Quantum-Chemical Structure-Affinity Studies on Kynurenic Acid Derivatives as Gly/NMDA Receptor Ligands

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ABSTRACT: A quantum-chemical structure–affinity relationship for kynurenic acid derivatives (KA) acting at the Gly/NMDA site is presented. The results of this study strongly suggest that these molecules bind to this site through three mechanisms: (1) a H bond between the N atom and an OH group of the receptor; (2) a $\pi-\pi$ orbital interaction between the empty MOs of the aromatic ring of KA derivatives and occupied MOs of the receptor; and (3) an orbital interaction between the carboxylate group and an electron-accepting site of the receptor. © 1999 John Wiley & Sons, Inc. Int J Quant Chem 71: 505–511, 1999

Introduction

The *N*-methyl-D-aspartate (NMDA) receptor seems to play a key role in several abnormal brain processes, such as in Alzheimer's disease, Huntington's disease, epilepsy, and cerebral ischemia [1–5]. To be activated, the NMDA receptor requires the occupation of two distinct recognition sites by glutamate and glycine (the latter at the so-called Gly/NMDA site) [6, 7]. Glycine acts as an endogenous coagonist at its site [8, 9]. Following the discovery of the stimulatory action of glycine at the NMDA receptor, it was found that kynurenic acid (KA, Fig. 1), a weak and nonselective NMDA antagonist, blocked this effect of glycine [10, 11]. KA has very weak affinity for the Gly/NMDA site and is not selective, having a similar potency as that of an antagonist at both NMDA and non-NMDA receptors (kainate and AMPA). The chemical modification of KA, however, has produced compounds with very high affinity [12–16].

Recently, a structure–activity study of 5- and 7-substituted KA derivatives was presented [13]. The biological property analyzed was the functional antagonist potency assessed by the determination of the apparent dissociation constants for antagonism of the depolarization induced by NMDA. The results suggested a requirement for optimally sized, hydrophobic 5- and 7-substituents, with bulk tolerance being greater at the 5-position [13].

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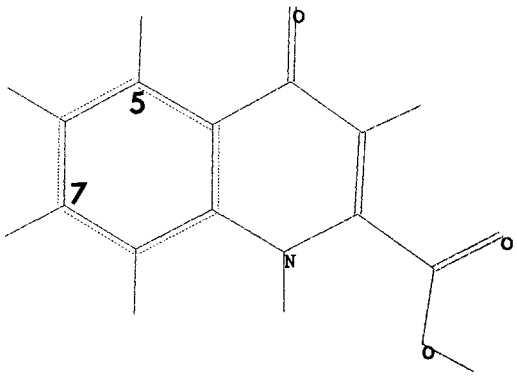

FIGURE 1. Kynurenic acid (KA).

The remarkable importance of these compounds requires more research on their structure–activity relationships (SAR). In this article, we report our results of an SAR study of a group of KA derivatives substituted in the phenyl ring. This study was focused on the affinity of these compounds for the Gly/NMDA site.

Methods, Models, and Calculations

As the method has been discussed thoroughly elsewhere, we shall present only a general sketch. Briefly, the equilibrium constant K can be expressed as [17–20]

$$\log K = a + b \log M_D + c \log \sigma_D + d \log(I_1 I_2 I_3) + e\Delta E, \quad (1)$$

where a, b, c, d, and e are constants, D refers to the drug molecule, σ is the symmetry number, M is the drug's molecular mass, $I_1 I_2 I_3$ is the product of the three moments of inertia about the three principal axes of rotation, and ΔE is the drug-receptor interaction energy.

