Optimization of bicyclic lactam derivatives as NMDA receptor antagonists

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ABSTRACT

N-Methyl-D-aspartate (NMDA) receptors are fundamental for the normal function of the central nerve system, and play an important role in memory and learning. An overactivation of these receptors will lead to neuronal loss associated with major neurological disorders, such as Parkinson's disease, Alzheimer's disease, schizophrenia, and epilepsy. Herein, twenty two novel enantiopure bicyclic lactams were designed, synthesized, and evaluated as NMDA receptor antagonists. Most of the new compounds displayed NMDA receptor antagonism and, the most promising compound showed IC₅₀ value in the same order of magnitude of memantine, a NMDA receptor antagonist used in the clinic for the treatment of Alzheimer's disease. Further biological evaluation indicated that this compound is brain-permeable (determined by an *in vitro* assay) and non-hepatotoxic. All of these results indicate that the (3*S*,7*aS*)-7a-(4-Chlorophenyl)-3-(4-hydroxybenzyl)tetrahydropyrrolo[2,1-*b*]oxazol-5(6*H*)-one (**5b**) is a potential candidate for the treatment of pathologies associated with the overactivation of NMDA receptors.

Keywords: NMDA receptor; Antagonists; Bicyclic lactams; Nitrogen Heterocycles; Asymmetric synthesis.

Introduction

N-Methyl-D-aspartate (NMDA) receptors belong to the family of ionotropic glutamate receptors (iGluRs) and are localized in the cell membrane of neurons [1]. They are typically composed of two GluN1 and two GluN2 subunits, which are further subdivided into four types of subunits, labelled as GluN2A-GluN2D. Two GluN3 subunits (GluN3A and GluN3B) can also be incorporated into the NMDA receptor complex [2, 3]. To be activated, NMDA receptors require the binding of the co-agonists glycine and glutamate, combined with membrane depolarization, to relieve the blockade of Mg^{2+} and the opening of an ion conductive pore across the membrane bilayer.

NMDA receptors have a vital role in the normal development of the nerve system, being extremely important in sustaining healthy memory, learning, and cognition processes [4]. However, the overactivation of these ligand-gated ion channels leads to neuronal loss, which is associated with several neurodegenerative disorders, including Alzheimer's and Parkinson's diseases, and others pathologies, such as stroke, schizophrenia, and epilepsy [5]. For this reason, the development of effective NMDA receptor antagonists seems to be a promising therapeutic approach to fight these diseases where NMDA receptors are overactivated.

In the last 30 years, several potent NMDA receptor antagonists have been developed. Unfortunately, these compounds have presented serious side effects and so it has not been possible for these compounds to be used in the clinic [6, 7]. In contrast to high-affinity channel blockers, the FDA-approved amantadine and memantine (moderate NMDA channel blockers) are well tolerated in the clinic [8, 9].

The recent elucidation of the NMDA receptor structure [10, 11], together with the positive outcomes obtained with memantine in the treatment of Alzheimer's disease [12], has led to a sustained resurgent interest in the development of novel compounds

that can suppress NMDA receptors activity during glutamate-induced excitotoxicity episodes, while keeping its normal physiological role unharmed.

Figure 1. Chemical structure of bicyclic lactams **1a** and **2**.

In this research area, we have been studying aminoalcohol-derived bicyclic lactams as NMDA receptor antagonists. We have previously identified two new chemical scaffolds, the (*S*)-phenylalaninol oxazolopyrrolidone lactam **1a** [13] and the (*R*)-tryptophanol-derived oxazolopiperidone lactam **2** [14] (Figure 1), which are around 1.5-fold more active than amantadine. However, these compounds were still less potent than memantine. For these reasons, we decided to synthesize derivatives of **1a** with the goal of enhancing the NMDA receptor blockade.

To perform a SAR study, we synthesized and evaluated compounds containing different substituents at position C-3, different lactam ring sizes, and different substituents at position C-7a (Figure 2).

Figure 2. Synthetic strategy for the optimization of bicyclic lactam **1a**.

Scheme 1. Synthesis of bicyclic lactams **1a-h**, **1a'-h'** and **5a-b**.

Results and Discussion

Chemistry

In our previous study, the most active phenylalaninol oxazolopyrrolidone lactam detained a phenyl ring at position C-7a of the pyrrolidone [13]. The first derivatizations were focused on this phenyl ring, by introducing both electron-withdrawing and electron-donating groups. In addition, to validate the importance of having a pyrrolidone ring in lactam **1a**, we have synthesized derivatives **3** and **3'**, possessing a piperidone ring instead of pyrrolidone. Moreover, based on the most active compound that we obtained from the series of tryptophanol-derived lactams [14], compound **2**, we

decided to synthesize compounds **4** and **4'**, derived from the (*S*)-, and (*R*)-tryptophanol amino alcohols, respectively, that contain a phenyl ring at position 8a.

In order to evaluate the effect on biological activity of introducing a hydroxyl group at the aromatic ring (a substituent present at the aromatic ring of the NMDA receptor antagonist ifenprodil), we synthesized the equivalent (*S*)-tyrosinol derivatives of the hit compound **1a**, and of the most active compound **1c**. In particular, two oxazolopyrrolidone lactams were synthesized, one with a chlorine atom at *para* position (compound **5b**) and the other without any substituent at the phenyl group (compound **5a**).

To evaluate the importance of the stereochemistry in the biological activity, both series of enantiomers were synthesized for most of the compounds, starting from the corresponding (*S*)- or (*R*)-aminoalcohols.

The bicyclic lactams **1a-h**, **1a'-h'**, **3-4**, **3'-4'**, and **5a-b** were easily obtained by chiral-induced cyclocondensation reactions between the respective amino alcohol and commercially available ketoacids, from moderate to good yields, and excellent stereoselectivities (Schemes 1 and 2).

Scheme 2. Synthesis of bicyclic lactams **3-4** and **3'-4'**.

Compound **1i** (Scheme 3) was synthesized by Suzuki coupling reaction of compound **1d** with 4-chlorophenylboronic acid.

Scheme 3. Synthesis of bicyclic lactam **1i**.

Only one diastereoisomer was formed in the cyclocondensation reactions (confirmed by $^1\text{H-NMR}$). The absolute configuration of the stereogenic center formed during the cyclocondensation reaction was determined using $^{13}\text{C-NMR}$ spectroscopy. In particular, the chemical shift of C-7a appears around 101.9-103.2 ppm for compounds **1a-h** and **5a-b**, diastereoisomers with 3,7a-(*S,S*)-relationship. These chemical shift values are in accordance with those described in the literature for similar compounds [13-16].

Biological activity and SAR study

To evaluate the potential of the synthesized compounds as NMDA receptor antagonists, we measured the ability of compounds in inhibiting the intracellular Ca^{2+} increase, induced by NMDA, in *in vitro* cultures of embryonary rat cortical neurons, using the Ca^{2+} -sensitive, fluorescent dye Fluo-4 (Figure 3 and Table 1). This measurement is a reliable protocol to finely monitor intracellular Ca^{2+} oscillations induced by an ionotropic stimulus, such as the glutamatergic agonist NMDA, in presence or absence of selective NMDA receptors blockers, adaptable for evaluating a number of potential blockers in a high throughput screening fashion.

Table 1. Effect of bicyclic lactams **1a-h**, **1a'-h'**, **3**, **3'**, **4**, **4'** and **5a-b**, at the concentration of 100 μ M, in the NMDA-induced calcium, using *in vitro* cultures of embryonic rat cortical neurons.

Compound	NMDA 10 μ M
	% Ca^{2+} increase ^a
Memantine	33 $\pm$ 3 ^{***}
1a	43 $\pm$ 4 ^{***}
1b	46 $\pm$ 6 ^{***}
1c	19 $\pm$ 5 ^{***}
1d	20 $\pm$ 4 ^{***}
1e	42 $\pm$ 5 ^{***}
1f	61 $\pm$ 6 ^{***}
1g	95 $\pm$ 4
1h	55 $\pm$ 3 ^{***}
1i	93 $\pm$ 2
1a'	54 $\pm$ 9 ^{***}
1b'	51 $\pm$ 5 ^{***}
1c'	28 $\pm$ 5 ^{***}
1d'	22 $\pm$ 5 ^{***}
1e'	34 $\pm$ 5 ^{***}
1f'	71 $\pm$ 7 ^{***}
1g'	95 $\pm$ 4
1h'	53 $\pm$ 5 ^{***}
3	42 $\pm$ 5 ^{***}
3'	66 $\pm$ 9 ^{***}
4	35 $\pm$ 6 ^{***}
4'	34 $\pm$ 6 ^{***}
5a	50 $\pm$ 10 ^{***}
5b	25 $\pm$ 13 ^{***}

^a% Ca^{2+} increase respect to control cells stimulated with NMDA 10 μ M in the absence of compound. Data are shown as means $\pm$ SEM of at least four different

cell batches in triplicate. *** $p < 0.001$, comparing with control in the absence of compounds.

