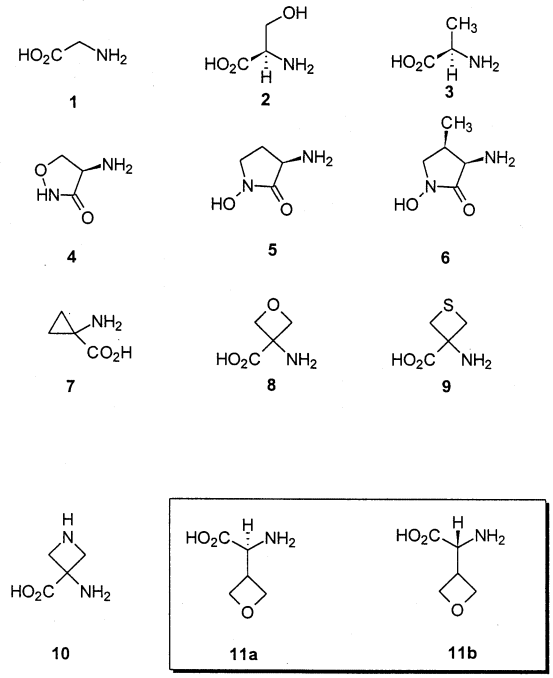

Chart 1.

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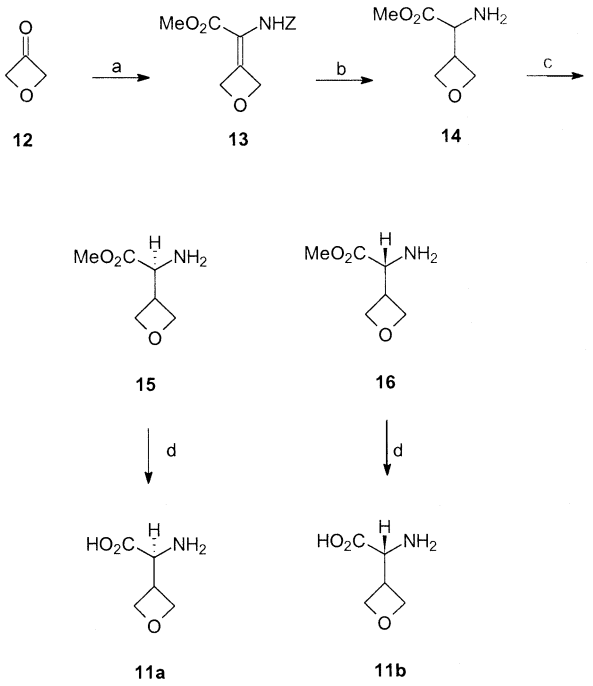
0014-827X/01/$ - see front matter
PII: S0014-827X(01)01101-6



Scheme 1. (a) Z-α-Phosphonoglycine, trimethyl ester, TMG. (b) H_2, Pearlman's catalyst. (c) (1) L-(+)-Tartrate/ D-(−)-tartrate, PhCHO; (2) $NaHCO_3$. (d) NaOH.

On the other hand, the NMDA receptor complex is involved in many aspects of neuronal plasticity and in the long term potentiation (LTP) of the synaptic activity, so that administration of glycine$_B$ agonists or partial agonists may results in improved brain functions, such as memory, learning and information storage, without reaching those levels of NMDA activation that result in excitotoxicity [3].

A great deal of work has been done over the last years on glycine$_B$ antagonists, starting from the endogenous glycine$_B$ antagonist kynurenic acid. Carboxytetrahydroquinolines, hydroxyquinolones, quinoxalinediones and carboxy-indole have provided a valuable source of extremely potent glycine$_B$ antagonists, many of which are featured with physico-chemical parameters suitable for in vivo testing. Currently, carboxy-indoles derivatives are under clinical trials for stroke [4]. Glycine$_B$ agonists and partial agonists have been proposed as potential cognitive enhancers and a wide variety of simple amino acid derivatives have been evaluated in a number of pharmacological models. D-serine (**2**) and D-alanine (**3**) are among the most potent glycine$_B$ agonists, together with the cyclic homologous 1-aminocyclopropanecarboxylic acid (ACPC, **4**). An increase in ring size usually decreases both affinity and efficacy, resulting in partial agonism. D-Cycloserine (**5**), a widely used antitubercolotic, is endowed with activity at the NMDA receptors and is the prototype of a glycine$_B$ partial agonist [5]. Manipulation of D-cycloserine has resulted in other derivatives, such as (R)-HA-966 (**6**) [6] or L-687,414 (**7**) [7] which have been under clinical trials as cognitive enhancers but now abandoned.

