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Original article

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

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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].

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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).

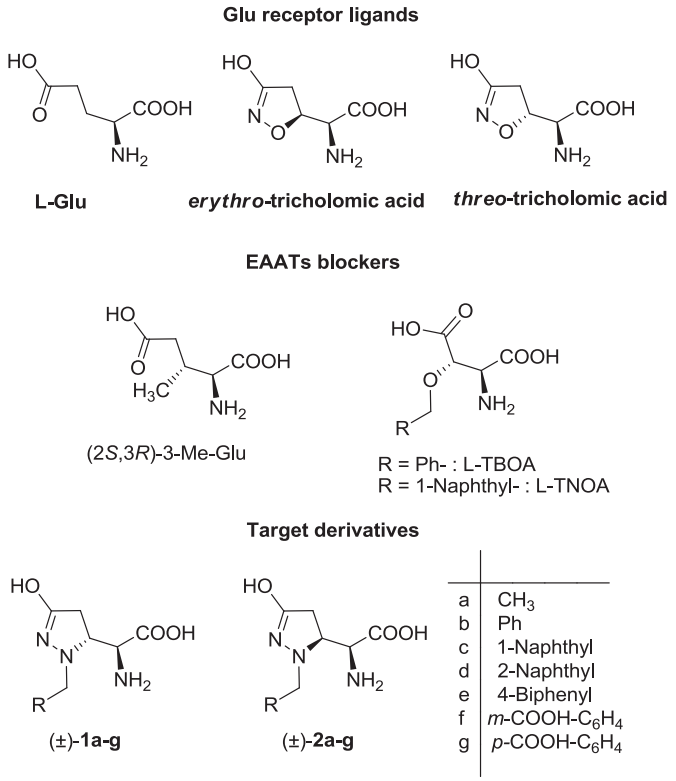
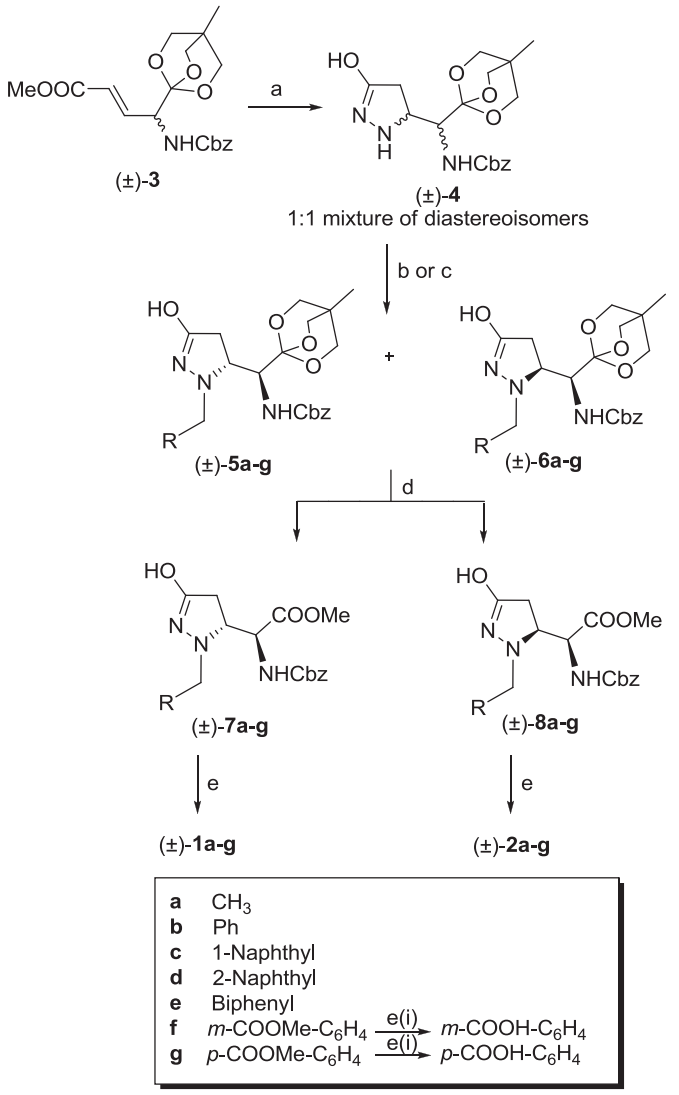


Fig. 1. Model and target compounds.



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.

Table 1
Affinity for iGluRs using rat cortical membranes.

Compound	[^{3}H]AMPA IC_{50} (μM)	[^{3}H]KA IC_{50} (μM)	[^{3}H]CGP 39653 K_i (μM)
(±)-*threo*-Tricholomic acid [9b]	19	6.0	73
L-*threo*-Tricholomic acid [9a]	>100	36 [4.45 ± 0.04]	95 [4.03 ± 0.05]
D-*threo*-Tricholomic acid [9a]	12 [4.92 ± 0.03]	11 [4.95 ± 0.04]	75 [4.13 ± 0.03]
(±)-**1a**	>100	>100	>100
(±)-**1b**	>100	>100	>100
(±)-**1c**	>100	80 [4.10 ± 0.05]	>100
(±)-**1d**	25 [4.60 ± 0.02]	>100	>100
(±)-**1e**	>100	>100	>100
(±)-**1f**	83 [4.08 ± 0.02]	22 [4.67 ± 0.09]	>100
(±)-**1g**	>100	>100	>100
(±)-*erythro*-Tricholomic acid [9b]	1.4	0.76	1.5
L-*erythro*-Tricholomic acid [9a]	0.95 [6.02 ± 0.01]	0.29 [6.55 ± 0.06]	41 [4.40 ± 0.07]
D-*erythro*-Tricholomic acid [9a]	>100	>100	0.67 [6.19 ± 0.05]
(±)-**2a**	>100	>100	>100
(±)-**2b**	>100	>100	>100
(±)-**2c**	>100	62 [4.21 ± 0.02]	>100
(±)-**2d**	>100	83 [4.08 ± 0.03]	>100
(±)-**2e**	>100	>100	>100
(±)-**2f**	>100	>100	>100
(±)-**2g**	>100	>100	>100

Data are given as mean [pIC_{50} ± SEM or pK_i ± SEM] of at least three independent experiments.

All derivatives were submitted to binding experiments, which were carried out on rat brain synaptic membranes of cortex, and the results are reported in Table 1.

These data show that, depending on the nature of the substituent, some of the new synthesized ligands are able to interact with either AMPA or KA receptors, with affinities in the mid-micromolar range. Notably, the 1-methylnaphthyl derivatives (±)-**1c** and (±)-**2c** bind to KA receptors with IC_{50}s of 80 μM and 62 μM, respectively, whereas in the case of the 2-methylnaphthyl derivatives (±)-**1d** and (±)-**2d** the binding preference depends on the relative stereochemistry: compound (±)-**1d** binds to AMPA receptors (IC_{50} = 25 μM), whereas its diastereoisomer (±)-**2d** weakly binds to KA receptors (IC_{50} = 83 μM). Also in the case of derivatives (±)-**1f** and (±)-**2f** the affinity was dependent on the relative stereochemistry: (±)-**1f** is a mixed AMPA/KA ligand, whereas its diastereoisomer (±)-**2f** is devoid of any affinity for iGluRs.

