

Electrostatic and conformational analysis of piracetam and cyclo-L-prolylglycine to explain their potency difference

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Structural Data Access

The reference cyclo-L-prolylglycine molecule and the piracetam conformations aligned to it for MEP similarity assessment are available at:

http://qsar.chem.msu.ru/~shulga/CPG_piracetam_MEP/

Conformation generation

Conformations for piracetam and CPG were generated in the DataWarrior v6.05 package^{S1} using the adaptive collisions avoidance algorithm, followed by optimization in the MMFF94s+ force field (the number of conformer generation attempts equal to 1000). To generate a representative set of conformations of piracetam, the search was carried out twice with different values of dielectric constant, ϵ , which affects the degree of influence of intramolecular electrostatic interactions. Two values of ϵ were used simulating vacuum ($\epsilon = 1$) and protein environment ($\epsilon = 4$). The choice of two dielectric constants allows us to understand the composition of conformational space in general and to find different types of conformations that can occur in different conditions. For the case of low dielectric constant ($\epsilon = 1$), four conformations of piracetam containing an intramolecular hydrogen bond were obtained (Figure 2 in the paper). The conformations differ in the spatial “chirality” of the conformer, as well as in the position of methylene carbon in position 4 of the pyrrolidone cycle. For $\epsilon = 4$, ten more diverse conformations were obtained, in which the intramolecular hydrogen bond was either weakly expressed according to geometric criteria, or completely absent. Such conformations correspond more closely to the relatively polar environment of the molecule within the receptors, and even more so in an aqueous solution. The same procedure for the CPG molecule resulted in only two conformations due to the rigid geometry of the molecule. The position of the methylene carbon in position 4 of the pyrrolidine fragment of the molecule (the most

distant from the six-membered cycle) has a slight variation. The electrostatic potential of the two conformers of CPG is almost identical, therefore, the more energetically favorable conformer was used for further analysis.

Molecular electrostatic potential

All described conformations of piracetam and CPG were used to calculate the quantum chemical molecular electrostatic potential (QC MEP) for subsequent comparison. The calculation was carried out in the RHF/6-31G* approximation, which is a standard practice for modeling electrostatic interactions in classical molecular mechanics force fields, *i.e.*, GAFF/AMBER^{S2,S3} and OPLS3e/4.^{S4,S5} The calculations were performed using the Firefly QC 8.2.0 software^{S6} on 5 layers of the Connolly surface (spheres of 1.4-2.2 vdW radii) with a point density of 4 Å⁻² (equivalent to 1.12 Bohr⁻²).

The comparison of MEPs of different conformers of the two molecules was carried out by an expert analysis in the PyMOL package,^{S7} after visualization of the quantum-chemically calculated MEP using the specific plug-in.^{S8}

Molecular dynamics

To assess the population of conformations of piracetam molecule in an aqueous solution, molecular dynamics (MD) simulation in the GROMACS v2023 package^{S9} was performed using the GAFF force field^{S2} for modeling small molecules and the TIP3P water model. To calculate the atomic charges, two methods were used, namely RESP^{S10} and DENR,^{S11,S12} which give charges consistent with the GAFF force field due to the good reproduction of MEP for molecules at the RHF/6-31G* level.

The AmberTools24 package^{S13} was used to obtain the force field parameters. Separate simulations were performed for each charge calculation method - RESP and DENR - for the piracetam molecule. The piracetam molecule in a periodic dodecahedral box was surrounded by TIP3P water molecules so that the nearest edge of the box from the molecule was at least 10 Å. The initial system was subjected to 50,000 energy minimization steps, followed by a cycle of 100 ps of NVT ensemble simulation (T=300K), 100 ps of NPT ensemble (T=300K - V-rescale, P=1 atm - Berendsen) and subsequent equilibration for 10 ns in the NPT ensemble. The next 20 ns of the NPT ensemble were used for conformational analysis, by extracting trajectory snapshots every 10 ps, which led to a 2001 snapshot for each set of atomic charges of the piracetam molecule.