The interaction energy is evaluated through the Klopman–Peradejordi–Gómez (KPG) approach as [19]

$$\Delta E = W + \sum_i \left[E_i Q_i + F_i S_i^E + G_i S_i^N \right] + \sum_i \sum_m \left[H_i(m) D_i(m) + J_i(m) S_i^E(m) \right] + \sum_i \sum_{m'} \left[R_i(m') D_i(m') + T_i(m') S_i^N(m') \right], \quad (2)$$

where W, E, F, G, H, J, R, and T are constants and Q_i, S_i^E, and S_i^N are, respectively, the net charge, the electrophilic superdelocalizability, and the nucleophilic superdelocalizability of atom i. The index $m(m')$ refers to the contribution to the above properties of the occupied (virtual) molecular orbital $m(m')$. $D_i(m)$ is the electronic density of atom i at molecular orbital (MO) m (or m'). Equation (2) was derived assuming that the only important component of ΔE is the change in electronic energy. Only drug-related terms appear in Eqs. (1) and (2).

By inserting Eq. (2) into Eq. (1), we obtain an equation expressing the relationship between biological activity and reactivity parameters of the drug molecules only. When employed within an *in vacuo* CNDO/2 level of parametrization, this approach has produced excellent QSAR results for very different biologically active molecules [21–25].

As the quantum-chemical reactivity indices can be calculated at any desired level of the theory [because Eqs. (1) and (2) do not depend on any particular calculation method], any method giving good results in calculating reactivity indices whose variation explains the variation of the affinity within a given drug family is acceptable. Because of our earlier good results in other systems [21–25], the numerical values for the electronic parameters were obtained from molecular wave functions calculated within the MO theory at the CNDO/2 level after full AM1 geometry optimization. AM1 was chosen for the geometry optimization because this method predicts reliable heats of formation. Finally, we took care that the numerical values of the nucleophilic superdelocalizabilities behaved well in the sense that their values were always positive [26].

The affinity constants were measured using very well defined experimental conditions in which the polarity of the binding domain of the NMDA receptor is an unknown but constant number. Therefore, it is necessary to carry out an SAR study with reactivity indices arising from calculations with different medium polarities to find the equation best reflecting the experimental results. Electrostatic environmental effects were incorporated through an extended version of the Generalized Born formula, which also includes steric hindrance effects upon desolvation of the atomic centers of the molecular system. For more details about the theory, we refer the reader to the literature [27, 28, and references therein]. This representation of environmental effects gave good results within the

CNDO/2 framework when applied to ion-pair formation [28] and receptor [29] and catecholamine storage [30] modeling.

The selected molecules are shown in Table I. The IC_{50} values were transformed to equilibrium constants, log K_i, accordingly to [31]

$$\log K_i = \log(0.294 IC_{50} - 0.0147). \qquad (3)$$

We must stress here that the number of selected molecules is formally restricted by this transformation; no other reported IC_{50} values can be incorporated into this set.

The molecules were studied in their protonated form (see below) in the presence or absence of a polarizable medium. In the last case, weakly ($\varepsilon = 3$) and highly ($\varepsilon = 30$) polarizable media were considered. The statistical fitting of Eq. (1) was performed using a stepwise regression technique with log K_i and the functional antagonistic potency as the dependent variables. The reactivity indices of a common skeleton composed of atoms 1–17 of Figure 2 were used as independent variables. The statistical analysis procedure may be summarized as follows: Starting from a simple formulation $Y = c + f(X_i)$, where c is a constant and X_i is the independent variable most correlated with Y, the model is improved by the addition of another variable to give $Y = c + f(X_i, X_j)$. At this moment, the first variable could have a negligible influence upon X. In this case, the program extracts it from the equation. In the next stage, a new variable is added and, eventually, a variable to the equation could leave the equation. The program works in this way until it finishes by stabilizing itself in such a way that no variable can enter or leave the system. The conditions for the inclusion or extraction of one variable are determined by the result of the F test. For establishing different conditions for the F test result and for multicollinearity, we may explore all the possible relevant equations.

TABLE I
Experimental and calculated affinities for KA derivatives.

