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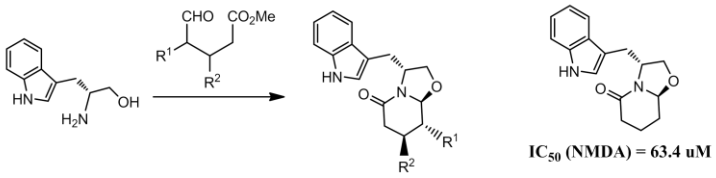

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Tryptophanol-derived oxazolopiperidone lactams: identification of a hit compound as NMDA receptor antagonist

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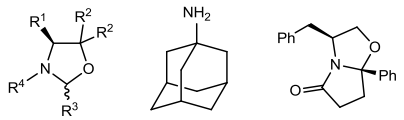
ABSTRACT

N-Methyl-D-Aspartate receptors (NMDAR) exacerbated activation leads to neuron death through a phenomenon called excitotoxicity. These receptors are implicated in several neurological diseases (eg. Alzheimer and Parkinson) and thus represent an important therapeutic target. We herein describe the study of enantiopure tryptophanol-derived oxazolopiperidone lactams as NMDA receptor antagonists. The most active hit exhibited an IC_{50} of 63.4 μM in cultured rat cerebellar granule neurons thus being 1.5 fold more active than clinically approved NMDA antagonist amantadine (IC_{50} = 92 μM).

Neurodegenerative pathologies represent a challenge for modern medicines especially in developed countries where life expectancy is higher. Patients suffering from Alzheimer's and Parkinson's diseases are confronted with the lack of adequate therapies that can provide a partial or complete reversal of neural death.[1] In this context, new therapeutical targets or new treatment strategies to fight mental dementia have attracted much research efforts from both academia and pharmaceutical companies in the recent years.[2] The neurotransmitter glutamate plays a pivotal role in the synaptic plasticity since its accumulation in the synaptic cleft activates the *N*-Methyl-D-Aspartate receptor (NMDAR), a subtype of ionotropic glutamate receptors (iGluR).[3] The NMDAR allows the influx of calcium ions (Ca^{2+}) to the neuron, thus reestablishing the membrane potential after the synapse. However, if there is a rapid increase in intracellular Ca^{2+} levels, it can lead to neuronal death trough mitochondrial dysfunction, a process that has been named excitotoxicity.[4] These neuronal receptors are heteromers formed by the assembly of different combinations of three or four NR1, NR2A-NR2D and NR3A-NR3B subunits which form Ca^{2+} permeable transmembrane channels. At the NR1 subunits is where the NMDAR co-agonist glycine will bind. On the other hand glutamate will bind to the NR2 subunits. Besides the need for the ~~simultaneous b~~inding of two agonists other unique features of NMDAR activation are its voltage-dependence and when resting its ion pore is permanently blocked by extracellular Mg^{2+}.[5]

In fact, the development of biologically active compounds that block NMDAR excessive activity and simultaneously maintain their physiological role unharmed is incredibly challenging due to the complex pharmacology and molecular architecture of these receptors. For this reason, blocking NMDARs completely with high affinity antagonists has already been shown not to be therapeutically useful.



1 **Amantadine** **2**

Figure 1. Known *N*-methyl-D-aspartate receptor antagonists.

In the last decades, several types of NMDAR antagonists with a wide range of action mechanisms have been reported but unfortunately only few exhibited proper pharmacokinetics and tolerable side effects in human trials.[6] A successful example is

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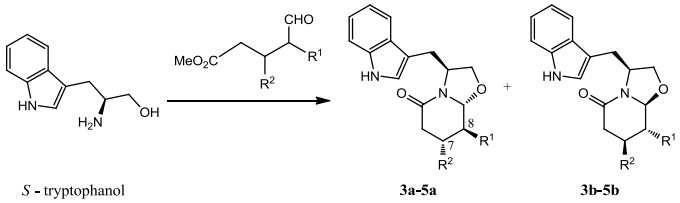
amantadine, a low-affinity NMDA channel blocker, currently used to mitigate levodopa-induced dyskinesia in Parkinson's disease.[7]

