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Structure and activity studies of glycine receptor ligands. Part 7. Structural remarks on arylidene–imidazoline-4-one glycinates and glycinamides

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Abstract

To improve the physicochemical properties (lipophilicity) of the potential ligands of glycine binding site of NMDA receptor, esters and amides of glycine derivatives of arylidene–imidazolidine-4-ones were obtained. The analysis of their properties (X-ray crystallography, NMR spectral data and theoretical calculations) was performed with respect to the existence of the probable tautomeric forms. Their possible interaction with hypothetical active points of the receptor (taking into the account the model of glycine binding site of NMDA receptor) was discussed on the basis of simulation with *IsoStar*. program. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Glycine receptor ligands; X-ray structure; Isostar

1. Introduction

As a part of our research program aimed at finding new ligands of the glycine — NMDA binding site, the glycine derivatives of arylidene–imidazoline-4-ones were prepared [1,2]. Compounds belonging to type **I** (Scheme1) have shown noticeable affinity to that receptor [1].

The preliminary analysis of structure-activity relationship has shown that the active arylidene–imidazolone glycines (I) possess $\log P \sim 1$. It is known that compounds acting at CNS should possess $\log P$ values of ca. 2 [3]. So, with the objective to improve the activity of this series of compound, increasing the $\log P$ value by chemical modification was postulated. At the same time, we were working on the three-dimensional model of ligand–receptor interaction, based on crystallographic data and molecular modelling results [4]. Moreover, it was presumed that an active form of imidazolone-glycines and -glycinates [4–6] could be probably of the **b**-tautomer form (Scheme 2).

The carboxyl group seemed to be the interesting region for the modification. Consequently, ester **II-2** was obtained and studied, including X-ray structure analysis [4]. Recently, additional ester **II-1** and series of amide derivatives **III** and **IV** were prepared (Table 1) and selected for pharmacological studies. Subsequently, structure and activity studies incorporating newly synthesised derivatives, particularly amides **III** and **IV**, were planned. Due to crystallisation

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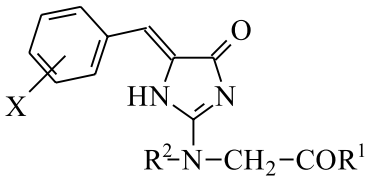


	R^1	R^2	ref.
I	OH	H	[1]
II	OC_2H_5	H	[4]
III	NH–R	H	present paper
IV	NH–R	CH_3CO	present paper

Scheme 1.

problems presented below the discussion is based on the molecular modelling and quantum chemistry calculations.

2. Materials and methods

2.1. Chemical part

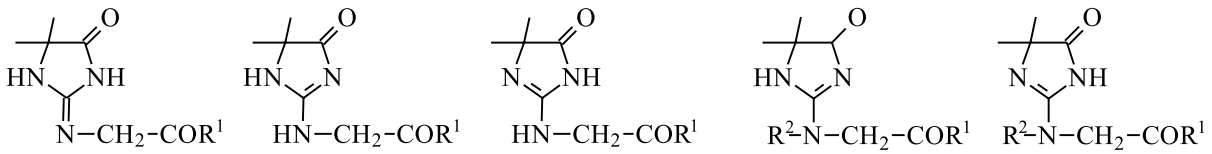
2.1.1. General procedure

As starting materials for the designed compounds, chlorosubstituted arylidene derivatives of 2-thiohydantoin were used [1,7], which reacted with methyl iodide giving the methylthioderivatives (Scheme 3). The methylthioderivatives were converted into aminoacids **I** upon reaction with glycine in refluxing acetic acid.

(a) Esters **II** were prepared according to previous work [8] as a result of acid catalysed esterification of **I**.
(b) For the amides **III**, **IV** synthesis, as starting material, the bicyclic acetyl imidazo[2,1-b]imidazoline-3,5-dione was used. This compound was obtained as the result of **I** cyclisation upon influence of acetic anhydride [8]. The bicyclic derivative was cleaved with two fold excess of ammonia [8] and different amines: (un)substituted-benzyl amines, 2-phenylethylamine, and 2-(*N*-morpholine) ethylamine. The reactions were carried out in heterogeneous conditions, in toluene suspensions. Upon action of substituted ethylamines, the deacetylated derivatives of group **III** were obtained. In the reactions with benzylamines, mainly the acetyl derivatives of group **IV** were obtained. In the case of *o*-chlorobenzylamine both types of compounds **III-3** and **IV-5** were isolated. The purity of the compounds obtained was checked by means of the thin layer chromatography. Their structures were confirmed on basis of elemental and spectral analyses (^{1}H-NMR).