Conformational analysis based on MD trajectory data in an aqueous solution was performed using the gmx utility (GROMOS clustering algorithm). The RMSD threshold value was changed to 0.48 Å using the “-cutoff 0.048” option. Before the conformational analysis, the ligand was centered in the cell to avoid artifacts of periodic conditions. For the subsequent analysis, clusterization output was used, which contains representative centers of the obtained clusters, as well as the composition of each cluster based on MD snapshots, which makes it possible to estimate the population of each conformation under modeling conditions.

Alignment procedure

We have conducted pairwise superposition of pharmacophoric features of piracetam and CPG molecules (different piracetam conformations and single CPG conformation). The alignment was done using the pair_fit function from PyMol. We have made 2 variants of alignment by optimal fitting of selected atoms in some piracetam conformation and CPG; both variants are shown on Figure S1.

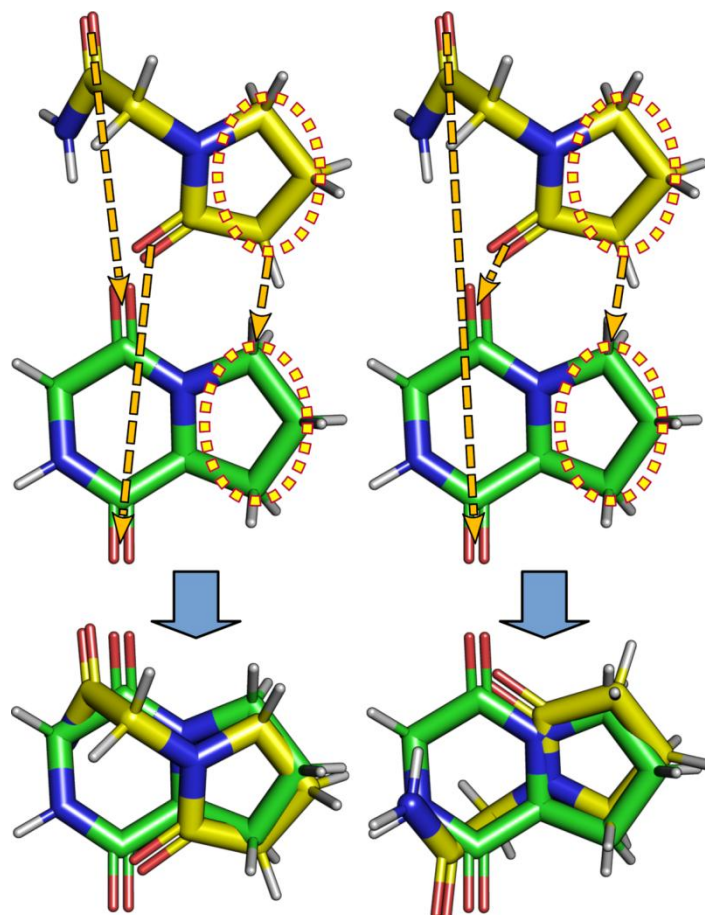


Figure S1. A scheme of two variants of pharmacophoric alignment of piracetam and cyclo-L-prolylglycine molecules.

Evaluation of MEP similarity

We qualitatively assessed the measure of MEP similarity of piracetam and CPG. At the first step, all piracetam conformers were superimposed with a single CPG conformer by their pharmacophoric features as described above. Then, for every superposition of two molecules (*i.e.*, for every combination of piracetam conformer and CPG molecule), a MEP grid was uniformly sampled on a 5 layer grid (1.4-2.2 vdW with a step of 0.2 vdW) around the “supermolecule”. Then, QC MEP (RHF/6-31G*) was calculated separately for the CPG and corresponding piracetam conformer on the sampled grid (*i.e.*, on the same set of points for the both molecules). In the end, the similarity of QC MEP of the piracetam’ conformer and CPG molecule was assessed through Pearson correlation coefficient (eq. S1), RMSD (eq. S2) and MAE (eq. S3). The results are presented in Table S1.

