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Structure and activity studies of glycine receptor ligands. Part 8. Arylidene-imidazoline-4-one aminoacids

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Abstract

Based on chemical and preliminary biological experiments (inhibition to glycine receptor), structure and activity relationship of arylidene-imidazoline-4-one amino acids has been studied. In the course of our work, the simulation of the hydrogen bonds formation between ligand molecule and hypothetical receptor has been designed. Computed interactions are going to simulate possible ligand–receptor interaction with selected amino acids (in this investigation—with basic lysine and acidic aspartic acid). Obtained model estimates roughly the binding energy of the amino acids with ligand molecules. The proposed amino acids binding energies approximately agree with activity of the isomeric benzylidene-imidazoline-4-one glycines and α-alanines which decreases in the order of *m*-Cl > *p*-Cl > *o*-Cl substituents in benzylidene moiety. Additionally, the lowering of activity is caused by lipophilic pocket volume.

Keywords: Glycine receptor ligands; Anticonvulsant activity; Molecular modelling; Model of the ligand–receptor interaction

1. Introduction

The objective of our latest research was to find new ligands for the glycine–NMDA binding site (iGluRs). In order to achieve this goal, the glycine derivatives of arylidene-imidazoline-4-ones were investigated [1–6]. It was found that some glycines have shown the noticeable affinity to iGluRs receptor [1]. To improve the affinity of those compounds, the increase of log *P* values [7] by chemical modification has been postulated. Additionally, it was clear from our study [6] that the substitutions in carboxyl group (proceeding to amides and esters) have been deceptive and the unsubstituted COO^- group was found to be essential for observable activity [8,9]. The model of ligand–receptor interaction for studied derivatives was also proposed (Fig. 1) [6].

Recently, based on our current results, only several 5-substituted-imidazoline-4-one amino acids have been selected, namely three series of the derivatives marked as **A**–**C** on Scheme 1. Based on chemical and preliminary biological investigation (Table 1), structure and activity studies on imidazoline-4-one amino acids were conducted.

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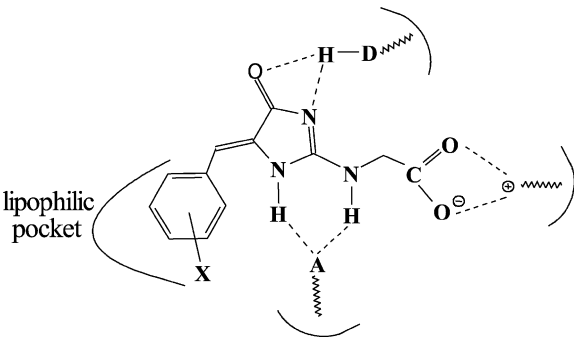


Fig. 1. The model of the iGluRs binding site for arylidene-imidazoline-4-one glycines.

Presented below discussion is based on molecular modelling using quantum chemistry calculations and includes simulation model of hydrogen bonds formation between basic molecule and hypothetical receptor. We hope that this ligand-receptor interaction with selected amino acids will also provide some information on the iGluRs.

2. Materials and methods

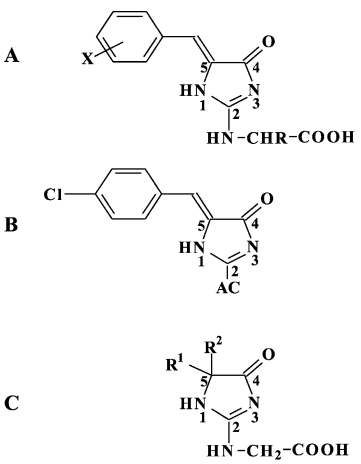
2.1. Chemical part

2.1.1. General procedure

As starting materials for the designed compounds, the 5-substituted derivatives of 2-thiohydantoins were used (Scheme 2). 5-(Z)-arylidene derivatives were obtained as the result of the well-known Knoevenagel condensation of 2-thiohydantoin with suitable aldehydes carried out in acetic acid in the presence of sodium acetate [10]. 5-Benzylo-2-thiohydantoin was synthesised through 1-acetyl intermediate in the reaction of phenylalanine with ammonium thiocyanate in the presence of acetic acid and acetic anhydride, which was followed by hydrolysis with 10% hydrochloric acid, as it was previously described [11]. 2-Thiohydantoin derivatives reacted, according to the method worked up by us earlier [1,12], with methyl iodide to give the methylthio derivatives which were condensed into the target compounds upon the reaction with (un)substituted glycine or alanine derivatives.

2.1.2. Experimental

Melting points (uncorrected) were determined on Mel.-Temp. II (LD Inc., USA) apparatus. ^{1}H-NMR spectra were recorded on a Gemini 200, Bruker 250, VARIAN MERCURY 300 MHz or Bruker DPX 400 Avance spectrometer, chemical shifts are reported in δ[ppm] values relative to internal reference of TMS. ^{13}C-NMR spectra were recorded on a Gemini 200 spectrometer. IR spectra were measured in KBr pellets with FT IR 410 spectrometer (Jasco). MS for compounds **13** and **17** were recorded on Finnigan MAT CH-7A spectrometer (ionisation) energy—70 eV). Elemental analyses were performed for C, H, N and the results differed no more than $\pm$0.4% from the theoretical values. TLC was conducted on Al



X = *o, m, p* – substituent in glycines (R=H) or α-alanines (R = CH_3)

AC = modified glycine or α-alanine

$R^1 = R^2$ = aromatic ring – $[CH=C(R^3)]_n$ – CH=; where: n=1,0; R^3 = H, CH_3

R^1, R^2 = phenyl, phenyl; benzyl, H.

Scheme 1.

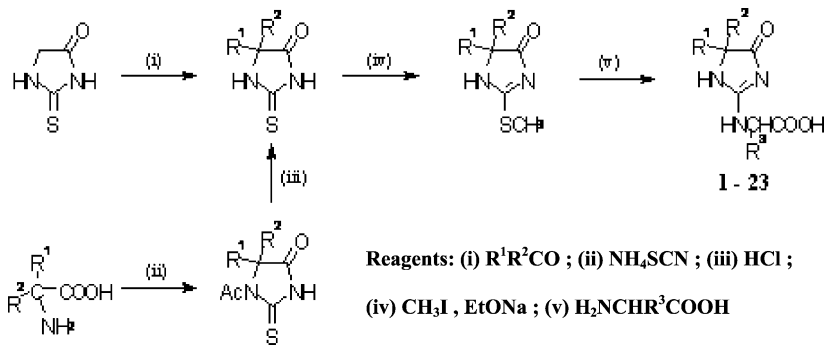
Table 1
Chemical and pharmacological details of 5-substituted-imidazolidyno-4-one amino acids (atoms numbering as in Scheme 1)

