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Dedicated to Professor Ionel Haiduc
on the occasion of his 70th anniversary

CONFORMATIONAL ANALYSIS WITH SEMIEMPIRICAL METHODS FOR A QUINOXALINE UREA DERIVATIVE, ACTIVE ON BENZODIAZEPINE RECEPTOR

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The structure of possible low energy conformers of a quinoxaline urea derivative, 5-(3,5-dimethyl-piperazine-1-carbonyl)-7-fluoro-4,5-dihydro-imidazo[1,5-*a*]quinoxaline-3-carboxylic acid *tert*-butyl ester that acts as high potent antagonist on the central benzodiazepine receptor has been obtained with the AMBER94 force field and the AM1 semiempirical MO method. The work discusses the variation of different geometrical parameters (bond angles and dihedrals, distances between atoms on the backbone and atoms placed on substituents) and the variation of physico-chemical properties calculated with AM1 method as dipole moments, E_{HOMO} and E_{LUMO}. AM1 rotation barriers suggest that for better interaction with the amino acid residues form the active site only limited interconversions between conformers are possible.

INTRODUCTION

Ligands that interact with benzodiazepine receptor (BzR) and modulate allosterically the action of gamma-amino butyric acid (GABA) on neuronal chloride channel can have a large spectrum of intrinsic activity. Full agonists ("GABA-positive" ligands) have anxiolytic,[1-9] anticonvulsant,[10-14] and hypnotic[15-16] effects. Pharmacological actions due to antagonists and inverse agonists ("GABA-negative" ligands) are proconvulsant and anxiogenical. Partial agonists lie within these opposed activities and may have reduced benzodiazepine-mediated side effects such as physical dependence, amnesia[17-19] oversedation (anxiolytics), and muscle relaxation.[20-24]

Classification of different $GABA_A$-benzodiazepine receptors and effects of different specific ligands have been reviewed.[25-26] In order to obtain ligands with better pharmacological profile different groups synthesized a large number of compounds.[27-34] Among them the derivatives of quinoxaline-ureas have shown high affinity for the benzodiazepine site on $GABA_A$ receptor. The identification of possible conformations for one of the most active compounds from this class obtained by Jacobson and coll.,[28] namely, 5-(3,5-dimethyl-piperazine-1-carbonyl)-7-fluoro-4,5-dihydro-imidazo[1,5-*a*]quino-xaline-3-carboxylic acid *tert*-butyl ester can give information about steric and electrostatic interactions with amino acid residues from the receptor active site. We report the results of a conformational analysis performed for this quinoxaline derivative.

METHODS

3D-geometries of conformers have been optimized with the AMBER94 force field from the HyperChem7.52 package[35] and conformational analysis was carried out with the Conformational Search module from the samesoftware. Four dihedrals: C_4-C_3-C_{15}-O_{16}, C_3-C_{15}-O_{16}-C_{17}, C_7-N_6-C_{22}-N_{23}, and N_6-C_{22}-N_{23}-C_{24} have been varied with steps of 30°. These correspond to four rotatable bonds marked by arrows in figure 1. In order to obtain a larger set of geometries the energy criterium was set to 418.4 kJ/mol (100 kcal/mol).

First 100 conformations with the lowest energies were kept. The number of optimizations was set to 5000. The AMBER geometries obtained for 100 conformers with the lowest energies above the best conformer were optimized at AM1 level, using AM1 Hamiltonian in standard parametrization from HyperChem7.52 package. Optimization was performed with the Polak-Ribiere conjugate gradient algorithm using the RMS gradient norm of 0.04184 kJ/Åmol (0.01 kcal/Åmol) and the SCF convergence of 10^{-5}.

RESULTS AND DISCUSSION

The structure of the quinoxaline-urea derivative, the numbering of heavy atoms and the dihedrals varied in conformational search are displayed in figure 1.

From the lowest 100 conformations resulted from the optimization with AMBER94 method only 24 are distinct and they are collected in Table 1 together with the corresponding values of energies and dihedrals.