A docking study was performed to investigate the binding mode and address the observed differences in binding affinities across the iGluR classes. A database comprising (±)-**1a**–**g**, (±)-**2a**–**g** and the four stereoisomers of tricholomic acid was constructed. For all 32 compounds the distance between the amino acid and distal carboxylic acid functionality corresponds to the distance measured in (*S*)-Glu which dictates agonist activity [13]. The 32 compounds were therefore docked into the agonist and partial agonist states of GluA2-LBD (PDB code 1tfj and 1fw0 respectively) and homology models of GluK5 agonist and partial agonist states built from the corresponding GluK1-LBD X-ray structures (PDB code 1txf and 3c32, respectively). While the docking algorithm failed to produce reasonable binding modes of the 32 compounds in GluK5, the full agonist state of GluA2 was successful. The quality of the scoring function S (free energy in kcal/mol) was first assessed by evaluating the four stereoisomers of tricholomic acid. Following the ranking of binding affinities highest scoring was returned for Glu-binding mode of L-*erythro*-tricholomic acid, followed by D-*threo*-, L-*threo*- and D-*erythro* tricholomic acid (*S* = −9.2 (IC_{50} = 0.95 μM), −8.6 (IC_{50} = 12 μM), −8.5 (IC_{50} > 100 μM) and −7.9 (IC_{50} > 100 μM), respectively) (Fig. 2A). For **1a**–**g** and **2a**–**g**, the results were less clear and the medium range micromolar affinity of **1d** over **1a**–**c**, **e**–**g** could not be explained. However, calculated binding mode of the 2*S*,5*R* stereoisomers of series 1 imposes a ring flip of the heterocycle to relieve steric clash of the *N*1-substituent with residue Gly141 of the receptor (Fig. 2B). It can be hypothesized that the lack

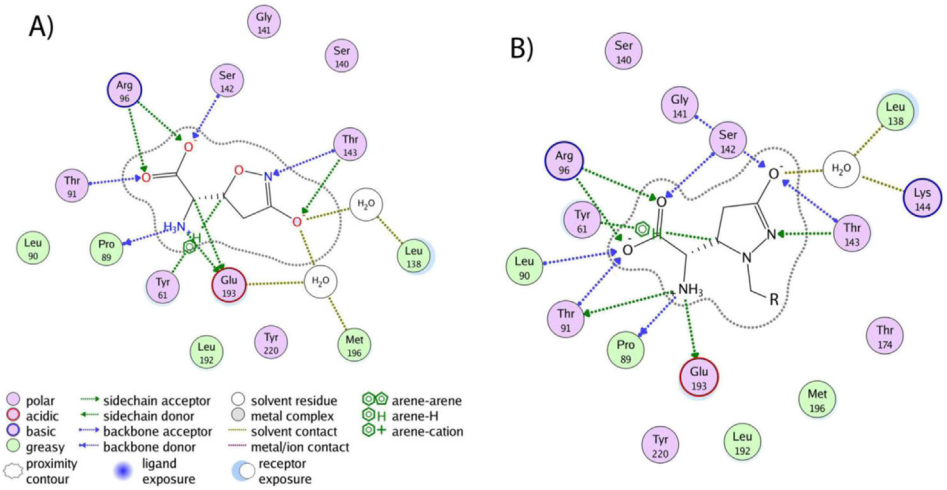


Fig. 2. A) In silico calculated binding mode of L-*erythro*-tricholomic acid in GluA2 being comparable to the binding mode of (*S*)-Glu determined by X-ray crystallography. B) In silico calculated binding mode of (2*S*,5*R*)-**1d** (*R* = 2-naphthyl). The heterocyclic skeleton is reoriented by rotation of the C2–C3 bond as to alleviate steric class between the *N*-substituent with Gly141 residue.

of binding affinity for this series could be due to disfavored disruption of the water matrix without installing favorable interactions between the *N*1-substituent and the receptor.

As anticipated, we have also tested our new derivatives as potential inhibitors of glutamate transporters EAAT1-3. In detail, the inhibition of the compounds at cloned human EAAT1-3 stably expressed in HEK293 cells was determined in a [^{3}H]-D-Asp uptake assay performed as previously described [14] using L-Glu and DL-TBOA as reference ligands. All the tested analogs proved to be inactive up to 300 or 1000 μM concentrations.

3. Conclusion

The results presented herein highlight that the 3-hydroxy-pyrazoline ring can be used in place of the 3-hydroxy-isoxazoline found in the natural product tricholomic acid to design new AMPA and KA receptor ligands. The insertion of bulky substituents at the *N*1 position of the pyrazoline ring may influence the preference of the ligand for either AMPA or KA receptors. The downside was a general decrease in the affinity, in particular if compared to the natural enantiomer L-*erythro*-tricholomic acid. We have also highlighted the impact of the relative stereochemistry of the two stereogenic centers on the biological activity. Efforts are ongoing to resolve the racemic mixture of (±)-**1f** to assess the contribute of each enantiomer to the observed mixed AMPA/KA binding profile.

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 constants (*J*) are expressed in Hz. Rotary power determinations were carried out using a Jasco P-1010 spectropolarimeter, coupled with a Haake N3-B thermostat. TLC analyses were performed on commercial silica gel 60 F_{254} aluminum sheets; spots were further evidenced by spraying with a dilute alkaline potassium permanganate solution or ninhydrin. Melting points were determined on a model B 540 Büchi apparatus and are uncorrected. MS analyses were performed on a Varian 320-MS triple quadrupole mass spectrometer with ESI source. Microanalyses (C, H, N) of new compounds were within ±0.4% of theoretical values.

Compounds (±)-**4**, (±)-**7a**, (±)-**8a**, (±)-**7b**, and (±)-**8b** were prepared as previously described [12].

4.2. General procedures

*A. General procedure for the synthesis of derivatives (±)-**5c**–**g** and (±)-**6c**–**g***: Compound (±)-**4** (1.00 mmol) was dissolved in dry THF (6 mL). The appropriate arylmethyl halide (1.00 mmol), K_2CO_3 (1.00 mmol) and NaI (0.10 mmol) were added to the solution. The reaction mixture was heated at 85 °C under microwave irradiation for 2 h. After disappearance of the starting material, the solvent was evaporated under vacuum and the residue was diluted with EtOAc (6 mL). The organic layer was washed with 1% NH_4Cl (6 mL), dried over anhydrous Na_2SO_4, filtered and evaporated to dryness. The product was isolated by column chromatography as a 1:1 mixture of diastereoisomers (±)-**5c**–**g** and (±)-**6c**–**g**.