		Imidazolidyno-4-one substituents		Pharmacological data		log *P*	log *D* (at pH = 7.4)	Ref
		At C5	Amino acid	Inhibition (%)	ASP			
A	**1**	*p*-Cl–Ph–CH=	Glycine	51	2	1.00	−0.53	[1]
	2	*p*-NO_2–Ph–CH=	Glycine	18	3	0.19	−1.31	[1]
	3	*p*-OCH_3–Ph–CH=	Glycine	32	2	0.23	−1.24	[1]
	4	Ph–CH=	Glycine	59	1	0.27	−1.21	[1]
	5	*p*-Br–Ph–CH=	Glycine	0.4	2	1.16	−0.37	[1]
	6	*p*-Cl–Ph–CH=	α-Alanine	28.6	3	0.85	−0.65	
	7	*m*-Cl–Ph–CH=	Glycine	90	1	1.05	−0.48	[1]
	8	*m*-NO_2–Ph–CH=	Glycine	30	3	0.25	−1.25	[1]
	9	*m*-C–Ph–CH=	α-Alanine	90.3	3	0.93	−0.60	
	10	*o*-Cl–Ph–CH=	Glycine	18	3	0.98	−0.55	[1]
B	**11**	*p*-Cl–Ph–CH=	*p*-Cl-Ph-α-alanine	23	1	3.20	−0.15	
	12	*p*-Cl–Ph–CH=	*o*-Cl-Ph-α-alanine		2	3.17	−0.17	
	13	*p*-Cl–Ph–CH=	*p*-F-Ph-α-alanine	17	3	2.62	−0.61	
	14	*p*-Cl–Ph–CH=	Ph-α-alanine	34.9	3	2.47	−0.58	
	15	*p*-Cl–Ph–CH=	Indoilo-α-alanine	27.7	2	2.69	−0.33	
	16	*p*-Cl–Ph–CH=	Ph-glycine		1	2.02	−0.53	
C	**17**	Pyridine–CH=	Glycine	16.5	3	−0.94	−2.29	
	18	α-Naphthalene–CH=	Glycine		4	1.49	−0.05	
	19	β-Naphthalene–CH=	Glycine		2	1.49	−0.05	
	20	Ph–CH=CH_2–CH=	Glycine	24	3	0.92	−0.58	
	21	Ph–CH=CH(CH_3)–CH=	Glycine		3	1.40	−0.13	
	22	Ph–, Ph–	Glycine	-1.1	3	0.97	−0.46	[1,3,5]
	23	Ph–CH_2–, H–	Glycine	10.5	3	0.03	−1.30	

Sheets, 0.2 mm layer of silica gel ($60F_{254}$ Merck). The developing solvent system used was $CHCl_3$: i-PrOH: NH_3 aq (9:11:2).

N-[5-(Z)-(4-chlorobenzylidene)-4-oxo-2-imidazolidinyl]alanine (***6***): $C_{13}H_{12}N_3O_3Cl$ (293.72); mp 250–251 °C (DMF + H_2O); Yield 44%; $R_f = 0.11$; ^{1}H-NMR (200 MHz) $\sigma = 1.43$ (d, $J = 7.23$ Hz, 3H, CH_3); 4.48 (br.d, $J = 7.40$ Hz, 1H, C*H*CH_3); 6.31 (s, 1H, CH =); 7.39 (d, $J = 8.56$ Hz, 2H, H-3′, H-5′); 8.02 (br. d, $J = 7.27$ Hz, 2H, H-2′, H-6′); 10.61 (br.s, 1H, 3-NH); IR (KBr) γ: 3336 (OH), 3128 (NH), 2924 (CH), 1752, 1748, 1696 (C=O), 1680, 1670, 1648 (ArCH=), 1594 (C=N), 1552, 1452, 1088, 1012, 824, 668, 630 cm^{-1}.

N-[5-(Z)-(3-chlorobenzylidene)-4-oxo-2-imidazolidinyl]alanine (***9***): $C_{13}H_{12}N_3O_3Cl$ (293.72); cream



Scheme 2.

amorphous powder; mp 232–234 °C (DMF + H_2O); Yield 58%; $R_f = 0.14$; ^{1}H-NMR (200 MHz) $\sigma = 1.45$ (d, J = 7.23 Hz, 3H, CH_3); 4.46 (d, J = 5.46 Hz, 1H, C*H*CH_3); 6.30 (s, 1H, CH =); 7.23–7.39 (m, 2H, H-4′, H-5′); 7.86 (br.s, 1H, H-6′); 8.20 (br.s, 1H, H-2′); 10.74 (br. s, 1H, 3-NH); IR (KBr) ?: 2981 (CH), 1765, 1730 (C=O), 1671 (ArCH=), 1603, 1566, 1456, 1386, 1363, 1305, 1083, 1059, 904, 857, 779 cm^{-1}.

N-[5-(Z)-(4-chlorobenzylidene)-4-oxo-2-imidazolidinyl]-(4-chlorophenyl)alanine (***11***): $C_{19}H_{15}N_3O_3Cl_2$ (404.26); vivid yellow amorphous powder, mp 238–239 °C (from DMF); Yield 73%; $R_f = 0.13$; ^{1}H-NMR (250 MHz) $\sigma = 3.03–3.16$ (m, 1H, HC*H*–CH), 3.25 (dd, *J* = 13.90 Hz, 5.04 Hz, 1H, *H*CH–CH); 4.67 (br.s, 1H, C*H*–HCH)); 6.30 (s, 1H, CH =); 7.27–7.42(m, 6H, H-3′, H-5′, H-2″, H-3″, H-5″, H-6″), 7.82 (br. s, 1H, *H*NCH); 8.03 (br. d; *J* = 7.60 Hz, H-2′, H-6′); 10.62 (br. s, 1H, 3-NH); IR (KBr) γ: 3422 (OH), 2921 (CH), 1700 (C=O), 1614 (ArCH=), 1490, 1376, 1089, 1016, 808, 710, 667 cm^{-1}.

N-[5-(Z)-(4-chlorobenzylidene)-4-oxo-2-imidazolidinyl]-(2-chlorophenyl)alanine (***12***): $C_{19}H_{15}N_3O_3Cl_2$ (404.26); cream yellow amorphous powder, mp 242–244 °C (from DMF + H_2O); Yield 50%; $R_f = 0.30$; ^{1}H-NMR (300 MHz) $\sigma = 3.09–3.17$ (m, 2H, CHCH_2); 4.83 (br.s, 1H, C*H*CH_2); 6.22 (s, 1H, ArCH=); 7.21 (d, *J* = 3.58 Hz, H-3″, H-5″); 7.36 (d, *J* = 7.98 Hz, 4H, H-3′, H-5′, H-4″, H-6″); 7.98 (d, *J* = 7.98 Hz, 2H, H-2′, H-6′); 10.65 (br. s, 1H, 3-NH); IR (KBr) γ: 3389 (OH), 3054 (NH), 2929 (CH); 1756, 1698 (C=O), 1664 (ArCH=), 1600, 1377, 1092, 1050, 823, 764, 660 cm^{-1}.