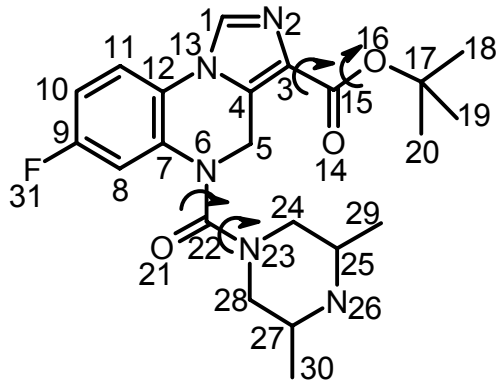

Fig. 1 – Structure of 5-(3,5-dimethyl-piperazine-1-carbonyl)-7-fluoro-4,5-dihydroimidazo-[1,5-*a*]quinoxaline-3-carboxylic acid *tert*-butyl ester, and the atom numbering; arrows mark bonds rotated in conformational analysis.

Table 1

AMBER distinct conformers with lowest energies and values of varied dihedrals

No	Energy	C_4-C_3-C_{15}-O_{16}	C_3-C_{15}-O_{16}-C_{17}	C_7-N_6-C_{22}-N_{23}	N_6-C_{22}-N_{23}-C_{24}
1	23.853	14.983	-179.086	-33.585	-36.349
2	23.854	115.630	-172.853	38.138	32.704
3	23.879	-116.436	172.759	-36.955	175.448
5	23.920	-13.361	179.497	33.448	-161.512
10	23.982	-22.354	-179.000	-52.694	-172.166
11	23.987	9.052	179.954	-33.309	-36.520
12	23.994	-141.323	176.250	-49.126	-177.406
17	24.045	-170.185	-179.438	-33.397	-36.627
18	24.050	22.450	179.170	53.688	22.841
23	24.096	169.713	179.589	33.437	-161.329
29	24.128	-12.392	179.227	33.418	35.438
30	24.134	-167.349	-179.608	-34.024	-36.035
31	24.139	14.612	-179.260	-33.515	165.628
32	24.140	138.790	-175.738	49.653	25.132
53	24.307	168.965	179.425	33.338	35.400
61	24.346	-39.567	179.579	-38.116	174.132
62	24.349	-170.657	-179.601	-33.217	165.076
64	24.379	40.093	-179.393	37.775	37.610
68	24.402	152.641	-178.431	103.664	-30.640
69	24.405	38.313	-171.226	-50.431	-178.075
70	24.416	-152.024	178.176	-102.719	-157.742
82	24.506	167.089	-179.897	34.559	34.973
88	24.557	-37.184	170.742	53.305	26.604
94	24.659	22.251	-175.716	-105.573	-158.811

Energy is expressed in kcal/mol and dihedrals in degrees

The condensed rings of structure from figure 1 are slightly deviated from planar geometry. The dihedral between phenyl and imidazole rings, C_7-C_{12}-N_{13}-C_4 is around 8-12°.

From table 1 one can see the large range of values for dihedral C_4-C_3-C_{15}-O_{16}: 9°; ±12-15°, ±22°, ±37-40°, ±115-116°; ±138-141°; ±152°, ±167-170°, while the dihedral C_3-C_{15}-O_{16}-C_{17} can have values only around ±170-180°.

Dihedral C_7-N_6-C_{22}-N_{23} can have a limited range of values: ±33-39°, ±49-53° and ±102-105°, while the N_6-C_{22}-N_{23}-C_{24} dihedral can take values around ±22-26°, ±30-37°, ±157-161° and ±172-178°.

The results obtained from geometry optimization of the 100 lowest AMBER conformers using the AM1 semiempirical MO method are displayed in Table 2.

Table 2

AM1 distinct conformers with the lowest energies, dipole moment, HOMO and LUMO energy, and the values of dihedrals and distances between atoms supposed to interact with amino acid residues from the receptor active site

