

1 Synthesis of phenylalalinol-derived oxazolopyrrolidone  
2 lactams and evaluation as NMDA receptor antagonists

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5

6 Dedication – Dedicated to Professor Sundaresan Prabhakar on occasion of  
7 his 75th anniversary.

8

9 **Abstract** N-methyl-D-aspartate (NMDA) receptor antagonists are known  
10 to rescue neuronal cell death caused by excessive activation of glutamate  
11 receptors. This phenomenon, known as excitotoxicity, is implicated in the  
12 pathogenesis of several neurodegenerative disorders including ischemia,  
13 Alzheimer's disease, Parkinson's disease, and Huntington's disease.  
14 Unfortunately, some antagonists of NMDA receptor have been tested in  
15 clinical trials with discouraging results. However, recent advances in the  
16 physiology and pharmacology of the NMDA receptor have kept the interest  
17 alive to modulate NMDA receptors for therapeutic intervention.  
18 We present here the synthesis of a small library of phenylalalinol-derived  
19 oxazolopyrrolidone lactams and their evaluation as NMDA receptor  
20 antagonists. The compounds were easily synthesized in yields up to 92%.  
21 In addition, one of the compounds has an  $IC_{50}$  of 62  $\mu M$  and offers  
22 potential to develop more potent NMDA receptor antagonists.

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**Keywords** Amino alcohols • Chiral auxiliaries • NMDA receptor antagonists • pyrrolidones • lactams

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## 1 **Introduction**

2 *N*-Methyl-*D*-aspartate receptors (NMDAR) are part of the ionotropic  
3 glutamate receptors family. After activation by their co-agonists, glycine  
4 and glutamate, they allow the neural influx of Ca<sup>2+</sup> through a membrane  
5 ion pore thus playing an important role in the postsynaptic depolarization  
6 [1-4].

7       Apart of their involvement on synaptic plasticity, which has been  
8 postulated as the neurochemical basis of learning and memory, NMDA  
9 receptors have been implicated in neuronal death [5]. High levels of  
10 glutamate have been found in brain trauma and other neurodegenerative  
11 diseases, so it is thought that NMDA receptors are potential targets for  
12 neuroprotective compounds [6]. In fact, the adamantanes amantadine and  
13 memantine develop their neuroprotective effects through blockade at the  
14 NMDA receptor. Specifically, memantine is authorized in Western  
15 countries and used therapeutically to slow-down the progression of  
16 Alzheimer's disease [7].

17       In 2009, some oral active oxazolidine derivatives were described to  
18 act as NMDA antagonists by preventing the binding of the NMDAR  
19 ligands [8]. Based on this information and due to our interest in the  
20 synthesis of oxazolo lactams [9-10] we decided to extend our research to  
21 the synthesis of enantiopure oxazolopyrrolidone lactams using (*S*)-

1 phenylalaninol as a chiral inductor. Since the biological activity is greatly  
2 affected by the absolute stereo-outcome of the compounds, a series of (*R*)-  
3 phenylalaninol derivatives was also prepared.