N-[5-(Z)-(4-chlorobenzylidene)-4-oxo-2-imidazolidinyl]-4-fluorophenylalanine (***13***): $C_{19}H_{15}N_3O_3$-ClF(387.80); lemon yellow amorphous powder, mp 236–237 °C (from DMF + H_2O); Yield 28%; $R_f = 0.22$; ^{1}H-NMR (400 MHz) $\sigma = 3.24$ (d, J = 4.19 Hz, 1H, *H*CH–CH); 3.26 (d, J = 4.44 Hz, 1H, HC*H*–CH); 4.68 (br.s, 1H, HCH–C*H*); 6.30 (s, 1H, ArCH=); 7.12 (t, *J* = 8.21 Hz, 2H, H-2″, H-6″); 7.30 (t, *J* = 5.57 Hz, 2H, H-3″, H-5″); 7.40 (d, *J* = 7.58 Hz, 2H, H-3′, H-5′); 7.52 (br. s, 1H, N*H*CH_2); 8.03 (br. s, 2H, H-2′, H-6′); 10.52 (br. s, 1H, 3-NH); 13.03 (br. s, 1H, COOH); IR (KBr) γ: 3440 (OH, NH), 1768, 1728, 1712, 1696 (C=O), 1648 (ArCH=), 1564 (C=N), 1528, 1504, 1220, 668, 648, 612 cm^{-1}; MS (*m/z*): 387 (3), 268 (16), 344 (14), 342 (63), 218 (76), 162 (23), 125 (68), 108 (97), 97 (100).

N-[5-(Z)-(4-chlorobenzylidene)-4-oxo-2-imidazolidinyl]-phenylalanine (***14***): $C_{19}H_{16}N_3O_3Cl$ (369.80); cream coloured amorphous powder, mp 245–247 °C (from DMF + H_2O); Yield 24%; $R_f = 0.19$; ^{1}H-NMR (250 MHz) $\sigma = 2.96–3.16$ (m, 1H, *H*CH–CH); 3.26 (dd, J = 13.87 Hz, 4.90 Hz, 1H, HC*H*–CH); 4.70 (br.s, 1H, HCH–C*H*); 6.31 (s, 1H, ArCH=); 7.18–7.34 (m, 5H, Ph–H); 7.40 (d, *J* = 8.51 Hz, 2H, H-3′, H-5′); 7.81 (br. s, 1H, N*H*CH_2); 8.04 (br. d, 2H, H-2′, H-6′); 10.56 (br. s, 1H, 3-NH); IR (KBr) γ: 3424 (OH), 3118, 3028 (NH),1741, 1702 (C=O), 1655 (ArCH=), 1587 (C=N), 1379, 1311, 1090, 699, 660 cm^{-1}.

N-[5-(Z)-(4-chlorobenzylidene)-4-oxo-2-imidazolidinyl]-tryptophan (***15***): $C_{21}H_{17}N_4O_3Cl$ (408.85); vivid yellow amorphous powder, mp 253–254 °C (from DMF + H_2O); Yield 63%; $R_f = 0.17$; ^{1}H-NMR (200 MHz) $\sigma = 3.21–3.37$ (m, 2H, CH–CH_2); 4.78 (br.s, 1H, C*H*CH_2); 6.31 (s, 1H, CH=); 6.95–7.11 (m, 2H, H-5″, H-6″); 7.17 (d, J = 2.23 Hz, 1H, H-2″); 7.35 (d, J = 7.76 Hz, 1H, H-7″); 7.39 (d, J = 8.60 Hz, 2H, H-3′, H-5′); 7.56 (d, J = 7.51 Hz, 1H, H-4″); 8.05 (d, J = 7.55 Hz, 2H, H-2′, H-6′); 8.21 (d, J = 8.62 Hz, 1H, N*H*CH_2); 10.49 (br.s, 1H, 3-NH); 10.90 (br.s, 1H, NHindol); IR (KBr) γ: 3384 (OH, NH), 1768, 1758, 1712, 1700 (C=O), 1648 (CH=), 1552, 1358, 1090, 816, 748, 674 cm^{-1}.

N-[5-(Z)-(4-chlorobenzylidene)-4-oxo-2-imidazolidinyl]phenylglycine (***16***): $C_{18}H_{14}N_3O_3Cl$ (355.78); cream yellow amorphous powder, mp 240–242°C (from DMF + H_2O); Yield 50%; $R_f = 0.21$; ^{1}H-NMR (200 MHz) $\sigma = 5.56$ (s, 1H, NHC*H*); 6.35 (s, 1H, ArCH=); 7.30–7.53 (m, 7H, H-3′, H-5′, 5 × Ph–H); 8.07 (br.s, 2H, H-2′, H-6′); 8.34 (br.s, 1H, N*H*CH); 10.36 (br.s, 1H, 3-NH); IR (KBr) γ: 3169, 3066 (OH, NH), 2812 (CH), 1759, 1708 (C=O), 1666 (ArCH=), 1590, 1378, 1181, 1090, 1023, 1011, 697 cm^{-1}.

N-[5-(pyridyl-3)-methylene-4-oxo-2-imidazolidinyl]glycine (***17***): $C_{11}H_{10}N_4O_3$ (246.23); bright yellow amorphous powder; mp 265–266 °C; Yield 41%; $R_f = 0.05$; ^{1}H-NMR (300 MHz) $\sigma = 4.04$ (s, 1H, NHCH_2) 6.69 (s, 1H, CH=); 7.04–7.15 (m, 3H, H-2′, H-3′, H-6′); 7.39 (d, *J* = 7.69 Hz, 1H, H-5′); 7.86 (d, *J* = 7.42 Hz, 1H, H-4′); 8.10 (br.s, 1H, N*H*CH_2); 11.51 (s, 1H, 3-NH); ^{13}H-NMR (75 MHz) $\sigma =$ 42.64 (*C*H_2COOH); 122.88 (C-5); 123.38 (ArCH=); 131.79

(C-5′); 135.96 (C-6′); 136.12 (C-2′); 147.46 (C-4); 147.89 (C-3); 150.49 (COOH); 150.74 (C=O); 170.88 (C=N); IR (KBr) γ: 3340 (OH), 3078 (NH), 1731 (C=O), 1682 (C=O), 1621 (ArCH=), 1600 (C=N), 1524, 1371, 1265, 1119,874, 805, 761, 684 cm^{-1}; MS (*m/z*): 246 ($M^{+\cdot}$,4), 230 (12), 228 (21), 200 (24), 188 (10), 145 (7), 118 (40), 93 (46), 77 (3), 44 (100).