Molecule[a]	R_5	R_7	log K[b]	log K[c]
1	H	H	1.081	1.081
2	Cl	H	0.180	0.268
3	Me	H	−0.220	0.024
4	Et	H	0.145	−0.314
5	H	Cl	−0.824	−0.878
6	H	Br	−0.692	−0.508
7	H	Me	−0.289	−0.036
8	Cl	Cl	−1.356	−1.491
9	Br	Br	−1.975	−1.729
10	Me	Me	−0.841	−0.774
11	Br	Me	−0.645	−0.492
12	Et	Cl	−1.209	−1.182
13	Cl	Et	0.154	0.022
14	Me	Br	−0.510	−0.685
15	Br	Et	−0.033	−0.273
16	Et	Br	−2.328	−2.354
17	Cl	Cl	−0.161	−0.143

[a] R_6 = H in all the molecules but molecule 17 for which R_6 = Cl.
[b] [13].
[c] Calculated with Eq. (4).

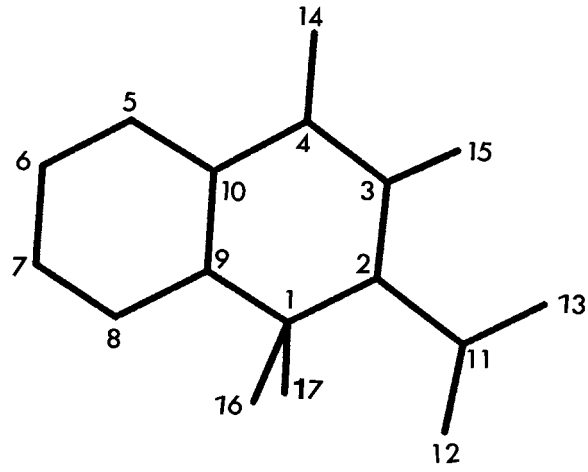

FIGURE 2. Atom numbering for the common skeleton.

Results and Discussion

THE RECOGNITION PHARMACOPHORE

The molecular electrostatic potential (MEP) is a good pictorial representation of the kind of electrostatic interaction that is dominant in the earlier steps of the drug-recognition process. These interactions occur in the region where there is an accumulation, recognition, and guiding of the drug molecule toward the receptor through long-range interactions (ionic in this case). For this reason, the MEP map generated around the pharmacophorically significant parts of the KA derivatives must resemble in shape the potential generated around the natural ligand, that is, glycine. In Figure 3, we show the MEP map of zwitterionic glycine, the

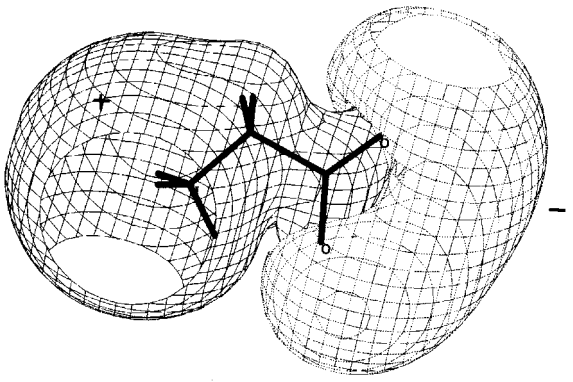

FIGURE 3. Molecular electrostatic potential (MEP) map for zwitterionic glycine.

form present at the receptor level.* We may see around the carboxylate moiety a large area of negative potential (marked hereafter −) while the cationic head presents a similar large area of positive potential (marked + hereafter). In Figures 4–6, we show, respectively, the MEP map of the neutral, anionic, and zwitterionic forms of KA. The comparison of these figures with Figure 3 strongly suggests that the zwitterionic form of KA is the one interacting with the Gly/NMDA site.