Figure 3. Effect of bicyclic lactams **1a-h**, **1a'-h'**, **3**, **3'**, **4**, **4'** and **5a-b** in the NMDA-induced Ca²⁺ increase using *in vitro* cultures of embryonic rat cortical neurons. Data are shown as means $\pm$ SEM of at least four different independent experiments in triplicate. *** $p < 0.001$, comparing with control in the absence of compounds (white bar).

In our first screening, phenylalaninol-derived bicyclic lactams **1a-h** and **1a'-h'** were evaluated at the concentration of 100 μ M. Most compounds significantly decreased Ca²⁺ entry. In particular, bicyclic lactams **1c-d**, and **1c'-d'** blocked over 70% the Ca²⁺ influx, and were more active than memantine (positive control used) and the hit compound **1a**. In this embryonic cell culture, seeded at a density of 30000 cells per well, memantine blocks the Ca²⁺ increase induced by NMDA 10 μ M with an IC₅₀ value of 47 μ M. These results suggest that a halogen atom at *para* position of the phenyl group (chlorine and bromine atoms) has an important role for the activity. Halogens are atoms able to fit hydrophobic pockets conveniently, as well as featuring other types of interactions, such as the so-called “halogen bond”, as hypothesized in this particular situation. The isosteric analogue of **1a**, that is the fluorinated **1b**, exerts a similar blockade to **1a**, whilst when we occupy a bulkier space with chlorine or bromine,

present in **1c** and **1d**, the blockade is enhanced. It is important to note that the nature of the binding pocket is basically hydrophobic, as methoxy group (-OCH₃), present in **1f** and **1f'**, which is thought to be isosteric of the bromine atom, generates a weaker blocking effect than its isosteric bromine derivatives or the non-substituted head compound **1a**. The insertion of a fluorine atom at the *meta* position (compounds **1h** and **1h'**) augmented slightly the blockade effect when compared to the corresponding non-halogenated counterparts **1f** and **1f'**. The existence of a hydrophobic binding pocket is also confirmed by the fact that derivative **1e'**, possessing a methyl group at *para* position, also caused a blocking effect better than compound **1a'**. Compounds **1f** and **1f'**, with a methoxy group, revealed low impacts over the Ca²⁺ influx, whilst the presence of a methylsulfonyl group (-SO₂CH₃, compounds **1g** and **1g'**) led to the complete loss of activity.

As previously observed by us and others, the stereochemistry is an important feature for the NMDA receptor antagonist activity [13, 14, 17, 18]. Interestingly, for this chemical family, the chlorinated (*S*)-phenylalaninol derivative **1c** revealed to be more active than the corresponding (*R*)-phenylalaninol enantiomer **1c'**. However, this effect was not so evident for the brominated (*S*)-phenylalaninol derivative **1d**.

The pyrrolidone ring seems to be important for the blockage of Ca²⁺ entry. In fact, compound **3'**, containing a piperidone ring, led to higher influx of Ca²⁺, compared with the corresponding pyrrolidone derivative **1a'**. However, as the eutomers **3** and **1a** similarly affected Ca²⁺ influx further studies are needed to better clarify this conclusion.

Interestingly, the (*R*)-phenylalaninol derivative **3'** (Figure 3) was much less active than the corresponding (*R*)-tryptophanol derivative **4'**, containing an indole moiety instead of a phenyl group in its chemical structure. However, for the (*S*)-

phenylalaninol derivative **3**, the replacement of this phenyl group by an indole group (i.e. compound **4**) did not overly influence the level of activity as NMDA blocker.

After the first screening, we further synthesized some more derivatives of the most promising antagonists, i.e. compounds **1c** and **1d**. Compound **1i** was synthesized to evaluate the effect of increasing the distance between the oxazolopyrrolidone skeleton moiety and the 4-chlorophenyl substituent on the biological activity study. However, this structural modification significantly decreased the blockade of the NMDA receptor.

We have also evaluated the effect of increasing polarity in the structure of compound **1c**. Starting from (*S*)-tyrosinol (amino alcohol with a hydroxyl group at the *para* position of the phenyl ring), we obtained two oxazolopyrrolidone lactams, one with a chlorine atom at the *para* position (compound **5b**) and the other without substituents at the phenyl ring (compound **5a**) (Scheme 1). The effect of compound **5a** in the NMDA-induced Ca^{2+} influx increase was not relevant. Very interestingly, the presence of a hydroxyl group and the chlorine atom at the oxazolopyrrolidone structure, i.e. compound **5b**, led to a significant increase of the Ca^{2+} influx. This result further confirms that the presence of the hydroxyl group in the phenyl ring is important for the interaction with the NMDA receptor, as observed previously for other NMDA receptor antagonists described in literature [8, 19].

The IC_{50} values (Table 2) were determined for the most active compounds (**1c-d** and **5b**). Compounds **1c** and **1d** had IC_{50} values almost in the same order of magnitude of memantine (IC_{50} value of 47 μM) and were 2.3 and 2.5 times more active than the hit compound **1a** (IC_{50} value of 91 μM), with IC_{50} values of 39 and 36 μM , respectively. More remarkably, compound **5b** presented an IC_{50} value of 27 μM . These improvements are not only of interest because of the augmented blocking effect of the

NMDA-induced Ca^{2+} increases, but also these new derivatives can be considered longer-lasting potential drugs, as their *para* positions at phenyl rings are substituted, thus avoiding the metabolic actions of CYP enzymes.

Table 2. IC_{50} values (inhibitory concentration able to reduce the NMDA-induced Ca^{2+} increase by 50%, calculated by measuring the Ca^{2+} in presence of at least 4 different concentrations of compound, from 1 to 100 μM) and permeability values from the PAMPA-BBB assay P_e ($10^{-6} \text{ cm s}^{-1}$) of compounds **1c-d** and **5b**.

Compound	NMDA (10 μM) ^a	PAMPA-BBB assay ^b	
	IC_{50} (μM)	P_e ($10^{-6} \text{ cm s}^{-1}$)	CNS Prediction
1c	39 ± 8	66.2 ± 5.1	cns +
1d	36 ± 3	14.1 ± 0.7	cns +
5b	27 ± 1	24.5 ± 1.9	cns +
1a	91 ± 6	—	—
Memantine	47 ± 3	—	—

^aThe values are expressed as means $\pm$ SEM of at least four different cell batches in triplicate. ^bResults are the mean $\pm$ SD of three independent experiments.

***In Vitro* Toxicity and Blood–Brain Barrier Permeation Assays**

The brain penetration of the three most promising compounds (compounds **1c**, **1d**, and **5b**) was predicted using the *in vitro* PAMPA-BBB assay described by Di et al. [20], and partially modified by Rodríguez-Franco et al. for assaying molecules with limited water-solubility [21-23]. The permeability of compounds (P_e) through a lipid extract of porcine brain was determined at room temperature by using PBS:ethanol (70:30) as solvent and results are gathered in table 2. In the same assay, 10 commercial drugs with known CNS penetration were also tested and their values were compared to reported values, giving a good linear correlation, $P_e (\text{exp.}) = 1.30 P_e (\text{bibl.}) + 8.61$ ($R^2 = 0.87$). From this equation and following the pattern previously established in the bibliography, we could predict compounds as follows: cns + (high BBB permeation) if P_e ($10^{-6} \text{ cm s}^{-1}$)

> 13.8; cns – (low BBB permeation) if P_e (10^{-6} cm s⁻¹) < 11.2; and cns +/- (uncertain BBB permeation) if P_e (10^{-6} cm s⁻¹) is between these values (Table 2). All tested compounds showed good CNS-permeability in this *in vitro* model and thus, it is expected they could reach their biological targets in the CNS.

The *in vitro* cytotoxicity of compounds **1c**, **1d**, and **5b** was also evaluated (Table 3) in the human hepatocellular carcinoma HepG2 cell line (an established, well-characterized toxicological model) [25]. No decrease of cell viability was observed up to 100 μ M concentration of the three tested compounds, indicating that these compounds are non-hepatotoxic for this concentration.

Table 3. *In vitro* activity of compounds **1c-d** and **5b** in HepG2 cell line.