2.1.2. Experimental

Uncorrected mp's were determined on Wagner Munz apparatus. ^{1}H-NMR spectra were recorded on Gemini 200 or Bruker VM250 in d_6DMSO, chemical shifts are reported in [ppm] values relative to internal



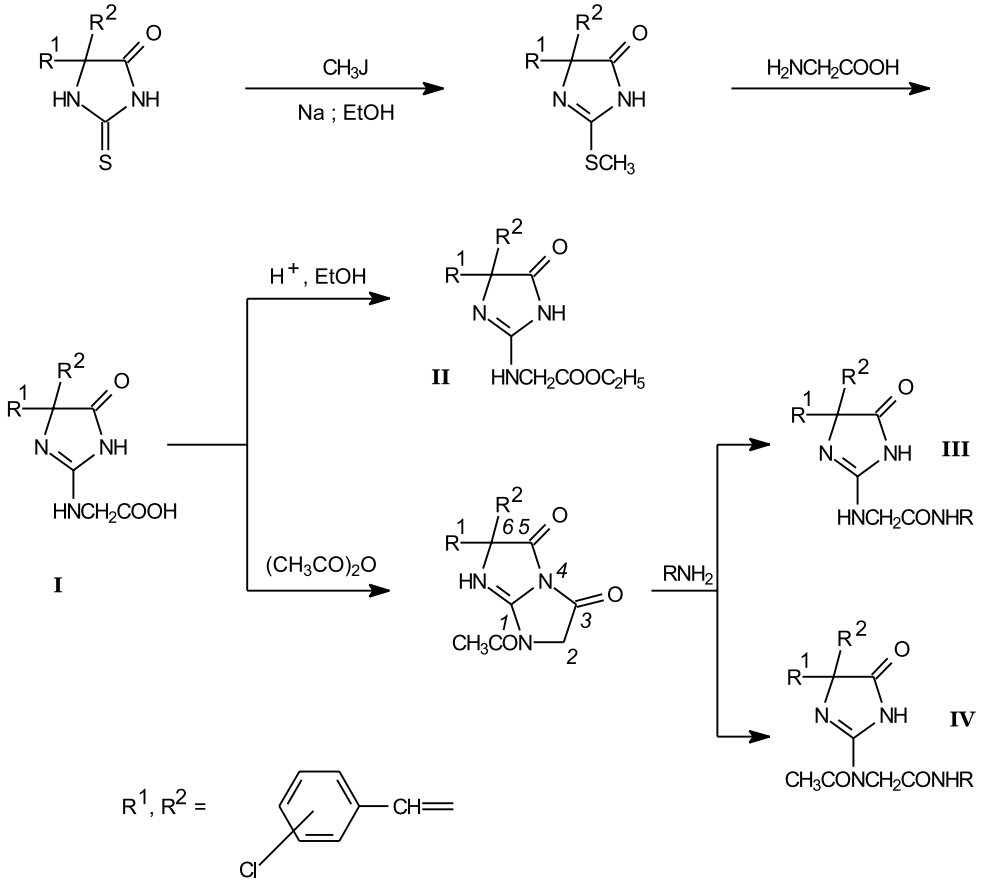
a **b** **c** **b** **c**

for **I, II** and **III** for **IV**

Scheme 2.

Table 1
Chemical details and biological evaluation results for esters and amide derivatives of the types **II–IV** from Scheme 1

	X	R^2	R^1	Inhibition (%)	ASP	log *P*	Ref.
II-1	*m*-Cl	H	OC_2H_5	40	**III**	2.09	
II-2	*p*-Cl	H	OC_2H_5	1.7	**I**	2.03	[8]
III-1	*p*-Cl	H	NH–CH_2CH_2–Morph	14	**III**	− 0.31	
III-2	*p*-Cl	H	NH–CH_2CH_2–Ph	6	**III**	2.64	
III-3	*p*-Cl	H	NH–CH_2CH_2–*o*-Cl–Ph	-	-	2.67	
IV-1	*p*-Cl	CH_3CO	NH_2	6.4	**III**	− 0.17	[8]
IV-2	*p*-Cl	CH_3CO	NH-CH_2-Ph	9.2	**I**	1.64	[8]
IV-3	*p*-Cl	CH_3CO	NH–CH_2–*p*-Cl–Ph	12	**I**	2.36	
IV-4	*p*-Cl	CH_3CO	NH-CH_2-*m*-Cl-Ph	-	**III**	2.29	
IV-5	*p*-Cl	CH_3CO	NH-CH_2-*o*-Cl-Ph	-	**I**	2.22	
IV-6	*p*-Cl	CH_3CO	NH–CH_2–*p*-F–Ph	11	**III**	1.79	
IV-7	*p*-Cl	CH_3CO	NH–CH_2–*p*-OCH_3–Ph	4	**III**	1.53	



Scheme 3.

reference of TMS. Infrared spectra were measured with FT IR 410 spectrometer (Jasco) in KBr pellets. The elemental analyses were performed for C, H, N and results were within ±0.4% of theoretical values.