QSAR RESULTS

More than 150 SAR equations were analyzed, and the results can be summarized as follows: The only statistically acceptable equation relating the Gly/NMDA site affinity to the reactivity indices was found for the case of a high-polarity medium ($\varepsilon = 30$). For molecules 1–17, the corresponding equation is

$$\begin{aligned}\log K_i = {} & 53.0044 + 0.7670 S_8^N(SLUMO) \\ & - 0.1633 S_9^N(SLUMO) + 7.5470 S_1^E \\ & + 1.1364 D_{12}(SHOMO) \\ & + 0.4235 S_{17}^N(NLUMO), \end{aligned} \quad (4)$$

with $n = 17$, mean $SD = 0.23$, and $R = 0.97$. In this equation, $S_8^N(SLUMO)$, $S_9^N(SLUMO)$, S_1^E, $D_{12}(SHOMO)$, and $S_{17}^N(NLUMO)$ are, respectively, the contribution of atom 8 to the nucleophilic superdelocalizability of the third virtual MO, the contribution of atom 9 to the nucleophilic superde-

* All the potential maps were generated with Hyperchem V.5.0 (Hypercube Inc. 419 Phillip Street, Waterloo, Ontario, Canada). The electrostatic contour value is 0.105, with a starting value of −0.1682 and an increment of 0.05. All the maps are in the heavy atom plane.

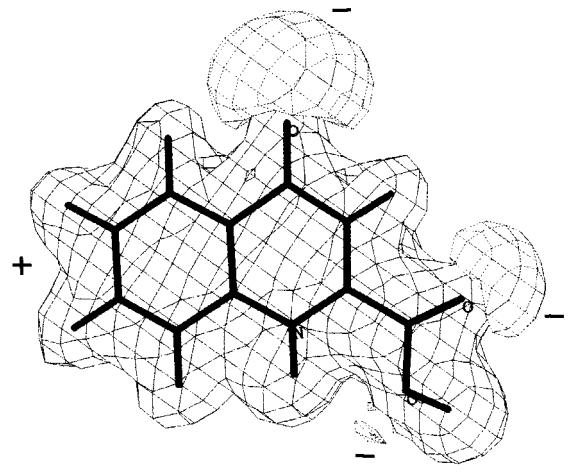

FIGURE 4. MEP map for neutral kynurenic acid.

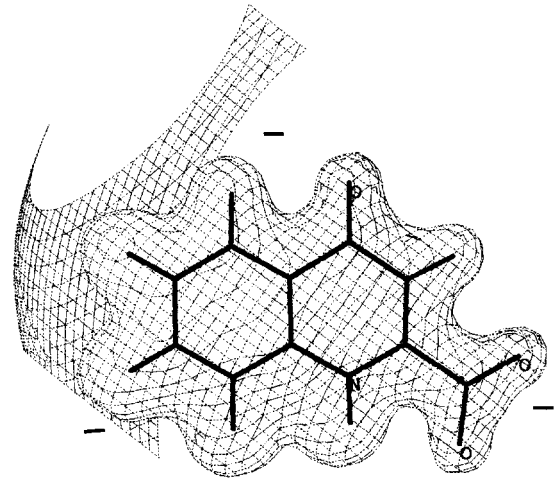

FIGURE 5. MEP map for anionic KA.

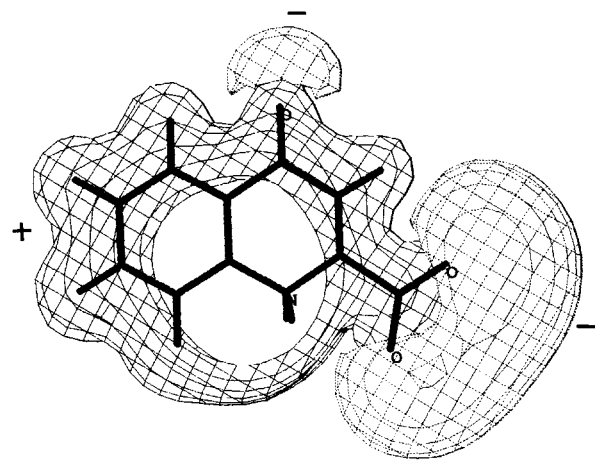

FIGURE 6. MEP map for zwitterionic KA.