Compound	25 μ M	50 μ M	100 μ M
1c	92.1 $\pm$ 4.2	92.8 $\pm$ 3.1	93.7 $\pm$ 3.4
1d	93.7 $\pm$ 1.5	90.2 $\pm$ 2.6	93.4 $\pm$ 2.7
5b	96.5 $\pm$ 2.2	93.6 $\pm$ 3.0	95.5 $\pm$ 1.4

^aThe values are expressed as means $\pm$ SD for at least 3 independent experiments with 3 replicates each.

Conclusions

A series of enantiopure amino alcohol-derived oxazolopyrrolidones and oxazolopiperidones were synthesized and evaluated as NMDA receptor antagonists in embryonic rat cortical neurons. Quite remarkably, compounds **1c**, **1d**, and **5b** displayed improved activity over the hit compound **1a**. The (*S*)-derivatives were more active than the (*R*)-counterparts as NMDA receptor antagonists. Moreover, halogens at the *para* position of phenyl group in position 7a are preferred to methyl, methoxy, and methylsulfonyl groups. The presence of a hydroxyl group (compound **5b**) favors the activity as antagonist, while the introduction of an aromatic ring between the

oxazolopyrrolidone skeleton and the 4-chlorophenyl substituent (compound **1i**) results in loss of activity.

In conclusion, several evidences are provided in this work supporting the therapeutic potential of bicyclic lactam **5b** as NMDA receptor antagonist and its potential applicability in the treatment of neurological diseases where NMDA receptors are over activated.

Experimental Section

Chemistry

General: All reagents and solvents were obtained from commercial suppliers and were used without further purification. Triethylamine (NEt₃) was dried over KOH under nitrogen atmosphere. Melting points were determined using a Kofler camera Bock monoscope M. Thin layer chromatography was performed using Merck Silica Gel 60 F254 plates and visualized by UV light. Merck Silica Gel (200-400 mesh) was used for flash column chromatography. ¹H and ¹³C-NMR spectra were recorded on a Bruker Fourier 300. ¹H and ¹³C NMR chemical shifts are reported in parts per million (ppm, δ) referenced to the solvent used. Proton coupling constants (*J*) are expressed in hertz (Hz). Multiplicities are given as: s (singlet), d (doublet), dd (double doublet), t (triplet), q (quartet), and m (multiplet). Elemental analyses were performed in a Flash 2000 CHNS-O ThermoScientific elemental analyzer, at Liquid Chromatography and Mass Spectrometry Laboratory, Faculty of Pharmacy of Lisbon University, and are within $\pm 0.4\%$ of theoretical values.

General Synthetic Procedure for obtaining 1a-h, 1a'-h', 3, 3', 4, 4', and 5a-b

To a solution of the amino alcohol (1.0 equiv.) in toluene was added the appropriate keto acid (1.1 equiv). The mixture was heated to reflux for 10-24 h under Dean-Stark apparatus. The solvent was removed under reduced pressure and the residue obtained was dissolved in ethyl acetate (EtOAc). The organic phase was washed with saturated aqueous solutions of NaHCO₃ and NaCl, dried with anhydrous Mg₂SO₄, filtered and evaporated. The obtained residue was purified by flash chromatography using EtOAc/*n*-hexane as eluent.

(3S,7aS)-3-Benzyl-7a-phenyltetrahydropyrrolo[2,1-b]oxazol-5(6H)-one (1a): Following the general procedure, to a solution of (*S*)-phenylalaninol (0.100 g, 0.661 mmol) in toluene (5 mL) was added 3-benzoyl propionic acid (0.130 g, 0.734 mmol). Reaction time: 16 h. The compound was purified by flash chromatography (EtOAc/*n*-hexane 1:2) to afford the title compound as a pale yellow oil (0.160 g, 82 %); ¹H-NMR (300 MHz, MeOD) δ 7.53 – 7.46 (m, 2H, ArH), 7.46 – 7.31 (m, 3H, ArH), 7.24 – 7.08 (m, 3H, ArH), 7.07 – 7.01 (m, 2H, ArH), 4.41 – 4.27 (m, 1H, H-3), 4.16 (dd, *J* = 8.7, 7.4 Hz, 1H, H-2), 3.57 (dd, *J* = 8.8, 6.8 Hz, 1H, H-2), 2.93 – 2.76 (m, 1H, CH₂), 2.70 (dd, *J* = 13.7, 7.1 Hz, 1H, ArCH₂), 2.56 – 2.39 (m, 2H, CH₂), 2.34 – 2.25 (m, 1H, ArCH₂), 2.23 – 2.15 (m, 1H, CH₂), as described in literature [13].

(3R,7aR)-3-Benzyl-7a-phenyltetrahydropyrrolo[2,1-b]oxazol-5(6H)-one (1a'): Following the general procedure, to a solution of (*R*)-phenylalaninol (0.101 g, 0.667 mmol) in toluene (5 mL) was added 3-benzoyl propionic acid (0.131 g, 0.734 mmol). Reaction time: 24 h. The compound was purified by flash chromatography (EtOAc/*n*-hexane 1:2) to afford the title compound as a pale yellow oil (0.157 g, 80 %); ¹H-NMR spectra was found to be identical to the one obtained for compound **1a** [13].

(3S,7aS)-3-Benzyl-7a-(4-fluorophenyl)tetrahydropyrrolo[2,1-b]oxazol-5(6H)-one (1b): Following the general procedure, to a solution of (*S*)-phenylalaninol (0.100 g, 0.661

mmol) in toluene (5 mL) was added 3-(4-fluorobenzoyl) propionic acid (0.143 g, 0.728 mmol). Reaction time: 23 h. The compound was purified by flash chromatography (EtOAc/*n*-hexane 1:2) to afford the title compound as a yellow oil (0.188 g, 91 %); $[\alpha]_D^{22} = +6.0^\circ$ ($c = 1.1$, CH₂Cl₂); ¹H-NMR (300 MHz, CDCl₃) δ 7.53 – 7.46 (m, 2H, ArH), 7.29 – 7.20 (m, 3H, ArH), 7.15 – 7.09 (m, 4H, ArH), 4.45 (dq, $J = 9.3, 7.0$ Hz, 1H, H-3), 4.15 (dd, $J = 8.8, 7.4$ Hz, 1H, H-2), 3.58 (dd, $J = 8.8, 7.0$ Hz, 1H), 2.95 (dd, $J = 13.7, 6.2$ Hz, 1H), 2.87 – 2.66 (m, 1H), 2.63 – 2.45 (m, 2H), 2.36 – 2.27 (m, 1H), 2.24 – 2.17 (m, 1H); ¹³C-NMR (75 MHz, CDCl₃) δ 179.98 (C=O), 162.79 (d, $J = 246.98$ HZ, ArCF), 138.60 (ArCq), 137.23 (ArCq), 129.02 (ArCH), 128.67 (ArCH), 127.15 (ArCH), 127.04 (ArCH), 126.81 (ArCH), 115.87 (ArCH), 115.59 (ArCH), 102.07 (C-7a), 72.38 (C-2), 56.64 (C-3), 40.05 (ArCH₂), 35.21 (CH₂), 32.59 (CH₂); Anal. Calcd. (C₁₉H₁₈FNO₂•0.25H₂O): C, 72.30%; H, 5.92%; N, 4.44%. Found C, 71.93%; H, 5.84%; N, 4.31%.

(3R,7aR)-3-Benzyl-7a-(4-fluorophenyl)tetrahydropyrrolo[2,1-*b*]oxazol-5(6H)-one (**1b'**):

Following the general procedure, to a solution of (*R*)-phenylalaninol (0.101 g, 0.667 mmol) in toluene (5 mL) was added 3-(4-fluorobenzoyl) propionic acid (0.144 g, 0.734 mmol). Reaction time: 22 h. The compound was purified by flash chromatography (EtOAc/*n*-hexane 1:2) to afford the title compound as a yellow oil (0.172 g, 83 %); $[\alpha]_D^{22} = -5.4^\circ$ ($c = 1.1$, CH₂Cl₂); ¹H-NMR spectra was found to be identical to the one obtained for compound **1b**; Anal. Calcd. (C₁₉H₁₈FNO₂•0.2H₂O): C, 72.45%; H, 5.90%; N, 4.45%. Found C, 72.74%; H, 6.49%; N, 4.94%.

