European Journal of Medicinal Chemistry 75 (2014) 151–158

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis of (3-hydroxy-pyrazolin-5-yl)glycine based ligands interacting with ionotropic glutamate receptors

Andrea Pinto [a,*], Lucia Tamborini [a], Federica Mastronardi [a], Roberta Ettari [a], Yeliz Safoz [a,b], Lennart Bunch [b], Birgitte Nielsen [b], Anders A. Jensen [b], Carlo De Micheli [a], Paola Conti [a]

[a] Dipartimento di Scienze Farmaceutiche, Università degli Studi di Milano, Via Mangiagalli 25, 20133 Milano, Italy
[b] Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Universitetsparken 2, 2100 Copenhagen OE, Denmark

ARTICLE INFO

Article history:
Received 20 June 2013
Received in revised form
27 November 2013
Accepted 20 January 2014
Available online 25 January 2014

Keywords:
L-Glutamic acid
Ionotropic glutamate receptors
Excitatory amino acid transporters
Pyrazoline

ABSTRACT

Following the concept that increasing the molecular complexity may enhance the receptor selectivity, we replaced the 3-hydroxy-isoxazoline ring of model compound tricholomic acid with a 3-hydroxy-pyrazoline ring, which could be variously decorated at the *N*1 position, inserting groups characterized by different electronic and steric properties. Binding assays on rat brain synaptic membranes showed that, depending on the nature of the substituent, some of the new synthesized ligands interacted with either AMPA or KA receptors, with affinities in the mid-micromolar range.

1. Introduction

L-Glutamate (L-Glu) is the main excitatory neurotransmitter in the mammalian central nervous system (CNS). It is involved in the modulation of many physiological processes i.e. learning, memory, and synaptic plasticity [1]. Dysfunction in the glutamatergic neurotransmission has been associated with several neurological disorders such as epilepsy, cerebral ischemia, stroke, hypoxia, and schizophrenia as well as chronic neurodegenerative pathologies such as neuropathic pain, amyotrophic lateral sclerosis, Huntington's chorea, Parkinson's, and Alzheimer's diseases [2].

Glutamate is stored in synaptic vesicles in the nerve terminal and is released by calcium-dependent exocytosis. Once released into the synaptic cleft, glutamate is acting through both ionotropic and metabotropic glutamate receptors (iGluRs and mGluRs, respectively). The iGluRs are tetrameric ligand-gated ion channels that mediate the fast excitatory transmission by fluxing cations (Na^+, K^+ or Ca^{++}) and thereby causing membrane depolarization of the cell membrane and excitation of the neurons. The iGluRs are divided into three classes based on sequence homology and ligand selectivity: *N*-methyl-D-aspartic acid (NMDA), (*RS*)-2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid (AMPA), and kainic acid (KA) receptors. These classes of receptors are further subdivided into subtypes: GluN1, GluN2A-D and GluN3A-B for NMDA receptors, GluA1-4 for AMPA receptors and GluK1-5 for KA receptors [3]. The mGluRs are G-protein-coupled receptors and mediate the slow excitatory transmission through second messenger systems. Eight subtypes of the mGluRs have been characterized, termed mGluR1-8 [4]. Glutamate levels are kept below neurotoxic concentration by an uptake transport system, the excitatory amino acid transporters (EAATs), which are localized both at the synaptic nerve terminals and at glial cells [5].

To study the role and function of one specific receptor or transporter subtype in a given neurological process, agonists or antagonists/inhibitors able to selectively interact with specific subtypes are key pharmacological tool compounds [6]; in this respect, natural products can serve as lead compounds for the design of new subtype selective ligands [7]. L-*erythro*-Tricholomic acid, a natural compound extracted from the poisonous mushroom *Tricholoma muscarium* [8], represents a partially rigidified analog of the endogenous neurotransmitter L-Glu, in which the distal carboxylate is bioisosterically replaced by the 3-hydroxy-isoxazoline ring. L-Tricholomic acid is an agonist at the AMPA and KA receptors, whereas its D-enantiomer interacts selectively with the NMDA receptors; moreover both the L- and D-*threo*-diastereoisomers are weak and non-selective GluR ligands [9].