TABLE II
Results of the Student's *t*-test for the significance of the variables appearing in Eq. (4).

Variable	*t*	*P*
$S_8^N(SLUMO)$	12.29	< 0.0005
$S_9^N(SLUMO)$	2.26	0.0225
S_1^E	7.11	< 0.0005
$D_{12}(SHOMO)$	3.40	0.0030
$S_{17}^N(NLUMO)$	2.12	0.0287

localizability of the third virtual MO, the total electrophilic superdelocalizability of atom 1, the electronic density of atom 12 at the third occupied MO, and the contribution of atom 17 to the nucleophilic superdelocalizability of the second virtual MO. The analysis of variance of Eq. (4) gives $F(5, 11) = 41.620$ ($P < 0.000$S), showing that this equation is highly significant. The predicted K values are shown in Table I. The result of the Student's test for the significance of variables appearing in this equation is presented in Table II. The squared correlation coefficient matrix for these variables is presented in Table III.

To obtain a more precise knowledge of the factors controlling the interaction of specifically substituted molecules, we carried out the same procedure for a group of only 5,7-disubstituted KA derivatives. The resulting equation is

$$\log K_i = 13.9905 - 3.7167S_7^E - 0.2323S_5^N(SLUMO) + 30.1853S_{17}^E, \quad (5)$$

with $n = 10$, mean $SD = 0.22$, and $R = 0.98$. Here, S_7^E, $S_5^N(SLUMO)$, and S_{17}^E are, respectively, the total electrophilic superdelocalizability of atom 7, the contribution of atom 5 to the nucleophilic superdelocalizability of the third virtual MO, and the

TABLE III
Squared correlation coefficient matrix for the variables appearing in Eq. (4).

	S_8^N (*SLUMO*)	S_9^N (*SLUMO*)	S_1^E	D_{12} (*SHOMO*)
S_9^N (*SLUMO*)	0.26	1.0		
S_1^E	0.34	0.00	1.0	
D_{12} (*SHOMO*)	0.01	0.00	0.03	1.0
S_{17}^N (*NLUMO*)	0.10	0.12	0.05	0.02

TABLE IV
Experimental and calculated affinities for 5,7-disubstituted kynurenic acid derivatives.

Molecule	log K [a]	log K [b]
1	1.081	1.236
8	−1.356	−1.612
9	−1.975	−1.836
10	−0.841	−0.830
11	−0.645	−0.570
12	−1.209	−1.193
13	0.154	−0.202
14	−0.510	−0.382
15	−0.033	−0.086
16	−2.328	−2.180

total electrophilic superdelocalizability of atom 17. The analysis of variance of Eq. (5) gives $F(3, 6) = 64.480$ ($P < 0.0005$), showing also that this equation is highly significant. The predicted K values are shown in Table IV. The result of the Student's test for the significance of variables appearing in this equation is presented in Table V. The squared correlation coefficient matrix for these variables is presented in Table VI.

Our results show that the Gly/NMDA receptor affinity variation is related to the variation of a definite set of molecular reactivity indices. This implies a common interaction mechanism between all the drugs analyzed and the Gly/NMDA sites. The first thing to note is that, as the best SAR equations were obtained for $\varepsilon = 30$, the receptor area is a polarized one. This fact is consistent with interaction with the zwitterionic form of KA derivatives.

The analysis of Eq. (4) shows that high receptor affinity is associated with high values of S_1^E and $S_9^N(SLUMO)$ and with low values for $S_8^N(SLUMO)$, $D_{12}(SHOMO)$, and $S_{17}^N(NLUMO)$. The appearance of S_1^E in Eq. (4) indicates that the variation of log K depends on the reactivity of the N atom with electrophilic components of the receptor. This sug-

TABLE V
Results of the Student's *t*-test for the significance of variables appearing in Eq. (5).