(3S,7aS)-3-Benzyl-7a-(4-chlorophenyl)tetrahydropyrrolo[2,1-*b*]oxazol-5(6H)- one (**1c**):

Following the general procedure, to a solution of (*S*)-phenylalaninol (0.100 g, 0.661 mmol) in toluene (5 mL) was added 3-(4-chlorobenzoyl) propionic acid (0.155 g, 0.728 mmol). Reaction time: 23 h. The compound was purified by flash chromatography

(EtOAc/*n*-hexane 1:2) to give the title compound as a yellow oil in (0.205 g, 95 %); $[\alpha]_D^{22} = +6.0^\circ$ ($c = 1.4$, CH₂Cl₂); ¹H-NMR (300 MHz, CDCl₃) δ 7.37 – 7.29 (m, 4H, ArH), 7.19 – 7.08 (m, 3H, ArH), 7.01 – 6.99 (m, 2H, ArH), 4.34 (dq, $J = 9.3, 7.0$ Hz, 1H, H-3), 4.05 (dd, $J = 8.8, 7.4$ Hz, 1H, H-2), 3.47 (dd, $J = 8.9, 7.0$ Hz, 1H, H-2), 2.84 (dd, $J = 13.7, 6.2$ Hz, 1H, ArCH₂), 2.78 – 2.65 (m, 1H), 2.53 – 2.35 (m, 2H), 2.26 – 2.18 (m, 1H), 2.13 – 2.05 (m, 1H); ¹³C-NMR (75 MHz, CDCl₃) δ 179.93 (C=O), 141.39 (ArCq), 137.16 (ArCq), 134.37 (ArCq), 129.04 (ArCH), 129.00 (ArCH), 128.66 (ArCH), 126.81 (ArCH), 126.72 (ArCH), 101.96 (C-7a), 72.41 (C-2), 56.66 (C-3), 40.04 (ArCH₂), 35.07 (CH₂), 32.54 (CH₂); Anal. Calcd. (C₁₉H₁₈ClNO₂): C, 69.62%; H, 5.53%; N, 4.27%. Found C, 69.57%; H, 5.51%; N, 4.44%.

(3R,7aR)-3-Benzyl-7a-(4-chlorophenyl)tetrahydropyrrolo[2,1-b]oxazol-5(6H)-one

(1c'): Following the general procedure, to a solution of (*R*)-phenylalaninol (0.100 g, 0.661 mmol) in toluene (5 mL) was added 3-(4-chlorobenzoyl) propionic acid (0.155 g, 0.728 mmol). Reaction time: 24 h. The compound was purified by flash chromatography (EtOAc/*n*-hexane 1:3) to afford the title compound as a yellow oil (0.180 g, 83 %); $[\alpha]_D^{22} = -5.0^\circ$ ($c = 1.4$, CH₂Cl₂); ¹H-NMR spectra was found to be identical to the one obtained for compound **1c**; Anal. Calcd. (C₁₉H₁₈ClNO₂): C, 69.62%; H, 5.53%; N, 4.27%. Found C, 69.72%; H, 5.58%; N, 4.39%.

(3S,7aS)-3-Benzyl-7a-(4-bromophenyl)tetrahydropyrrolo[2,1-b]oxazol-5(6H)-one (1d):

Following the general procedure, to a solution of (*S*)-phenylalaninol (0.100 g, 0.661 mmol) in toluene (5 mL) was added 3-(4-bromobenzoyl) propionic acid (0.187 g, 0.725 mmol). Reaction time: 25 h. The compound was purified by flash chromatography (EtOAc/*n*-hexane 1:2) to give the title compound as an orange oil (0.246 g, 99.8 %); $[\alpha]_D^{22} = +5.4^\circ$ ($c = 1.0$, CH₂Cl₂); ¹H-NMR (300 MHz, CDCl₃) δ 7.57 – 7.53 (m, 2H, ArH), 7.40 – 7.37 (m, 2H, ArH), 7.28 – 7.19 (m, 3H, ArH), 7.10 – 7.08 (m, 2H, ArH),

4.44 (dq, $J = 9.3, 7.0$ Hz, 1H, H-3), 4.13 – 4.09 (dd, $J = 8.2, 7.2$ Hz, 1H, H-2), 3.55 (dd, $J = 8.9, 7.0$ Hz, 1H, H-2), 2.92 (dd, $J = 13.7, 6.2$ Hz, 1H, ArCH₂), 2.87 – 2.73 (m, 1H), 2.62 – 2.43 (m, 2H), 2.32 (dd, $J = 13.7, 6.2$ Hz, 1H), 2.24 – 2.14 (m, 1H); ¹³C-NMR (75 MHz, CDCl₃) δ 179.94 (C=O), 141.96 (ArCq), 137.17 (ArCq), 132.02 (ArCH), 129.02 (ArCH), 128.68 (ArCH), 127.07 (ArCH), 126.83 (ArCH), 122.56 (ArCq), 102.02 (C-7a), 72.44 (C-2), 56.70 (C-3), 40.06 (ArCH₂), 35.06 (CH₂), 32.55 (CH₂); Anal. Calcd. (C₁₉H₁₈BrNO₂•0.4H₂O): C, 60.13%; H, 5.00%; N, 3.69%. Found C, 60.01%; H, 4.90%; N, 4.00%

(3R,7aR)-3-Benzyl-7a-(4-bromophenyl)tetrahydropyrrolo[2,1-b]oxazol-5(6H)-one

(1d'): Following the general procedure, to a solution of (*R*)-phenylalaninol (0.101 g, 0.669 mmol) in toluene (5 mL) was added 3-(4-bromobenzoyl) propionic acid (0.189 g, 0.736 mmol). Reaction time: 26 h. The compound was purified by flash chromatography (EtOAc/*n*-hexane 1:3) to afford the title compound as a yellow oil (0.189 g, 75.2 %); $[\alpha]_D^{22} = -4.7^\circ$ ($c = 1.1$, CH₂Cl₂); ¹H-NMR spectra was found to be identical to the one obtained for compound **1d**; Anal. Calcd. (C₁₉H₁₈BrNO₂): C, 61.30%; H, 4.87%; N, 3.76%. Found C, 61.19%; H, 4.94%; N, 3.71%.

(3S,7aS)-3-Benzyl-7a-(p-tolyl)tetrahydropyrrolo[2,1-b]oxazol-5(6H)-one **(1e)**:

Following the general procedure, to a solution of (*S*)-phenylalaninol (0.100 g, 0.661 mmol) in toluene (5 mL) was added 3-(4-methylbenzoyl) propionic acid (0.140 g, 0.728 mmol). Reaction time: 25 h. The compound was purified by flash chromatography (EtOAc/*n*-hexane 1:2) to give the title compound as a yellow oil (0.189 g, 93.2 %); $[\alpha]_D^{22} = +1.4^\circ$ ($c = 1.5$, CH₂Cl₂); ¹H-NMR (300 MHz, CDCl₃) δ 7.40 – 7.38 (m, 2H, ArH), 7.27 – 7.18 (m, 5H, ArH), 7.11 – 7.08 (m, 2H, ArH), 4.41 (dq, $J = 9.3, 7.0$ Hz, 1H, H-3), 4.12 – 4.07 (dd, $J = 8.8, 7.0$ Hz, 1H, H-2), 3.58 (dd, $J = 8.8, 7.0$ Hz, 1H, H-2), 2.96 (dd, $J = 13.7, 6.2$ Hz, 1H, ArCH₂), 2.88 – 2.75 (m, 1H), 2.60 – 2.43 (m, 2H), 2.40

(s, 3H, CH₃), 2.35 – 2.17 (m, 2H). ¹³C-NMR (75 MHz, CDCl₃) δ 179.98 (C=O), 139.73 (ArCq), 138.20 (ArCq), 137.50 (ArCq), 129.49 (ArCH), 129.06 (ArCH), 128.62 (ArCH), 126.71 (ArCH), 125.17 (ArCH), 102.44 (C-7a), 72.39 (C-2), 56.59 (C-3), 40.07 (ArCH₂), 35.32 (CH₂), 32.74 (CH₂); Anal. Calcd. (C₂₀H₂₁NO₂•0.15H₂O): C, 77.52%; H, 6.94%; N, 4.52%. Found C, 77.35%; H, 6.95%; N, 4.44%.

(3R,7aR)-3-Benzyl-7a-(p-tolyl)tetrahydropyrrolo[2,1-b]oxazol-5(6H)-one (**1e'**):

Following the general procedure, to a solution of (*R*)-phenylalaninol (0.100 g, 0.661 mmol) in toluene (5 mL) was added 3-(4-methylbenzoyl) propionic acid (0.140 g, 0.728 mmol). Reaction time: 26 h. The compound was purified by flash chromatography (EtOAc/*n*-hexane 1:2) to give the title compound as a yellow oil (0.152 g, 75 %); [α]²²_D = -3.2 ° (*c* = 1.4, CH₂Cl₂); ¹H-NMR spectra was found to be identical to the one obtained for compound **1e**. Anal. Calcd. (C₂₀H₂₁NO₂): C, 78.15%; H, 6.89%; N, 4.56%. Found C, 78.10%; H, 7.13%; N, 4.76%.