* Corresponding author.
E-mail address: andrea.pinto@unimi.it (A. Pinto).

0223-5234/$ – see front matter © 2014 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.ejmech.2014.01.029

Following the concept that increasing the molecular complexity can lead to an increase in receptor selectivity [10], we replaced the 3-hydroxy-isoxazoline ring of tricholomic acid with the 3-hydroxy-pyrazoline ring, which can be variously decorated at the *N*1 position with groups of increasing steric bulkiness [compounds (±)-**1a**–**g** and (±)-**2a**–**g**, Fig. 1].

In addition, we used the aromatic ring as a spacer to add a further acidic function to the molecule in order to favor possible additional/alternative ionic or hydrogen bonding interactions within the binding pocket. The new derivatives were compared to the model compounds *erythro*- and *threo*-tricholomic acid in terms of affinity and selectivity for the different ionotropic receptors. Moreover, because it is known that the insertion of bulky substituents in the aspartate/glutamate skeleton may generate molecules that act as blockers of the EAATs [11], as in the case of *threo* benzyloxy aspartic acid (TBOA, Fig. 1), we also evaluated the interaction of our new ligands with human recombinant EAAT subtypes EAAT1-3.

2. Results and discussion

Compound (±)-**4** was identified as a versatile intermediate for the generation of all the planned derivatives. We previously reported that compound (±)-**4** can be obtained, as a mixture of racemic diastereoisomers, from the one-pot condensation/intramolecular cyclization of hydrazine with the α,β-unsaturated ester (±)-**3** [12], and that treating (±)-**4** with acetaldehyde in the presence of sodium borohydride affords the *N*-ethyl derivatives (±)-**5a** and (±)-**6a** [12]. We have also reported that a benzyl-substituent can be inserted at the *N*1, by treating (±)-**4** with benzylbromide and a catalytic amount of NaI (compounds (±)-**5b** and (±)-**6b**) [12]. The OBO ester can then conveniently be converted into the corresponding methyl ester and, at this stage, the mixture of diastereoisomers (±)-**7** and (±)-**8** can be separated by silica gel column chromatography. Following the same procedure used for the preparations of (±)-**7b** and (±)-**8b**, a series of new *N*1-arylmethyl derivatives was synthesized by treating (±)-**4** with the desired arylmethyl halide, in the presence of K_2CO_3 in THF heating at 85 °C under microwave irradiation (derivatives (±)-**5c**–**g** and (±)-**6c**–**g**). The couples of diastereoisomers (±)-**5a**–**g** and (±)-**6a**–**g** were in all cases separated by column chromatography after conversion into the corresponding methyl esters (±)-**7a**–**g** and (±)-**8a**–**g**.

The assignment of the relative configuration (2*S**,5′*R**) to diastereoisomers (±)-**7b**–**g** and (2*S**,5′*S**) to diastereoisomers (±)-**8b**–**g** was based on the comparison of the ^{1}H NMR spectra of each diastereoisomer with that of compounds (±)-**7a** and (±)-**8a**, respectively, whose relative configuration was previously unambiguously assigned by X-ray analysis [12].

Final amino acids (±)-(2*S**,5′*R**)-**1a**–**g** and (±)-(2*S**,5′*S**)-**2a**–**g** were obtained after alkaline hydrolysis of the methyl ester followed by cleavage of the Cbz protecting group with hydrobromic acid in a solution of acetic acid. In the case of derivatives (±)-**7f**,**g** and (±)-**8f**,**g**, both ester functions were hydrolyzed by treatment with 0.5 N NaOH (Scheme 1).