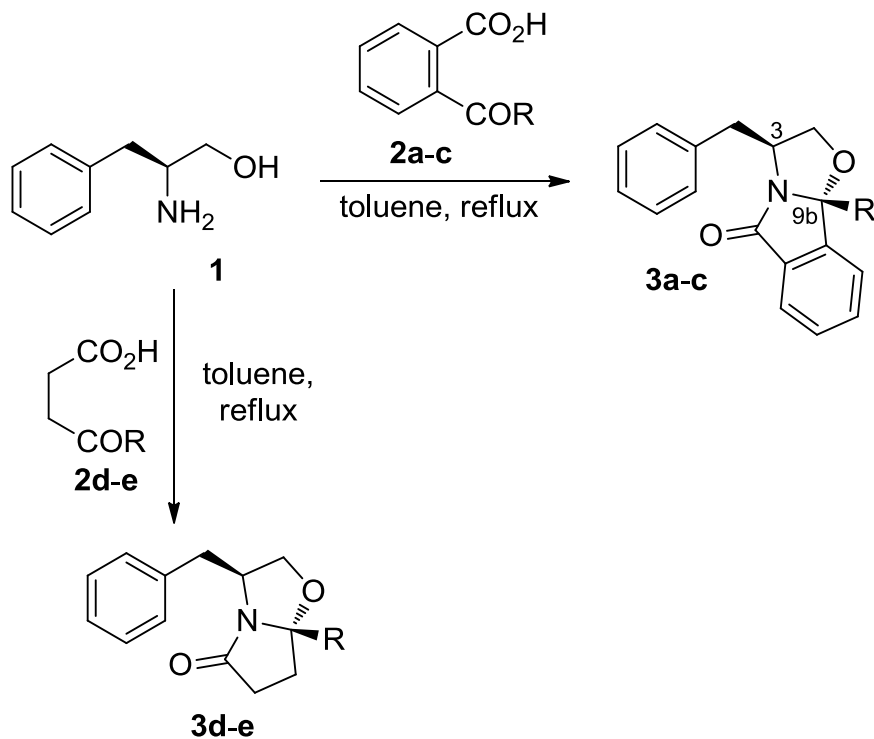
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## 5 **Results and Discussion**

6

### 7 *Synthesis*

8 Recently, our research group has been interested in the synthesis of  
9 phenylalaninol-derived oxazolopyrrolidone lactams to be evaluated as  
10 NMDA receptor antagonists. The first series of compounds was  
11 synthesized by cyclocondensation of (*S*)-phenylalaninol **1** with oxoacids  
12 **2a-e** (Scheme 1). In turn, tricyclic lactams **3a-c** were prepared from 2-  
13 acylbenzoic acid derivatives **2a-c** via reflux in toluene under Dean-Stark  
14 conditions (Table 1). Starting from oxoacids **2d-e** and using the same  
15 reaction conditions we obtained the bicyclic lactams **3d-e** in 72-73% yields  
16 (Table 1). In all cases, only one diastereoisomer product was observed.

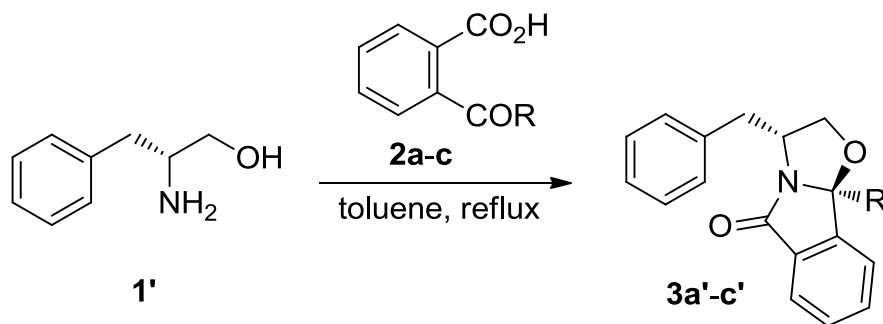


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2 **Scheme 1**

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4 To study the effect of the corresponding enantiomers as antagonists  
5 at the NMDA receptor, lactams **3a'-c'** were also synthesized starting from  
6 (*R*)-phenylalaninol with 62-85% yields (Scheme 2, Table 1).



7

8 **Scheme 2**

9

1 **Table 1** Reaction of phenylalaninol enantiomers **1** and **1'** with oxo acids **2**.

