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

Nuno A. L. Pereira ● Francesc X. Sureda ● Mireia Turch ● Mercedes Amat ● Joan Bosch ● Maria M. M. Santos

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

Abstract N-methyl-D-aspartate (NMDA) receptor antagonists are known to rescue neuronal cell death caused by excessive activation of glutamate receptors. This phenomenon, known as excitotoxicity, is implicated in the pathogenesis of several neurodegenerative disorders including ischemia, Alzheimer's disease, Parkinson's disease, and Huntington's disease. Unfortunately, some antagonists of NMDA receptor have been tested in clinical trials with discouraging results. However, recent advances in the physiology and pharmacology of the NMDA receptor have kept the interest alive to modulate NMDA receptors for therapeutic intervention.

We present here the synthesis of a small library of phenylalalinol-derived oxazolopyrrolidone lactams and their evaluation as NMDA receptor antagonists. The compounds were easily synthesized in yields up to 92%. In addition, one of the compounds has an IC_{50} of 62 μM and offers potential to develop more potent NMDA receptor antagonists.

Keywords Amino alcohols ● Chiral auxiliaries ● NMDA receptor antagonists ● pyrrolidones ● lactams

M. M. M. Santos (✉)
Research Institute for Medicines and Pharmaceutical Sciences (iMed.UL), Faculty of Pharmacy, University of Lisbon. Av. Prof. Gama Pinto, 1649-003 Lisbon, Portugal
e-mail: mariasantos@ff.ul.pt
Telephone: +351 217946451
Fax: +351 217946470

N. A. L. Pereira ● M. M. M. Santos (✉)
Research Institute for Medicines and Pharmaceutical Sciences (iMed.UL), Faculty of Pharmacy, University of Lisbon. Av. Prof. Gama Pinto, 1649-003 Lisbon, Portugal

F. X. Sureda ● M. Turch
Pharmacology Unit, Faculty of Medecine and Health Sciences, Universitat Rovira i Virgili, c./St. Llorenç 21, 43201 Reus (Tarragona), Spain

M. Amat ● J. Bosch
Laboratory of Organic Chemistry, Faculty of Pharmacy and Institute of Biomedicine (IBUB), University of Barcelona, Av. Joan XXIII, s/n, 08028 Barcelona, Spain

Introduction

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

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

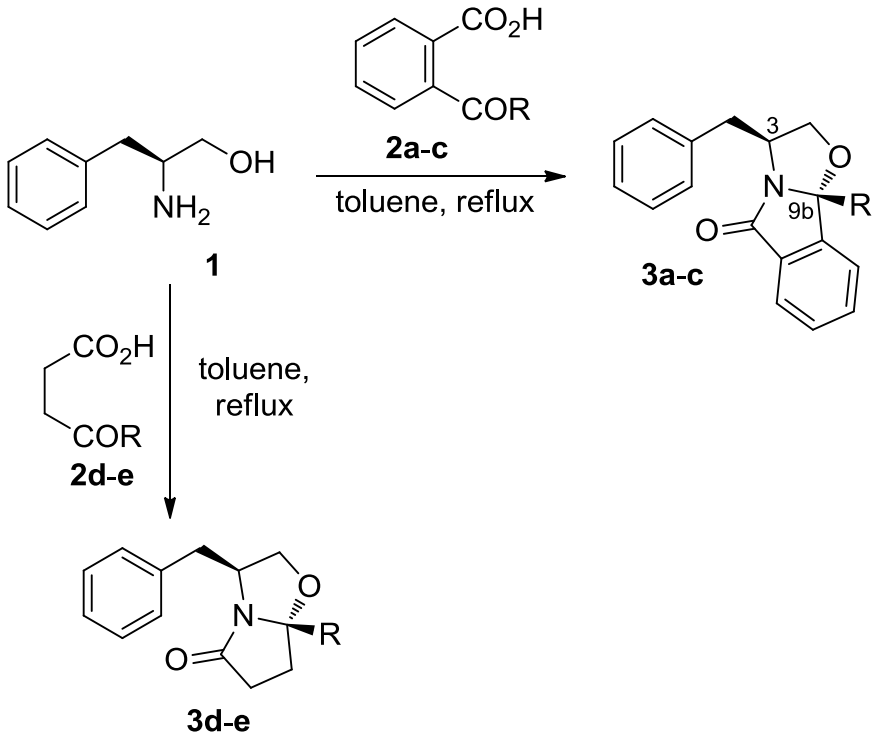
In 2009, some oral active oxazolidine derivatives were described to act as NMDA antagonists by preventing the binding of the NMDAR ligands [8]. Based on this information and due to our interest in the synthesis of oxazolo lactams [9-10] we decided to extend our research to the synthesis of enantiopure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