Minor chemical modifications have been shown to dramatically alter the binding and the functional properties of glycine analogs, thus making the understanding of the topological requirements of the glycine agonist site of the NMDA receptor an intriguing problem. In 1991, Kozikoski et al. reported the synthesis and the biological evaluation of a series of four-membered ring amino acids as rigid glycine or glycine-bioisoster analogs (**8**–**10**) [8]. Despite their structural similarity with known glycine partial agonists, the above derivatives showed no effect at the glycine$_B$ site. Aiming at further elucidating the topology of the glycine agonist site of the NMDA receptor and in the frame of a research project devoted to the design and synthesis of non proteinogenic amino acids, we report here the synthesis of (+) and (−)-3-oxetanylglycine (**11a** and **11b**, respectively), a cyclic derivative of homoserine endowed with a size-limited side chain and with a hydrogen bond accepting atom. The incorporation of the oxygen atom into a strained four membered ring may allow to mimic the disposition of the polar atom in the D-serine, although compounds **11a** and **11b** are formally analogs of the inactive homoserine.

2. Results and discussion

Enantiomerically pure (+) and (−)-3-oxetanylglycine were prepared according to Scheme 1 starting from the known 3-oxetanone (**12**) [8].

A Wittig–Horner type reaction [9] involving the condensation of the heterocyle ketone **12** with *Z*-α-phosphonoglycine-trimethyl ester [10] in the presence of tetramethylguanidine (THF, −78°C) afforded the protected didehydro-α-amino ester **13** in 32% isolated yield. Catalytic hydrogenation (Pearlman's catalyst, MeOH–CH_2Cl_2, r.t., 1 atm) of **13** gave the corresponding racemic 3-oxetanylglycine methyl ester (**14**) with 77% yield. Asymmetric resolution of the chiral center was achieved through the initial reaction with L-(+)tartaric acid followed by treatment with benzaldehyde to give, after 3 days, a colorless solid constituted by (+)-3-oxetanlglycine metyl ester-L-hemitartrate (20% yield), readily deprotected (sat. $NaHCO_3$) to (+)-3-oxetanylglycine methyl ester (**15**, 73% yield). Alkaline hydrolysis (0.3 N NaOH, H_2O–MeOH, r.t.) of the ester **15**, followed by ion exchange chromatography on Dowex 50X2-200 resin and elution with 10% pyridine afforded (+)-3-oxetanylglycine (**11a**) in 50% yield. Analogously, treatment of the racemic methyl ester **14** with D-(−)-tartaric acid followed by reaction with benzaldehyde afforded, after 5 days, the corresponding (−)-3-oxetanylglycine methyl ester-D-hemitartrate

(52% yield) which was deprotected (sat. $NaHCO_3$) to (−)-3-oxetanylglycine methyl ester (**16**, 91% yield). Alkaline hydrolysis (0.3 N NaOH, H_2O–MeOH, r.t.) of the ester **16**, followed by ion exchange chromatography on Dowex 50X2-200 resin and elution with 10% pyridine afforded (−)-3-oxetanylglycine (**11b**) in 54% yield.

The absolute configuration of compounds **11a** and **11b** remains to be determined and will be communicated in the due course.

Compounds **11a** and **11b** were preliminarily evaluated for their capacity to displace [^{3}H]-glycine from rat cortical membranes. Briefly, synaptosomal membranes from rat cerebral cortex were washed five times with pH 7.4 buffer and brine and were preincubated with [^{3}H]-glycine 20 nM. Non-specific binding was evaluated by adding glycine (**1**) 1 mM. Neither compound **11a** nor **11b** showed significant binding at concentrations up to 100 μM.

The complete lack of activity of the two oxetanylglycine derivatives **11a** and **11b** disproves our working hypothesis on the possibility that the four-membered ring could orient the endocyclic oxygen in such a way to productively interact with hydrogen bonding donor site(s) of the receptor. Alternatively, the strain introduced by the small ring may unfavorably alter the electronic character of the oxygen atom, thus preventing it from interact with the receptor.