*N-[5-(naphthyl-1)-methylene-4-oxo-2-imidazolidinyl]glycine (**18**)*: $C_{16}H_{13}N_3O_3$ (295.28);light yellow amorphous powder, mp 281–282 °C; yield 59%; $R_f = 0.11$; ^{1}H-NMR (200 MHz) $\sigma = 4.11$ (s, 2H, NHCH_2); 7.09 (s, 1H, CH=); 7.51–7.63 (m, 3H, H-3′, H-6′, H-7′); 7.85 (d, J = 7.99 Hz, 1H, H-5′); 7.94 (dd, J = 7.90 Hz, 1.77 Hz, 1H, H-4′); 8.21 (br. s, 1H, H-2,); 8.83 (br. s, 1H, H-8′); IR (KBr) γ: 3424 (OH); 3180 (NH), 2944 (CH), 2720, 1754 (C=O), 1728, 1704 (C=O), 1658, 1648 (CH=), 1392, 1296, 1244, 1224, 1040, 978, 884, 780, 652 cm^{-1}.

*N-[5-(naphthyl-2)-methylene-4-oxo-2-imidazolidinyl]glycine (**19**)*: $C_{16}H_{13}N_3O_3$ (295.28); cream yellow amorphous powder, mp 279–281 °C (from DMF + H_2O); yield 68%; $R_f = 0.12$; ^{1}H-NMR (250 MHz) $\sigma = 4.13$ (s, 2H, NHCH_2); 6.51 (s, 1H, CH=); 7.46–7.53 (m, 2H, H-6′, H-7′); 7.84–7.89 (m, 3H, H-4′, H-5′, H-8′); 8.28 (br.s, 2H, N*H*CH_2, H-1′); 8.46 (br.s, 1H, H-3′); 10.90 (br.s, 3-NH); IR (KBr) γ: 3336 (OH); 3212 (NH), 2932 (CH), 1756, 1742, 1712 (C=O), 1648 (CH=), 1606, 1512, 1396, 1340, 1292, 1112, 1028, 874, 826, 772 cm^{-1}

*N-(5-cinnamylidene-4-oxo-2-imidazolidinyl)glycine (**20**)*: $C_{14}H_{13}N_3O_3$ (271.28); yellow amorphous powder; mp 287–288 °C; yield 85%; $R_{f=}$0.10; ^{1}H-NMR (200 MHz) $\sigma = 1.91$ (s, 0.33 × 3H, CH_3-COOH); 4.04 (s, 2H, NHCH_2); 6.21 (d, $J = 11.54$ Hz, 1H, ArCH=); 6.88 (d, $J = 15.66$ Hz, 1H, CH=); 7.26 (d, $J = 11.70$ Hz, 1H, CH=); 7.27–7.48 (m, 3H, H-3′,H-4′,H-5′); 7.52 (d, $J =$ 1.43 Hz, 2H, H-2′,H-6′); IR (KBr) γ: 3436 (OH,NH), 2916 (CH), 1732, 1712, 1696 (C=O), 1680, 1648, 1552, 1512, 1500, 1452, 1356, 1298, 978, 668 cm^{-1}.

*N-[5-(2-methyl)-cinnamylidene-4-oxo-2-imidazolidinyl]glycine (**21**)*: $C_{15}H_{15}N_3O_3$ (285.31); cream yellow amorphous powder; mp 238–239 °C; Yield 58%; $R_f = 0.11$; ^{1}H-NMR (200 MHz) $\sigma = 2.27$ (s, 3H, CH_3); 4.02 (s, 2H, NHCH_2); 6.12 (s, 1H, ArCH=); 6.93 (s, 1H, CH=) 7.24–7.28 (m, 1H, H-4′); 7.37 (d, $J = 4.29$ Hz, 4H, H-2′, H-3′, H-5′ H-6′); 10.63 (br.s, 1H, 3-NH); IR (KBr) γ: 3432 (OH), 3068 (NH), 2916 (CH), 1756 (C=O), 1702 (C=O), 1648 (CH=), 1620 (C=N), 1528, 1388, 1264, 1192, 1040, 840, 750 cm^{-1}.

*N-(5-benzyl-4-oxo-2-imidazolidinyl)glycine (**23**)*: $C_{12}H_{13}N_3O_3$ (247.25); cream yellow amorphous powder; mp 233–234 °C (CH_3COOH); Yield 30%; $R_f = 0.09$; ^{1}H-NMR (200 MHz) $\sigma = 1.91$ (s,0.66 × 3H, CH_3COOH); 2.61–2.72 (m, 1H, CH); 3.03 (dd, $J = 14.02$ Hz, $J = 4.10$ Hz, 1H, CH); 3.86 (s, 2H, NHCH_2); 4.08 (br.s, 1H, CH), 7.16–7.27 (m, 5H, Ph–H); IR (KBr) γ: 3408 (OH), 3284, 3076 (NH), 2976, 2912 (CH), 1768, 1712, 1708, 1696 (C=O), 1680, 1612, 1552, 1294, 1262, 1030, 702, 618 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. [13]. Affinities of tested compounds 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; the results are presented in Table 1.

2.2.2. Anticonvulsant assays

All animal anticonvulsant and neurotoxicity assays were conduced by the Antiepileptic Drug Development (ADD) Program, Epilepsy Branch, Neurological Disorders Program, National Institutes of Neurological and Communicative Disorders and Stroke (NINCDS) according to testing procedures which have been described earlier [14,15]. Phase I, and the threshold tonic extension (TTE) testing procedures. Phase I of the evaluation consisted of three tests: maximal electroshock (MES), subcutaneous pentylenetetrazol (ScMet), and rotorod test for neurological toxicity (Tox). Alls compounds apart were injected intraperitoneally into mice as suspensions in 0.5% methylcellulose. Small groups of animals were used (1–8) at dose levels of 30, 100 and 300 mg/kg. These data are presented in Table 2. The classifications were as follows (Table 2): (1) anticonvulsant activity at 100 mg/kg or less; (2) anticonvulsant activity at doses greater than 100 mg/kg; (3) compound inactive at 300 mg/kg.

Table 2
Anticonvulsant activity in the MES, ScMet test and acute neurological toxicity in the rotorod test after intraperitoneal administration to mice. The data are expressed as animals protected/animals tested