In 2009, a group of oxazolidines **1** was described as NMDAR antagonists by preventing the binding of NMDAR ligands through a yet unknown mechanism of action.[8] Based on this information, we decided to test a small library of phenylalaninol-derived oxazolopyrrolidones, which contain an oxazolidine ring but have a more rigid structure than compounds **1**. In fact, we discovered a hit compound **2** with an IC_{50} of 62 µM in cultured cerebellar granule neurons (Fig. 1).[9] Following our first encouraging results in the development of novel chemotypes of NMDAR antagonists, we have decided to explore if related derivatives have enhanced activity.

Here, we present our biological activity screening results with a series of tryptophanol-derived oxazolopiperidones. These compounds can be synthesized through chiral-induced cyclocondensation reactions between enantiopure tryptophanol and racemic or prochiral δ-oxo-esters in moderate to good yields.[10] Compounds **3**-**5** were synthesized by cyclocondensation reaction of *S*-tryptophanol and the appropriate δ-oxo-esters in yields up to 81% (Scheme 1).[11]

The NMDA receptor blocking activity of compounds **3a**-**5a** and **3b**-**5b** was evaluated by measuring the compounds ability to inhibit the intracellular calcium increase, induced by NMDA, in *in vitro* cultures of neurons (Table 1).[12] In our first screening of *S*-tryptophanol-derived oxazolopiperidones, only compounds without any substituent in the piperidine ring, compounds **3a** and **3b**, exhibited some activity. Compounds **4a**-**5a** and **4b**-**5b** were inactive (IC_{50} > 450 µM) pointing out the receptor intolerance for methylene-ester and ethyl chains at C-7 and C-8, respectively.



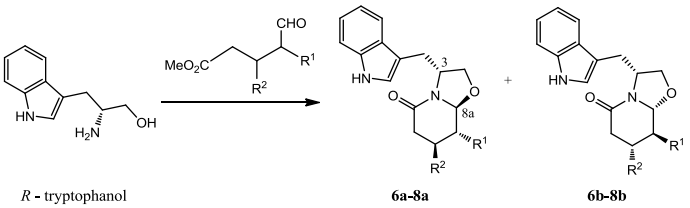
Scheme 1. Synthesis of *S*-tryptophanol-derived oxazolopiperidones. Reagents and conditions: 1.1eq of δ-oxo-esters, toluene, 16h Δ, inert atmosphere and Dean-Stark apparatus.

Table 1. NMDAR antagonist activity of compounds **3a**-**5a** and **3b**-**5b**.

Compound	R^1	R^2	NMDA (100 µM) IC_{50} (µM) [a]
3a	H	H	429.2±142.4
3b	H	H	305.1±37.0
4a	Ethyl	H	>450
4b	Ethyl	H	>450
5a	H	CH_2CO_2Me	>450
5b	H	CH_2CO_2Me	>450
Amantadine	—	—	92.0±29.1

[a]Results are the mean of three independent experiments.

Bearing in mind the importance of the absolute stereochemistry of biologically active compounds, we have also prepared a series of *R*-tryptophanol-derived oxazolopiperidones (Scheme 2).



Scheme 2. Synthesis of *R*-tryptophanol-derived oxazolopiperidones. Reagents and conditions: 1,1eq. of δ-oxo-esters, toluene, 16h Δ, inert atmosphere and Dean-Stark apparatus.