TLC was conducted on Al sheets, 0.2 mm layer of silica gel ($60F_{254}$, Merck). The developing systems used were (A) $CHCl_3$/izopropanol/NH_3 aq. 9:11:2; (B) $CHCl_3$/acetone: 1:1.

2.1.2.1. Ethyl-N-acetyl-N-[5-(Z)-(3-chlorobenzylidene)-4-oxo-2-imidazolidinyl]glycinate **II-1**. The suspension of *N*-[5-(*Z*)-(3-chlorobenzylidene)-4-oxo-2-imidazolidinyl] glycine [1] (2.8 g, 0.01 mol) in 100 ml of ethanol was refluxed for 5 h with 1.5 ml of conc. H_2SO_4. After cooling the precipitate was filtered out and recrystallised from ethanol. $C_{14}H_{14}N_3O_3$ (307.73); mp 231–234°C (EtOH); Yield 44%; R_f(A) = 0.80; ^{1}H-NMR (200 MHz) $\sigma = 1.22$ (t, $J = 7.09$ Hz, 3H, CH_3); 4.13 (s, 2H, HNCH_2); 4.16 (q, $J = 7.10$ Hz, CH_2CH$_3$); 6.34 (s, 1H, CH=); 7.33 (m, 2H, H-4, H-5); 7.82 (br.d, $J = 4.64$ Hz, 1H, H-6); 8.21 (br.s, 1H, H-2); IR (KBr) ν: 3330, 3173 (NH), 1736 (C_4=O), 1694 (COO), 1671 (ArCH=), 1599, 1507, 1219, 1120, 900, 783, 686 cm^{-1}.

2.1.2.2. N-[5-(Z)-(4-chlorobenzylidene)-4-oxo-2-imidazolidinyl]glycin [2-(N-morpholinoethyl)]amide **III-1**. To the stirred suspension of 1-acetyl-6-(*Z*)-(4-chlorobenzylidene) 2,3,5,6-tetrahydroimidazo-[2,1-b]imidazole-3,5-dione [8] (0.303 g, 1 mmol in 10 ml of toluene *N*-(2-aminoethyl)-morpholine (0.260 g, 2 mmol) was added. The mixture was refluxed for 5 h. The precipitate was filtered off and recrystallised from ethanol. $C_{20}H_{24}N_5O_4Cl$ (433.99); mp 254–256°C (EtOH); yield 43%; ^{1}H-NMR (250 MHz) $\sigma = 2.33$ (m, 6H, $CH_2N(CH_2)_2$); 3.22 (m, 2H, NHCH_2CH$_2$); 3.52 (m, 4H, O$(CH_2)_2$); 3.94 (d, $J = 5.0$ Hz, 2H, NHCH_2CO); 6.28 (s, 1H, CH=); 7.35 (d, $J = 7.5$ Hz, 2H, H-3′, H-5′); 7.54 (t, $J = 5.0$ Hz, 1H, $NHCH_2$); 8.05 (d, $J = 7.5$ Hz, 2H, H-2′, H-6′); 8.09 (t, 1H, CONH); 10.24 (br.s, 1H, (8%) 1-NH); 10.70 (br.s, 1H, (92%) 3-NH); IR (KBr) ν: 3398, 3293 (NH), 2941, 2890, 2856, 2810 (CH_2), 1726 (CH_4=O), 1690 (C=O), 1661 (ArCH=), 1596, 1503, 1259, 1117, 1084, 1037, 679 cm^{-1}.

2.1.2.3. N-[5-(Z)-(4-chlorobenzylidene)-4-oxo-2-imidazolidinyl]glycin[2-(phenylethyl)]amide **III-2**. **III-2** was obtained as described for **III-1**. $C_{20}H_{19}N_4O_2Cl$ (382.84); mp 237–240°C (DMF); yield 68%; R_f(A) = 0.70; ^{1}H-NMR (200 MHz) $\sigma = 2.75$ (t, $J = 7.70$ Hz, 2H, CH_2Ph); 3.33 (def t, $J = 6.81$ Hz, 2H, NHCH_2CH$_2$); 3.99 (s, 2H, HNCH_2); 7.11–7.28 (m, 5H, H-2′, H-3′, H-4′, H-5′, H-6′); 7.38 (d, $J = 8.33$ Hz, 2H, H-3, H-5); 7.56 (br.s, 1H, NH); 8.09 (d, $J = 8.00$ Hz, 2H, H-2′, H-6′); 8.22 (def t, $J = 5.29$ Hz, 1H, CONH); 10.35 [s, 1H, (9%) 1-NH; 10.71 (s, 1H, (91%) 3-NH); IR (KBr) ν: 3282 (NH), 1704 (C_4=O), 1674 (C=O), 1652 (ArCH=), 1607, 1507, 1282, 1088, 864, 820, 682 cm^{-1}.