No	ΔH_f	DM	E_{HOMO}	E_{LUMO}	D1	D2	D3	D4	d1	d2	d3	d4
45	-61.689	4.321	-9.14	-0.96	-1.17	179.51	-9.16	-59.45	6.248	6.221	4.651	4.845
82	-61.688	4.345	-9.15	-0.96	-0.79	179.64	8.01	-165.35	6.267	6.205	4.656	4.847
47	-61.501	5.163	-9.19	-0.99	179.07	178.27	6.54	-164.61	7.723	6.188	6.476	4.845
95	-61.501	5.128	-9.42	-1.15	-176.93	-177.88	-6.36	-60.77	7.677	6.179	6.452	4.851
68	-61.236	3.555	-8.99	-0.84	4.95	179.81	79.15	167.60	6.125	6.515	5.221	5.108
91	-61.234	3.538	-8.98	-0.83	-4.81	179.59	-80.45	-17.51	6.107	6.519	5.242	5.121
51	-61.170	2.570	-8.93	-0.78	4.80	179.58	120.70	167.42	5.543	6.675	5.818	5.777
78	-61.148	3.091	-8.96	-0.80	-5.29	179.39	-100.59	-16.32	5.887	6.537	5.510	5.386
86	-61.123	3.002	-8.95	-0.80	4.92	-179.08	104.81	167.21	5.825	6.554	5.577	5.458
30	-60.871	3.253	-9.12	-0.94	-4.21	179.81	-20.78	172.08	6.212	6.333	4.729	4.890
56	-60.870	3.262	-9.11	-0.94	3.90	-179.67	21.39	50.32	6.219	6.340	4.737	4.889
7	-60.636	3.840	-9.20	-0.98	177.84	178.39	12.51	56.51	7.696	6.244	6.524	4.885
11	-60.636	3.849	-9.20	-0.98	-178.51	-178.32	-12.70	167.86	7.700	6.246	6.530	4.885
77	-59.987	2.812	-9.06	-0.88	176.99	-179.93	-101.54	5.02	6.568	6.940	7.799	6.058
88	-59.987	2.810	-9.07	-0.88	-176.81	179.96	101.44	161.04	6.566	6.940	7.798	6.057

ΔH_f (AM1 heat of formation) in kcal/mol; DM – dipole moment (Debye); E_{HOMO} and E_{LUMO} – energy of the highest occupied and the lowest unoccupied molecular orbitals (eV); D1, D2, D3 and D4 dihedral angles (°): D1 – C_4-C_3-C_{15}-O_{16}, D2 – C_3-C_{15}-O_{16}-C_{17}, D3 – C_7-N_6-C_{22}-N_{23}, D4 –N_6-C_{22}-N_{23}-C_{24}; d1, d2 distances (Å): d1 – N_{23}-O_{14}, d2 – N_2-O_{21}, d3 – O_{14}-O_{21}, d4 – N_{23}-F_{31}.

Of the 100 conformers obtained from conformational search using the AMBER94 force field by optimizing them with the AM1 MO method there remained only 15 distinct conformers (Table 2) ranked in ascending order of their heat of formation. For keeping the correspondence between the AMBER and AM1 conformers, an AM1 conformer obtained from a starting AMBER conformer has the same number as the AMBER conformer. By using the same numbering way, the different ranking of the conformers' energies resulted from the two methods is directly evidenced.

From Table 2 one can observe a slight correlation between the heat of formation and the dipole moment: the conformers with the lowest energies have the dipole moments around 4 – 5 Debye, while the conformers with the highest energies have the dipole moments around 3 Debye. The AM1 values of the HOMO energies between -8.93 and -9.20 eV and of the LUMO energies between -0.78 and -1.15 suggest that the conformers could interact with the benzodiazepine receptor as electron acceptors.

The dihedral C_4-C_3-C_{15}-O_{16} can have values 0±5°, ±176-179°. In general, conformers with dihedral C_4-C_3-C_{15}-O_{16} around 0° have lower energy than the conformers with dihedral C_4-C_3-C_{15}-O_{16} around 180°. Like in AMBER optimized structures, the dihedral C_3-C_{15}-O_{16}-C_{17} can have values only around ±178-180°. The C_7-N_6-C_{22}-N_{23} dihedral can have values in the following ranges ±6-9°, ±12°, ±20-21°, ±79-80°, ±100-104° and ±120°. The N_6-C_{22}-N_{23}-C_{24} dihedral can have values around ±5°, ±16-17°, ±50, ±56-60° and ±161-172°.