Aminoalcohol	R	Reaction time/h	Product	Yield (%)
( <i>S</i> )-phenylalaninol	H	16	<b>3a</b>	70
( <i>S</i> )-phenylalaninol	Me	16	<b>3b</b>	92
( <i>S</i> )-phenylalaninol	Ph	16	<b>3c</b>	85
( <i>S</i> )-phenylalaninol	Me	48	<b>3d</b>	73
( <i>S</i> )-phenylalaninol	Ph	48	<b>3e</b>	72
( <i>R</i> )-phenylalaninol	H	16	<b>3a'</b>	71
( <i>R</i> )-phenylalaninol	Me	16	<b>3b'</b>	85
( <i>R</i> )-phenylalaninol	Ph	16	<b>3c'</b>	74

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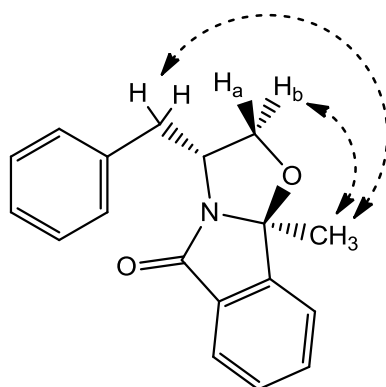
3 *NMR spectroscopy*

4 The most important features of the <sup>1</sup>H NMR spectra of these compounds  
5 are the resonances of the H-3, H-2, and CH<sub>2</sub>Ar protons. The H-3 signal  
6 appears as a multiplet around 4.33–4.48 ppm. The diastereotopic H-2  
7 protons appear as double of doublets around 4.08-4.33 ppm and 3.58-4.07  
8 ppm. The methylene CH<sub>2</sub>Ar protons appear as double of doublets around  
9 2.94-3.19 ppm and 2.30-2.99 ppm.

10 Furthermore, in the <sup>13</sup>C NMR spectra of compounds **3a-c** the newly formed  
11 C-9b chiral center appears around 100ppm. This signal moves downfield as

1 the electronic demand of the substituent is increased:  $\delta = 90.78$  (H); 98.93  
2 (CH<sub>3</sub>); 101.04 (Ph) ppm.

3 Compound 3b' underwent NOESY experiments and it was possible to  
4 observe the correlations depicted in figure 1. As expected and accordingly  
5 with published results with very similar compounds [9] synthesized via  
6 cyclocondensation with an enantiopure aminoalcohols, the stereo-outcome  
7 doesn't seem to be affected by the size of the keto-acid R substituent.



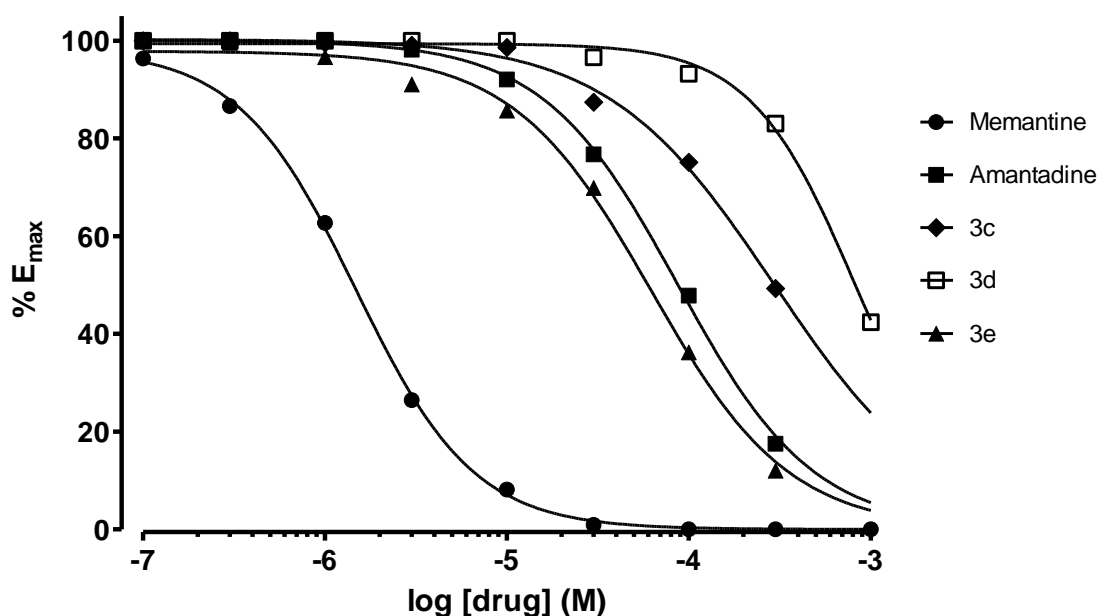
13 Figure 1.- NOESY correlations observed for **3b'**.