	Dose (mg/kg)	MES		ScMet		Rotorod			Dose (mg/kg)	MES		ScMet		Rotorod	
		0.5 h	4 h	0.5 h	4 h	0.5 h	4 h			0.5 h	4 h	0.5 h	4 h	0.5 h	4 h
6	30	0/1	0/1	0/1	0/1	0/4	0/2	**16**	30	0/1	0/1	1/5	0/1	0/4	0/2
	100	0/3	0/3	0/1	0/1	1/8	0/4		100	0/3	0/3	1/5	0/1	0/8	0/4
	300	0/1	0/1	0/1	0/1	0/4	0/2		300	0/1	0/1	0/1	0/1	0/4	0/2
9	30	0/1	0/1	0/1	0/1	0/4	0/2	**17**	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	0/3	0/3	0/1	0/1	0/8	0/4		100	0/3	0/3	0/1	0/1	0/8	0/4
	300	0/1	0/1	0/1	0/1	0/4	0/2		300	0/1	0/1	0/1	0/1	0/4	0/2
11	30	0/1	0/1	0/1	0/1	0/4	0/2	**18**	30	0/1	0/1	2/5	2/5	0/4	0/2
	100	0/3	1/3	0/1	0/1	0/8	0/4		100	0/3	1/3	0/1	0/1	0/8	0/4
	300	0/1	1/1	0/1	0/1	0/4	1/2		300	0/1	1/1	1/1	1/1	2/4	2/2
12	30	0/1	0/1	0/1	0/1	1/4	0/2	**19**	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	0/3[a]	0/3	0/1	0/1	2/8[b]	0/4		100	0/3	0/3	0/1	0/1	0/8	0/4
	300	1/1	1/1	0/1	0/1	4/4	2/2		300	0/1	0/1	1/5	2/5	0/4	0/2
13	30	0/1	0/1	0/1	0/1	0/4	0/2	**20**	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	0/3	0/3	0/1	0/1	0/8	0/4		100	0/3	0/3	0/1	0/1	0/8	0/4
	300	0/1	0/1	0/1	0/1	0/4	0/2		300	0/1	0/1	0/1	0/1	0/4	0/2
14	30	0/1	0/1	0/1	0/1	0/4	0/2	**21**	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	0/3	0/3	0/1	0/1	0/8	0/4		100	0/3	0/3	0/1	0/1	0/8	1/4
	300	0/1	0/1	0/1	0/1	0/4	0/2		300	0/1	0/1	1/5	0/1	3/4	1/2
15	30	0/1	0/1	0/1	0/1	0/4	0/2	**23**	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	0/3[c]	0/3	0/1	0/1	0/8[d]	0/4		100	0/3	0/3	0/1	0/1	0/8	0/4
	300	0/1	1/1	0/1	0/1	0/4	0/2		300	0/1	0/1	0/1	0/1	0/4	0/2

[a] At 15 min 0/3; 1 h 0/3; 2 h 0/3; 6 h 0/3.
[b] At 15 min 0/3; 1 h 1/3; 2 h 0/3; 6 h 0/3.
[c] At 2 h 1/3; 6 h 0/3.
[d] At 2 h 0/3; 6 h 0/3.

2.3. Computational procedure

All molecules were built on the basis of the crystallographic structure of p-Cl-phenyl-imidazoline-4-one ethyl glycinate [5] and roughly optimised using PCMODEL.6 program [16]. MO calculations were carried out by AM1 method using MOPAC program (version 6.0) [17] in water environment (dielectric constant 78.4). The structures of complexes with small molecules (water and ammonia) and/or with amino acids were obtained by DOC procedure from PCMODEL.6 and were subsequently optimised by AM1. The values of log *P* and log *D* were calculated by means of PALLAS (version 1.2) program [18].

3. Results and discussion

3.1. Reviewing the chemical materials

The discussion in this paper relates to 23 amino acidic derivatives of imidazoline-4-ones glycines and/or α-alanines, for which chemical details and chosen properties are collected in Table 1. In general, all objects under consideration can be divided into two groups: simple amino acids (set **A**-compounds **1**–**10** and set **C**-compounds **17**–**23** in Table 1) and modified ones (set **B**-compounds **11**–**16** in Table 1). The amino acid modification was employed for lipophilicity increase (Table 1). In fact, the values of log *P* for all simple amino acids were approximately equal to 1,

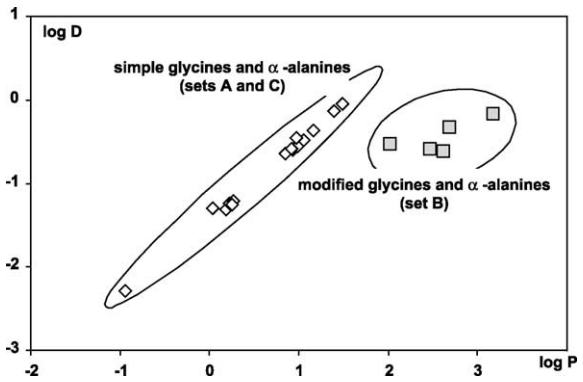


Fig. 2. Scattegram of log *P* vs. log *D* (at pH = 7.4).

while those for the modified ones—ranged from 2 to 3. It is well illustrated by Fig. 2, which presents the dependence of log *P* vs. log *D* (at pH = 7.4) for two sets of derivatives. Therefore, our search for imidazoline-4-ones with log $P \sim 3$ has been well designed (set **B**). Unfortunately, so far we have not been able to improve compound affinity.

To support our discussion, simple amino acids from Table 1 have been divided into two sets—**A** and **C**. The natures of C5 atom, its substitution and/or hybridisation were the reason for distinguishing between both sets. Consequently, the set **A** gathers previously studied [1] differently substituted benzylidene-imidazoline-4-one glycines (**1**–**5**, **7**, **8**, **10** in Table 1) and two new α-alanine analogues (**6** and **9**). The 5-substituted-[4-oxo-2-imidazolidinyl]-glycines from set **C** were studied in order to estimate the lipophilic pocket size. Subsequently, in all derivatives **17**–**23,** various aromatic groups and various linking spacers have been located at C5. As follows, this set collects compounds **17**–**19** accompanied with standard [–CH=] spacer (as in **A** and **B** sets) and various aromatic cycles. In order to explore the lipophilic pocket length, compounds with longer [–CH=CR^3–CH=] linking spacer joining phenyl and imidazole rings (**20** and **21**) were investigated (see Scheme 1). In the end, the change in hybridisation from sp^2 to sp^3 at C5 atom in species **4** produced a new derivative **23**. That last compound has been designed as perceptive analogue of the derivatives from set **A**. Derivative **23** differs from **4** only in hybridisation of C5 and, consequently, possesses [–CH_2–] group as the linking spacer. Thus, two derivatives, **22** (5,5-diphenyl-imidazolone-4-one glycine [1,5]) and **23**, form a group of species having C5 atom in sp^3 hybridisation.

3.2. Pharmacological properties

Selected compounds, whose chemical details are described in Table 1, were evaluated in vitro for their affinity to the glycine-binding site of NMDA receptor (iGluRs). The in vivo preliminary pharmacological testing results of all newly synthesised compounds are presented in Tables 2 and 3. The MES assay has predictive value for agents of potential therapeutic value in the treatment of grand mal epilepsy, whereas the ScMet test is for those likely to be effective against petit mal. The TTE test is a clinically non-selective electroconvulsive seizure model, which detects compounds that raise seizure threshold as well as those, which prevent seizure spread. In addition, this test can identify certain compounds that are inactive in both the MES and the ScMet tests.

The results from anticonvulsant assays for the newly obtained compounds **6**, **9**, **11**–**21,** and **23** are summarised in Tables 2 and 3. Compounds **11**, **12**, **15**, and **18** have shown protective activity in the MES test for mice, compounds **16**, **18**, **19**, and **21** were active in the ScMet test, compounds **6**, **13**, **14**, **17**, **19**, **20**, **21** and **23** were active in the TTE test, neurotoxicity has been shown by compounds **6**, **11**, **12**, **18**, and **21**.