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

Results and Discussion

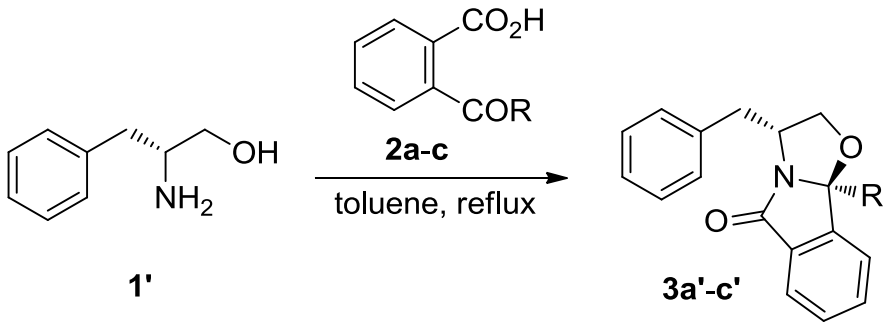
Synthesis

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



Scheme 1

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



Scheme 2

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

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

NMR spectroscopy

The most important features of the ^{1}H NMR spectra of these compounds are the resonances of the H-3, H-2, and CH_2Ar protons. The H-3 signal appears as a multiplet around 4.33–4.48 ppm. The diastereotopic H-2 protons appear as double of doublets around 4.08-4.33 ppm and 3.58-4.07 ppm. The methylene CH_2Ar protons appear as double of doublets around 2.94-3.19 ppm and 2.30-2.99 ppm.

Furthermore, in the ^{13}C NMR spectra of compounds **3a-c** the newly formed C-9b chiral center appears around 100ppm. This signal moves downfield as

the electronic demand of the substituent is increased: δ = 90.78 (H); 98.93 (CH_3); 101.04 (Ph) ppm.

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

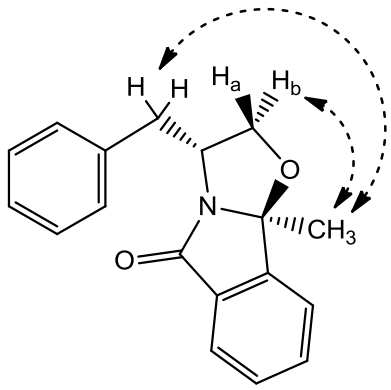


Figure 1.- NOESY correlations observed for **3b'**.

NMDA receptor antagonist activity

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

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

More importantly, the phenyl derivative **3e** is more potent as NMDA receptor antagonism than amantadine (92.0 µM).

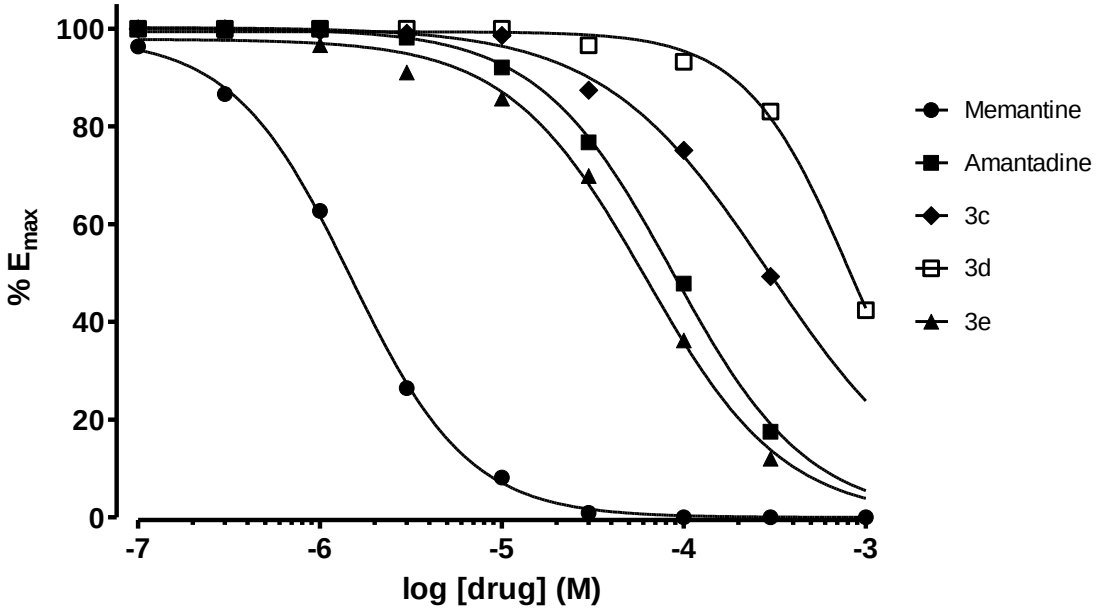


Figure 2.- Inhibitory effect of the synthesized compounds and the adamantanes memantine and amantadine on NMDA-induced intracellular

calcium increase in cultured cerebellar granule neurons. The compounds were tested from 0.1 μM up to the highest possible concentration. Data are the mean of three different experiments, carried out on three different batches of cultured cells.