#### 15 *NMDA receptor antagonist activity*

16 The NMDA receptor activity of the compounds was evaluated by  
17 measuring their ability to inhibit the intracellular calcium increase induced  
18 by NMDA in cultured cerebellar granule neurons. Addition of glutamate or  
19 NMDA (100  $\mu$ M) in the presence of glycine (10  $\mu$ M) produced a robust  
20 and stable increase in intracellular calcium that was challenged with  
21 cumulative additions of the compounds to be tested.

1 In our assays, memantine (used as a positive control) yielded an  $IC_{50}$  value  
2 in the low micromolar range (1.48  $\mu M$ ). As it is shown on figure 2, only  
3 three out of the eight synthesized compounds showed an inhibitory activity  
4 higher than the 50% of the maximal effect. Specifically, **3c** and **3d** showed  
5 an  $IC_{50}$  in the high micromolar range ( $> 250 \mu M$ ), while **3e** showed a  
6 higher potency as a NMDA antagonist, giving an  $IC_{50}$  of 62.0  $\mu M$ . Related  
7 to compound **3c**, which showed an  $IC_{50}$  of 309.7  $\mu M$ , the enantiomer **3c'**  
8 was inactive, so it seems that the stereochemistry at the 3 position is  
9 important for activity.

10 More importantly, the phenyl derivative **3e** is more potent as NMDA  
11 receptor antagonism than amantadine (92.0  $\mu M$ ).



12

13 Figure 2.- Inhibitory effect of the synthesized compounds and the  
14 adamantanes memantine and amantadine on NMDA-induced intracellular



1 calcium increase in cultured cerebellar granule neurons. The compounds  
2 were tested from 0.1  $\mu\text{M}$  up to the highest possible concentration. Data are  
3 the mean of three different experiments, carried out on three different  
4 batches of cultured cells.

5  
6 In summary, we have synthesized and fully characterized several  
7 phenylalalinol-derived oxazolopyrrolidone lactams. In addition we describe  
8 here the potential use of lactam **3e** as a hit compound to develop NMDA  
9 receptor antagonists. The data now obtained provides a basis for exploring  
10 if related derivatives have enhanced activity. The synthesis and biological  
11 evaluation of more **3e** related compounds are in progress.

12

## 13 **Experimental**

14

### 15 **Chemistry**

16 All reagents and solvents were obtained from commercial suppliers and  
17 were used without further purification. Melting points were determined  
18 using a Kofler camera Bock monoscope M. The infrared spectra were  
19 collected on a Shimadzu IRAffinity-1 FTIR infrared spectrophotometer.  
20 Low resolution mass spectra (MS) were performed in LCLEM, Faculdade  
21 de Farmácia, Universidade de Lisboa. Merck Silica Gel 60 F<sub>254</sub> plates were

1 used as analytical TLC; flash column chromatography was performed on  
2 Merck Silica Gel (200-400 mesh).  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded  
3 on a Bruker 400MHz Ultra-Shield. Proton nuclear magnetic resonance  
4 spectra were recorded at 400 MHz. Carbon nuclear magnetic resonance  
5 spectra were recorded at 100 MHz.  $^1\text{H}$  and  $^{13}\text{C}$  NMR chemical shifts are  
6 expressed in  $\delta$  (ppm) referenced to the solvent used and the proton coupling  
7 constants  $J$  in hertz (Hz). Spectras were assigned using appropriate COSY,  
8 DEPT and HMQC sequences.

