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Synthesis and pharmacological evaluation of conformationally constrained glutamic acid higher homologues

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ABSTRACT

Homologation of glutamic acid chain together with conformational constraint is a commonly used strategy to achieve selectivity towards different types of glutamate receptors. In the present work, starting from two potent and selective unnatural amino acids previously developed by us, we investigated the effects on the activity/selectivity profile produced by a further increase in the distance between the amino acidic moiety and the distal carboxylate group. Interestingly, the insertion of an aromatic ring as a spacer produced a low micromolar affinity NMDA ligand that might represent a lead for the development of a new class of NMDA antagonists.

1. Introduction

Glutamate (L-Glu), the main excitatory transmitter within the mammalian central nervous system (CNS), is released by neuron terminals in a calcium dependent manner, in response to depolarization.[1] It is well known that glutamate synaptic release is involved in the modulation of many physiological processes, i.e., sensations and pain perception and cognitive function regulation. Furthermore, L-Glu plays a key role in synaptic plasticity.[2] On the other hand, L-Glu plays a crucial role in acute and chronic neurodegenerative diseases (i.e., cerebral ischemia, traumatic brain injury, spinal injury, epilepsy, ALS, Parkinson's, Alzheimer's and Huntington's diseases).[3] Glutamate operates through different classes of receptors divided into two large families, depending on the signal transduction mechanism: ionotropic receptors (iGluRs) and metabotropic EAA receptors (mGluRs).[4] Each family is composed by different receptor types and subtypes, grouped on the basis of their sequence homology, coupling mechanisms and pharmacology. Ionotropic glutamate receptors are tetramers composed of 4 subunits forming the transmembrane ionic channel permeable to Na^+, K^+ and Ca^{2+} ions. The three heterogeneous classes of iGluRs are: *N*-methyl-D-aspartic acid (NMDA) receptors, (*RS*)-2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid (AMPA) receptors and kainic acid (KA) receptors. The availability of highly selective ligands for the different receptor types and subtypes represents a primary target to understand their physiological role and their pharmacological relevance. Also from a therapeutic point of view, the more interesting compounds are undoubtedly those characterized by high selectivity for a specific receptor and receptor subtype, since this feature allows minimizing the possible side effects encountered with unselective compounds. The possible strategies useful to achieve receptor selectivity include the conformational rigidification,[5] the increase of the molecular complexity[6] and the bioisosteric substitution,[7] in particular on the distal carboxylate group, which can be efficaciously substituted with various groups, e.g., phosphonate, tetrazole and 3-hydroxyisoxazole. Moreover, the amino acidic chain homologation is usually exploited to modify a ligand profile from agonist to antagonist.[8]

Combining some of these strategies, i.e., the conformational rigidification and the amino acidic chain homologation, we have previously synthesized two amino acids (±)-**F-94b** and (±)-**F-94c**, characterized by the presence of a homologated glutamic acid chain tethered in a monocyclic structure (Fig. 1).[9]

Starting from model compounds (±)-**F-94b** and (±)-**F-94c**, which behave as potent and selective NMDA antagonists (K_i = 0.21 μM and 0.96 μM, respectively), we designed a set of new amino acids characterized by a further increase in the distance between the two pharmacophoric entities, realized through the insertion of an ethenyl, ethylene or phenylene spacer (Fig. 2, compounds (±)-**1a**/**b**–(±)-**5a**/**b**). Worth noting, the aromatic ring was functionalized in the *ortho*, *meta* or *para* position in order to evaluate the most

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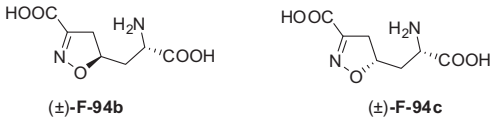

Figure 1. Structure of model compounds (±)-**F-94b** and (±)-**F-94c**.

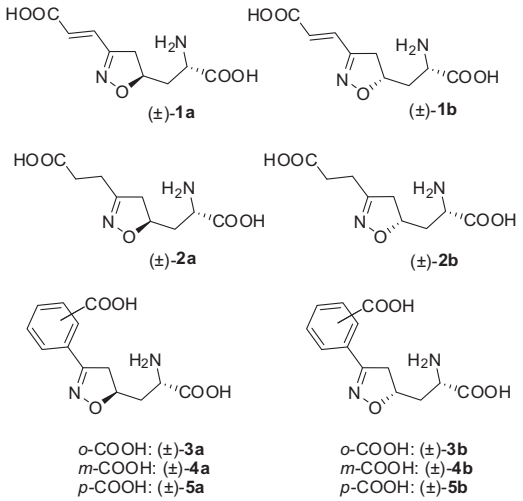

Figure 2. Structures of the target amino acids.

suitable distance and orientation of the distal acidic group with respect to the α-amino acidic group.