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

Experimental

Chemistry

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

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

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

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

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

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

(3S,9bR)-3-benzyl-9b-methyl-2,3-dihydrooxazolo[2,3-a]isoindol-5(9bH)-one (**3b**, $C_{18}H_{17}NO_2$)

Starting from 330 mg of (*S*)-2-amino-3-phenylpropan-1-ol in 30 mL of toluene. The obtained residue was purified by column chromatography (AcOEt:*n*-hexane 3:7) affording 560 mg (92%) of a colorless oil. **3b**. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.69 (d, J= 7.4 Hz, 1H, H-Ar), 7.54 (m, 1H, H-Ar); 7.45 (m, 2H, H-Ar); 7.27 (m, 4H, H-Ar); 7.20 (m, 1H, H-Ar); 4.39 (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, 1H, H-2), 3.21 (dd, *J* = 13.8, 5.8 Hz, 1H, CH_2-Ph), 2.95 (dd, *J* = 13.8, 8.6 Hz, 1H, CH_2-Ph), 1.69 (s, 3H, CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 174.20 (C=O), 147.26 (Cq), 137.27 (Cq), 133.22 (CH-Ar), 131.60 (Cq), 130.16 (CH-Ar), 129.45 (2CH-Ar), 128.59 (2CH-Ar), 126.80 (CH-Ar), 124.33 (CH-Ar), 122.10 (CH-Ar), 98.93 (C-9b), 74.06 (CH_2), 56.77 (CH), 40.89 (CH_2-Ph), 23.02 (CH_3) ppm; IR (NaCl): $\bar{\nu}$ = 1715 (C=O) cm^{-1}; MS (ESI, CP 3.0 kV, SP 30V): *m/z calc.* = 279 $[M]^+$, *m/z found* 280 $[M+H]^+$; R_f (ethyl acetate: *n*-hexane 1:1) = 0.769.

(3S,9bR)-3-benzyl-9b-phenyl-2,3-dihydrooxazolo[2,3-a]isoindol-5(9bH)-one (**3c**, $C_{23}H_{19}NO_2$)

Starting from 100 mg of (*S*)-2-amino-3-phenylpropan-1-ol in 7 mL of toluene. The obtained residue was purified by column chromatography (AcOEt:*n*-hexane 1:9). Recrystallization from diethyl ether/*n*-hexane afforded 193 mg (86%) **3c**. M.p.: 92-94 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.80 – 7.75 (m, 1H, H-Ar), 7.68 – 7.62 (m, 2H, H-Ar), 7.50 – 7.37 (m, 5H, H-Ar), 7.31 – 7.20 (m, 4H, H-Ar), 7.17 – 7.14 (m, 2H, H-Ar), 4.66 – 4.52 (m, 1H, H-3), 4.44 (dd, *J* = 8.6, 7.5 Hz, 1H, H-2), 3.96 (dd, *J* = 8.7, 6.6 Hz, 1H, H-2), 3.02 (dd, *J* = 13.8, 6.8 Hz, 1H, CH2-Ph), 2.51 (dd, *J* = 13.8, 8.7 Hz, 1H, CH2-Ph). ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ = 174.52 (C=O), 147.27 (Cq), 138.95 (Cq), 137.61 (Cq), 133.41 (CH-Ar), 131.10 (Cq), 130.23 (CH-Ar), 129.11 (2CH-Ar), 128.94 (2CH-Ar), 128.86 (CH-Ar), 128.64 (2CH-Ar), 126.77 (CH-Ar), 125.96 (2CH-Ar), 124.52 (CH-Ar), 123.56 (CH-Ar), 101.04 (C-9b), 75.91 (CH_2), 56.80 (CH), 40.54 (CH_2-Ph) ppm; IR (KBr): $\overline{\nu}$ = 1721 (C=O) cm^{-1} ; MS (ESI, CP 3.0 kV, SP 30V): *m/z calc.* = 341 $[M]^+$, *m/z found* = 342 $[M+H]^+$; R_f (ethyl acetate: *n*-hexane 3:7) = 0.607.