Table 3
Anticonvulsant activity in the TTE test. The data are expressed as animals protected/animals tested

Compound	Dose (mg/kg)	Time					
		0.25 h	0.5 h	1.0 h	2.0 h	4.0 h	6.0 h
6	100	1/4	0/4	1/4	0/4	3/4	–
13	100	1/4	0/4	0/4	0/4	0/4	0/4
14	100	1/4	0/4	0/4	0/4	0/4	–
16	100	0/4	0/4	0/4	0/4	0/4	–
17	100	0/4	1/4	0/4	0/4	0/4	–
19	100	0/4	0/4	0/4	1/4	0/4	–
20	100	0/4	0/4	1/4	3/4	2/4	–
21	100	0/4	0/4	0/4	1/4	1/4	–
23	100	1/4	1/4	1/4	0/4	0/4	0/4

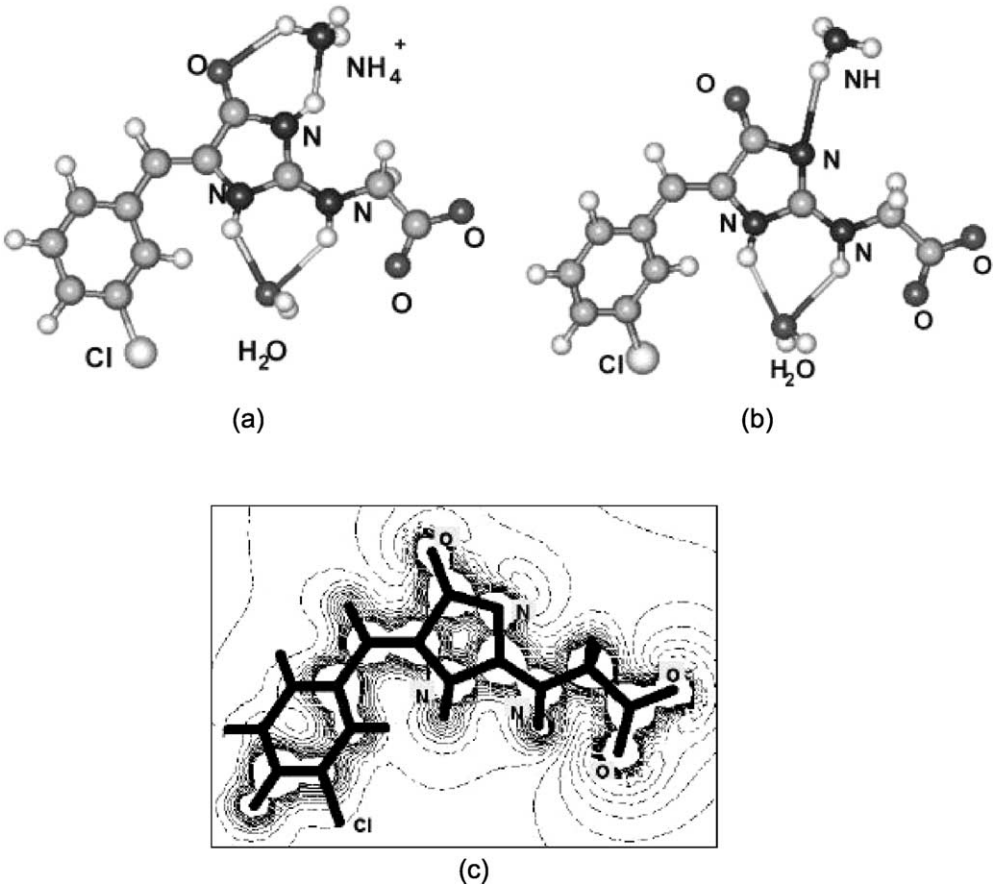


Fig. 3. The complex based on H-bonds interactions for [(Z)-5-(*m*-chlorol-benzylidene)-4-oxo-2-imidazolidinyl]-glycine **7**. (a) With water and ammonia ion; (b) with water and ammonia; (c) MEP distribution in the plane of imidazole; dotted lines with negative potential.

3.3. Structure–activity studies of benzylidene-imidazolidyno-4-one amino acids

As it was postulated in the introduction, we adapted receptor binding model with iGluRs (Fig. 1) to benzylidene-imidazolidyno-4-one glycines [6]. Analysing this model, it seems possible and useful to simulate the complex formation of a selected molecule with at least H-bond donor and acceptor (the proposed strategy can be classified as 4D QSAR [19] methodology). To achieve this objective, in first stage we were trying to simulate interactions between only two small particles: water (H-bond acceptor) and ammonia ion (H-bond donor). After the docking procedure (employing molecular mechanics formalism), the energy of the joined molecules was minimised by semiempirical methods (AM1). The final complex of the most active derivative [(Z)-5-(*m*-Cl-benzylidene)-4-oxo-2-imidazolidinyl]-glycine **7** is presented in Fig. 3(a). Water molecule, corresponding to H-bond acceptor from Fig. 1(b), binds both N–H groups. Ammonia ion NH_4^+ forms two H-bonds with both potential acceptors (Fig. 3(a)) from the ligand molecule. It should be noticed that ammonia molecule—also H-bond donor—is linked only to one of two possible acceptors (Fig. 3(b)). It is understandable from the distribution of molecular electrostatic potential round the ligand molecule (MEP-calculated after AM1 optimisation) depicted in Fig. 3(c). We can find two MEP minima designed as potential H-bond acceptors: one near the endocyclic nitrogen and the other one at the carboxyl oxygen. However, only

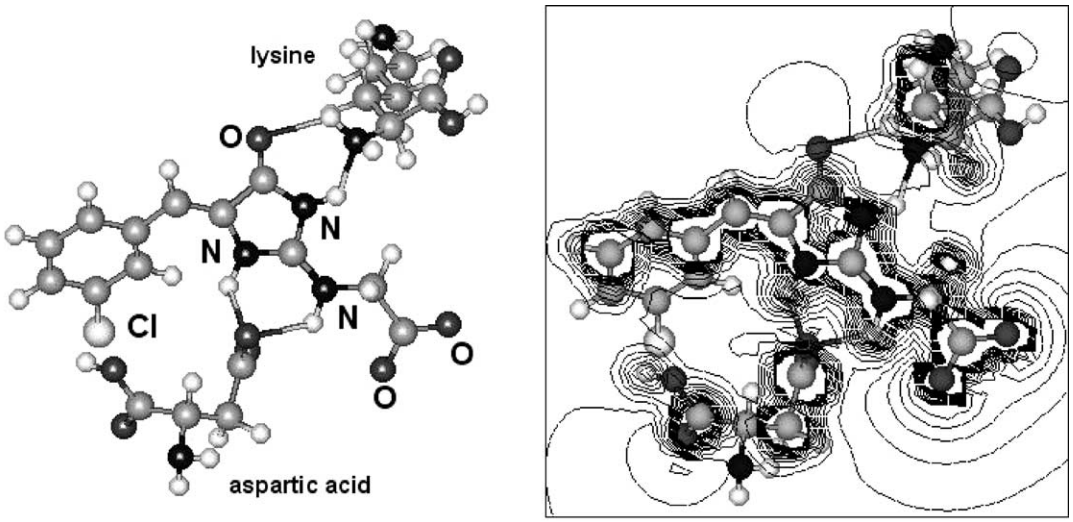


Fig. 4. (a) The complex of [(Z)-5-(*m*-chlorol-benzylidene)-4-oxo-2-imidazolidinyl]-glycine **7** with basic lysine and acidic aspartic acid; (b) MEP distribution in the plane of imidazole.

the deeper one at nitrogen was useful in the formation of the complex with ammonia.