9  
10 **General procedure for the cyclocondensation reaction of (*S*)-2-**  
11 **amino-3-phenylpropan-1-ol 1 with keto-acids 2a-e:**

12 To a stirred solution of (*S*)-2-amino-3-phenylpropan-1-ol in boiling  
13 toluene under inert atmosphere and a Dean-Stark apparatus, was added 1,1  
14 eq. of the desired oxo-acid. The mixture was refluxed until total  
15 consumption of the starting aminoalcohol. The solvent was evaporated and  
16 the crude residue was purified by column chromatography using ethyl  
17 acetate/*n*-hexane as eluent. The solid products were recrystallized in diethyl  
18 ether/*n*-hexane.

19 (*3S,9bR*)-3-benzyl-2,3-dihydrooxazolo[2,3-*a*]isoindol-5(9*bH*)-one  
20 (**3a**)

1 Starting from 90 mg of (*S*)-2-amino-3-phenylpropan-1-ol in 10 mL  
2 of toluene. The obtained residue was purified by column chromatography  
3 (AcOEt: *n*-hexane 3:7). Recrystallization from diethyl ether/*n*-hexane  
4 afforded 110 mg (70%) **3a**. <sup>1</sup>H NMR spectra was found to be identical with  
5 the one described in Ref. [11].

6 (*3S,9bR*)-3-benzyl-9b-methyl-2,3-dihydrooxazolo[2,3-*a*]isoindol-  
7 5(*9bH*)-one (**3b**, C<sub>18</sub>H<sub>17</sub>NO<sub>2</sub>)

8 Starting from 330 mg of (*S*)-2-amino-3-phenylpropan-1-ol in 30 mL  
9 of toluene. The obtained residue was purified by column chromatography  
10 (AcOEt:*n*-hexane 3:7) affording 560 mg (92%) of a colorless oil. **3b**. <sup>1</sup>H  
11 NMR (400 MHz, CDCl<sub>3</sub>): δ = 7.69 (d, *J* = 7.4 Hz, 1H, H-Ar), 7.54 (m, 1H,  
12 H-Ar); 7.45 (m, 2H, H-Ar); 7.27 (m, 4H, H-Ar); 7.20 (m, 1H, H-Ar); 4.39  
13 (m, 1H, H-3); 4.21 (dd, *J* = 8.9, 7.4 Hz, 1H, H-2), 4.07 (dd, *J* = 8.9, 6.5 Hz,  
14 1H, H-2), 3.21 (dd, *J* = 13.8, 5.8 Hz, 1H, CH<sub>2</sub>-Ph), 2.95 (dd, *J* = 13.8, 8.6  
15 Hz, 1H, CH<sub>2</sub>-Ph), 1.69 (s, 3H, CH<sub>3</sub>) ppm; <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ  
16 = 174.20 (C=O), 147.26 (Cq), 137.27 (Cq), 133.22 (CH-Ar), 131.60 (Cq),  
17 130.16 (CH-Ar), 129.45 (2CH-Ar), 128.59 (2CH-Ar), 126.80 (CH-Ar),  
18 124.33 (CH-Ar), 122.10 (CH-Ar), 98.93 (C-9b), 74.06 (CH<sub>2</sub>), 56.77 (CH),  
19 40.89 (CH<sub>2</sub>-Ph), 23.02 (CH<sub>3</sub>) ppm; IR (NaCl):  $\bar{\nu}$  = 1715 (C=O) cm<sup>-1</sup>; MS  
20 (ESI, CP 3.0 kV, SP 30V): *m/z calc.* = 279 [M]<sup>+</sup>, *m/z found* 280 [M+H]<sup>+</sup>; R<sub>f</sub>  
21 (ethyl acetate: *n*-hexane 1:1) = 0.769.