2.1.2.4. N-[5-(Z)-(4-chlorobenzylidene)-4-oxo-2-imidazolidinyl]glycin-2-chlorobenzamide **III-3**. To the DMF filtrate after **IV-5** separation water was added. The obtained precipitate was recrystallised from DMF/H_2O to give **III-3**. $C_{19}H_{16}N_4O_2Cl_2$ (403.26); mp 228–230°C (DMF/H_2O); yield 41%; R_f(B) = 0.41; ^{1}H-NMR (250 MHz) $\sigma = 4.15$ (d, $J = 4.75$ Hz, 2H, NHCH_2); 4.48 (d, $J = 5.75$ Hz, 2H, NHCH_2CO); 6.39 (s, 1H, CH=); 6.99–7.07 (m, 1H, H-5″); 7.19–7.31 (m, 1H, H-4″); 7.43 (d, $J = 8.75$ Hz, 2H, H-3′, H-5′); 7.38–7.62 (m, 2H, H-3″, H-6″); 7.76 (br.s, 1H, N*H*CH$_2$); 8.17 (d, $J = 8.5$ Hz, 2H, H-2′, H-6′); 8.69 (t, $J = 5.75$ Hz, 1H, N*H*CH$_2$CO); 10.35 (s, 1H, (8%) 1-NH); 10.88 (s, 1H, (92%) 3-NH); IR (KBr) ν: 3301, 3085 (NH), 1706 (C_4=O), 1662 (C=O), 1618 (ArCH=), 1515, 1278, 1247, 1117, 1089, 818, 748, 683 cm^{-1}.

2.1.2.5. N-acetyl-N-[5-(Z)-(4-chlorobenzylidene)-4-oxo-2-imidazolidynyl]glycin-4-chlorobenzamide **IV-3**. **IV-3** was obtained as **IV-5**. $C_{21}H_{18}N_4O_3Cl_2$ (445.29); mp 257–258°C (DMF); yield 56%; ^{1}H-NMR (200 MHz) $\sigma = 2.40$ (s, 3H, CH_3CO); 4.32 (d, $J = 5.74$ Hz, 2H, NHCH$_2$); 4.68 (s, 2H, NCH$_2$); 6.83 (s, 1H, CH=); 7.15 (d, $J = 8.39$ Hz, 2H, H-3′, H-5″); 7.25 (d, $J = 8.44$ Hz, 2H, H-2″, H-6″); 7.35 (d, $J = 8.45$, 2H, H-3′, H-5′); 8.12 (d, $J = 8.49$ Hz, 2H, H-2′, H-6′); 8.87 (t, $J = 5.50$ Hz, 1H, N*H*CH$_2$); 11.30 (s, 1H, 1-NH); IR (KBr) ν: 3332, 3287 (NH), 1721 (C_4=O), 1688 (C=O), 1644 (ArCH=), 1554, 1464, 1213, 1090, 992, 818, 670 cm^{-1}.

2.1.2.6. N-acetyl-N-[5-(Z)-(4-chlorobenzylidene)-4-oxo-2-imidazolidinyl]glycin-3-chlorobenzamide **IV-4**. **IV-4** was obtained as **IV-5**. $C_{21}H_{18}N_4O_3Cl_2$ (445.29); mp 236–238°C (DMF); yield 28%; R_f(B) =

0.65; ^{1}H-NMR (250 MHz) $\sigma = 2.40$ (s, 3H, CH_3CO); 4.34 (d, $J = 5.80$ Hz, 2H, NHCH_2); 4.68 (s, 2H, NCH_2); 6.83 (s, 1H, CH=); 7.13–7.33 (m, 3H, H-4″, H-5″, H-6″); 7.20 (s, 1H, H-2″); 7.35 (d, $J = 7.50$ Hz, 2H, H-3′, H-5′); 8.12 (d, $J = 7.50$ Hz, 2H, H-2′, H-6′); 8.89 (t, $J = 6.2$ Hz, 1H, N*H*CH_2); 11.31 (s, 1H, 1-NH).

2.1.2.7. N-acetyl-N-[5-(Z)-(4-chlorobenzylidene)-4-oxo-2-imidazolidinyl]glycin-2-chlorobenzamide **IV-5**. The suspension of 1-acetyl-6-(*Z*)-(4-chlorobenzylidene)-2,3,5,6-tetrahydroimidazo-[2,1-b]-imidazole-3,5-dione [8] (1.51 g, 5 mmol) and 2-chlorobenzylamine (1.41 g, 10 mmol) in 50 ml of toluene was refluxed for 5 hr. The precipitate was filtred off and recrystallised from DMF to give **IV-5**. $C_{21}H_{18}N_4O_3Cl_2$ (445.29); mp 248–250°C (DMF); yield 30%; ^{1}H-NMR (250 MHz) $\sigma = 2.47$ (s, 3H, CH_3CO); 4.46 (d, $J = 5.50$ Hz, 2H, CH_2NH); 4.80 (s, 2H, CH_2CO); 6.91 (s, 1H, CH=); 7.03 (dt., J = 7.5, 1.25 Hz, 1H, H-5″); 7.27 (dt, $J = 7.75$, 1.75 Hz, 1H, H-4″); 7.38–7.48 (m, 2H, H-3″, H = 6″); 7.46 (d, $J = 8.50$ Hz, 2H, H-3′, H-5′), 8.23 (d, $J = 8.75$ Hz, 2H, H-2′, H-6′); 8.95 (t, $J = 5.0$ Hz, 1H, N*H*CH_2); 11.38 (s, 1H, 1-NH); IR (KBr) ν: 3317, 3073 (NH), 2981, 2930 (CH_2), 1728 (C_4=O), 1692 (C=O), 1644 (ArCH=), 1548, 1213, 1085, 992, 824, 752 cm^{-1}.