Promising results, presented in Fig. 3, have stimulated us to construct a complex of the [(Z)-5-(*m*-Cl-benzylidene)-4-oxo-2-imidazolidinyl]-glycine **7** with selected amino acids. It is clear that the chosen amino acids should be present in the corresponding receptor [9,20]. In this example, basic lysine and acidic aspartic acid were designated as the species for complex formation. Finally, the complex given in Fig. 4(a) was obtained and the corresponding MEP distribution is presented in Fig. 4(b). If this model is realistic, the energy of the complex formation (or binding energy of the amino acids with ligand molecules) should more or less correlate with the inhibition to the receptor [21]. Three isomers **1**, **7**, and **10** with *p*-, *m*-, *o*-Cl substituents in benzylidene and different inhibition (Table 1) are convenient examples to confirm model implicitly. The thermodynamic stability data (in the form of H_f—heat of formation) of all three ligands H_f (1) and corresponding complexes H_f (2) are gathered in Table 4. These values indicate that the complex of the most active

Table 4
Thermodynamic parameters for selected [(Z)-5-(chlorol-benzylidene)-4-oxo-2-imidazoli-dinyl]-glycines and α-alanines and their corresponding complexes with basic lysine and acidic aspartic acid

Compound	Inhibition (%)	Heat of formation [kcal/mol]		
		H_f(1)	H_f (2)	$\Delta H_f = H_f$ (1) − H_f (2)
1	51	−74.76	−370.70	−296.01
7	90	−73.95	−370.39	−296.44
10	18	−73.33	−369.30	−295.97
6	28.6	−78.07	−374.55	−296.48
9	90.3	−75.39	−372.37	−296.98

derivative **7** (90% of inhibition) is also the most stable thermodynamically. Thus, we are able to assume that the following difference:

$$\Delta H_f = H_f(2) - H_f \quad (1)$$

corresponds to the binding energy of the amino acids with ligand molecules. It was found that the inhibition and ΔH_f for three chlorine isomeric derivatives (**1**, **7**, and **10**) exhibit logical dependence visible in Fig. 5.

During the investigation, the docking procedure was applied for simple as well as for isomeric α-alanines **9** and **6**. Numerical results of those calculations are included into Table 4. After supplementation of the data for three isomeric glycines with those for two isomeric α-alanines (**9** and **6**), analogous logical dependence between inhibition and ΔH_f was obtained (Fig. 5). These two examples have confirmed that the substitution pattern in benzylidene is very important. The binding energy of the amino acids with ligand molecules in the form of ΔH_f (Table 4) approximately agrees with activity of the isomeric molecules. The activity of these compounds is affected by the substituent position and decreases in the order *m*-Cl > *p*-Cl > *o*-Cl.

It should be emphasised that the procedure described above is based on comparison of the heat of formations for group of isomeric species. In Table 1 only two such groups of derivatives can be found, both described above.

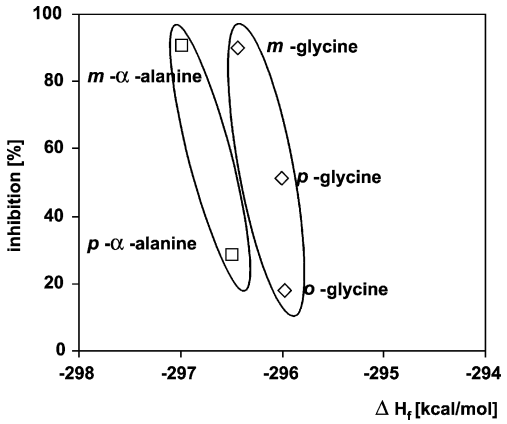


Fig. 5. Relationship between inhibition to iGluRs and binding energy in complex with basic lysine and acidic aspartic acid for isomeric [Z-(Cl-substituted-benzylidene)-4-oxo-2-imidazolidinyl]-glycines and α-alanines.

3.4. Structures and properties of non-benzylidene-imidazolidyno-4-one glycines

The compounds from set **C** (all glycines) have been designed to focus our attention to substituent(s) at C5 atom. In all **17**–**23** glycines from this set, benzylidene rings are unsubstituted. For this reason compound **4** was selected as a reference molecule for the subsequent structure–activity analysis.

Despite the differences in C5 atom hybridisation and/or the number of atoms in the spacer between aromatic and imidazole rings, the volume of whole substituent(s) at C5 is presumed to be important for binding. To validate such assumption, the volumes of molecules from set **C** and of respective substituents at C5 have been calculated (Table 5). One can see that only volumes of **17** and **23** agree with the volume of the reference molecule **4**. Thus, presumably the activity lowering for **18**–**22** was a direct result of the volume limitation in lipophilic pocket. Nevertheless, based on contemporary knowledge, the deeper discussion on this subject is impossible at this moment.

The molecules **17** and **23** are especially interesting. Despite comparable molecule volume, these derivatives are significantly less active than the reference one **4** (Table 1). To explain this observation, conformational analyses of both molecules were performed firstly. It was established that there are

Table 5
The volume of the substituent(s) at C5 for derivatives from sets **C**. The compound **4** was added as reference molecule

Compound	Substituent at C5	Volume (Å^3) of	
		Molecule	Substituent
4	Phenyl–CH=	278	116
17	Pyridine–CH=	273	111
18	α-Naphtalene–CH=	340	178
19	β-naphtalene–CH=	339	177
20	Ph–CH=CH_2–CH=	318	156
21	Ph–CH=CH(CH_3)–CH=	339	177
22	Ph, Ph	356	194
23	Ph–CH_2–, H	288	126