(3S,7aS)-3-Benzyl-7a-(4-methoxyphenyl)tetrahydropyrrolo[2,1-b]oxazol-5(6H)-one

(1f): Following the general procedure, to a solution of (*S*)-phenylalaninol (0.100 g, 0.661 mmol) in toluene (5 mL) was added 3-(4-methoxybenzoyl) propionic acid (0.153 g, 0.735 mmol). Reaction time: 23 h. The compound was purified by flash chromatography (EtOAc/*n*-hexane 1:2) to give the title compound as an yellow oil (0.171 g, 80 %); [α]²²_D = +1.6 ° (*c* = 0.5, CH₂Cl₂); ¹H-NMR (300 MHz, CDCl₃) δ 7.43 – 7.40 (m, 2H, ArH), 7.27 – 7.18 (m, 3H, ArH), 7.11 – 7.08 (m, 2H, ArH), 6.96 – 6.93 (m, 2H, ArH), 4.41 (dq, *J* = 9.3, 7.0 Hz, 1H, H-3), 4.12 (dd, *J* = 8.8, 7.0 Hz, 1H, H-2), 3.85 (s, 3H, OCH₃), 3.58 (dd, *J* = 8.8, 7.0 Hz, 1H, H-2), 2.96 (dd, *J* = 13.7, 6.2 Hz, 1H, ArCH₂), 2.88 – 2.74 (m, 1H), 2.60 – 2.43 (m, 2H), 2.36 – 2.16 (m, 2H); ¹³C-NMR (75 MHz, CDCl₃) δ 179.99 (C=O), 159.67 (ArCq), 137.45 (ArCq), 134.72 (ArCq), 129.04 (ArCH), 128.61 (ArCH), 126.70 (ArCH), 126.52 (ArCH), 114.07 (ArCH), 102.32 (C-

7a), 72.34 (C-2), 56.56 (C-3), 55.46 (OCH₃), 40.06 (ArCH₂), 35.31 (CH₂), 32.70 (CH₂); Anal. Calcd. (C₂₀H₂₁NO₃•0.15H₂O): C, 73.66%; H, 6.60%; N, 4.30%. Found C, 73.32%; H, 6.63%; N, 4.32%.

(3R,7aR)-3-Benzyl-7a-(4-methoxyphenyl)tetrahydropyrrolo[2,1-b]oxazol-5(6H)-one

(1f'): Following the general procedure, to a solution of (*R*)-phenylalaninol (0.102 g, 0.675 mmol) in toluene (5 mL) was added 3-(4-methoxybenzoyl) propionic acid (0.153 g, 0.735 mmol). Reaction time: 22 h. The compound was purified by flash chromatography (EtOAc/*n*-hexane 1:2) to give the title compound as a yellow oil (0.160 g, 73 %); $[\alpha]_D^{22} = -2.4^\circ$ ($c = 0.6$, CH₂Cl₂); ¹H-NMR spectra was found to be identical to the one obtained for compound **1f**; Anal. Calcd. (C₂₀H₂₁NO₃): C, 74.28%; H, 6.55%; N, 4.33%. Found C, 73.97%; H, 6.57%; N, 4.33%.

(3S,7aS)-3-Benzyl-7a-(4-(methylsulfonyl)phenyl)tetrahydropyrrolo[2,1-b]oxazol-5(6H)-one

(1g): Following the general procedure, to a solution of (*S*)-phenylalaninol (0.100 g, 0.661 mmol) in toluene (5 mL) was added 3-(4-methylsulfonylbenzoyl) propionic acid (0.186 g, 0.727 mmol). Reaction time: 22 h. The compound was purified by flash chromatography (EtOAc/*n*-hexane 3:1) and recrystallized from EtOAc/*n*-hexane to give the title compound as a white crystalline solid (0.216 g, 88 %); $[\alpha]_D^{22} = +10.5^\circ$ ($c = 1.0$ CH₂Cl₂); mp: 159-161 °C; ¹H-NMR (300 MHz, CDCl₃) δ 7.95 – 7.91 (m, 2H, ArH), 7.67 – 7.62 (m, 2H, ArH), 7.20 – 7.12 (m, 3H, ArH), 7.02 – 6.99 (m, 2H, ArH), 4.40 (dq, $J = 9.1, 6.9$ Hz, 1H, H-3), 4.11 (dd, $J = 8.9, 7.3$ Hz, 1H, H-2), 3.49 (dd, $J = 8.9, 7.0$ Hz, 1H, H-2), 3.05 (s, 3H, SO₂CH₃), 2.83 (dd, $J = 13.7, 6.3$ Hz, 1H, ArCH₂), 2.77-2.68 (m, 1H), 2.58 – 2.41 (m, 2H), 2.25 (dd, $J = 13.9, 8.8$ Hz, 1H), 2.17 – 2.07 (m, 1H); ¹³C-NMR (75 MHz, MeOD) δ 182.22 (C=O), 150.16 (ArCq), 142.11 (ArCq), 138.49 (ArCq), 130.08 (ArCH), 129.53 (ArCH), 129.06 (ArCH), 127.69 (ArCH), 127.47 (ArCH), 103.23 (C-7a), 73.45 (C-2), 58.22 (C-3), 44.29 (SO₂CH₃), 40.56 (ArCH₂),

36.00 (CH₂), 33.21 (CH₂); Anal. Calcd. (C₂₀H₂₁NO₄S•0.05H₂O): C, 64.51%; H, 5.72%; N, 3.76%. Found C, 64.24%; H, 5.62%; N, 3.89%.

(3R,7aR)-3-Benzyl-7a-(4-(methylsulfonyl)phenyl)tetrahydropyrrolo[2,1-*b*]oxazol-

5(6H)-one (1g'): Following the general procedure, to a solution of (*R*)-phenylalaninol (0.101 g, 0.665 mmol) in toluene (5 mL) was added 3-(4-methylsulfonylbenzoyl) propionic acid (0.188 g, 0.732 mmol). Reaction time: 24 h. The compound was purified by flash chromatography (EtOAc/*n*-hexane 2:1) and recrystallized from EtOAc/*n*-hexane to give white crystalline solid (0.171 g, 69 %); $[\alpha]_D^{22} = -13.6^\circ$ (*c* = 1.0 CH₂Cl₂); mp: 160-161 °C; ¹H-NMR spectra was found to be identical to the one obtained for compound **1g**. Anal. Calcd. (C₂₀H₂₁NO₄S): C, 64.67%; H, 5.70%; N, 3.77%. Found C, 65.01%; H, 5.81%; N, 3.80%.

(3S,7aS)-3-Benzyl-7a-(3-fluoro-4-methoxyphenyl)tetrahydropyrrolo[2,1-*b*]oxazol-

5(6H)-one (1h): Following the general procedure, to a solution of (*S*)-phenylalaninol (0.100 g, 0.661 mmol) in toluene (5 mL) was added 3-(3-fluoro-4-methoxybenzoyl) propionic acid (0.165 g, 0.727 mmol). Reaction time: 20 h. The compound was purified by flash chromatography (EtOAc/*n*-hexane 1:2) to give the title compound as a yellow oil (0.198 g, 88 %); $[\alpha]_D^{22} = +5.1^\circ$ (*c* = 1.3, CH₂Cl₂); ¹H-NMR (300 MHz, CDCl₃) δ 7.25 – 7.17 (m, 4H, ArH), 7.16 – 7.10 (m, 2H, ArH), 7.08 – 7.05 (m, 2H, ArH), 4.33 (dq, *J* = 9.3, 7.1 Hz, 1H, H-3), 4.19 – 4.13 (m, 1H, H-2), 3.88 (s, 3H, OCH₃), 3.61-3.55 (m, 1H, H-2), 2.90-2.78 (m, 2H, ArCH₂), 2.52 – 2.32 (m, 3H), 2.23 – 2.13 (m, 1H); ¹³C-NMR (75 MHz, CDCl₃) δ 179.88 (C=O), 152.48 (d, *J* = 238.5 Hz, ArCF), 147.64 (d, *J* = 10.7 Hz, ArCOMe), 137.21 (ArCq), 135.79 (d, *J* = 5.25 Hz, ArCq), 128.99 (ArCH), 128.61 (ArCH), 126.75 (ArCH), 120.96 (d, *J* = 3.75 Hz, ArCH), 113.42 (d, *J* = 4.5 Hz, ArCH), 113.36 (ArCH), 113.19 (ArCH), 101.74 (C-7a), 72.39 (C-2), 56.62 (OCH₃), 56.40 (C-3), 40.03 (ArCH₂), 35.09 (CH₂), 32.53 (CH₂); Anal. Calcd.

(C₂₀H₂₀FNO₃•0.3H₂O): C, 69.26%; H, 6.00%; N, 4.04%. Found C, 69.04%; H, 5.77%; N, 4.21%.