*B. General procedure for the synthesis of derivatives (±)-**7c**–**g** and (±)-**8c**–**g***: **Step 1**. To a solution of the 1:1 mixture (±)-**5**/(±)-**6** (1.00 mmol) in MeOH (6 mL) and water (1.0 mL), a catalytic amount of PPTS (0.10 mmol) was added. The reaction mixture was stirred for 1 h at room temperature until completion. After evaporation of MeOH under reduced pressure, the residue was dissolved in EtOAc (4 mL) and washed with water (2 × 4 mL). The organic phase was dried over Na_2SO_4, filtered and concentrated in vacuum. **Step 2**. The crude obtained from the previous step (1.00 mmol) was dissolved in MeOH (4 mL) and K_2CO_3 (0.22 mmol) was added. The reaction mixture was stirred for 3 h at room temperature. The solvent was evaporated in vacuum, the residue was dissolved in EtOAc (4 mL) and washed with 3% NH_4Cl (4 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated to dryness. Purification via column chromatography allowed the separation of the two diastereoisomers (±)-**7c**–**g** and (±)-**8c**–**g**.

*C. General procedure for the synthesis of derivatives (±)-**1a**–**g** and (±)-**2a**–**g***: **Step 1**. The protected intermediate (±)-**7a**–**g** or (±)-**8a**–**g** (0.50 mmol) was dissolved in dioxane (3.0 mL) and treated with 0.5 N aqueous NaOH (3.0 mL). The reaction was stirred for 1.5 h at room temperature. The disappearance of the starting material was monitored by TLC. After evaporation of dioxane, the aqueous layer was washed with Et_2O, acidified to pH 2 with 2 N aqueous HCl, and extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4 and, after evaporation of the solvent, the carboxylic acid was obtained and directly used in the next step. **Step 2**. The carboxylic acid (0.50 mmol) was dissolved in a 33% solution of HBr in AcOH (4.4 mL). The reaction was stirred for 1 h at room temperature. The volatiles were removed under vacuum, the residue was dissolved in water and submitted to cation exchange chromatography using Amberlite IR-120 H. The acidic solution was slowly eluted onto the resin, and then the column was washed with water until the pH was neutral. The compound was eluted off the resin with 1 N aqueous ammonia, and the product-containing fractions (detected with ninhydrin stain on a TLC plate) were combined. The solvent was freeze–dried to give the final amino acid. Alternatively, the final amino acid was not submitted to cation exchange chromatography and was obtained as HBr salt by direct crystallization from ^{i}PrOH/Et_2O.

4.2.1. Benzyl (S*)-((R*)-3-hydroxy-1-(naphthalen-1-ylmethyl)-4,5-dihydro-1H-pyrazol-5-yl)(4-methyl-2,6,7-trioxabicyclo[2.2.2]octan-1-yl)methylcarbamate [(±)-5c] and benzyl (S*)-((S*)-3-hydroxy-1-(naphthalen-1-ylmethyl)-4,5-dihydro-1H-pyrazol-5-yl)(4-methyl-2,6,7-trioxabicyclo[2.2.2]octan-1-yl)methylcarbamate [(±)-6c]

The 1:1 mixture of diastereoisomers (±)-**5c** and (±)-**6c** was obtained in 60% yield following the general procedure A.

White foam. Column chromatography (silica gel, cyclohexane/EtOAc, 1:1 to 1:4). MS: 518.3 $[M + H]^+$.

4.2.2. Benzyl (S*)-((R*)-3-hydroxy-1-(naphthalen-2-ylmethyl)-4,5-dihydro-1H-pyrazol-5-yl)(4-methyl-2,6,7-trioxabicyclo[2.2.2]octan-1-yl)methylcarbamate [(±)-5d] and benzyl (S*)-((S*)-3-hydroxy-1-(naphthalen-2-ylmethyl)-4,5-dihydro-1H-pyrazol-5-yl)(4-methyl-2,6,7-trioxabicyclo[2.2.2]octan-1-yl)methylcarbamate [(±)-6d]

The 1:1 mixture of diastereoisomers (±)-**5d** and (±)-**6d** was obtained in 64% yield following the general procedure A. White foam. Column chromatography (silica gel, cyclohexane/EtOAc, 1:1, 100% EtOAc gradient). MS: 518.4 $[M + H]^+$.

4.2.3. Benzyl (S*)-((R*)-1-(biphenyl-4-ylmethyl)-3-hydroxy-4,5-dihydro-1H-pyrazol-5-yl)(4-methyl-2,6,7-trioxabicyclo[2.2.2]octan-1-yl)methylcarbamate [(±)-5e] and (S*)-((S*)-1-(biphenyl-4-ylmethyl)-3-hydroxy-4,5-dihydro-1H-pyrazol-5-yl)(4-methyl-2,6,7-trioxabicyclo[2.2.2]octan-1-yl)methylcarbamate [(±)-6e]

The 1:1 mixture of diastereoisomers (±)-**5e** and (±)-**6e** was obtained in 72% yield following the general procedure A. White foam. Column chromatography (silica gel, cyclohexane/EtOAc, 7:3, 100% EtOAc gradient). MS: 544.3 $[M + H]^+$.

4.2.4. Methyl 3-(((R)-5-((S*)-(benzyloxycarbonylamino)(4-methyl-2,6,7-trioxabicyclo[2.2.2]octan-1-yl)methyl)-3-hydroxy-4,5-dihydro-1H-pyrazol-1-yl)methyl)benzoate (±)-**5f** and methyl 3-(((S*)-5-((S*)-(benzyloxycarbonylamino)(4-methyl-2,6,7-trioxabicyclo[2.2.2]octan-1-yl)methyl)-3-hydroxy-4,5-dihydro-1H-pyrazol-1-yl)methyl)benzoate (±)-**6f***

The 1:1 mixture of diastereoisomers (±)-**5f** and (±)-**6f** was obtained in 80% yield following the general procedure A. White foam. Column chromatography (silica gel, 100% EtOAc). MS: 526.3 $[M + H]^+$.

4.2.5. Methyl 4-(((R)-5-((S*)-(benzyloxycarbonylamino)(4-methyl-2,6,7-trioxabicyclo[2.2.2]octan-1-yl)methyl)-3-hydroxy-4,5-dihydro-1H-pyrazol-1-yl)methyl)benzoate (±)-**5g** and methyl 4-(((S*)-5-((S*)-(benzyloxycarbonylamino)(4-methyl-2,6,7-trioxabicyclo[2.2.2]octan-1-yl)methyl)-3-hydroxy-4,5-dihydro-1H-pyrazol-1-yl)methyl)benzoate (±)-**6g***

The 1:1 mixture of diastereoisomers (±)-**5g** and (±)-**6g** was obtained in 79% yield following the general procedure A. White foam. Column chromatography (silica gel, 100% EtOAc). MS: 526.3 $[M + H]^+$.

4.2.6. (S)-Methyl 2-(benzyloxycarbonylamino)-2-((R*)-3-hydroxy-1-(naphthalen-1-ylmethyl)-4,5-dihydro-1H-pyrazol-5-yl)acetate [(±)-**7c**] and (S*)-methyl 2-(benzyloxycarbonylamino)-2-((S*)-3-hydroxy-1-(naphthalen-1-ylmethyl)-4,5-dihydro-1H-pyrazol-5-yl) acetate [(±)-**8c**]*

Compounds (±)-**7c** and (±)-**8c** were obtained following the general procedure B starting from the mixture (±)-**5c**/(±)-**6c**. Column chromatography (silica gel, eluent: cyclohexane/EtOAc, 1:1, 3:7 gradient).