$$R = \frac{1}{N_{MEP\ points}} \sum_i \frac{MEP_i^{(1)}}{\sigma^{(1)}} \frac{MEP_i^{(2)}}{\sigma^{(2)}}; \sigma = \sqrt{\frac{1}{N_{MEP\ points}} \sum_i MEP_i^2}, \quad (S1)$$

$$RMSD = \sqrt{\frac{1}{N_{MEP\ points}} \sum_i \left(MEP_i^{(2)} - MEP_i^{(1)} \right)^2}, \quad (S2)$$

$$MAE = \frac{1}{N_{MEP\ points}} \sum_i \left| MEP_i^{(2)} - MEP_i^{(1)} \right|, \quad (S3)$$

where R is a Pearson correlation coefficient with a mean value forced to zero to preserve the physical meaning (positive $MEP^{(1)}$ matches positive $MEP^{(2)}$ etc.); $RMSD$ is a root mean square deviation; MAE is a mean average error.

Table S1. The statistical data of MEP comparison for alignment of cyclo-L-prolylglycine with different conformations of piracetam. R is a Pearson correlation coefficient, $RMSD$ is residual mean square distance, MAE is mean absolute error, max R is maximum value of Pearson correlation coefficient among two types of alignment (using different atoms in CPG and piracetam for alignment).

Conformation	Intramolecular HB	Chirality (if any)	R	$RMSD$	MAE	max R
c_1-1	-	0	0.053973	0.023986	0.018126	0.363869
c_1-2	-	0	0.363869	0.020339	0.014522	0.363869
c_2-1	-	0	0.309820	0.021338	0.015543	0.309820
c_2-2	-	0	0.066361	0.023926	0.018034	0.309820
c_3-1	-	0	0.255020	0.022869	0.019055	0.354922
c_3-2	-	0	0.354922	0.021274	0.016870	0.354922
c_4-1	-	0	0.387986	0.020793	0.016836	0.387986
c_4-2	-	0	0.300485	0.022078	0.018146	0.387986
c_5-1	-	0	0.466678	0.017305	0.014033	0.690808
c_5-2	-	0	0.690808	0.013336	0.010540	0.690808
c_6-1	-	0	0.014851	0.026750	0.020866	0.191213
c_6-2	-	0	0.191213	0.025025	0.018280	0.191213
c_7-1	-	0	0.640285	0.014274	0.011166	0.640285
c_7-2	-	0	0.478031	0.017028	0.013681	0.640285

Conformation	Intramolecular HB	Chirality (if any)	<i>R</i>	<i>RMSD</i>	<i>MAE</i>	max <i>R</i>
c_8-1	+	“proper”	0.585956	0.015001	0.011737	0.585956
c_8-2	+	“proper”	0.463079	0.017191	0.014244	0.585956
c_9-1	-	0	0.037404	0.027983	0.021593	0.037404
c_9-2	-	0	-0.003407	0.028645	0.021817	0.037404
c_10-1	-	0	-0.121257	0.027420	0.020506	0.116120
c_10-2	-	0	0.116120	0.025100	0.018047	0.116120
c_11-1	+	“inverted”	0.324923	0.020090	0.016861	0.477089
c_11-2	+	“inverted”	0.477089	0.017529	0.013954	0.477089
c_12-1	-	0	0.678266	0.013344	0.009732	0.678266
c_12-2	-	0	0.476779	0.016772	0.012394	0.678266
c_13-1	-	0	0.506841	0.015991	0.012145	0.745483
c_13-2	-	0	0.745483	0.011571	0.008365	0.745483
c_14-1	-	0	0.093020	0.026062	0.019029	0.093020
c_14-2	-	0	-0.119851	0.027860	0.021029	0.093020
c_15-1	-	0	-0.003423	0.028436	0.021615	-0.003423
c_15-2	-	0	-0.051623	0.028779	0.021545	-0.003423
c_16-1	-	0	0.003130	0.023913	0.018276	0.345461
c_16-2	-	0	0.345461	0.020217	0.014481	0.345461
c_17-1	-	0	0.076634	0.024107	0.017832	0.076634
c_17-2	-	0	-0.107020	0.025346	0.019344	0.076634
c_18-1	-	0	0.398016	0.018494	0.014747	0.550755
c_18-2	-	0	0.550755	0.016375	0.012937	0.550755
c_19-1	-	0	0.680888	0.012614	0.009563	0.680888
c_19-2	-	0	0.490600	0.015874	0.012253	0.680888
c_20-1	-	0	0.044299	0.024149	0.018316	0.331086
c_20-2	-	0	0.331086	0.020777	0.015193	0.331086
c_21-1	-	0	0.179573	0.024351	0.019746	0.183449
c_21-2	-	0	0.183449	0.024234	0.018942	0.183449
c_22-1	-	0	0.371682	0.021641	0.016927	0.371682
c_22-2	-	0	0.194785	0.023874	0.018885	0.371682
c_23-1	-	0	0.498226	0.015995	0.012105	0.748339
c_23-2	-	0	0.748339	0.011100	0.007746	0.748339
1_1-1	+	“inverted”	0.653980	0.012829	0.009905	0.653980
1_1-2	+	“inverted”	0.648425	0.012728	0.009731	0.653980