((3R,7aR)-3-Benzyl-7a-(3-fluoro-4-methoxyphenyl)tetrahydropyrrolo[2,1-b]oxazol-

5(6H)-one (1h'): Following the general procedure, to a solution of (*R*)-phenylalaninol (0.100 g, 0.661 mmol) in toluene (5 mL) was added 3-(3-fluoro-4-methoxybenzoyl) propionic acid (0.165 g, 0.727 mmol). Reaction time: 24 h. The compound was purified by flash chromatography (EtOAc/*n*-hexane 2:3) to give the title compound as a yellow oil (0.184 g, 82 %); $[\alpha]^{22}_{\text{D}} = -4.2^{\circ}$ ($c = 1.3$, CH₂Cl₂); ¹H-NMR spectra was found to be identical to the one obtained for compound **1h**. Anal. Calcd. (C₂₀H₂₀FNO₃): C, 70.37%; H, 5.91%; N, 4.10%. Found C, 70.14%; H, 6.44%; N, 4.22%.

(3S,8aS)-3-Benzyl-8a-phenylhexahydro-5H-oxazolo[3,2-a]pyridin-5-one (3): Following the general procedure, to a solution of (*S*)-phenylalaninol (0.100 g, 0.661 mmol) in toluene (5 mL) was added 4-benzoylbutyric acid (0.140 g, 0.728 mmol). Reaction time: 23 h. The compound was purified by flash chromatography (EtOAc/*n*-hexane 1:2) and recrystallized from EtOAc/*n*-hexane to give white crystalline solid (0.176 g, 87 %); $[\alpha]^{22}_{\text{D}} = -5.2^{\circ}$ ($c = 1.2$ CH₂Cl₂); mp: 138-140 °C; ¹H-NMR (300 MHz, CDCl₃) δ 7.25 – 7.19 (m, 5H, ArH), 7.05 – 6.95 (m, 3H, ArH), 6.88 – 6.85 (m, 2H, ArH), 4.34 – 4.23 (m, 1H, H-3), 3.70 (dd, *J* = 9.0, 7.7 Hz, 1H, H-2), 3.38 (dd, *J* = 12.9, 3.6 Hz, 1H, H-2), 3.11 (t, *J* = 8.9 Hz, 1H), 2.44 (dd, *J* = 18.6, 6.9 Hz, 1H, ArCH₂), 2.24 (ddd, *J* = 18.7, 11.1, 7.7 Hz, 1H), 2.03 – 1.95 (m, 1H), 1.74 – 1.64 (m, 1H), 1.58 – 1.50 (m, 2H), 1.47 – 1.23 (m, 1H); ¹³C-NMR (75 MHz, CDCl₃) δ 170.19 (C=O), 141.77 (ArCq), 137.86 (ArCq), 128.97 (ArCH), 128.63 (ArCH), 128.43 (ArCH), 126.74 (ArCH), 126.64 (ArCH), 96.47 (C-8a), 68.63 (C-2), 57.60 (C-3), 39.20 (ArCH₂), 36.49 (CH₂), 31.06 (CH₂), 15.91 (CH₂); Anal. Calcd. (C₂₀H₂₁NO₂): C, 78.15%; H, 6.89%; N, 4.56%. Found C, 77.81%; H, 6.97%; N, 4.53%.

(3R,8aR)-3-Benzyl-8a-phenyltetrahydro-2H-oxazolo[3,2-a]pyridin-5(3H)-one (**3'**):

Following the general procedure, to a solution of (*R*)-phenylalaninol (0.100 g, 0.661 mmol) in toluene (5 mL) was added 4-benzoylbutyric acid (0.140 g, 0.728 mmol). Reaction time: 23 h. The compound was purified by flash chromatography (EtOAc/*n*-hexane 2:3) and recrystallized from EtOAc/*n*-hexane to give white crystalline solid (0.176 g, 86 %); $[\alpha]_D^{22} = +7.1^\circ$ ($c = 1.2$, CH₂Cl₂); mp: 139-141 °C; ¹H-NMR spectra was found to be identical to the one obtained for compound **3**. Anal. Calcd. (C₂₀H₂₁NO₂): C, 78.15%; H, 6.89%; N, 4.56%. Found C, 78.53%; H, 6.90 %; N, 4.66%.

(3S,8aS)-3-((1*H*-Indol-3-yl)methyl)-8a-phenylhexahydro-5H-oxazolo[3,2-a]pyridin-5-

one (**4**): Following the general procedure, to a solution of (*S*)-tryptophanol (0.100 g, 0.53 mmol) in toluene (5 mL) was added 4-benzoylbutyric acid (0.113 g, 0.59 mmol). Reaction time: 21 h. The compound was purified by flash chromatography (EtOAc/*n*-hexane 1:1) and recrystallized from EtOAc/*n*-hexane to give white crystalline solid (0.132 g, 72 %); $[\alpha]_D^{22} = -29.2^\circ$ ($c = 1.0$, CH₂Cl₂); mp: 165-167 °C; ¹H NMR (300 MHz, CDCl₃) δ 7.95 (s, 1H, NH), 7.65 (d, $J = 7.9$ Hz, 1H, ArH), 7.52 – 7.37 (m, 5H, ArH), 7.34 – 7.22 (m, 1H, ArH), 7.22 – 7.04 (m, 4H, ArH), 6.83 (d, $J = 2.1$ Hz, 1H, ArH), 4.74 – 4.59 (m, 1H, H-3), 3.94 (dd, $J = 9.0, 7.7$ Hz, 1H, H-2), 3.68 (ddd, $J = 13.7, 3.5, 1.0$ Hz, 1H), 3.35 (t, $J = 8.7$ Hz, 1H), 2.69 (dd, $J = 18.7, 7.1$ Hz, 1H, ArCH₂), 2.56 – 2.38 (m, 2H), 2.22 (dt, $J = 12.5, 3.0$ Hz, 1H), 1.92 (ddd, $J = 14.1, 12.6, 3.6$ Hz, 1H), 1.77 (dddd, $J = 9.8, 7.6, 3.7, 2.2$ Hz, 1H), 1.66 – 1.45 (m, 1H); ¹³C NMR (75 MHz, MeOD) δ 172.59 (C=O), 142.94 (ArCq), 138.06 (ArCq), 129.67 (ArCq), 129.42 (ArCH), 128.57 (ArCH), 127.84 (ArCH), 123.34 (ArCH), 122.46 (ArCH), 119.69 (ArCH), 119.47 (ArCH), 112.22 (ArCH), 111.61 (ArCq), 97.71 (C-9b), 69.97 (C-2), 58.04 (C-3), 37.33 (ArCH₂), 31.58 (CH₂), 29.72 (CH₂), 16.65 (CH₂); Anal. Calcd. (C₂₂H₂₂N₂O₂): C, 76.28%; H, 6.40%; N, 8.09%. Found C, 76.05%; H, 6.55%; N, 8.23%.

(3R,8aR)-3-((1*H*-Indol-3-yl)methyl)-8*a*-phenylhexahydro-5*H*-oxazolo[3,2-*a*]pyridin-5-one (**4'**): Following the general procedure, to a solution of (*R*)-tryptophanol (0.100 g, 0.53 mmol) in toluene (5 mL) was added 4-benzoylbutyric acid (0.113 g, 0.59 mmol). Reaction time: 21 h. The compound was purified by flash chromatography (EtOAc/*n*-hexane 1:1) and recrystallized from EtOAc/*n*-hexane to give white crystalline solid (0.136g, 74.5%); $[\alpha]_D^{22} = +32.3^\circ$ ($c = 1.0$, CH₂Cl₂); mp: 166-168 °C; ¹H-NMR spectra was found to be identical to the one obtained for compound **4**. Anal. Calcd. (C₂₂H₂₂N₂O₂): C, 76.28%; H, 6.40%; N, 8.09%. Found C, 76.24%; H, 6.17%; N, 8.24%.