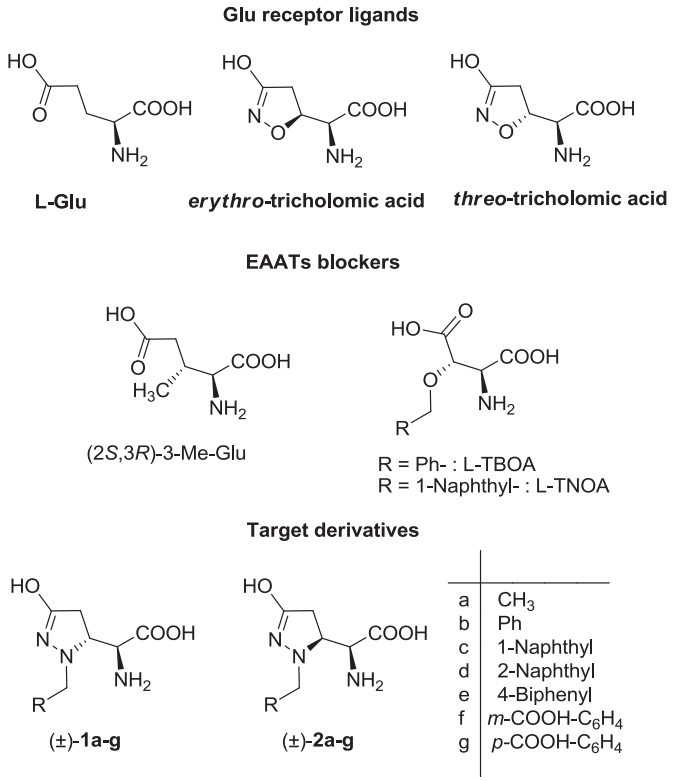
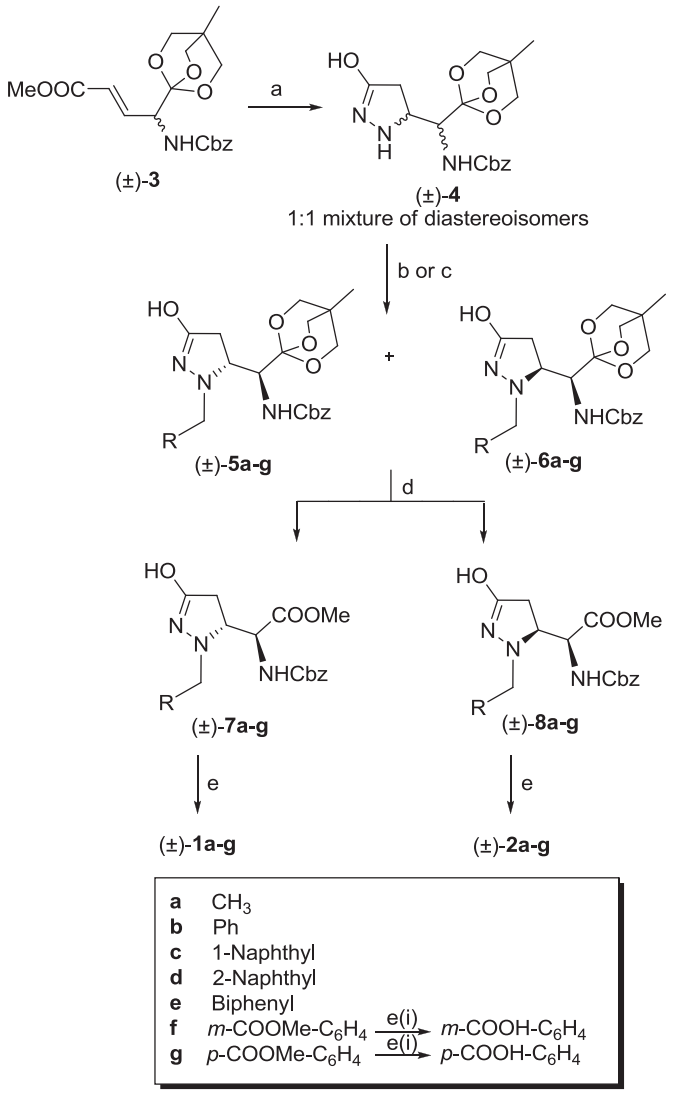


Fig. 1. Model and target compounds.



Scheme 1. Synthesis of the target compounds. Reagents and conditions. a: $NH_2NH_2{}^*H_2O$, EtOH, Δ; b: $ArCH_2Br$, K_2CO_3, NaI, THF, 85 °C μW; c: CH_3CHO, $NaBH_4$, MeOH [12]; d: i) PPTS, MeOH, H_2O, ii) K_2CO_3, MeOH; e: i) 0.5 N NaOH/H_2O, dioxane, rt, ii) 33% HBr/AcOH.