Variable	*t*	*P*
S_7^E	13.80	< 0.0005
$S_5^N(SLUMO)$	3.84	0.0043
S_{17}^E	5.41	0.0008

TABLE VI
Squared correlation coefficient matrix for the variables appearing in Eq. (5).

	S_7^E	S_5^N (SLUMO)
$S_5^N(SLUMO)$	0.17	1.0
S_{17}^E	0.14	0.02

gests a charge transfer from the N atom toward the receptor. The requirement of a low value for $S_{17}^N(NLUMO)$ (i.e., a low electron-accepting capacity for atom 17) are indicative of a possible interaction with a slightly nucleophilic center of the receptor. This suggests the possibility of the formation of a H bond between the N atom of KA derivatives and a residue of the receptor. This has been suggested by experimentalists [13]. Given that the groups able to form a H bond in proteins are —OH,—SH, and —NH_2, we may speculate that the target is the —OH group because of the electron-donating capacity of the N atom.

The appearance of $S_8^N(SLUMO)$ and, with a lesser significance, of $S_9^N(SLUMO)$ suggests an interaction between atoms 8 and 9 and one or more nucleophilic centers of the receptor through the formation of a charge-transfer complex in which the aromatic ring is acting as an electron acceptor. Given that the SLUMO is a π orbital, we may think of an interaction with occupied π MOs of the receptor. The importance of the occupied and empty MOs other than the frontier orbitals has been stressed [32–35]. The fact that the interaction is better described by the third empty MO does not mean that the NLUMO and the LUMO do not participate in it, but that their variation is not statistically significant to describe the variation of receptor affinity. The requirement of a low value for $D_{12}(SHOMO)$ can be interpreted within the above scheme as the necessity that the SHOMO, a π MO, not be localized on atom 12, thus allowing the penetration of a receptor MO in this area. This could be due to a possible interaction between an occupied π MO of the receptor with an empty π MO of KA derivatives centered on atom 12.

An electrostatic interaction between the carboxylate group and a complementary site of the receptor has been suggested [7]. Our results suggest that this may not be the case but that the carboxylate group may contribute to a charge-transfer complex. This is consistent for the case of some quinoxalindiones with a high Gly/NMDA affinity having a carbonyl group instead of a carboxyl [13].

For the 5,7-disubstituted KA derivatives [Eq. (5)], the analysis is the following: The appearance of S_7^E suggests a charge transfer from atom 7 to an electron-deficient center of the receptor involving the aromatic ring. Nevertheless, as high affinity requires a low electron-donating ability of atom 7, it seems that the substituent that is attached to atom 7 must be a good electron acceptor. Experimentalists have suggested the same idea [13]. The appearance of S_{17}^E is indicative of a charge transfer from atom 17 to the receptor. Moreover, the fact that high affinity is associated with a high electron-donating ability of this atom (which is perfectly coherent with a low electron-accepting ability) confirms the above suggestion of a H bond.

The appearance of $S_5^N(SLUMO)$ again suggests the interaction between a nucleophilic center of the receptor and at least atom 5. This interaction might be through formation of a charge-transfer complex involving the SLUMO (of π character) centered on atom 5. This could be again indicative of a π–π orbital interaction. Given that high receptor affinity demands that atom 5 have a high electron-accepting ability, this may suggest that its substituent can alter the electronic structure of the aromatic ring if it has a strong electron-attracting character. This is coincident with the experimental observation that high receptor affinities are associated with a halogen at position 5 [13].

In conclusion, the KA derivatives present the following interaction mechanism with the Gly/NMDA site:

1. Formation of a H bond between the N atom and an OH group of the receptor.
2. A π–π orbital interaction between the KA aromatic ring and a complementary aromatic site of the receptor. KA derivatives contribute to this interaction with their empty π MOs. This interaction is facilitated by the presence, at positions 5 and/or 7, of electron-attracting groups.
3. An orbital interaction between at least one O atom of the carboxylate group and one electron-accepting site of the receptor.

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