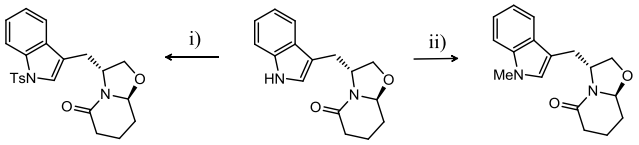
This new series of enantiopure derivatives were also screened for NMDAR antagonistic activity and results are summarized in Table 2. As observed for the *S*-tryptophanol derivatives the introduction of an ethyl group (**7a-7b**) or a CH_2CO_2Me chain (**8a-8b**) in the piperidine ring leads to complete loss of activity. Very interestingly, there is a large preference of the NMDA receptor for the derivatives containing an unsubstituted piperidine ring (compounds **3a**, **3b** and **6a**). The *R*-tryptophanol-derived oxazolopiperidone **6a**[13] showed the highest potency as a NMDAR antagonist (IC_{50} of 63.4 µM, see Table 2 and Fig. 2) and was 1.5 fold more active than amantadine (IC_{50} = 92 µM), a known NMDAR antagonist. The diastereoisomers **3b** and **3a**, exhibited IC_{50}'s of 305 µM and 429 µM, being approximately 5 fold and 7 fold less potent as NMDAR antagonists than compound **6a**. Together, these results suggest that an absolute stereochemistry of *R* at position C-3 and *S* at position C-8a is an important requisite for antagonistic activity in this class of compounds.

Table 2. NMDA antagonistic activity of compounds **6a-8a** and **6b-8b**.

Compound yield	R^1	R^2	NMDA (100 µM) IC_{50} (µM) [a]
6a (11%)	H	H	63.4±9.0
6b (49%)	H	H	>450
7a (12%)	Ethyl	H	>450
7b (58%)	Ethyl	H	>450
8a (13%)	H	CH_2CO_2Me	>450
8b (57%)	H	CH_2CO_2Me	>450
Amantadine	—	—	92.0±29.1

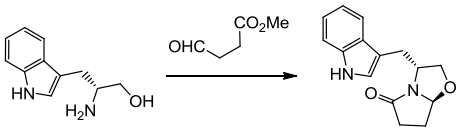
[a]Results are the mean of three independent experiments.

Finally, we decided to synthesize derivatives of compound **6a** to better understand the structural requisites for NMDA receptor antagonistic activity. Two compounds with the nitrogen atom of the indole moiety protected were synthesized (Scheme 3). Compound **9**, bearing a tosyl group at the indole nitrogen, was synthesized using a phase-transfer reaction.[14] Compound **10**, bearing a methyl group at the indole nitrogen, was obtained using sodium hydride and iodomethane.[15]



9 **6a** **10**

Scheme 3. Synthesis of compounds **9** and **10**. Reagents and conditions: **i)**: TsCl, CH_2Cl_2, NaOH (aq., 30% w/v), TBAI (cat.), 0ºC, 16h. **ii)**: NaH, MeI, DMF, 0ºC.



R-tryptophanol **11**

Scheme 4. Synthesis of compound **11**. Reagents and conditions: 1,1eq. methyl 4-oxobutanoate, toluene, 16h, Δ, inert atmosphere and Dean-Stark apparatus.

In order to explore the piperidone ring contraction effect on NMDAR antagonism activity we synthesized compound **11** through a cyclocondensation reaction of *R*-tryptophanol and methyl 4-oxobutanoate (Scheme 4).[16] This last set of structurally related analogs of compound **6a** was evaluated for NMDAR antagonism and results are depicted at Table 3. The introduction of tosyl or methyl substituents at the NH-indole position resulted in complete inactivity of compounds **9** and **10**. This observation suggests that there might be a need for a polarized N-H bond for the establishment of receptor interactions. Compound **11**, bearing a pyrrolidone ring, despite maintaining the absolute stereoutcome of compound **6a** is 5 fold less potent (IC_{50} = 341.2 µM).

Table 3. NMDA antagonistic activity of compounds **9**-**11**.

Compound	NMDA (100 µM) IC_{50} µM[a]
9	>450
10	>450
11	341.2 ±21.1
Amantadine	92.0±29.1

[a]Results are a mean of three independent experiments.

In conclusion, as far as our knowledge goes, herein we have identified for the first time the tryptophanol-derived oxazolopiperidone lactam scaffold for the development of novel NMDA receptor antagonists. The compounds are easily synthesized from enantiopure tryptophanol and racemic δ-oxo-esters in only one synthetic step and good yields. In particular, we discovered that tryptophanol-derived oxazolopiperidone lactam **6a** is 1.5 fold more active than clinically approved NMDA antagonist amantadine. Further studies are being conducted to optimize this compound as NMDA receptor antagonist in order to develop new oxazolo-piperidone leads with potential activity in neurodegenerative diseases.