1           (3*S*,9*bR*)-3-benzyl-9*b*-phenyl-2,3-dihydrooxazolo[2,3-*a*]isoindol-  
2 5(9*bH*)-one (**3c**, C<sub>23</sub>H<sub>19</sub>NO<sub>2</sub>)

3           Starting from 100 mg of (*S*)-2-amino-3-phenylpropan-1-ol in 7 mL  
4 of toluene. The obtained residue was purified by column chromatography  
5 (AcOEt:*n*-hexane 1:9). Recrystallization from diethyl ether/*n*-hexane  
6 afforded 193 mg (86%) **3c**. M.p.: 92-94 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ  
7 = 7.80 – 7.75 (m, 1H, H-Ar), 7.68 – 7.62 (m, 2H, H-Ar), 7.50 – 7.37 (m,  
8 5H, H-Ar), 7.31 – 7.20 (m, 4H, H-Ar), 7.17 – 7.14 (m, 2H, H-Ar), 4.66 –  
9 4.52 (m, 1H, H-3), 4.44 (dd, *J* = 8.6, 7.5 Hz, 1H, H-2), 3.96 (dd, *J* = 8.7,  
10 6.6 Hz, 1H, H-2), 3.02 (dd, *J* = 13.8, 6.8 Hz, 1H, CH<sub>2</sub>-Ph), 2.51 (dd, *J* =  
11 13.8, 8.7 Hz, 1H, CH<sub>2</sub>-Ph). ppm; <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>): δ = 174.52  
12 (C=O), 147.27 (Cq), 138.95 (Cq), 137.61 (Cq), 133.41 (CH-Ar), 131.10  
13 (Cq), 130.23 (CH-Ar), 129.11 (2CH-Ar), 128.94 (2CH-Ar), 128.86 (CH-  
14 Ar), 128.64 (2CH-Ar), 126.77 (CH-Ar), 125.96 (2CH-Ar), 124.52 (CH-  
15 Ar), 123.56 (CH-Ar), 101.04 (C-9*b*), 75.91 (CH<sub>2</sub>), 56.80 (CH), 40.54 (CH<sub>2</sub>-  
16 Ph) ppm; IR (KBr):  $\bar{\nu}$  = 1721 (C=O) cm<sup>-1</sup>; MS (ESI, CP 3.0 kV, SP  
17 30V): *m/z* calc. = 341 [M]<sup>+</sup>, *m/z* found = 342 [M+H]<sup>+</sup>; R<sub>f</sub> (ethyl acetate: *n*-  
18 hexane 3:7) = 0.607.

19           (3*S*,7*aR*)-3-benzyl-7*a*-methyltetrahydropyrrolo[2,1-*b*]oxazol-5(6*H*)-  
20 one (**3d**)

1 Starting from 100 mg of (*S*)-2-amino-3-phenylpropan-1-ol in 10 mL  
2 of toluene. The obtained residue was purified by column chromatography  
3 (AcOEt:*n*-hexane 1:1) affording 111 mg (73%) of a colorless oil. **3d**. <sup>1</sup>H  
4 NMR spectra was found to be identical with the one described in Ref. [12]  
5 (*3S,7aS*)-3-benzyl-7a-phenyltetrahydropyrrolo[2,1-*b*]oxazol-5(6*H*)-  
6 *one* (**3e**, C<sub>19</sub>H<sub>19</sub>NO<sub>2</sub>)