Compound (±)-**7c**: 35% yield. Crystallized from EtOAc/*n*-hexane as white prisms. R_f: 0.60 (EtOAc); mp: dec >152 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): 2.45 (dd, J = 2.5, 17.5, 1H); 3.12 (dd, J = 9.4, 17.5, 1H); 3.45 (s, 3H); 4.12–4.23 (m, 2H); 4.35 (d, J = 12.1, 1H); 4.50 (dd, J = 2.5, 9.4, 1H); 5.04 (d, J = 12.1, 1H); 5.14 (d, J = 12.1, 1H); 5.45 (d, J = 9.4, 1H); 6.76 (s, 1H); 7.24–7.55 (m, 9H); 7.82–7.88 (m, 2H); 8.00–8.10 (m, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): 31.3, 52.7,, 58.5, 62.7, 63.8, 67.7, 124.4, 125.5, 126.4, 126.6, 128.3, 128.4, 128.8, 128.9, 129.3, 129.7, 131.0, 132.1, 134.2, 136.1, 157.1, 170.6, 173.4; MS: 448.2 $[M + H]^+$; Anal. calcd for $C_{25}H_{25}N_3O_5$: C 67.10, H 5.63, N 9.39, found: C 67.41, H 5.80, N 9.61.

Compound (±)-**8c**: 35% yield. Crystallized from *n*-hexane as colorless prisms. R_f: 0.52 (EtOAc); mp: dec >158 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): 2.58 (d, J = 17.2, 1H); 3.10 (dd, J = 8.8, 17.2, 1H); 3.66 (s, 3H); 3.81–3.86 (m, 1H); 4.21–4.70 (m, 3H); 4.86 (d, J = 12.1, 1H); 5.00 (d, J = 12.1, 1H); 5.34 (d, J = 8.5, 1H); 6.79 (s, 1H); 7.20–7.60 (m, 9H); 7.79 (d, J = 8.0, 2H); 8.15 (d, J = 8.5, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): 31.2, 52.8, 56.4, 63.1, 63.8, 67.2, 124.3, 125.4, 126.3, 126.9, 128.3, 128.4, 128.7, 129.0, 129.1, 129.7, 131.1, 132.2, 134.2, 136.2, 155.5, 170.3, 173.6; MS: 448.2 $[M + H]^+$; Anal. calcd for $C_{25}H_{25}N_3O_5$: C 67.10, H 5.63, N 9.39, found: C 67.38, H 5.82, N 9.58.

4.2.7. (S)-Methyl 2-(benzyloxycarbonylamino)-2-((R*)-3-hydroxy-1-(naphthalen-2-ylmethyl)-4,5-dihydro-1H-pyrazol-5-yl)acetate [(±)-**7d**] and (S*)-methyl 2-(benzyloxycarbonylamino)-2-((S*)-3-hydroxy-1-(naphthalen-2-ylmethyl)-4,5-dihydro-1H-pyrazol-5-yl) acetate [(±)-**8d**]*

Compounds (±)-**7d** and (±)-**8d** were synthesized following the general procedure B starting from the mixture (±)-**5d**/(±)-**6d**.

Compound (±)-**7d**: 38% yield. Crystallized from EtOAc and *n*-hexane as colorless rod-like crystals. R_f: 0.57 (CH_2Cl_2/MeOH, 9:1); mp: 141–143 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): 2.40 (dd, J = 2.6, 17.3, 1H); 2.97 (dd, J = 9.4, 17.3, 1H); 3.55 (s, 3H); 3.92 (d, J = 12.3, 1H); 4.01 (d, J = 12.3, 1H); 4.02–4.09 (m, 1H); 4.48 (dd, J = 3.2, 9.7, 1H); 5.09 (d, J = 12.2, 1H); 5.15 (d, J = 12.2, 1H); 5.51 (d, J = 9.4, 1H); 6.82 (bs, 1H); 7.31–7.38 (m, 6H); 7.48–7.53 (m, 2H); 7.68 (s, 1H); 7.79–7.84 (m, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): 31.7, 52.8, 58.4, 62.3, 65.2, 67.7, 126.6, 126.7, 127.3, 127.9, 128.1, 128.3, 128.5, 128.7, 128.8, 128.9, 132.9, 133.3, 133.4, 136.2, 157.2, 170.5, 173.5; MS: 448.3 $[M + H]^+$; Anal. calcd for $C_{25}H_{25}N_3O_5$: C 67.10, H 5.63, N 9.39, found: C 66.88, H 5.38, N 9.12.

Compound (±)-**8d**: 38% yield. Crystallized from EtOAc and *n*-hexane as colorless rod-like crystals. R_f: 0.46 (CH_2Cl_2/MeOH, 9:1); mp: 139–140 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): 2.53 (d, J = 17.1, 1H); 2.94 (dd, J = 9.7, 17.1, 1H); 3.72 (s, 3H); 3.80–3.85 (m, 1H); 3.98 (d, J = 12.2, 1H); 4.03 (d, J = 12.2, 1H); 4.28 (dd, J = 3.8, 7.9, 1H); 4.64 (d, J = 12.2, 1H); 4.92 (d, J = 12.2, 1H); 5.59 (d, J = 7.9, 1H); 6.81 (bs, 1H); 7.21–7.27 (m, 2H); 7.29–7.34 (m, 3H); 7.43–7.52 (m, 3H); 7.70 (s, 1H); 7.78–7.85 (m, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): 31.5, 52.8, 56.6, 63.4, 64.8, 67.0, 126.6, 126.6, 127.3, 128.0, 128.1, 128.2, 128.4, 128.7, 128.8, 128.9, 133.0, 133.3, 133.4, 136.3, 155.6, 170.3, 173.8; MS: 448.3 $[M + H]^+$; Anal. calcd for $C_{25}H_{25}N_3O_5$: C 67.10, H 5.63, N 9.39, found: C 66.90, H 5.42, N 9.15.