2.1.2.8. N-acetyl-N-[5-(Z)-(4-chlorobenzylidene)-4-oxo-2-imidazolidinyl]glycin-4-fluorobenzamide **IV-6**. **IV-6**was obtained as **IV-5**. $C_{21}H_{18}N_4O_3ClF$ (428.84); mp 255–258°C (DMF); yield 61%; R_f(A) = 0.85; ^{1}H-NMR (200 MHz) $\sigma = 2.40$ (s, 3H, CH_3CO); 4.32 (d, $J = 5.76$ Hz, 2H, NHCH_2); 4.68 (s, 2H, NCH_2); 6.84 (s, 1H, CH=), 6.95 (dt, $J = 9.00$, 2.33 Hz, 2H, H-2″, H-6″); 7.27 (dt, $J = 9.00$, 2.23 Hz, 2H, H-3″, H-5″); 7.35 (d, $J = 8.62$ Hz, 2H, H-3′, H-5′); 8.12 (d, $J = 8.52$ Hz, 2H, H-2′, H-6′); 8.87 (t, $J = 5.96$ Hz, 1H, N*H*CH_2); 11.32 (s, 1H, 1-NH); IR (KBr) ν: 3321 (NH), 1729 (C_4=O), 1694 (C=O), 1664 (ArCH=), 1548, 1213, 994, 822, 670 cm^{-1}.

2.1.2.9. N-acetyl-N-[5-(Z)-(4-chlorobenzylidene)-4-oxo-2-imidazolidinyl]glycin-4-methoxybenzamide **IV-7**. **IV-7** was obtained as **IV-5**. $C_{22}H_{21}N_4O_4Cl$ (440.88); mp 248–250°C (DMF); yield 68%; R_f(A) = 0.85; ^{1}H-NMR (250 MHz) $\sigma = 2.37$ (s, 3H, CH_3CO); 3.64 (s, 3H, OCH_3); 4.24 (d, $J = 7.50$ Hz, 2H, NHCH_2); 4.64 (s, 2H, NCH_2); 6.69 (dd, $J = 7.5$, 2.5 Hz, 2H, H-2″, H-6″); 6.81 (s, 1H, CH=); 7.15 (dd, $J = 7.5$, 2.5 Hz, 2H, H-3″, H-5″); 7.32 (dd, $J = 8.5$, 2.0 Hz, 2H, H-3′, H-5′); 8.08 (dd, $J = 8.5$, 2.0 Hz, 2H, H-2′, H-6′); 8.75 (t, $J = 7.50$ Hz, 1H, N*H*CH_2); 11.28 (s, 1H, 1-NH); IR (KBr) ν: 3308, 3286 (NH), 1726 (C_4=O), 1690 (C=O), 1648 (ArCH=), 1550, 1250, 1212, 1084, 992, 825, 811 cm^{-1}.

2.2. Pharmacological part

2.2.1. Receptor binding determinations

Radioligand binding studies were performed according to the method described by Grimwood et al. [9]. Affinities of tested compounds **II-1**, **II-2**; **III-1**, **III-2**; **IV-1**–**IV-3**, **IV-6**, **IV-7** used at concentrations of 100 μM, for the glycine site of the NMDA receptor were determined by displacement of the glycine site antagonist [^{3}H]-L-689,560 binding to rat cortex (hippocampus membranes). The percent of inhibition of the ligand was estimated and the results are presented in Table 1.

2.2.2. Anticonvulsant assays

All animal anticonvulsant and neurotoxicity assays were conducted by the Antiepileptic Drug Development Program, Epilepsy Branch, Neurological Disorders Program, National Institutes of Neurological and Communicative Disorders and Stroke (NINCDS) according to the testing procedures described earlier [16,17]. Phase I of anticonvulsant screening project (ASP), involved three tests: maximal electroshock seizure (MES), subcutaneous pentylenetetrazol (ScMet), and neurological toxicity (Tox). All compounds apart from **III-3** were injected intraperitoneally into mice as suspensions in 0.5% methylcellulose. Small groups of animals were used (1–8) and dose levels were 30, 100 and 300 mg/kg. These data are presented in Table 1. The classifications were as follows: I: anticonvulsant activity at 100 mg/kg or less; II: anticonvulsant activity at doses larger than 100 mg/kg; III: compound inactive at 300 mg/kg.