7 Starting from 100 mg of (*S*)-2-amino-3-phenylpropan-1-ol in 10 mL  
8 of toluene. The obtained residue was purified by column chromatography  
9 (AcOEt:*n*-hexane 3:7). Recrystallization from diethyl ether/*n*-hexane  
10 afforded 140 mg (72%) **3e**. M.p.: 55-56 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ  
11 7.51 (d, *J* = 7.4 Hz, 2H, H-Ar), 7.44 – 7.38 (m, 3H, H-Ar), 7.28 – 7.21 (m,  
12 3H, H-Ar), 7.08 (d, *J* = 7.3 Hz, 2H, H-Ar), 4.50 – 4.35 (m, 1H, H-3), 4.13  
13 (t, *J* = 8.1 Hz, 1H, H-2), 3.65 – 3.49 (m, 1H, H-2), 2.94 (dd, *J* = 13.7, 6.2  
14 Hz, 1H, CH<sub>2</sub>-Ph), 2.89 – 2.77 (m, 1H, H-6), 2.63 – 2.45 (m, 2H, H-6 & H-  
15 7), 2.35 – 2.18 (m, 2H, CH<sub>2</sub>-Ph & H-7) ppm; <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  
16 δ 179.87 (C=O), 142.55 (Cq), 137.26 (Cq), 128.90 (2CH-Ar), 128.70  
17 (2CH-Ar), 128.49 (2CH-Ar), 128.31 (CH-Ar), 126.60 (CH-Ar), 125.07  
18 (2CH-Ar), 102.27 (C-7a), 72.26 (CH<sub>2</sub>), 56.44 (CH), 39.92 (CH<sub>2</sub>-Ph), 35.05  
19 (C-7), 32.57 (C-6) ppm; IR (KBr):  $\bar{\nu}$  = 1721 (C=O) cm<sup>-1</sup>; MS (ESI, CP 3.0  
20 kV, SP 30V): *m/z* calc. = 293 [M]<sup>+</sup>, *m/z* found = 294 [M+H]<sup>+</sup>; R<sub>f</sub> (ethyl  
21 acetate: *n*-hexane 3:7) = 0.313.

1

2           **General procedure for the cyclocondensation reaction of (*R*)-2-**  
3 **amino-3-phenylpropan-1-ol 1' with keto-acids 2a-c:**

4           To a stirred solution of (*R*)-2-amino-3-phenylpropan-1-ol in boiling  
5 toluene under inert atmosphere and a Dean-Stark apparatus, was added 1,1  
6 eq. of the desired oxo-acid. The mixture was refluxed until total  
7 consumption of the starting aminoalcohol. The solvent was evaporated and  
8 the crude residue was purified by column chromatography using ethyl  
9 acetate/*n*-hexane as eluent.

10           (*3R,9bS*)-3-benzyl-2,3-dihydrooxazolo[2,3-*a*]isoindol-5(*9bH*)-one

11 (**3a'**)

12           Starting from 100 mg of (*R*)-2-amino-3-phenylpropan-1-ol in 15 mL  
13 of toluene. The obtained residue was purified by column chromatography  
14 (AcOEt:*n*-hexane 2:8). Recrystallization from diethyl ether/*n*-hexane  
15 afforded 125 mg (71%) **3a'**. <sup>1</sup>H NMR spectra was found to be identical  
16 with the one described in Ref. [11].

17           (*3R,9bS*)-3-benzyl-9*b*-methyl-2,3-dihydrooxazolo[2,3-*a*]isoindol-

18 5(*9bH*)-one (**3b'**, C<sub>18</sub>H<sub>17</sub>NO<sub>2</sub>)

19           Starting from 100 mg of (*R*)-2-amino-3-phenylpropan-1-ol in 15 mL  
20 of toluene. The obtained residue was purified by column chromatography  
21 (AcOEt:*n*-hexane 2:8) affording 157 mg (85%) of a colorless oil. <sup>1</sup>H NMR,

1  $^{13}\text{C}$  NMR and IR spectra were found to be identical with the ones described  
2 for compound **3b**.