4.2.8. (S)-Methyl 2-(benzyloxycarbonylamino)-2-((R*)-1-(biphenyl-4-ylmethyl)-3-hydroxy-4,5-dihydro-1H-pyrazol-5-yl) acetate [(±)-**7e**] and (S*)-methyl 2-(benzyloxycarbonylamino)-2-((S*)-1-(biphenyl-4-ylmethyl)-3-hydroxy-4,5-dihydro-1H-pyrazol-5-yl)acetate [(±)-**8e**]*

Compounds (±)-**7e** and (±)-**8e** were synthesized following the general procedure B starting from the mixture (±)-**5e**/(±)-**6e.**

Compound (±)-**7e**: 40% yield. Crystallized from EtOAc and *n*-hexane as white prisms. R_f: 0.59 (EtOAc); mp: 138–141 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): 2.41 (dd, J = 2.4, 17.3, 1H); 2.94 (dd, J = 9.4, 17.3, 1H); 3.65 (s, 3H); 3.80 (d, J = 12.3, 1H); 3.89 (d, J = 12.3, 1H); 3.98–4.05 (m, 1H); 4.48 (dd, J = 3.2, 9.4, 1H); 5.10 (d, J = 12.3, 1H); 5.16 (d, J = 12.3, 1H); 5.53 (d, J = 9.4, 1H); 6.94 (bs, 1H); 7.26–7.39 (m, 8H); 7.42–7.48 (m, 2H); 7.54–7.59 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz): 31.7, 52.9, 58.3, 62.5, 64.8, 67.8, 127.3, 127.7, 127.8, 128.3, 128.5, 128.8, 129.1, 130.3, 134.2, 136.2, 140.6, 141.5, 157.1, 170.5, 173.3; MS: 474.3 $[M + H]^+$; Anal. calcd for $C_{27}H_{27}N_3O_5$: C 68.48, H 5.75, N 16.89, found: C 68.70, H 5.80, N 17.00.

Compound (±)-**8e**: 40% yield. Crystallized from EtOAc and *n*-hexane as white prisms. R_f: 0.50 (EtOAc); mp: 178–181 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): 2.52 (d, J = 16.9, 1H); 2.88 (dd, J = 9.1, 16.9, 1H); 3.73 (s, 3H); 3.76–3.81 (m, 1H); 3.86 (d, J = 12.4, 1H); 3.92 (d, J = 12.4, 1H); 4.28–4.34 (m, 1H); 4.91 (d, J = 12.1, 1H); 5.05 (d, J = 12.1, 1H); 5.65 (d, J = 7.6, 1H); 6.98 (bs, 1H); 7.21–7.38 (m, 7H); 7.40–7.48 (m, 2H); 7.53–7.60 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz): 31.5, 52.9, 56.6, 63.8, 64.4, 67.4, 127.3, 127.7, 127.8, 128.3, 128.5, 128.8, 129.1, 130.2, 134.4, 136.2, 140.6, 141.4, 155.7, 170.3, 173.6; MS: 474.3 $[M + H]^+$; Anal. calcd for $C_{27}H_{27}N_3O_5$: C 68.48, H 5.75, N 16.89, found: C 68.75, H 5.83, N 17.02.

4.2.9. Methyl 3-(((R)-5-((S*)-1-(benzyloxycarbonylamino)-2-methoxy-2-oxoethyl)-3-hydroxy-4,5-dihydro-1H-pyrazol-1-yl) methyl)benzoate [(±)-**7f**] and methyl 3-(((S*)-5-((S*)-1-(benzyloxycarbonylamino)-2-methoxy-2-oxoethyl)-3-hydroxy-4,5-dihydro-1H-pyrazol-1-yl)methyl)benzoate [(±)-**8f**]*

Compounds (±)-**7f** and (±)-**8f** were synthesized following the general procedure B starting from the mixture (±)-**5f**/(±)-**6f**.

Compound (±)-**7f**: 38% yield. Crystallized from *n*-hexane/EtOAc as white prisms. R_f: 0.50 (AcOEt); mp: 155–156 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): 2.41 (dd, J = 2.5, 17.6, 1H); 2.88 (dd, J = 9.4, 17.6, 1H); 3.66 (s, 3H); 3.84 (d, J = 12.7, 1H); 3.91 (s, 3H); 3.93 (d, J = 12.7, 1H); 4.00–4.07 (m, 1H); 4.50 (dd, J = 3.0, 9.6, 1H); 5.06 (d, J = 12.4, 1H); 5.15 (d, J = 12.4, 1H); 5.61 (d, J = 9.6, 1H); 7.10 (bs, 1H); 7.30–7.38 (m, 5H); 7.39–7.49 (m, 2H); 7.92 (s, 1H); 7.98 (d, J = 6.9, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): 31.5, 52.6, 53.0, 58.2, 62.7, 64.6, 67.8, 128.3, 128.5,

128.8, 129.2, 129.8, 130.9, 131.0, 134.4, 135.5, 136.1, 157.1, 166.8, 170.4, 173.4; MS: 456.2 $[M + H]^+$; Anal. calcd for $C_{23}H_{25}N_3O_7$: C 60.65, H 5.53, N 9.23, found: C 60.89, H 5.79, N 9.46.

Compound (±)-**8f**: 38% yield. Crystallized from *n*-hexane/EtOAc as white prisms. R_f: 0.40 (AcOEt); mp: 154–156 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): 2.52 (d, J = 17.3, 1H); 2.89 (dd, J = 9.4, 17.3, 1H); 3.65–3.80 (m, 1H), 3.72 (s, 3H); 3.88 (d, J = 12.0, 1H); 3.89 (s, 3H); 3.93 (d, J = 12.0, 1H); 4.32 (dd, J = 4.4, 8.5, 1H); 4.94 (d, J = 12.3, 1H); 5.05 (d, J = 12.3, 1H); 5.65 (d, J = 8.5, 1H); 7.10 (bs, 1H); 7.28–7.44 (m, 5H); 7.48–7.54 (m, 2H); 7.94–7.99 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): 31.4, 52.5, 53.0, 56.4, 64.0, 64.2, 67.4, 128.4, 128.5, 128.8, 129.2, 129.8, 130.9, 131.0, 134.3, 135.7, 136.2, 155.6, 166.8, 170.2, 173.5; MS: 456.2 $[M + H]^+$; Anal. calcd for $C_{23}H_{25}N_3O_7$: C 60.65, H 5.53, N 9.23, found: C 60.93, H 5.81, N 9.46.

4.2.10. Methyl 4-(((R*)-5-((S*)-1-(benzyloxycarbonylamino)-2-methoxy-2-oxoethyl)-3-hydroxy-4,5-dihydro-1H-pyrazol-1-yl) methyl)benzoate [(±)-7g] and methyl 4-(((S*)-5-((S*)-1-(benzyloxycarbonylamino)-2-methoxy-2-oxoethyl)-3-hydroxy-4,5-dihydro-1H-pyrazol-1-yl)methyl)benzoate [(±)-8g]

Compounds (±)-**7g** and (±)-**8g** were synthesized following the general procedure B starting from the mixture (±)-**5g**/(±)-**6g**.

Compound (±)-**7g**: 36% yield. Crystallized from *n*-hexane/EtOAc as white prisms. R_f: 0.6 (EtOAc); mp: 157–159 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): 2.41 (dd, J = 2.1, 17.6, 1H); 2.86 (dd, J = 9.4, 17.6, 1H); 3.66 (s, 3H); 3.85 (d, J = 13.0, 1H); 3.92 (s, 3H); 3.94 (d, J = 13.0, 1H); 3.98–4.06 (m, 1H); 4.51 (dd, J = 3.2, 9.4, 1H); 5.07 (d, J = 12.3, 1H); 5.15 (d, J = 12.3, 1H); 5.60 (d, J = 9.4, 1H); 7.10 (bs, 1H); 7.30–7.39 (m, 7H); 8.05 (d, J = 8.2, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): 31.5, 52.5, 53.0, 58.2, 62.8, 64.7, 67.8, 128.3, 128.5, 128.8, 129.7, 130.3, 130.4, 136.1, 140.4, 157.1, 166.8, 170.5, 173.5; MS: 456.2 $[M + H]^+$; Anal. calcd for $C_{23}H_{25}N_3O_7$: C 60.65, H 5.53, N 9.23, found: C 60.48, H 5.38, N 9.00.