Conformation	Intramolecular HB	Chirality (if any)	<i>R</i>	<i>RMSD</i>	<i>MAE</i>	max <i>R</i>
1_2-1	+	“proper”	0.612186	0.013816	0.011018	0.612186
1_2-2	+	“proper”	0.537748	0.015216	0.012277	0.612186
1_3-1	+	“inverted”	0.576858	0.014441	0.011606	0.594643
1_3-2	+	“inverted”	0.594643	0.014160	0.010898	0.594643
1_4-1	+	“proper”	0.650918	0.012697	0.010125	0.650918
1_4-2	+	“proper”	0.611207	0.013621	0.010637	0.650918
4_1-1	-	0	0.220564	0.019994	0.015181	0.544676
4_1-2	-	0	0.544676	0.015517	0.011576	0.544676
4_10-1	-	0	0.031581	0.023338	0.017621	0.341781
4_10-2	-	0	0.341781	0.019740	0.014498	0.341781
4_2-1	-	0	0.551411	0.016316	0.013369	0.551411
4_2-2	-	0	0.473973	0.017866	0.014608	0.551411
4_3-1	-	0	0.436697	0.018210	0.015134	0.589932
4_3-2	-	0	0.589932	0.015675	0.012314	0.589932
4_4-1	-	0	0.305551	0.020380	0.015078	0.305551
4_4-2	-	0	0.064083	0.022944	0.017327	0.305551
4_5-1	-	0	0.615423	0.015056	0.011986	0.615423
4_5-2	-	0	0.442485	0.018126	0.014729	0.615423
4_6-1	-	0	0.225438	0.020034	0.015310	0.562144
4_6-2	-	0	0.562144	0.015260	0.011272	0.562144
4_7-1	-	0	0.512676	0.016176	0.012192	0.512676
4_7-2	-	0	0.268426	0.019318	0.014655	0.512676
4_8-1	-	0	0.471792	0.017993	0.014984	0.547002
4_8-2	-	0	0.547002	0.016533	0.013441	0.547002
4_9-1	-	0	0.489077	0.016538	0.012467	0.489077
4_9-2	-	0	0.266918	0.019306	0.014621	0.489077

Alignment to piracetam conformations in crystal structures of complexes with AMPA receptor

In order to compare the piracetam conformations found in experiment with our analysis we chose to compare the PDB crystal structure 3LSX,^{S14} which has been previously used to estimate the CPG possibility to bind in a similar manner as piracetam with extracellular agonist-binding S1S2 domain GluA3i of the AMPA receptor.^{S15} PDB:3LSX contains two different conformations of piracetam and the original paper^{S14} notes that the electron density of piracetam is rather weak, that could mean either the piracetam position is not well resolved or the overall binding is too weak to fill all the crystal units of S1S2 AMPA receptor domain. Thus, the derived conclusions should be taken not as the definite check but rather as an additional supporting evidence.

The two extracted conformations (Figure S2) were superimposed as rigid bodies to the set of conformations from Table S1 in order to find the closest corresponding conformation from our analysis.