3 *(3R,9bS)-3-benzyl-9b-phenyl-2,3-dihydrooxazolo[2,3-a]isoindol-*  
4 *5(9bH)-one (3c', C<sub>23</sub>H<sub>19</sub>NO<sub>2</sub>)*

5 Starting from 100 mg of (*R*)-2-amino-3-phenylpropan-1-ol in 15 mL  
6 of toluene. The obtained residue was purified by column chromatography  
7 (AcOEt:*n*-hexane 2:8). Recrystallization from diethyl ether/*n*-hexane  
8 afforded 167 mg (74%) of **3c'**.  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR and IR spectra were  
9 found to be identical with the ones described for compound **3c**.

10

#### 11 **NMDA receptor antagonist activity**

12 The activity of the synthesized compounds as NMDA receptor antagonists  
13 was evaluated using primary cultures of rat cerebellar neurons, as described  
14 previously [13]. Briefly, cultures were prepared from 7-8 day-old Wistar  
15 rats (Charles River, France). Cerebella were dissected, minced and  
16 trypsinized, and after several sedimentations, cells were plated on poly-  
17 lysinized coverslips placed in 24-well plates at a density of  $1 \cdot 10^6$  cells/mL.  
18 Plates were kept at 37°C in a cell incubator (Heraeus, Germany). After 16-  
19 18h, 10  $\mu\text{M}$  cytosine arabinoside (Sigma-Aldrich, USA) was added to  
20 avoid excessive proliferation of astrocytes. Cultures prepared in this

1 manner are ready to be used in the NMDA receptor activity assays from the  
2 7th to the 11th day in vitro.

3 Activity at the NMDA receptor was assessed using the calcium-  
4 sensitive probe Fura-2 (Invitrogen, USA). After incubation with 6  $\mu\text{M}$   
5 Fura-2 acetoxymethyl ester (Fura-2 AM) for 30-45 min at 37°C, a coverslip  
6 was transferred to a plastic holder that was inserted in a quartz cuvette for  
7 fluorescence measurements. Recordings of Fura-2 fluorescence were  
8 performed using a PerkinElmer LS50B luminiscence spectrometer, both at  
9 340 and 380 nm excitation wavelengths, and at 510 nm of emission. The  
10 ratio of  $F_{340}/F_{380}$  (R) is proportional to intracellular calcium. All the  
11 measurements were made at 37°C and under mild stirring. Once the  
12 recording was started, glycine (10  $\mu\text{M}$ ) and NMDA (100  $\mu\text{M}$ ) were added  
13 to the cuvette, at 50 and 100 s respectively. This produced a sustained  
14 increase in  $F_{340}/F_{380}$ , indicating that the NMDA receptors were activated  
15 and that the intracellular calcium concentration was high. This intracellular  
16 calcium increase was challenged with cumulative concentrations of the  
17 compounds under investigation, (from  $1 \cdot 10^{-7}$  M up to up to  $3 \cdot 10^{-4}$  M). If  
18 the compounds would act as antagonists at the NMDA receptor this would  
19 be detected as a decrease in the value  $F_{340}/F_{380}$ . Experiments were  
20 performed in triplicate. Memantine was used as a positive control.



1           When a minimum of 50% of inhibition was reached, the IC<sub>50</sub> value  
2 was calculated using non-linear regression with GraphPad Prism 5.0.

3

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11

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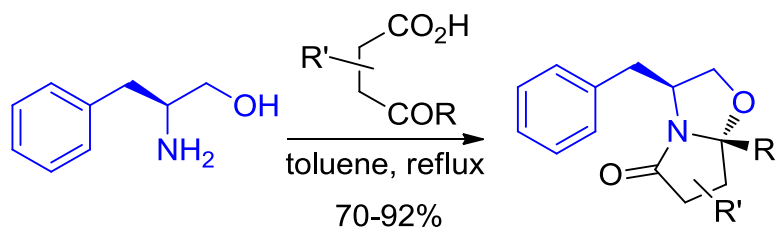
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2 Graphics for use in the Table of Contents



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