2.3. Computational procedure

All molecules were modelled based on the crystallographic structure of **II-2** [4] and roughly optimised using PCMODEL.6 program [10]. MO calculations were carried out with AM1 method [10] using MOPAC program (version 6.0) [11] in water environment

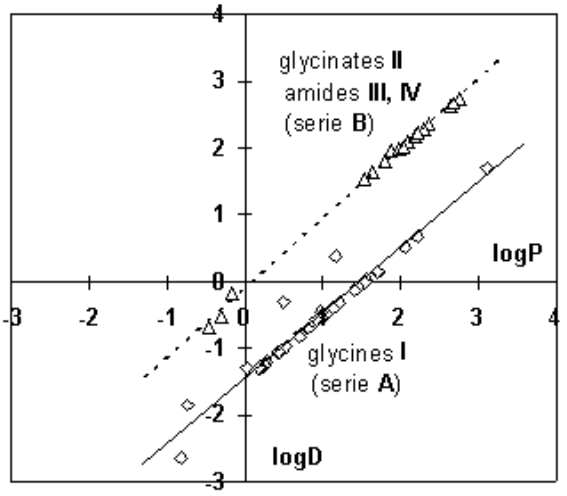


Fig. 1. Scattergram for log *D* vis log *P*.

(dielectric constant 78.4). The values of log *P* were calculated by means of PALLAS (version 1.2) program [12].

3. Results and discussion

The differences in chemical nature of all the investigated compounds (Scheme 1) become clear after looking at Fig. 1. So, all arylidene–imidazolone glycine derivatives studied until now are classified in two groups. The first group incorporates acidic glycines **I** (set **A**) with variously substituted aromatic ring, the second one — glycinates **II**, and amides **III** and **IV** (set **B**). Synthesis and pharmacological test results of set **B** were a direct outcome of our search for modified arylidene–imidazolone glycines (set **A**) with increased log *P* values [3]. However, independent of log *P* value, the compounds from set **B** are explicitly less interesting than parent glycines **I**. In the *in vitro* binding assays, all modified compounds of series **B** possess lower inhibition to the receptor, only the ester **II-1** (from family of *m*-Cl arylidene derivatives) showed noticeable affinity to the receptor (40% of inhibition), yet, it was still lower than that of the parent molecule **I-1** (90% of inhibition)[1]. Hence, just two acid–ester pairs are insufficient for general conclusion, the activities of acids (Table 1, **I-1** and **I-2**) are almost exactly 50 units (%) higher than that of esters (**II-1** and **II-2**). At the same time, the activities of both *m*-Cl-substituted species are almost 40 units (%) higher than that of *p*-Cl ones. Remaining compounds (**III** and **IV** amides) are practically inactive, independently of log *P* value (averaged inhibition ~8%).

During the *in vivo* anticonvulsant tests (Phase I ADD Program), all compounds in doses up to 300 mg/kg were devoid of neurotoxicity. In the group of esters (**II**), *p*-chloro substituted **II-2** was evaluated as active and classified to the class I ASP. Only three amides, namely: benzyl, *p*-chlorobenzyl, and *o*-chlorobenzyl amides were rated among the class I ASP. The other compounds were found to be inactive.

3.1. Imidazolone–glycines tautomerisation

Three resonance structures for imidazolone–glycine derivatives are possible (Scheme 2). It has been shown [4,5] that the concentration of **b**-tautomer is essential for the inhibition to the glycine receptor. In order to understand the relationship between structure and affinity to the receptor based on the model presented previously [4], this concentration should be estimated tentatively. This can be achieved via ^{1}H-NMR spectra analysis and/or based on theoretical calculations. It is also of special interest to compare the results from both analyses.

Glycinamides of type **III** may exist in all three tautomeric forms (Scheme 2). However, the spectral analyses revealed that these derivatives exist (in DMSO) in solution mainly in **c**-form. In the ^{1}H-NMR spectra, the double set of signals was related to protons attached to the imidazolone ring nitrogens at positions 1-NH and 3-NH. The singlets at 10.24–10.35 ppm were related to protons attached to the nitrogen at position 1 of the imidazolone ring (**b**-tautomer), and the singlets at 10.70–10.88 ppm — to more acidic protons attached to the nitrogen at position three of the imidazolone ring (**c**-tautomer). The signals of protons from the exocyclic NH groups of **b**-tautomers were very weak and hidden under the aromatic proton signals. The analysis of the signals' integration of 1-N and 3-N protons enabled us to conclude that in the described amides the **c**-tautomers were observed in predominance to the **b**-ones (ca. 9:1 ratio) (Table 2), contrary to the previously described