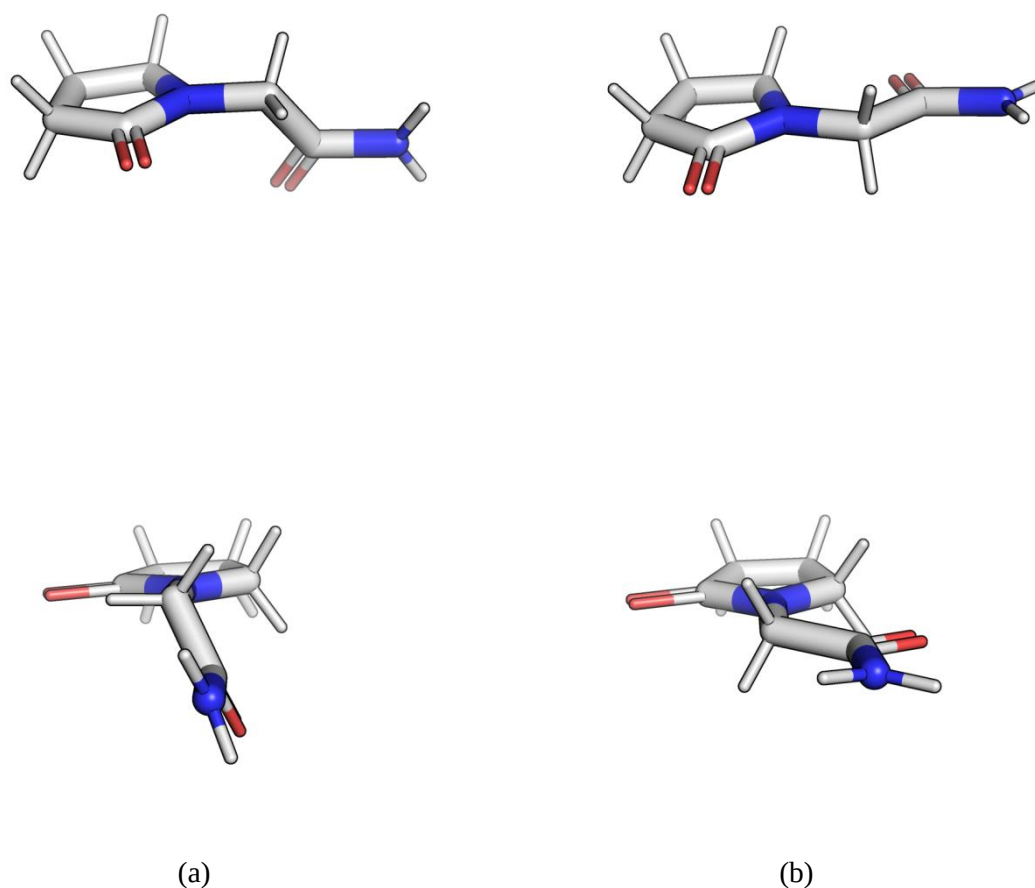


Figure S2. Two distinct conformations of piracetam complexed with the dimer of S1S2 domain of AMPA receptor from PDB:3LSX.^{S14}

The closest conformations revealed in our MD modeling of piracetam in water medium with RESP charges to each of the (a) and (b) conformations from PDB:3LSX are c_12 and c_23 (Figure S3), respectively. The corresponding statistical metrics are provided in Table S2.

It should be noted that despite the conformations taken from PDB are not similar to those containing intramolecular hydrogen bond (IHB) in piracetam, they both have even greater MEP correspondence to CPG (judging by Max R value from Table S2) and have even lower occupancy in water media than the IHB conformation (occupancy from Table S2). As pointed out by the authors of PDB:3LSX structural analysis,^{S14} low density of piracetam does not allow unambiguous detection of the piracetam conformation in the complex with AMPA receptor. Additionally, AMPA receptor is not the single target for rather small molecules such as piracetam and CPG in CNS. But in any case, it was shown that the occupancy of the conformations of piracetam that are the most electrostatically (judged by QC MEP correlation and residual error) similar to the fixed conformation of CPG are small in water media, and this can be used as an additional explanation of the 1000-fold higher activity of CPG compared to piracetam.