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

Starting from 100 mg of (*S*)-2-amino-3-phenylpropan-1-ol in 10 mL of toluene. The obtained residue was purified by column chromatography (AcOEt:*n*-hexane 1:1) affording 111 mg (73%) of a colorless oil. **3d**. ^{1}H NMR spectra was found to be identical with the one described in Ref. [12]

(3S,7aS)-3-benzyl-7a-phenyltetrahydropyrrolo[2,1-b]oxazol-5(6H)-one (**3e**, $C_{19}H_{19}NO_2$)

Starting from 100 mg of (*S*)-2-amino-3-phenylpropan-1-ol in 10 mL of toluene. The obtained residue was purified by column chromatography (AcOEt:*n*-hexane 3:7). Recrystallization from diethyl ether/*n*-hexane afforded 140 mg (72%) **3e**. M.p.: 55-56 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.51 (d, J = 7.4 Hz, 2H, H-Ar), 7.44 – 7.38 (m, 3H, H-Ar), 7.28 – 7.21 (m, 3H, H-Ar), 7.08 (d, J = 7.3 Hz, 2H, H-Ar), 4.50 – 4.35 (m, 1H, H-3), 4.13 (t, J = 8.1 Hz, 1H, H-2), 3.65 – 3.49 (m, 1H, H-2), 2.94 (dd, J = 13.7, 6.2 Hz, 1H, CH_2-Ph), 2.89 – 2.77 (m, 1H, H-6), 2.63 – 2.45 (m, 2H, H-6 & H-7), 2.35 – 2.18 (m, 2H, CH_2-Ph & H-7) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 179.87 (C=O), 142.55 (Cq), 137.26 (Cq), 128.90 (2CH-Ar), 128.70 (2CH-Ar), 128.49 (2CH-Ar), 128.31 (CH-Ar), 126.60 (CH-Ar), 125.07 (2CH-Ar), 102.27 (C-7a), 72.26 (CH_2), 56.44 (CH), 39.92 (CH_2-Ph), 35.05 (C-7), 32.57 (C-6) ppm; IR (KBr): $\bar{\nu}$ = 1721 (C=O) cm^{-1}; MS (ESI, CP 3.0 kV, SP 30V): *m/z calc.* = 293 $[M]^+$, *m/z found* = 294 $[M+H]^+$; R_f (ethyl acetate: *n*-hexane 3:7) = 0.313.

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

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

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

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

(3R,9bS)-3-benzyl-9b-methyl-2,3-dihydrooxazolo[2,3-a]isoindol-5(9bH)-one (**3b'**, $C_{18}H_{17}NO_2$)

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

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

(3R,9bS)-3-benzyl-9b-phenyl-2,3-dihydrooxazolo[2,3-a]isoindol-5(9bH)-one (**3c'**, $C_{23}H_{19}NO_2$)

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

NMDA receptor antagonist activity

The activity of the synthesized compounds as NMDA receptor antagonists was evaluated using primary cultures of rat cerebellar neurons, as described previously [13]. Briefly, cultures were prepared from 7-8 day-old Wistar rats (Charles River, France). Cerebella were dissected, minced and trypsinized, and after several sedimentations, cells were plated on poly-lysinized coverslips placed in 24-well plates at a density of 1•106 cells/mL. Plates were kept at 37°C in a cell incubator (Heraeus, Germany). After 16-18h, 10 µM cytosine arabinoside (Sigma-Aldrich, USA) was added to avoid excessive proliferation of astrocytes. Cultures prepared in this

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

Activity at the NMDA receptor was assessed using the calcium-sensitive probe Fura-2 (Invitrogen, USA). After incubation with 6 µM Fura-2 acetoxymethyl ester (Fura-2 AM) for 30-45 min at 37°C, a coverslip was transferred to a plastic holder that was inserted in a quartz cuvette for fluorescence measurements. Recordings of Fura-2 fluorescence were performed using a PerkinElmer LS50B luminiscence spectrometer, both at 340 and 380 nm excitation wavelengths, and at 510 nm of emission. The ratio of F340/F380 (R) is proportional to intracellular calcium. All the measurements were made at 37°C and under mild stirring. Once the recording was started, glycine (10 µM) and NMDA (100 µM) were added to the cuvette, at 50 and 100 s respectively. This produced a sustained increase in F_{340}/F_{380}, indicating that the NMDA receptors were activated and that the intracellular calcium concentration was high. This intracellular calcium increase was challenged with cumulative concentrations of the compounds under investigation, (from $1 \cdot 10^{-7}$ M up to up to $3 \cdot 10^{-4}$ M). If the compounds would act as antagonists at the NMDA receptor this would be detected as a decrease in the value F_{340}/F_{380}. Experiments were performed in triplicate. Memantine was used as a positive control.