Compound (±)-**8g**: 36% yield. Crystallized from *n*-hexane/EtOAc as white prisms. R_f: 0.51 (EtOAc); mp: 177–179 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): 2.51 (d, J = 17.3, 1H); 2.86 (dd, J = 9.1, 17.3, 1H); 3.71 (s, 3H); 3.71–3.80 (m, 1H); 3.88 (d, J = 13.0, 1H); 3.90 (s, 3H); 3.94 (d, J = 13.0, 1H); 4.30 (dd, J = 4.1, 7.9, 1H); 4.92 (d, J = 12.1, 1H); 5.07 (d, J = 12.1, 1H); 5.68 (d, J = 12.0, 1H); 7.12 (bs, 1H); 7.35–7.42 (m, 7H); 8.05 (d, J = 8.2, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): 31.5, 52.5, 53.0, 56.5, 64.0, 64.3, 67.4, 128.3, 128.5, 128.8, 129.7, 130.2, 130.3, 136.2, 140.5, 155.6, 166.9, 170.2, 173.7; MS: 456.2 $[M + H]^+$; Anal. calcd for $C_{23}H_{25}N_3O_7$: C 60.65, H 5.53, N 9.23, found: C 60.90, H 5.79, N 9.43.

4.2.11. (S*)-2-Amino-2-((R*)-1-ethyl-3-hydroxy-4,5-dihydro-1H-pyrazol-5-yl)acetic acid [(±)-1a]

Compound (±)-**1a** was synthesized following the general procedure C starting from intermediate (±)-**7a**.

White solid; 73% yield; R_f: 0.47 (*n*-butanol/H_2O/AcOH 4/2/1); mp: dec >106 °C; ^{1}H NMR (D_2O, 300 MHz): 0.95 (t, J = 7.3, 3H); 2.52 (dd, J = 1.8, 17.9, 1H); 2.76 (q, J = 7.3, 2H); 3.12 (dd, J = 8.2, 17.9, 1H); 3.51 (d, J = 8.5, 1H); 3.58 (ddd, J = 1.8, 8.2, 8.5, 1H); ^{13}C NMR (D_2O, 75 MHz): 11.2, 32.4, 53.4, 56.4, 61.4, 172.0, 175.5; MS: 188.0 $[M + H]^+$; Anal. calcd for $C_7H_{13}N_3O_3$: C 44.91, H 7.00, N 22.45, found: C 45.10, H 7.14, N 22.69.

4.2.12. (S*)-2-Amino-2-((S*)-1-ethyl-3-hydroxy-4,5-dihydro-1H-pyrazol-5-yl)acetic acid [(±)-2a]

Compound (±)-**2a** was synthesized following the general procedure C starting from intermediate (±)-**8a**.

White solid; 65% yield; R_f: 0.3 (*n*-butanol/H_2O/AcOH 4/2/1); mp: dec >100 °C; ^{1}H NMR (D_2O, 300 MHz): 0.96 (t, J = 7.2, 3H); 2.48 (dd, J = 3.8, 17.9, 1H); 2.68–2.84 (m, 2H); 2.96 (dd, J = 9.7, 17.9, 1H); 3.67 (d, J = 4.1, 1H); 3.73 (ddd, J = 3.8, 4.1, 9.7, 1H); ^{13}C NMR (D_2O, 75 MHz): 11.0, 31.2, 53.7, 56.2, 61.1, 171.2, 174.8; MS: 188.0 $[M + H]^+$; Anal. calcd for $C_7H_{13}N_3O_3$: C 44.91, H 7.00, N 22.45, found: C 45.07, H 7.11, N 22.60.

4.2.13. (S*)-2-Amino-2-((R*)-1-benzyl-3-hydroxy-4,5-dihydro-1H-pyrazol-5-yl)acetic acid [(±)-1b]

Compound (±)-**1b** was synthesized following the general procedure C starting from intermediate (±)-**7b**.

White solid; 61% yield; R_f: 0.7 (*n*-butanol/H_2O/AcOH 4/2/1); mp: dec >181 °C; ^{1}H NMR (D_2O, 300 MHz): 2.42 (dd, J = 1.9, 17.9, 1H); 2.68 (dd, J = 8.8, 17.9, 1H); 3.50 (d, J = 8.8, 1H); 3.72 (ddd, J = 1.9, 8.8, 8.8, 1H); 3.85 (d, J = 12.9, 1H); 3.95 (d, J = 12.9, 1H); 7.20–7.40 (m, 5H); ^{13}C NMR (D_2O, 75 MHz): 32.6, 56.3, 60.9, 62.5, 128.6, 129.0, 130.4, 134.9, 172.0, 175.7; MS: 250.1 $[M + H]^+$; Anal. calcd for $C_{12}H_{15}N_3O_3$: C 57.82, H 6.07, N 16.86, found: C 57.70, H 5.86, N 16.70.

4.2.14. (S*)-2-Amino-2-((S*)-1-benzyl-3-hydroxy-4,5-dihydro-1H-pyrazol-5-yl)acetic acid [(±)-2b]

Compound (±)-**2b** was synthesized following the general procedure C starting from intermediate (±)-**8b**.

White solid; 64% yield; R_f: 0.6 (*n*-butanol/H_2O/AcOH 4/2/1); mp: dec >235 °C; ^{1}H NMR (D_2O, 300 MHz): 2.40 (dd, J = 3.8, 17.7, 1H); 2.60 (dd, J = 9.4, 17.7, 1H); 3.55 (d, J = 4.1, 1H); 3.85 (ddd, J = 3.8, 4.1, 9.4, 1H); 3.87 (d, J = 12.9, 1H); 3.95 (d, J = 12.9, 1H); 7.28–7.35 (m, 5H); ^{13}C NMR (D_2O, 75 MHz): 31.4, 56.2, 60.5, 62.8, 128.7, 129.1, 130.3, 134.8, 171.2, 175.0. MS: 250.1 $[M + H]^+$; Anal. calcd for $C_{12}H_{15}N_3O_3$: C 57.82, H 6.07, N 16.86, found: C 57.65, H 5.80, N 16.68.

4.2.15. (S*)-2-Amino-2-((R*)-3-hydroxy-1-(naphthalen-1-ylmethyl)-4,5-dihydro-1H-pyrazol-5-yl)acetic acid hydrobromide [(±)-1c]

Compound (±)-**1c** was obtained as HBr salt following the general procedure C starting from intermediate (±)-**7c**.