(3S,7aS)-3-(4-Hydroxybenzyl)-7*a*-phenyltetrahydropyrrolo[2,1-*b*]oxazol-5(6*H*)-one (**5a**): Following the general procedure, to a solution of (*S*)-tyrosinol hydrochloride (0.061 g, 0.299 mmol) in toluene (3 mL) was added anhydrous triethylamine (45.9 μL, 0.329 mmol). At 60 °C was added 3-benzoylpropionic acid (0.064 g, 0.359 mmol). Reaction time: 24 h. The compound was purified by flash chromatography (EtOAc/*n*-hexane 1:2) to afford the title compound as a white powder (0.018 g, 19.4 %); mp: 163-164 °C; ¹H-NMR (300 MHz, CDCl₃) δ 7.53 – 7.49 (m, 2H, ArH), 7.47 – 7.36 (m, 3H, ArH), 6.99 – 6.95 (m, 2H, ArH), 6.76 – 6.71 (m, 2H, ArH), 5.63 (s, 1H, ArOH), 4.41 (dq, $J = 9.3, 7.0$ Hz, 1H, H-3), 4.18 (dd, $J = 8.7, 7.5$ Hz, 1H, H-2), 3.59 (dd, $J = 8.7, 6.8$ Hz, 1H, H-2), 2.92 – 2.74 (m, 2H), 2.64 – 2.46 (m, 2H), 2.32 – 2.20 (m, 2H); ¹³C-NMR (75 MHz, CDCl₃) δ 180.51 (C=O), 155.15 (ArCq), 142.44 (ArCq), 130.10 (ArCH), 128.96 (ArCq), 128.85 (ArCH), 128.50 (ArCH), 125.14 (ArCH), 115.54 (ArC), 102.53 (C-7a), 72.52 (C-2), 56.82 (C-3), 39.09 (ArCH₂), 35.18 (CH₂), 32.78 (CH₂); Anal. Calcd. (C₁₉H₁₉NO₃): C, 73.77%; H, 6.19%; N, 4.53%. Found C, 73.88%; H, 6.57%; N, 4.68%.

(3S,7aS)-7*a*-(4-Chlorophenyl)-3-(4-hydroxybenzyl)tetrahydropyrrolo[2,1-*b*]oxazol-5(6*H*)-one (**5b**): Following the general procedure, to a solution of (*S*)-tyrosinol

hydrochloride (0.032 g, 0.156 mmol) in toluene (2 mL) was added anhydrous TEA (24.0 μ L, 0.172 mmol). At 60 °C was added 3-(4-chlorobenzoyl) propionic acid (0.040 g, 0.187 mmol). Reaction time: 24 h. The compound was purified by flash chromatography (EtOAc/*n*-hexane 1:2) to afford the title compound as a white powder (0.016 g, 29.1 %); mp: 186-187 °C; ¹H-NMR (300 MHz, CDCl₃) δ 7.45 – 7.35 (m, 4H, ArH), 6.98 – 6.93 (m, 2H, ArH), 6.74 – 6.70 (m, 2H, ArH), 5.31 (s, OH), 4.44 – 4.34 (m, 1H, H-3), 4.15 (dd, *J* = 8.6, 7.6 Hz, 1H, H-2), 3.55 (dd, *J* = 8.7, 6.9 Hz, 1H, H-2), 2.88 – 2.74 (m, 2H), 2.62 – 2.43 (m, 2H), 2.30 – 2.14 (m, 2H); ¹³C-NMR (75 MHz, CDCl₃) δ 180.42 (C=O), 155.05 (ArCq), 141.19 (ArCq), 134.45 (ArCq), 130.10 (ArCH), 129.07 (ArCH), 128.80 (ArCq), 126.80 (ArCH), 115.58 (ArCH), 102.06 (C-7a), 72.45 (C-2), 56.90 (C-3), 39.11 (ArCH₂), 35.18 (CH₂), 32.64 (CH₂); Anal. Calcd. (C₁₉H₁₈ClNO₃): C, 66.38%; H, 5.28%; N, 4.07%. Found C, 66.19%; H, 5.22%; N, 3.94%.

Synthetic Procedure for obtaining (3*S*,7*aS*)-3-benzyl-7*a*-(4'-chloro-[1,1'-biphenyl]-4-yl)tetrahydropyrrolo[2,1-*b*]oxazol-5(6*H*)-one (1i)

To a solution of bicyclic lactam **1d** (0.051 g, 0.137 mmol, 1 equiv.) in dioxane (1.6 mL) was added Pd(PPh₃)₂Cl₂ (5 mg, 16.3 μ mol, 0.1 equiv.), Na₂CO₃ 1 M (420 μ L) and 4-chlorophenylboronic acid (0.027 g, 0.173 mmol, 1.2 equiv.). The resulting mixture was degassed and stirred at 100 °C during 4 h under N₂ atmosphere. After cooling to room temperature, the reaction mixture was diluted with CH₂Cl₂, filtered under celite and concentrated under pressure to give the crude product. The crude was absorbed on silica and purified by flash chromatography (EtOAc/*n*-hexane 2:3) to afford the title compound as a colorless oil (0.045 g, 82.0 %); ¹H-NMR (300 MHz, CDCl₃) δ 7.47 – 7.39 (m, 6H, ArH), 7.29 – 7.26 (m, 2H, ArH), 7.12 – 7.01 (m, 3H, ArH), 6.96 – 6.94 (m,

2H, ArH), 4.30 (dq, J = 9.0, 7.0 Hz, 1H, H-3), 4.01 (dd, J = 8.7, 7.5 Hz, 1H, H-2), 3.46 (dd, J = 8.8, 7.1 Hz, 1H, H-2), 2.82 (dd, J = 13.7, 6.2 Hz, 1H, ArCH₂), 2.75 – 2.62 (m, 1H), 2.48 – 2.32 (m, 2H), 2.24– 2.05 (m, 2H); ¹³C-NMR (75 MHz, CDCl₃) δ 179.95 (C=O), 142.08 (ArCq), 140.06 (ArCq), 138.96 (ArCq), 137.3q (ArCq), 133.81 (ArCq), 129.14 (ArCH), 129.02 (ArCH), 128.62 (ArCH), 128.45 (ArCH), 127.36 (ArCH), 126.75 (ArCH), 125.81 (ArCH), 102.25 (C-7a), 72.45 (C-2), 56.65 (C-3), 40.05 (ArCH₂), 35.21 (CH₂), 32.62 (CH₂); Anal. Calcd. (C₂₅H₂₂ClNO₂): C, 74.34%; H, 5.49%; N, 3.47%. Found C, 74.66%; H, 5.58%; N, 3.47%.

Biology

NMDA receptor antagonist activity: Pregnant rats (18 days) were sacrificed by decapitation under a procedure that follows the European Union Council Directive issued for these purposes, and approved by the Ethics Committee of the School of Medicine, University Autonoma of Madrid, Spain. Efforts to reduce the number of animals and their suffering were made. Embryos were immediately extracted from the womb by caesarean section and their brains were carefully dissected out. Meninges were removed, and a portion of the total cortex was isolated. The fragments obtained from several embryos were subjected to mechanic digestion for 5 min. Cells were then re-suspended in Neurobasal medium with 2% B-27 and seeded in 96-well black plates, at a density of 30.000 cells/well. Neuronal cultures were allowed to mature for 8 – 10 days and, when the microscope showed the existence of a dense neuronal network, experiments were performed. Cells were loaded with 3 μM of Fluo-4/AM for 45 min at 37 °C in Krebs-HEPES with the composition (in mM): 144 NaCl, 5.9 KCl, 2.5 MgCl₂, 10 glucose, 10 HEPES, 2 Ca²⁺; pH 7.4 at room temperature. During this time, Fluo-4 dye, in its acetoxymethyl ester form, easily crosses the cell membrane by passive

diffusion, where is hydrolyzed by intracellular esterases to the Ca^{2+} -sensitive, fully active Fluo-4 fluorescent dye in its carboxylate form, which is thus unable to leak back to the extracellular media. Then, cells were washed with Krebs-HEPES solution twice, removing the spare Fluo-4/AM still present in the extracellular medium. Compounds dissolved in Krebs-HEPES at the concentration desired were incubated for 10 min. In the case of assessing their IC_{50} , compounds were assayed at least at four different concentrations, ranging from 1 to 100 μM . A batch of cells were incubated only with vehicle and considered as the positive control of the maximal $[\text{Ca}^{2+}]_i$ expected upon the administration of NMDA. Application of NMDA 10 μM induced the opening of the NMDA-sensitive glutamate receptors and the subsequent $[\text{Ca}^{2+}]$ increase. The experiments were carried out at room temperature. Fluorescence was measured in a fluorescence microplate reader (FLUOstar Optima, BMG, Germany). Wavelengths of excitation and emission were 485 and 520 nm, respectively. At the end of the experiment, Triton X-100 (5%) was applied and the maximal fluorescence, i.e. F_{max} , in each well was obtained. Then, MnCl_2 1M was applied and the basal fluorescence, i.e. F_{min} , in each well was obtained. Data were represented as percentage of fluorescence increase with respect to control, according to the formula $\Delta F / \Delta F_{\text{control}} \times 100$, where ΔF and $\Delta F_{\text{control}}$ are the percent increases of fluorescence with respect to the $F_{\text{max}} - F_{\text{min}}$ interval in each well.