Table 2
Imidazolone-glycines tautomerization
H_f—heat of formation and p_i for tautomers **a**, **b** and **c** according to AM1 method

^{1}*H-NMR spectra analysis*						
Comp.no	a		b		c	
II-2	Dynamic state		Dynamic state		Dynamic state	
III-1	<1		1		9	
IV-1	–		Dominant		Not existing	
	H_f (kJ/mol)	p_i (%)	H_f(kJ/mol)	p_i (%)	H_f(kJ/mol)	p_i (%)
II-2	−71.38	62	−68.77	23	−67.63	15
III-1	8.97	62	11.45	24	12.73	14
IV-1	-	-	−56.05	35	−57.66	65

diphenyl glycinamides [2]. Glycinamides of type **IV** may exist only in the two tautomeric forms: **b** and **c** (Scheme 2). Only single signals for N–H protons were observed at the range 11.28–11.38 ppm. These signals were observed in even more deshielded region as more acidic protons of **c** tautomers of amides **III** are present (the signal range observed was 10.70–10.88 ppm). We presume that the only tautomers, which exist in DMSO solution, are **b** tautomers, where the 1-N–H protons are being under anisotropic influence of carbonyl group attached to the exocyclic nitrogen causing downfield shift of these signals.

Such predominance of one of the tautomeric forms was not observed for the esters of type **II**. The N–H protons were not detected, indicating the dynamic behaviour of the structures in the solution for structure **II-1** and presumably only the **c** tautomer of *p*-chloro substituted **II-2** existed in the solution (H–N proton observed at 10.95 ppm).

To calculate the percentage (p_i) of molecules in a given energetic state, non-degenerate Boltzmann distribution was used (second part of Table 2). The heat of formation H_f for each tautomer was calculated using the semiempirical AM1 method [11] in a simulated water environment (dielectric constant equals 78.4 units). As all studied amides (**III** and **IV**) belong to *p*-chloro-arylidene derivatives, also the calculations for ester **II-2** were performed for compatibility. From the series of amides, the molecules **III-2** and **IV-1** (Table 1) were selected for further modelling. The calculations for derivatives from set **B** (**II** and **III**) showed that the a tautomer is the most probable form in the aqueous environment (~62%). According to that calculations, there are about 23% of **b** tautomer and only 15% of **c**. In amides of type **IV** (only the two tautomeric forms are taken into account), the **c** tautomer is the most stable and the ratio **b/c** is about 1:2. Both tautomeric forms of *N*-acylated amide **IV-1** are stabilised by intramolecular N–H···O hydrogen bonds (Fig. 2).

The theoretical calculation results for glycine derivatives of all types (see Table 2) do not exactly agree with the conclusions based on the ^{1}H-NMR spectra analysis. These results (at physiological temperature 37°C) could be a direct outcome of different character of environment: ^{1}H-NMR spectra were measured in DMSO aprotonic solvent, while the calculations were performed in H_2O being a highly protonic one. However, in the equilibrium state in the solution all tautomeric forms may be present and, thus, none of them could be neglected. Therefore, this permits to conclude that the tautomers, essential for binding to the receptor, are coming into existence dynamically, during binding to the receptor. So, it is important to consider the other additional conditions, which divert significantly the inhibition to the receptor of the compounds from sets **A** and **B**.

3.2. Structure-activity relationships

Molecules from sets **A** and **B** differ from the viewpoint of responsibility for coulombic interactions between receptor and ligand. Therefore, considering only **b** tautomeric forms, we were able to limit the analyses of partial charges (from AM1 approximation in aqueous solution) just to the carboxylic group

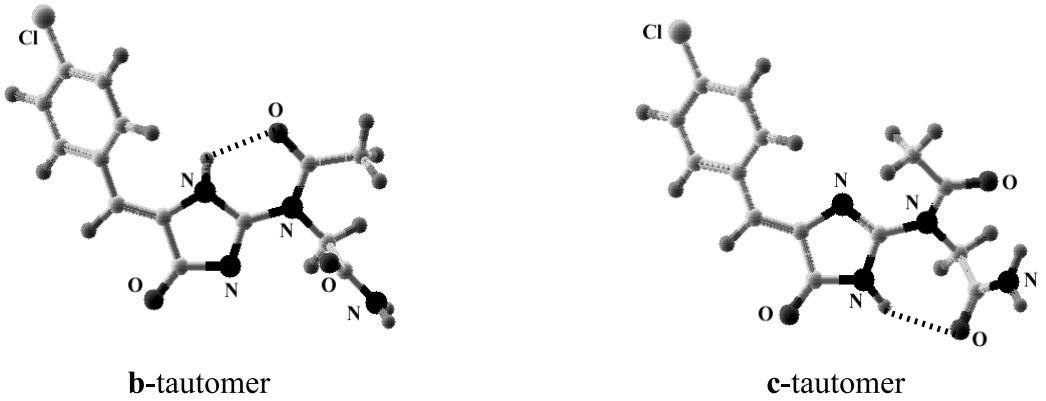


Fig. 2. Two tautomeric forms of *N*-acylated amide **IV-1**. Doted lines mark intramolecular H-bonds.

residue: two oxygens for **I** and **II**, and/or oxygen and nitrogen for **III** and **IV**, respectively (Table 3).