White solid; 75% yield; R_f: 0.7 (*n*-butanol/H_2O/AcOH 4/2/1); mp: dec >170 °C; ^{1}H NMR (DMSO-d_6): 2.30 (d, J = 17.1, 1H); 3.03 (dd, J = 8.2, 17.1, 1H); 3.70–3.84 (m, 1H); 3.93 (dd, J = 8.2, 8.2, 1H); 4.36 (d, J = 13.2, 1H); 4.42 (d, J = 13.2, 1H); 7.42–7.64 (m, 4H); 7.84–7.96 (m, 2H); 8.24–8.33 (m, 1H); 8.36 (bs, 1H); 9.32 (bs, 1H); ^{13}C NMR (DMSO-d_6): 31.8, 54.9, 61.0, 61.7, 125.1, 126.1, 126.3, 126.8, 129.0, 129.0, 129.4, 132.5, 132.6, 134.1, 169.6, 173.2; MS: $[M + H]^+$ = 300.1. Anal. calcd for $C_{16}H_{18}BrN_3O_3$: C 50.54, H 4.77, N 11.05, found: C 50.18, H 4.98, N 10.75.

4.2.16. (S*)-2-Amino-2-((S*)-3-hydroxy-1-(naphthalen-1-ylmethyl)-4,5-dihydro-1H-pyrazol-5-yl)acetic acid hydrobromide [(±)-2c]

Compound (±)-**2c** was obtained as HBr salt following the general procedure C starting from intermediate (±)-**8c**.

White solid; 68% yield; R_f: 0.6 (*n*-butanol/H_2O/AcOH 4/2/1); mp: dec >248 °C; ^{1}H NMR (DMSO-d_6): 2.33 (dd, J = 2.1, 17.0, 1H); 2.80 (dd, J = 9.1, 17.0, 1H); 3.80–3.88 (m, 1H); 4.00–4.08 (m, 1H); 4.34 (d, J = 13.2, 1H); 4.42 (d, J = 13.2, 1H); 7.44–7.64 (m, 4H); 7.84–7.96 (m, 2H); 8.26 (d, J = 7.6, 1H); 8.40 (bs, 1H); 9.40 (bs, 1H); ^{13}C NMR (DMSO-d_6): 31.7, 54.9, 61.0, 61.9, 125.2, 126.1, 126.4, 127.0, 129.0, 129.0, 129.6, 132.4, 132.6, 134.1, 169.3, 172.8; MS: $[M + H]^+$ = 300.1. Anal. calcd for $C_{16}H_{18}BrN_3O_3$: C 50.54, H 4.77, N 11.05, found: C 50.10, H 4.88, N 10.85.

4.2.17. (S*)-2-Amino-2-((R*)-3-hydroxy-1-(naphthalen-2-ylmethyl)-4,5-dihydro-1H-pyrazol-5-yl)acetic acid hydrobromide [(±)-1d]

Compound (±)-**1d** was obtained as HBr salt following the general procedure C starting from intermediate (±)-**7d**.

White solid; 65% yield; R_f: 0.7 (*n*-butanol/H_2O/AcOH 4/2/1); mp: dec >144 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): 2.25 (d, J = 17.3, 1H);

2.68–2.80 (m, 1H); 3.80–3.88 (m, 2H); 4.03 (d, $J = 13.2$, 1H); 4.16 (d, $J = 13.2$, 1H); 7.46–7.54 (m, 2H); 7.60 (d, $J = 8.5$, 1H); 7.84–7.94 (m, 4H); 8.40 (bs, 1H); 9.47 (bs, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz): 32.2, 54.8, 61.2, 63.4, 126.6, 126.8, 128.3, 128.5, 128.6, 128.7, 129.2, 133.2, 133.5, 134.3, 169.7, 172.9; MS: 300.1 $[M + H]^+$; Anal. calcd for $C_{16}H_{18}BrN_3O_3$: C 50.54, H 4.77, N 11.05, found: C 50.30, H 5.05, N 10.98.

4.2.18. (S*)-2-Amino-2-((S*)-3-hydroxy-1-(naphthalen-2-ylmethyl)-4,5-dihydro-1H-pyrazol-5-yl)acetic acid hydrobromide [(±)-2d]

Compound (±)-**2d** was obtained as HBr salt following the general procedure C starting from intermediate (±)-**8d**.

White solid; 63% yield; R_f: 0.6 (*n*-butanol/H_2O/AcOH 4/2/1); mp: dec >233 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): 2.27 (dd, $J = 2.1$, 17.2, 1H); 2.45–2.58 (m, 1H); 3.86–3.98 (m, 2H); 4.00 (d, $J = 13.2$, 1H); 4.10 (d, $J = 13.2$, 1H); 7.46–7.56 (m, 2H); 7.62 (d, $J = 9.4$, 1H); 7.84–7.96 (m, 4H); 8.38 (bs, 1H); 9.45 (bs, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz): 32.0, 54.8, 61.1, 63.5, 126.7, 126.8, 128.3, 128.4, 128.4, 128.7, 129.5, 133.3, 133.5, 134.0, 169.3, 172.8; MS: 300.1 $[M + H]^+$; Anal. calcd for $C_{16}H_{18}BrN_3O_3$: C 50.54, H 4.77, N 11.05, found: C 50.18, H 4.90, N 10.94.

4.2.19. (S*)-2-Amino-2-((R*)-1-(biphenyl-4-ylmethyl)-3-hydroxy-4,5-dihydro-1H-pyrazol-5-yl)acetic acid hydrobromide [(±)-1e]

Compound (±)-**1e** was obtained as HBr salt following the general procedure C starting from intermediate (±)-**7e**.

White solid; 68% yield; R_f: 0.7 (*n*-butanol/H_2O/AcOH 4/2/1); mp: dec >178 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): 2.33 (d, $J = 17.3$, 1H); 2.63 (dd, $J = 7.9$, 17.3, 1H); 3.62–3.76 (m, 2H); 3.90 (d, $J = 13.5$, 1H); 4.00 (d, $J = 13.5$, 1H); 7.35 (t, $J = 7.3$, 1H); 7.42–7.54 (m, 4H); 7.62–7.70 (m, 4H); 8.40 (bs, 1H); 9.44 (bs, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz): 32.4, 55.3, 61.4, 62.7, 127.2, 127.3, 128.2, 129.7, 131.1, 135.8, 140.0, 140.5, 169.7, 173.2; MS: 326.2 $[M + H]^+$; Anal. calcd for $C_{18}H_{20}BrN_3O_3$: C 53.21, H 4.95, N 10.34, found: C 53.00, H 5.10, N 10.13.

4.2.20. (S*)-2-Amino-2-((S*)-1-(biphenyl-4-ylmethyl)-3-hydroxy-4,5-dihydro-1H-pyrazol-5-yl)acetic acid hydrobromide [(±)-2e]

Compound (±)-**2e** was obtained as HBr salt following the general procedure C starting from intermediate (±)-**8e**.