2. Results and discussion

2.1. Chemistry

Intermediates (±)-**6a** and (±)-**6b**, prepared as previously reported by us,[9] were selectively reduced at the more activated ester function located at position 3 of the isoxazoline ring, by using sodium borohydride (Scheme 1). Intermediate alcohols (±)-**7a** and (±)-**7b** were then oxidized with pyridinium chlorochromate (PCC) to give the corresponding aldehydes that were directly submitted to a Wittig reaction with a stabilized pre-formed ylide [i.e., methyl(triphenylphosphoranylidene)acetate] yielding, exclusively, the *E*-olefins (±)-**8a** and (±)-**8b** in good yield. Compounds (±)-**8a** and (±)-**8b** were finally converted into the desired amino acids (±)-**1a** and (±)-**1b** by alkaline hydrolysis of the ester functions and standard Boc-deprotection with trifluoroacetic acid (Scheme 1).

Catalytic hydrogenation of the key intermediates (±)-**8a** and (±)-**8b** afforded the saturated derivatives (±)-**9a** and (±)-**9b** that were finally converted into the desired amino acids (±)-**2a** and (±)-**2b** using the conditions reported above (Scheme 1).

The synthesis of the target amino acids (±)-**3a**–(±)-**5a** and (±)-**3b**–(±)-**5b** was carried out exploiting the 1,3-dipolar cycloaddition reaction of dipolarophile (±)-**10**[9] with suitably functionalized nitriloxides generated in situ by dehydrohalogenation of chloro oximes **11**–**13**, prepared following a literature procedure (Scheme 2).[10] The 1,3-dipolar cycloaddition reaction produced in all cases a 1:1 mixture of the two 5-substituted diastereoisomers. The relative configuration to the couples of derivatives (±)-**14a**/(±)-**14b**, (±)-**15a**/(±)-**15b**, and (±)-**16a**/(±)-**16b** was assigned by comparing their ^{1}H NMR spectroscopic signals with those of the previously described intermediates (±)-**6a** and (±)-**6b**, whose structure was unequivocally determined by X-ray analysis.[9]

The pairs of diastereoisomers were separated by flash chromatography on silica gel and transformed into the final amino acids (±)-**3a**–(±)-**5a** and (±)-**3b**–(±)-**5b** using standard deprotection conditions.

2.2. Biology

The new compounds were assayed in vitro by means of receptor binding techniques. All binding assays were performed using rat brain synaptic membranes from male Sprague–Dawley rats, and tissue preparations were prepared as previously described.[11] Affinities for NMDA, AMPA, and KA receptors were determined using 2 nM [^{3}H]CGP39653,[12] 5 nM [^{3}H]AMPA,[13] and 5 nM [^{3}H]KA,[14] respectively, with minor modifications as previously described.[15] As reported in Table 1, all the new derivatives showed no significant affinity for AMPA and KA receptors (IC_{50} > 100 μM) while some of them showed a low micromolar affinity for NMDA receptors.

The data reported in Table 1 showed that the two-carbon homologation carried out on model compound (±)-**F-94b** and (±)-**F-94c**, leading to derivatives (±)-**2a** and (±)-**2b**, produced a marked reduction in affinity though the selectivity for NMDA receptor was maintained. Similarly to the model compounds, the more active stereoisomer is the one possessing an (α*S**,5*R**) relative stereochemistry, i.e., compound (±)-**2a** (K_i = 13 μM). When a conformational constraint was imposed by the introduction of a *trans*-configured double bond, the affinity for the NMDA receptor was completely lost.

Interestingly, the affinity was restored by the introduction of a phenyl ring bearing the carboxylic acid group in the *ortho* position. Notably, compound (±)-**3a** displays the best binding affinity (K_i = 3.6 μM) within this series. Although the number of carbon atoms linking the distal carboxylate group to the α-amino acidic moiety is the same as in compounds (±)-**2a** and (±)-**2b**, it is likely that the aromatic ring in compound (±)-**3a** allows a better fit of the γ-COOH within the binding pocket.

3. Conclusion

In the present work, attempts to develop high affinity NMDA receptor ligands have been described and discussed. Ten new amino acids were synthesized inspired by the NMDA antagonists **F-94b** and **F-94c**, previously developed by us. The target compounds were obtained in good yields and high chemical purities. The pharmacological testing has revealed that some of the new derivatives behave as selective NMDA ligands, and derivative (±)-**3a** was highlighted for its interesting binding affinity in the low micromolar range. The results obtained in our study contribute to enlarge the knowledge of the SAR of iGluR ligands thus supporting a further investigation to uncover more potent ligands. In particular, in our opinion, compound (±)-**3a** represents a new interesting hit to be further optimized due to the presence of an aromatic ring, at variance of model compounds, which offers the possibility to be further decorated. Guided by molecular modelling studies, we plan to insert on the aromatic moiety different substituents capable of giving additional interactions within the binding pocket. The outcome should be an increase in binding affinity and, hopefully, also an increase in selectivity for a specific NMDA receptor subtype.

4. Experimental

4.1. Material and methods

All reagents were purchased from Sigma. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Varian Mercury 300 (300 MHz) spectrometer. Chemical shifts (δ) are expressed in ppm, and coupling