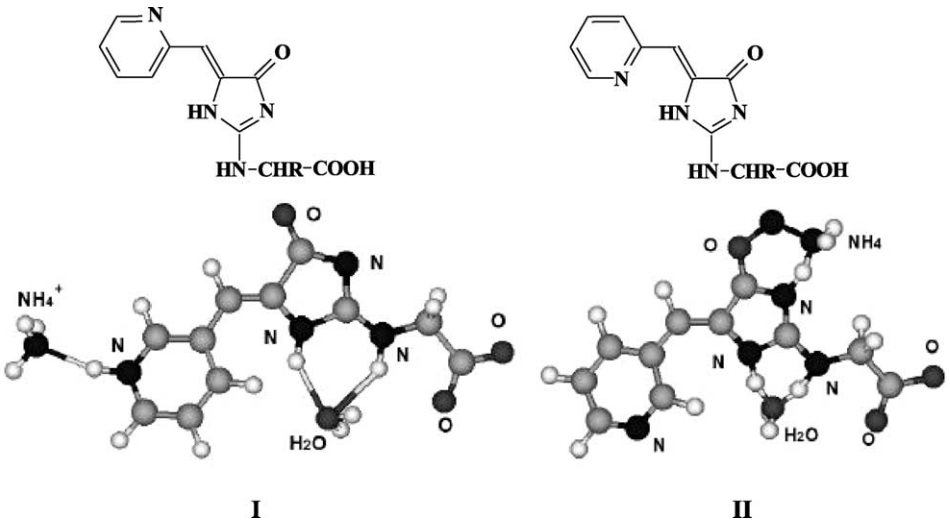


Fig. 6. Two possible (**I** and **II**) complexes of **17** with water and ammonia.

three possible conformations for molecule **23** of almost equal energy. However, all of them are far from planarity and none of them is superimposed with the reference molecule **4**. Therefore, molecule **23** cannot fulfil binding model environs. Now, it becomes clear why the benzyl analogue is less active than the benzylidene reference molecule. After conformational calculations for **17** it was established that the pyridil-3 substituent was able to adopt, with similar probability, two opposing orientations in the molecule (Fig. 6). Hence, the complexes simulation with water and ammonia ion should be performed for both conformers of **17**. As one can see, in complex with first form (I), the pyridine nitrogen participates as dominating H-bond acceptor. This complex does not agree with the model accepted previously for benzylidene reference derivatives (see Fig. 3). One can anticipate that two potential and different ways of H-bond formation unquestionably reflect-on the inhibition lowering with respect to the reference molecule **4**.

4. Conclusion

Model of the hydrogen bonds formation between ligand molecule and small chemical particles (water and ammonia), proposed in this paper, might be very useful in structure–activity studies. First of all, these small molecules are able to easily distinguish some pharmacophoric points in the molecule of the ligand. Subsequently, it can be utilised to simulate potential ligand–receptor interaction with chosen amino acids. In some cases (for example: isomeric molecule) heat of formations, being corresponding to binding energy of the amino acids with ligands, can provide a rational interpretation of discrepancy in biological properties of the studied isomeric derivatives. On the other hand, the model of the complex with small particles may be used as advantageous tool for preliminary selection of the studied molecules.

5. Elemental analyses

*N-[5-(Z)-(4-chlorobenzylidene)-4-oxo-2-imidazolidinyl]alanine (**6**)*: $C_{13}H_{12}N_3O_3Cl$. Calcd: C 53.15; H 4.12; N 14.31. Found: C 52.93, H 4.18, N 14.03.

*N-[5-(Z)-(3-chlorobenzylidene)-4-oxo-2-imidazolidinyl]alanine (**9**)*: $C_{13}H_{12}N_3O_3Cl$. Calcd: C 53.15; H 4.12; N 14.31. Found: C 53.34, H 4.00, N 14.18.

*N-[5-(Z)-(4-chlorobenzylidene)-4-oxo-2-imidazolidinyl]-(4-chlorophenyl)alanine (**11**)*: $C_{19}H_{15}N_3O_3Cl_2$. Calcd: C 56.45; H 3.74; N 10.39. Found: C 56.61, H 3.59, N 10.06.

N-[5-(Z)-(4-chlorobenzylidene)-4-oxo-2-imidazolidinyl]-(2-chlorophenyl)alanine (***12***): $C_{19}H_{15}N_3O_3Cl_2$. Calcd: C 56.45; H 3.74; N 10.39. Found: C 56.27, H 3.67, N 10.21.

N-[5-(Z)-(4-chlorobenzylidene)-4-oxo-2-imidazolidinyl]-4-fluorophenylalanine (***13***): $C_{19}H_{15}N_3O_3ClF$. Calcd: C 58.84; H 3.90; N 10.84. Found: C 58.61, H 4.07, N 10.90.

N-[5-(Z)-(4-chlorobenzylidene)-4-oxo-2-imidazolidinyl]-phenylalanine (***14***): $C_{19}H_{16}N_3O_3Cl$. Calcd: C 61.71; H 4.36; N 11.36. Found: C 61.42, H 4.51, N 11.05.

N-[5-(Z)-(4-chlorobenzylidene)-4-oxo-2-imidazolidinyl]-tryptophan (***15***): $C_{21}H_{17}N_4O_3Cl$. Calcd: C 61.69; H 4.19; N 13.71. Found: C 62.01, H 4.02, N 13.81.

N-[5-(Z)-(4-chlorobenzylidene)-4-oxo-2-imidazolidinyl]phenylglycine (***16***): $C_{18}H_{14}N_3O_3Cl$. Calcd: C 60.77; H 3.97; N 11.81. Found: C 60.44, H 3.77, N 11.46.

N-[5-(pyridyl-3)-methylene-4-oxo-2-imidazolidinyl]glycine (***17***): $C_{11}H_{10}N_4O_3$. Calcd: C 53.66; H 4.09; N 22.76. Found: C 53.97, H 4.20, N 22.97.

N-[5-(naphthyl-1)-methylene-4-oxo-2-imidazolidinyl]glycine (***18***): $C_{16}H_{13}N_3O_3$. Calcd: C 65.08; H 4.44; N 14.23. Found: C 64.87, H 4.56, N 14.30.

N-[5-(naphthyl-2)-methylene-4-oxo-2-imidazolidinyl]glycine (***19***): $C_{16}H_{13}N_3O_3$. Calcd: C 65.08; H 4.44; N 14.23. Found: C 65.39, H 4.57, N 13.92.

N-(5-cinnamylidene-4-oxo-2-imidazolidinyl)glycine (***20***): $C_{14}H_{13}N_3O_3 \times 0.33\ CH_3COOH$. Calcd: C 60.49; H 4.96; N 14.44. Found: C 60.26, H 5.19, N 14.10.

N-[5-(2-methyl)-cinnamylidene-4-oxo-2-imidazolidinyl]glycine (***21***): $C_{15}H_{15}N_3O_3$. Calcd: C 63.15; H 5.30; N 14.73. Found: C 63.41, H 5.24, N 14.97.

N-(5-benzyl-4-oxo-2-imidazolidinyl)glycine (***23***): $C_{12}H_{13}N_3O_3 \times 0.66\ CH_3COOH$. Calcd: C 55.76; H 5.49; N 14.65. Found: C 55.57, H 5.69, N 14.53.

Acknowledgements

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