Despite their lack of activity at the glycine site of the NMDA receptor complex, derivatives **11a** and **11b** may serve as novel building block for peptidomimetics. Indeed, the need of reducing many of the limitations associated with the therapeutic use of peptides is motivating a search of suitable non-proteinogenic replacements for coded amino acids [11,12]. In light of their peculiar steric characteristic such as a size limited ring and a reduced conformational mobility, as well as the presence of a hydrogen acceptor atom, amino acids **11a** and **11b** may be considered as potential surrogate for polar, coded amino acids such as threonine, serine, and asparagine [13].

3. Experimental

Melting points were determined by the capillary method on a Büchi 535 electrothermal apparatus. ^{1}H NMR and ^{13}C NMR spectra were taken on a Brucker AC 200 spectrometer as solutions in $CDCl_3$, unless otherwise indicated. Proton chemical shifts are reported in p.p.m. downfield from tetramethylsilane, except with D_2O which was also used as internal standard. Carbon chemical shifts are reported in ppm using MeOH as internal standard. (δ 49.0). Flash chromatography was performed on Merck silica gel (0.040–0.063 mm). Specific rotations were recorded on a Jasco Dip-360 digital polarimeter.

3.1. *N*-Benzyloxycarbonyl-α,β-didehydro-(3-oxetanyl)-glycine, methyl ester (**13**)

Tetramethylguanidine (0.63 ml, 5.00 mmol) was added to a magnetically stirred solution of *N*-benzyloxycarbonyl-(phosphonoglycine-trimethyl ester (1.72 g, 5.20 mmol) in dry THF (8 ml) at −70°C in a nitrogen atmosphere. Stirring was continued for 1 h after which 3-oxetanone (**12**, 0.360 g, 5.00 mmol) was added and the resulting mixture was maintained under stirring at room temperature overnight. The reaction mixture was then diluted with AcOEt (10 ml), filtered and the solid residue was carefully washed with AcOEt (60 ml). The combined organic phases were washed with 1% citric acid (2 × 20 ml), brine (20 ml), and dried over Na_2SO_4. Evaporation of the solvent yielded an oily residue (0.6 g) which was submitted to flash chromatography: elution with light petroleum–AcOEt 8:2 afforded **13** as a colorless solid (0.440 g, 32%), mp 121–123°C; ^{1}H NMR ($CDCl_3$) δ 3.75 (3H, s, CH_3O), 5.10 (2H, s, $PhCH_2$), 5.32–5.45 (4H, m, 2 × CH_2), 6.80 (1H, br s, NH), 7.33 (5H, s, aromatic's); ^{13}C NMR ($CDCl_3$) δ 52.53, 67.32, 78.53, 115.84, 128.12, 128.31, 128.51, 135.65, 139.87, 152.66, 163.48.

3.2. (±)-3-Oxetanylglycine, methyl ester (**14**)

Hydrogen was bubbled into a suspension of Pearlman's catalyst (0.020 g) and **13** (0.190 g, 0.68 mmol) in CH_2Cl_2–MeOH (30 ml, 1:1) at room temperature for 2 h under vigorous magnetic stirring. After filtration and evaporation of the solvent, the residue (0.15 g) was submitted to flash chromatography: elution with $CHCl_3$–MeOH (95:5) gave **14** as a pale yellow oil (0.076 g, 77%); ^{1}H NMR ($CDCl_3$) δ 1.65 (2H, s, NH_2), 3.05–3.25 (1H, m, CH_2CH), 3.72 (3H, s, CH_3), 3.75 (1H, d, $CHNH_2$), 4.55–4.85 (4H, m, 2xCH_2); ^{13}C NMR ($CDCl_3$) δ 38.76, 51.97, 56.36, 74.04, 74.24, 174.60.