When a minimum of 50% of inhibition was reached, the IC_{50} value was calculated using non-linear regression with GraphPad Prism 5.0.

Acknowledgements

Fundação para a Ciência e Tecnologia (Portugal) is acknowledged for financial support (PTDC/QUI-QUI/111664/2009; PEst-OE/SAU/UI4013/2011; REDE/1518/REM/2005). We also thank the Portuguese-Spanish- Integrated Action (E-07/11 and AIB2010PT-00324) and the Spanish and Ministerio de Ciencia e Innovación (MICINN) (CTQ2009-07021/BQU) for financial support.

References

1. Wollmuth LP, Talukdu I (2011) Local constraints in either the GluN1 or GluN2 subunit equally impair NMDA receptor pore opening. J Gen Physiol 138(2): 179-194.
2. Mayer ML (2006) Glutamate recetors at atomic resolution. Nature 440: 456–462.
3. Kaczor AA, Matosiuk D (2010) Molecular structure of ionotropic glutamate receptors. Curr Med Chem 17(24): 2608-2635.
4. Paoletti P (2011) Molecular basis of NMDA receptor functional diversity. Eur J Neurosci 33(8): 1351-1365.

5. Danysz W, Parsons CG (2012) Alzheimer's disease, beta-amyloid, glutamate, NMDA receptors and memantine – searching for the connections. Br J Pharmacol 167(2): 324-352.

6. Lau A, Tymianski M (2010) Glutamate receptors, neurotoxicity and neurodegeneration. Pflugers Arch 460:525-542.

7. Parsons CG, Stoffler A, Danysz W (2007) Memantine: a NMDA receptor antagonist that improves memory by restoration of homeostasis in the glutarnatergic system - too little activation is bad, too much is even worse. Neuropharmacology 53: 699-723.

8. Ip NY, Zhu H-J, Ip FC-F (2009) Oxazolidine as NMDA receptor antagonists. WO2009092324, July 30, 2009.

9. Santos MMM (2011) Tryptophanol-Derived Oxazolopiperidone Lactams: Valuable Building Blocks for the Enantioselective Synthesis of Piperidine-Containing Alkaloids. In: Heterocyclic Targets in Advanced Organic Synthesis, Research Signpost, India, 69-82.

10. Amat M, Pérez M, Bosch J (2011) Enantioselective Synthesis of Indole Alkaloids from Chiral Lactams. Synlett 2: 143-160.

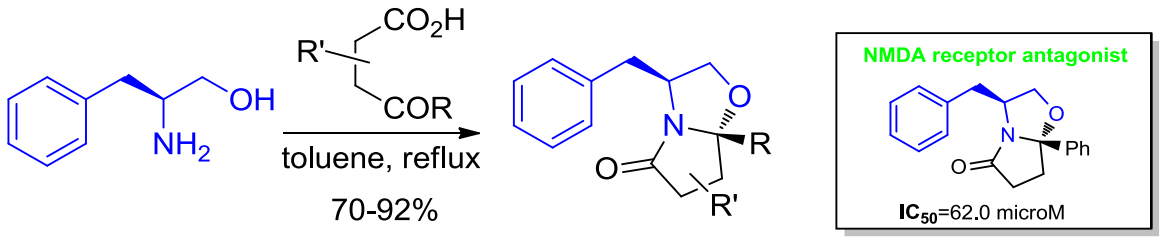
11. Sikoraiová J, Chihab-Eddine A, Marchalín S, Daïch A (2002) Diastereoselective Access to Chiral Non-Racemic [1,3]Oxazolo-[2,3-a]isoindol-5-one Ring Systems via O-Cationic Cyclization. J Heterocyclic Chem 39: 383-390.

12. Allin SM, James SL, Martin WP, Smith TAD, Elsegood, MRJ (2001) Stereoselective synthesis of the pyrroloisoquinoline ring system. J Chem Soc, Perkin Trans 1: 3029-3036.

13. Torres E, Duque MD, López-Querol M, Taylor MC, Naesens L, Ma C, Pinto LH, Sureda FX, Kelly JM, Vázquez S. (2012) Synthesis of benzopolycyclic cage amines: NMDA receptor antagonist, trypanocidal and antiviral activities. Bioorg Med Chem 20:942-948.

1

2 Graphics for use in the Table of Contents



3
4