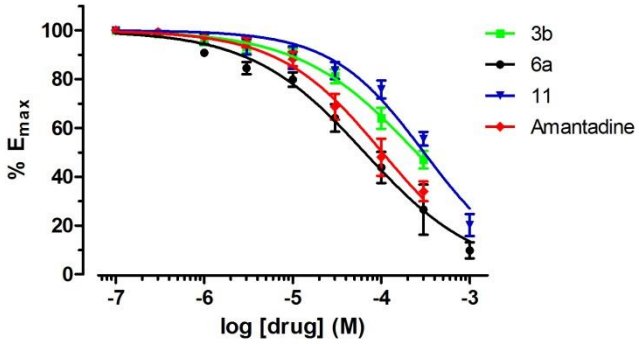


Figure 2. Inhibitory effect of compounds **3b**, **6a**, **11**, and amantadine on NMDA-induced intracellular calcium increase in cultured cerebellar granule neurons.

Acknowledgements

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12. 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 $1 \cdot 10^6$ 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 (Life Technologies, 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, NMDA (100 µM) was added to the cuvette, at 100 s respectively. This produced a sustained increase in F340/F380, 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 $1 \bullet 10^{-7}$ M up to up to $1 \bullet 10^{-3}$ M). If the compounds would act as antagonists at the NMDA receptor this would be detected as a decrease in the value F340/F380. Experiments were performed in triplicate. Amantadine 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.