For ionic **I-2**, charges of about −0.7e are located on both oxygens. However, for **II-2**, nature of oxygens is not identical having charges equal −0.46e and −0.30e, respectively. For **III-2** and **IV-1**, charges are significantly lower than for **I-1** and they equal, on average, −0.53e on oxygen and −0.30e on amide nitrogen (Table 3). The proton donoring object (NH − from CONH–R group) — in the coulombic attraction area of all amides — additionally hampered the ligand–receptor interactions. Moreover for **IV**, intramolecular H-bond (N–H···O) hinders the proton donoring group capable of forming N–H···A bond with the receptor (Fig. 2). Presumably, the affinity to the receptor of imidazoline–glycine derivatives depends mainly on the negative charge over the carboxylic group residue. Every substitution, lowering this charge and blocking ionisation, restrains the derivative contact with another chemical objects. These differences, intuitively clear and confirmed by the value of charges (Table 3), are visualised by scattergams, which summarise crystalographically confirmed areas of non-bonded contacts for COO^-, COOR, and $CONH_2$ groups with H–X (*X*=O, N) [13–15]. This representative scattegrams (Fig. 3 a–c) were created using *IsoStar*. program [12–15]. Areas of contact are explicitly different.

Table 3
The partial charges [e] in tautomer **b** at oxygens from COO residue for **I** and **II** or at oxygen and nitrogen from CON residue from **III** and **IV**

Partial charge at	**I-2**	**II-2**	**III-1**	**IV-1**
O1	−0.70	−0.46	−0.53	−0.52
O2 (or N)	−0.69	−0.30	−0.30	−0.30

4. Conclusions and recommendations for future work

The obtained **III** and **IV** amides (Table 1) are much less active in a series of *p*-Cl–arylidene–imidazoline–glycine derivatives than other compounds of the same origin. The *p*-Cl–arylidene–imidazoline–glycine **I-2** was chosen as the leading structure, because it has shown the affinity to the glycine binding site in NMDA receptor and was active in MES test (ASP class II); up to dosage of 300 mg/kg, and contrary to *m*-chloro derivative **I-1** it was devoid of neurotoxic effects. Nevertheless, their *m*-Cl analogue **II-1** would be also less active than parent acid. This means that there is the necessity to verify the direction in searching for the new improved ligands with glycine binding site in NMDA receptor.

We intend to obtain and test imidazoline–glycine derivatives with various aromatic substituents attached over the region of liphophilic pocket. So far, just one diphenyl analogue was found to be inactive [5]. At the same time, we are going to investigate other imidazoline–aminoacids. It should be emphasised that the research direction proposed here is the consequence of the thorough analysis of the

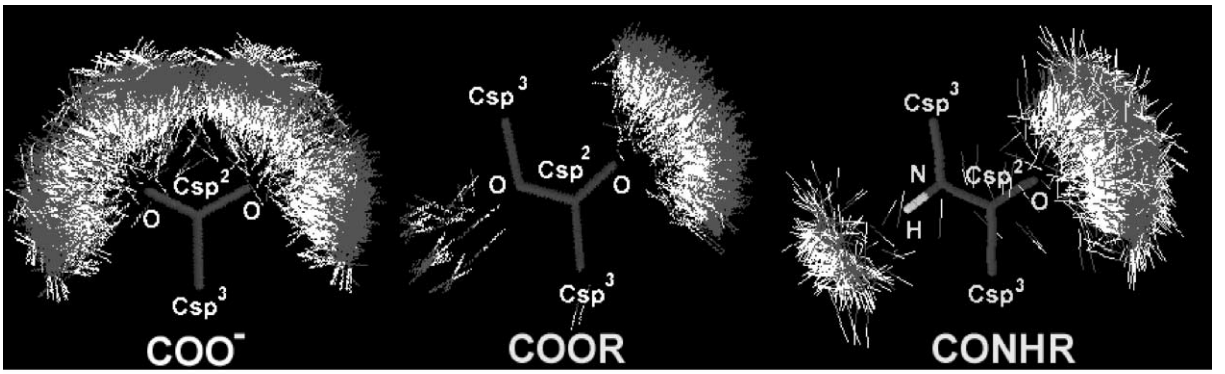


Fig. 3. Surfaces of crystalographically registered interactions with H–X (X=O, N) for: (a) COO^-; (b) COOR; (c) $CONH_2$.

model of interaction between arylideno-imidazoline–glicynes and the glycine binding site of NMDA receptor created by us [4].

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