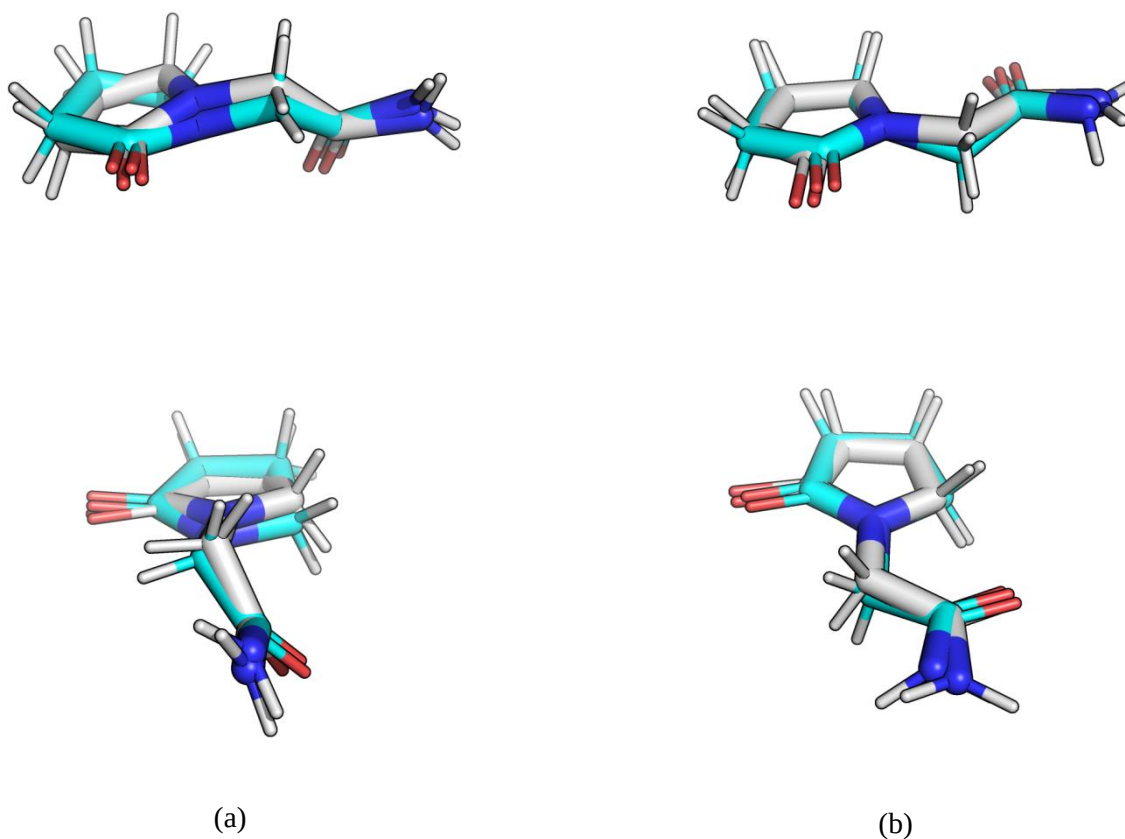


Figure S3. Alignment of the two distinct conformations of piracetam (a) and (b) complexed with S1S2 domain of AMPA receptor from PDB:3LSX^{S14} with the conformations from our cluster analysis of MD trajectory of piracetam in water medium with RESP charges. PDB piracetam conformations are in white, MD cluster conformations are in cyan.

Table S2. The statistical metrics of the aligned conformations of (a) and (b) from PDB:3LSX to the clusters revealed in MD trajectory analysis of piracetam in water medium.

Conformation from PDB	(a)	(b)
Closest cluster from MD	c_12	c_23
<i>RMSD</i> , Å	0.314	0.286
Max <i>R</i> from Table S1	0.678	0.748
Occupancy from MD, %	1.1	0.05

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