In Vitro Blood–Brain Barrier Permeation Assay (PAMPA-BBB): Prediction of the brain penetration was evaluated using a parallel artificial membrane permeation assay for the blood-brain barrier (PAMPA-BBB), in a similar manner as previously described [21-24]. Pipetting was performed with a semi-automatic pipettor (CyBi®-SELMA) and UV reading with a microplate spectrophotometer (Multiskan Spectrum, Thermo Electron Co.). Commercial drugs, phosphate buffered saline solution at pH 7.4 (PBS), and

dodecane were purchased from Sigma, Aldrich, Acros, and Fluka. Millex filter units (PVDF membrane, diameter 25 mm, pore size 0.45 μm) were acquired from Millipore. The porcine brain lipid (PBL) was obtained from Avanti Polar Lipids. The donor microplate was a 96-well filter plate (PVDF membrane, pore size 0.45 μm) and the acceptor microplate was an indented 96-well plate, both from Millipore. The acceptor 96-well microplate was filled with 200 μL of PBS:ethanol (70:30) and the filter surface of the donor microplate was impregnated with 4 μL of porcine brain lipid (PBL) in dodecane (20 mg mL^{-1}). Compounds were dissolved in PBS: ethanol (70:30) at 100 $\mu\text{g mL}^{-1}$, filtered through a Millex filter, and then added to the donor wells (200 μL). The donor filter plate was carefully put on the acceptor plate to form a sandwich, which was left undisturbed for 120 min at 25 $^{\circ}\text{C}$. After incubation, the donor plate is carefully removed and the concentration of compounds in the acceptor wells was determined by UV-Vis spectroscopy. Every sample is analyzed at five wavelengths, in four wells and at least in three independent runs, and the results are given as the mean $\pm$ standard deviation. In each experiment, 10 quality control standards of known CNS permeability were included to validate the analysis set.

In Vitro toxicity: The cytotoxicity was assessed using resazurin non-fluorescent dye (Alamar Blue assay) that is reduced by dehydrogenases of viable cells into resorufin a fluorescent dye [26]. The day before experiments cells obtained from the American Type Culture Collection HepG2, a human derived liver hepatocellular carcinoma cell line (ATCC® HB-8065™), were seeded at 2×10^4 cells per well in 96 well tissue culture plates, in 100 μL of RPMI 1640 culture medium supplemented with 10% fetal bovine serum, 100 units of penicillin G (sodium salt), 100 mg of streptomycin sulfate and 2mM L-glutamine, at a concentration that allows cells to grow exponentially during the assay.

Tested compounds were dissolved in dimethyl sulfoxide (DMSO) serially diluted in the culture medium and added to the cells. The final concentration of DMSO in culture medium during treatment did not exceed 0.5% (v/v), and the same concentration of DMSO was added to the control. Each compound concentration was tested in triplicate in three independent experiments. Cells were incubated at 37 °C in humidified 5% CO₂ atmosphere. After 28 h, resazurin dye solution was added to each well to a final concentration of 2.5 µg/mL, after 3 h of incubations the fluorescence was measured (excitation, 530 nm; emission, 590 nm) in a microplate reader (FLUOstar Omega, BMG Labtech, Germany).

The relative cell viability (%) compared to control cells was calculated by $[\text{Fluorescence}]_{\text{sample}} \times 100\% / [\text{Fluorescence}]_{\text{control}}$.

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Supplementary data

Supplementary data associated with this article can be found in the online version. These data include: ^1H and ^{13}C NMR spectra for compounds **1c**, **1d** and **5b**.

References

- [1] P. Stawski, H. Janovjak, D. Trauner, *Bioorg. Med. Chem.* **2010**, *18*, 7759-7772.
- [2] P. Paoletti, C. Bellone, Q. Zhou, *Nature Rev. Neurosci.* **2013**, *14*, 383-400.
- [3] N.G. Glasgow, B.S. Retchless, J.W. Johnson, *J. Physiol.* **2015**, *593*, 83-95.
- [4] R.G.M. Morris, *Neuropharmacology* **2013**, *74*, 32-40.
- [5] G.E. Hardingham, H. Bading, *Nature Rev. Neurosci.* **2010**, *11*, 682-696.
- [6] M. Koller, S. Urwyler, *Expert Opin. Ther. Patents* **2010**, *20*, 1683-1702.
- [7] R.M. Santangelo, T.M. Acker, S.S. Zimmerman, B.M. Katzman, K.L. Strong, S.F. Traynelis, D.C. Liotta, *Expert Opin Ther Patents* **2012**, *22*, 1337-1352.
- [8] K.L. Strong, Y. Jing, A.R. Prosser, S.F. Traynelis, D.C. Liotta, *Expert Opin Ther Patents* **2014**, *24*, 1349-1366.
- [9] W. Danysz, C.G. Parsons, *Br. J. Pharmacol.* **2012**, *167*, 324-352.
- [10] C.-H. Lee, W. Lü, J.C. Michel, A. Goehring, J. Du, X. Song, E. Gouaux, *Nature* **2014**, *511*, 191-197.
- [11] E. Karakas, H. Furukawa, *Science*, **2014**, *344*, 992-997.
- [12] C.G. Parsons, A. Stoeffler, W. Danysz, *Neuropharmacology*, **2007**, *56*, 699-723.
- [13] N.A.L. Pereira, F.X. Sureda, M. Turch, M. Amat, J. Bosch, M.M.M. Santos, *Monatsh. Chem.* **2013**, *144*, 473-477.
- [14] N.A.L. Pereira, F.X. Sureda, R. Esplugas, M. Pérez, M. Amat, M.M.M. Santos, *Bioorg. Med. Chem. Lett.* **2014**, *24*, 3333-3336.
- [15] N.A.L. Pereira, A. Monteiro, M. Machado, J. Gut, E. Molins, M.J. Perry, J. Dourado, R. Moreira, P.J. Rosenthal, M. Prudêncio, M.M.M. Santos, *ChemMedChem* **2015**, *10*, 2080-2089.
- [16] L.E. Burgess, A.I. Meyers, *J. Org. Chem.* **1992**, *57*, 1656-1662.
- [17] S.T. Moe, D.L. Smith, E.G. DelMar, S.M. Shimizu, B.C. Van Wagenen, M.F. Balandrin, Y.E. Chien, J.L. Raszkievicz, L.D. Artman, H.S. White, A.L. Mueller, *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2411-2415.

- [18] R.W. Carling, P.D. Leeson, A.M. Moseley, R. Baker, A.C. Foster, S. Grimwood, J.A. Kemp, G.R. Marshall, *J. Med. Chem.* **1992**, 35, 1942-1953.
- [19] S. Dey, D. Schepmann, B. Wuensch, *Bioorg. Med. Chem. Lett.* **2016**, 26, 889-893.
- [20] L. Di, E.H. Kerns, K. Fan, O.J. McConnell, G.T. Carter, *Eur. J. Med. Chem.* **2003**, 38, 223-232.
- [21] M.I. Rodriguez-Franco, M.I. Fernández-Bachiller, C. Pérez, B. Hernández-Ledesma, B. Bartolomé, *J. Med. Chem.* **2006**, 49, 459-462.
- [22] B. Lopez-Iglesias, C. Pérez, J.A. Morales-García, S. Alonso-Gil, A. Pérez-Castillo, A. Romero, M.G. López, M. Villarroya, S. Conde, M.I. Rodríguez-Franco, *J. Med. Chem.* **2014**, 57, 3773-3785.
- [23] M.I. Fernandez-Bachiller, C. Pérez, L. Monjas, J. Rademann, M.I. Rodríguez-Franco, *J. Med. Chem.* **2012**, 55, 1303-1317.
- [24] M. de la Fuente Revenga, N. Fernández-Sáez, C. Herrera-Arozamena, J.A. Morales-García, S. Alonso-Gil, A. Pérez-Castillo, D.-H. Caignard, S. Rivara, M.I. Rodríguez-Franco, *J. Med. Chem.* **2015**, 58, 4998-5014.
- [25] J. C. García- Cañaveras, J.V. Castell, M.T. Donato, A. Lahoz, *Sci. Rep.* **2016**, 6, 27239.
- [26] M. M. Nociari, A. Shalev, P. Benias, C. Russo, *J. Immunol. Methods* **1998**, 213, 157-167.

TABLE OF CONTENTS TEXT

Twenty two novel enantiopure bicyclic lactams were synthesized and evaluated as NMDA receptor antagonists. The most promising compound displayed an IC_{50} value in the same order of magnitude of memantine. *In vitro* assays indicate that this compound is brain permeable (determined by an *in vitro* assay) and non-hepatotoxic, being a potential candidate for the treatment of pathologies associated with the overactivation of NMDA receptors.