13. Compound **6a**: mp 153-154 ºC; $[a]_D^{20}$ = -7.9º (c = 2.2, CH_2Cl_2); ^{1}H NMR spectra was found to be identical with the one described in ref. 10a; Anal. calcd. for $C_{16}H_{18}N_2O_2 \bullet 0.15H_2O$: C 70.38, H 6.77, N 10.26, found: C 70.59, H 6.82, N 10.44.
14. Compound **9** (72% yield): mp 70–71 ºC; IR (KBr) 1645, 1449, 1369; δ_H (400MHz, $CDCl_3$) 7.98 (d, J = 8.2 Hz, 1H, ArH), 7.73 (d, J = 8.0 Hz, 2H, ArH), 7.63 (d, J = 7.8 Hz, 1H, ArH), 7.36 – 7.29 (m, 1H, ArH), 7.22 (m, 3H, ArH), 4.61 – 4.50 (m, 1H, H-3), 4.36 (dd, J = 8.9, 4.4 Hz, 1H, H-8a), 4.05 (t, J = 8.3 Hz, 1H, H-2), 3.58 (t, J = 8.3 Hz, 1H, H-2), 3.24 (dd, J = 14.2, 2.8 Hz, 1H, CH_2-Indole), 2.94 (dd, J = 14.3, 8.9 Hz, 1H, CH_2-Indole), 2.48 (dd, J = 17.9, 5.4 Hz, 1H, Halkyl), 2.36 – 2.22 (m, 4H, CH_3tosyl & Halkyl), 2.13 (m, 1H, H alkyl), 1.85 (m, 1H, Halkyl), 1.48 (m, 1H, Halkyl), 1.41 – 1.33 (m, 1H, Halkyl); δ_C (100MHz, $CDCl_3$) 168.99 (C=O), 144.96 (Cq), 135.23 (Cq), 135.06 (Cq), 131.00 (Cq), 129.85 (2x CAr), 126.72 (2x CAr), 125.00 (CAr), 123.87 (CAr), 123.34 (CAr), 119.92 (CAr), 118.29 (Cq), 113.73 (CAr), 87.41 (C-8a), 69.54 (C-2), 53.53 (C-3), 31.31 (Calkyl), 28.10 (Calkyl), 27.34 (CH_2-Indole), 21.58 (CH_3tosyl), 17.03 (Calkyl); Anal. Calcd. for $C_{23}H_{24}N_2O_4S \bullet 0.25H_2O$: C 64.39, H 5.77, N 6.53, S 7.46, found: C 64.24, H 5.62, N 6.47, S 7.47.
15. Compound **10** (83% yield): IR (NaCl) 1644; δ_H (400MHz, $CDCl_3$) 7.69 (d, J = 7.9 Hz, 1H, ArH), 7.28 (d, J = 8.2 Hz, 1H, ArH), 7.22 (t, J = 7.5 Hz, 1H, ArH), 7.11 (t, J = 7.4 Hz, 1H, ArH), 6.88 (s, 1H, H-2-indole), 4.61 (qd, J = 8.0, 3.2 Hz, 1H, H-3), 4.47 (dd, J = 9.0, 4.4 Hz, 1H, H-8a), 4.06 (t, J = 8.2 Hz, 1H, H-2), 3.75 (s, 3H, NCH_3), 3.68 (dd, J = 15.3, 6.7 Hz, 1H, H-2), 3.31 (dd, J = 14.3, 3.1 Hz, 1H, CH_2indole), 3.02 (dd, J = 14.2, 8.8 Hz, 1H, CH_2indole), 2.52 (dd, J = 18.0, 5.7 Hz, 1H, Halkyl), 2.31 (ddd, J = 18.1, 11.6, 6.5 Hz, 1H, Halkyl), 2.16 (m, 1H, Halkyl), 1.86 (m, 1H, Halkyl), 1.55 (m, 1H, Halkyl), 1.37 (ddd, J = 13.2, 10.8, 3.0 Hz, 1H, Halkyl); δ_C (101MHz, $CDCl_3$) 168.66 (C=O), 137.00 (Cq), 128.22 (Cq), 127.29 (CAr), 121.75 (CAr), 119.32 (CAr), 119.04 (CAr), 109.71 (C-q), 109.12 (CAr), 87.26 (C-8a), 69.72 (C-2), 54.55 (C-3), 32.67 (NCH_3), 31.40 (Calkyl), 28.20 (CH_2indole), 27.53 (Calkyl), 17.14 (Calkyl).
16. Compound **11** (33% yield): mp 160.5-161.5 °C; IR (KBr) 3272, 1673; $[a]_D^{20}$ = -62.7º (c = 2.1, CH_2Cl_2); δ_H (400MHz, $CDCl_3$) 8.20 (s, 1H, NH), 7.63 (d, J = 7.8 Hz, 1H, ArH), 7.35 (dd, J = 8.0, 0.9 Hz, 1H, ArH), 7.20 (m, 2H, ArH), 7.13 (t, J= 7.4 Hz, 1H, ArH) 5.04 (dd, J = 6.1, 2.5 Hz, 1H, H7a), 4.58 – 4.44 (m, 1H, H3), 4.13 (dd, J = 8.7, 7.2 Hz, 1H, H2), 3.70 (dd, J = 8.7, 6.2 Hz, 1H, H2), 3.14 (dd, J = 14.9, 6.2 Hz, 1H, CH_2Indole), 2.95 (dd, J = 15.0, 7.6 Hz, 1H, CH_2Indole), 2.65 (ddd, J = 17.6, 10.2, 7.3 Hz, 1H, H6), 2.51 (ddd, J = 17.6, 10.3, 4.7 Hz, 1H, H6), 2.31 (m, 1H, H7), 2.11 – 1.95 (m, 1H, H7); δ_C (101MHz, $CDCl_3$) 179.53 (C=O), 136.21 (Cq), 127.61 (Cq), 122.44 (CAr), 122.16 (CAr), 119.51 (CAr), 118.72 (CAr), 111.23 (Cq), 111.17 (CAr), 91.80 (C7a), 72.32 (C2), 54.61 (C3), 31.73 (CH_2indole), 29.18 (C6), 24.45 (C7); Anal. Calcd. for $C_{15}H_{16}N_2O_2$: C 70.29, H 6.31, N 10.93, found: C 70.26, H 6.36, N 10.93.