White solid; 62% yield; R_f: 0.7 (*n*-butanol/H_2O/AcOH 4/2/1); mp: dec >233 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): 2.25 (dd, $J = 2.1$, 17.0, 1H); 2.40 (dd, $J = 8.5$, 17.0, 1H); 3.86 (d, $J = 13.2$, 1H); 3.88–3.94 (m, 2H); 3.96 (d, $J = 13.2$, 1H); 7.35 (t, $J = 7.3$, 1H); 7.42–7.52 (m, 4H); 7.62–7.70 (m, 4H); 8.36 (bs, 1H); 9.45 (bs, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz): 32.2, 54.9, 60.9, 62.7, 127.2, 127.3, 128.2, 129.7, 131.4, 135.3, 140.1, 140.4, 169.3, 172.7; MS: 326.2 $[M + H]^+$; Anal. calcd for $C_{18}H_{20}BrN_3O_3$: C 53.21, H 4.95, N 10.34, found: C 53.03, H 5.05, N 10.21.

4.2.21. 3-(((R*)-5-((S*)-Amino(carboxy)methyl)-3-hydroxy-4,5-dihydro-1H-pyrazol-1-yl)methyl)benzoic acid hydrobromide [(±)-1f]

Compound (±)-**1f** was obtained as HBr salt following the general procedure C starting from intermediate (±)-**7f**.

White solid; 60% yield; R_f: 0.3 (*n*-butanol/H_2O/AcOH 4/2/1); mp: dec >165 °C; ^{1}H NMR (D_2O, 300 MHz): 2.42 (dd, $J = 1.5$, 17.9, 1H); 2.79 (dd, $J = 7.6$, 17.9, 1H); 3.80 (s, 1H); 3.82 (dd, $J = 1.5$, 7.6, 1H); 3.89 (d, $J = 13.2$, 1H); 3.97 (d, $J = 13.2$, 1H); 7.39 (t, $J = 7.6$, 1H); 7.71 (d, $J = 7.6$, 1H); 7.84–7.89 (m, 2H); ^{13}C NMR (D_2O, 75 MHz): 32.5, 55.2, 60.4, 62.1, 129.3, 129.8, 130.2, 131.2, 135.3, 135.4, 170.3, 170.4, 175.4; MS: 291.8 $[M - H]^-$; Anal. calcd for $C_{13}H_{16}BrN_3O_5$: C 41.73, H 4.31, N 11.23, found: C 41.61, H 4.54, N 10.92.

4.2.22. 3-(((S*)-5-((S*)-Amino(carboxy)methyl)-3-hydroxy-4,5-dihydro-1H-pyrazol-1-yl)methyl)benzoic acid hydrobromide [(±)-2f]

Compound (±)-**2f** was obtained as HBr salt following the general procedure C starting from intermediate (±)-**8f**.

White solid; 63% yield; R_f: 0.3 (*n*-butanol/H_2O/AcOH 4/2/1); mp: dec >183 °C; ^{1}H NMR (D_2O, 300 MHz): 2.40 (dd, $J = 2.3$, 17.9, 1H); 2.65 (dd, $J = 9.3$, 17.9, 1H); 3.82 (d, $J = 3.8$, 1H); 3.88 (d, $J = 13.2$, 1H); 3.90 (ddd, $J = 2.3$, 3.8, 9.3, 1H); 3.98 (d, $J = 13.2$, 1H); 7.40 (t, $J = 7.9$, 1H); 7.53 (d, $J = 7.9$, 1H); 7.85–7.91 (m, 2H); ^{13}C NMR (D_2O, 75 MHz): 31.6, 55.3, 60.8, 62.4, 129.3, 129.8, 130.3, 131.2, 135.2, 135.4, 169.9, 170.5, 175.7; MS: 291.8 $[M - H]^-$; Anal. calcd for $C_{13}H_{16}BrN_3O_5$: C 41.73, H 4.31, N 11.23, found: C 41.65, H 4.45, N 11.20.

4.2.23. 4-(((R*)-5-((S*)-Amino(carboxy)methyl)-3-hydroxy-4,5-dihydro-1H-pyrazol-1-yl)methyl)benzoic acid hydrobromide [(±)-1g]

Compound (±)-**1g** was obtained as HBr salt following the general procedure C starting from intermediate (±)-**7g**.

White solid; 66% yield; R_f: 0.3 (*n*-butanol/H_2O/AcOH 4/2/1); mp: dec >237 °C; ^{1}H NMR (D_2O, 300 MHz): 2.42 (dd, $J = 1.5$, 18.2, 1H); 2.79 (dd, $J = 7.9$, 18.2, 1H); 3.78–3.88 (m, 2H); 3.89 (d, $J = 13.2$, 1H); 3.99 (d, $J = 13.2$, 1H); 7.36 (d, $J = 8.2$, 2H); 7.86 (d, $J = 8.2$, 2H); ^{13}C NMR (D_2O, 75 MHz): 32.5, 55.2, 60.5, 62.3, 129.7, 130.1, 130.4, 140.5, 170.3, 170.5, 175.4; MS: 292.1 $[M - H]^-$; Anal. calcd for $C_{13}H_{16}BrN_3O_5$: C 41.73, H 4.31, N 11.23, found: C 41.52, H 4.35, N 11.08.

4.2.24. 4-(((S*)-5-((S*)-Amino(carboxy)methyl)-3-hydroxy-4,5-dihydro-1H-pyrazol-1-yl)methyl)benzoic acid hydrobromide [(±)-2g]

Compound (±)-**2g** was obtained as HBr salt following the general procedure C starting from intermediate (±)-**8g**.

White solid; 68% yield; R_f: 0.3 (*n*-butanol/H_2O/AcOH 4/2/1); mp: dec >225 °C; ^{1}H NMR (D_2O, 300 MHz): 2.41 (dd, $J = 1.8$, 18.2, 1H); 2.69 (dd, $J = 8.5$, 18.2, 1H); 3.86–4.02 (m, 4H); 7.39 (d, $J = 8.2$, 2H); 7.88 (d, $J = 8.2$, 2H); ^{13}C NMR (D_2O, 75 MHz): 31.7, 55.1, 60.9, 62.5, 129.7, 130.2, 130.3, 140.4, 169.6, 170.4, 175.7; MS: 291.7 $[M - H]^-$; Anal. calcd for $C_{13}H_{16}BrN_3O_5$: C 41.73, H 4.31, N 11.23, found: C 41.56, H 4.40, N 11.10.

4.3. Receptor binding assays

Affinities for native AMPA, KA, and NMDA receptors in rat cortical synaptosomes were determined using 5 nM [^{3}H]AMPA [15], 5 nM [^{3}H]KA [16], and 2 nM [^{3}H]CGP 39653 [17], respectively, with modifications as previously described [18]. Rat brain membrane preparations used in these receptor binding experiments were prepared according to a method previously described [19].

4.4. In silico study

The modeling study was performed using the software package MOE (Molecular Operating Environment, Chemical Computing Group, 2012) using the built-in mmff94x forcefield and the distance solvent model. Docking studies was performed using the standard setup, using *induced fit* as the method and exclusive water molecules. The receptor protein was prepared for docking by first running the algorithm Protonate 3D and scoring function *S* returned as Gibbs free energy in kcal/mol. Homology models were built using the standard setup.

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