3.3. (+)-3-Oxetanylglycine, methyl ester (**15**)

A solution of L-(+)-tartaric acid (0.497 g, 3.31 mmol) in dry EtOH (8 ml) was added to a magnetically stirred solution of **14** (0.480 g, 3.31 mmol) in dry Et_2O (10 ml). The white precipitate readily formed was then washed with EtOH (20 ml), Et_2O (20 ml) and dried under vacuum to give (±)-3-oxetanylglycine, methyl ester-L-hemitartrate (0.5 g). This compound was then suspended in dry EtOH (5 ml) containing benzaldehyde (0.35 ml, 0.33 mmol) and refluxed for 5 min. The resulting clear solution was allowed to cool to room temperature and was magnetically stirred for 3 days. A colorless solid was then collected by filtration and washed with EtOH (20 ml) to give (+)-3-oxetanyl-

glycine, methyl ester-L-hemitartrate (0.197 g, 20%). Treatment of the tartrate salt with saturated $NaHCO_3$ (10 ml) was followed by extraction with AcOEt (4 × 10 ml); the combined organic phases were dried over Na_2SO_4 and the solvent evaporated off to give the ester **15** (0.070 g, 73%); ^1H NMR ($CDCl_3$) δ 1.63 (2H, s, NH_2), 3.15 (1H, m, CH_2CH), 3.72 (3H, s, CH_3), 3.75 (1H, d, $CHNH_2$), 4.70 (4H, m, 2 × CH_2).

3.4. (+)-3-Oxetanylglycine (*11a*)

1 N NaOH (0.5 ml) was added to a solution of **15** (0.055 g, 0.38 mmol) in MeOH (1 ml) and the resulting mixture was magnetically stirred at room temperature for 4 h. The reaction mixture was neutralized with 3 N HCl, concentrated under vacuum to reduce the volume (2 ml) and submitted to ion exchange chromatography on Dowex 50x2-200 resin: elution with 10% pyridine afforded **11a** as white crystals (0.025 g, 50%), m.p. 159–161°C; $[\alpha]_D^{25} = +19.8$ (c 1, H_2O).

3.5. (−)-3-Oxetanylglycine, methyl ester (*16*)

A solution of D-(−)-tartaric acid (1.034 g, 6.69 mmol) in dry EtOH (10 ml) was added to a magnetically stirred solution of **14** (1.00 g, 6.69 mmol) in dry Et_2O (10 ml). The white precipitate readily formed was then washed with EtOH (20 ml), Et_2O (20 ml) and dried under vacuum to give (±)-3-oxetanylglycine, methyl ester-D-hemitartrate (1.98 g, 97%). This compound was then suspended in dry EtOH (8 ml) containing benzaldehyde (0.73 g, 6.89 mmol) and refluxed for 5 min. The resulting clear solution was allowed to cool to room temperature and was magnetically stirred for 5 days. A white solid was then collected by filtration and washed with EtOH (30 ml) to give (−)-3-oxetanylglycine, methyl ester-D-hemitartrate (1.068 g, 52%). Treatment of the tartrate salt with saturated $NaHCO_3$ (20 ml) was followed by extraction with AcOEt (4 × 10 ml); the combined organic phases were dried over Na_2SO_4 and the solvent evaporated off to give the ester **16** (0.48 g, 91%); ^1H NMR ($CDCl_3$) δ 1.62 (2H, s, NH_2), 3.18 (1H, m, CH_2CH), 3.63 (3H, s, CH_3), 3.65 (1H, d, $CHNH_2$), 4.70 (4H, m, 2 × CH_2).

3.6. (−)-3-Oxetanylglycine (*11b*)

1 N NaOH (1.25 ml) was added to a solution of **16** (0.130 g, 0.896 mmol) in MeOH (2.5 ml) and the resulting mixture was magnetically stirred at room temperature for 4 h. The reaction mixture was neutralized with 3 N HCl, concentrated under vacuum to reduce the volume (3 ml) and submitted to ion exchange chromatography on Dowex 50X2-200 resin: elution with 10% pyridine afforded **11b** as a colorless solid (0.062 g, 54%), mp 158.5–160.5°C; ^1H NMR (D_2O) δ 3.20–3.50 (1H, m, CH_2CH), 3.70–3.90 (1H, d, $CHNH_2$), 4.50–4.80 (4H, m, 2 × CH_2); ^{13}C NMR (D_2O) δ 35.62, 55.97, 74.38, 74.46, 172.46; $[\alpha]_D^{25} = -17.6$ (c 1, H_2O).

Acknowledgements

We gratefully acknowledge Professor Alessandro Galli, Università of Firenze, for testing derivatives **11a** and **11b**.

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