A comparison of the dihedral values resulted from geometry optimization with AMBER or AM1 methods can be seen in Table 3. There is a great difference between AMBER and AM1 conformers regarding the equilibrium values of C_4-C_3-C_{15}-O_{16} dihedrals. The AMBER force field allows a large spectrum of values, while the AM1 method allows only two possible values for the C_4-C_3-C_{15}-O_{16} dihedral. A reverse result was obtained for dihedrals C_7-N_6-C_{22}-N_{23} and N_6-C_{22}-N_{23}-C_{24}. The former has a larger spectrum of values in AM1

optimized conformers (±5-20, ±80-120, and ±160-180) than in AMBER optimized conformers (±30-60, ±102-105). Also, the same observation is valid for the second dihedral, N_6-C_{22}-N_{23}-C_{24}. It has also a larger number of possible values in AM1 conformers ((±5-20, ±50-60, and ±161-172) than in AMBER conformers (±20-40). Taking into account the known data from X-ray spectra for similar compounds, the AM1 method gives geometries more accurate than the AMBER method. As expected, the dihedral C_3-C_{15}-O_{16}-C_{17} can have values only around 180° both in AM1 and AMBER conformers.

Table 3

Values of varied dihedrals in AMBER and AM1 optimized conformers

C_4-C_3-C_{15}-O_{16}	AMBER	±10-40	±110-170	
C_4-C_3-C_{15}-O_{16}	AM1	0±5		±176-179
C_3-C_{15}-O_{16}-C_{17}	AMBER			±170-180
C3-C_{15}-O_{16}-C_{17}	AM1			±178-180
C_7-N6-C_{22}-N_{23}	AMBER	±30-60	±102-105	
C_7-N6-C_{22}-N_{23}	AM1	±5-20	±80-120	±160-180
N_6-C_{22}-N_{23}-C_{24}	AMBER	±20-40		
N_6-C_{22}-N_{23}-C_{24}	AM1	±5-20	±50-60	±161-172

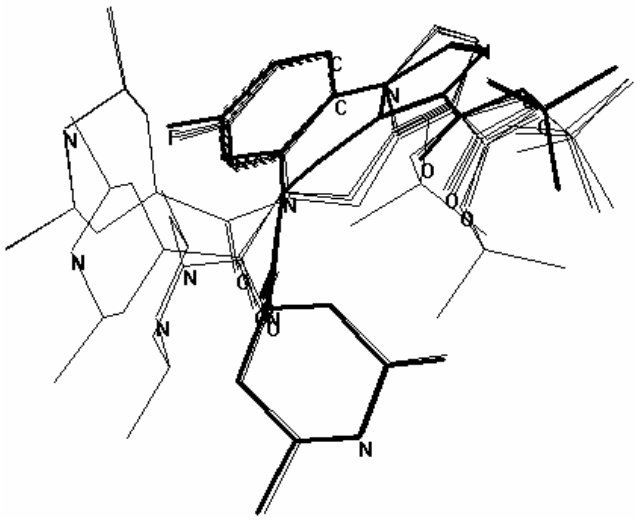

Fig. 2 – The superimposition of 7 AM1 conformers with energetic difference above the energy of the best conformer (thick lines) in the range of thermal energy. Hydrogen atoms are hidden for clarity.

From the superposition of the AM1 conformers having energies above the best conformer situated in the range of thermal energy (figure 2), one can observe the large conformational space occupied by piperazine rings on both sides of the molecular plane (represented by the three condensed rings).

From figure 2 and tables 1-3 one can see that the ligand has at least 15 AM1 conformers in the low minima on the potential energy surface and these conformers occupy a large conformational space.

The distances between the different heteroatoms that could be implicated in interactions with amino acid residues from the active site are shown in Table 3.

In order to estimate the energies necessary for a conformer to interconvert in other low energy conformers the barrier energies for rotating the flexible bonds have been calculated. The rotation barriers for dihedrals C_4-C_3-C_{15}-O_{16} and C_7-N_6-C_{22}-N_{23} are shown in figure 3. The C_4-C_3-C_{15}-O_{16} dihedral has two maxima around 90 and 255° and the corresponding energies are around 2.6 kcal/mol and 2.7 kcal/mol, respectively. Dihedral C_7-N_6-C_{22}-N_{23} has also two maxima around 75 and 225° and the corresponding energies are 122.7 and 36.6 kcal/mol, respectively.

In figure 4 are charted the rotation barriers for dihedrals C_3-C_{15}-O_{16}-C_{17} and N_6-C_{22}-N_{23}-C_{24}.

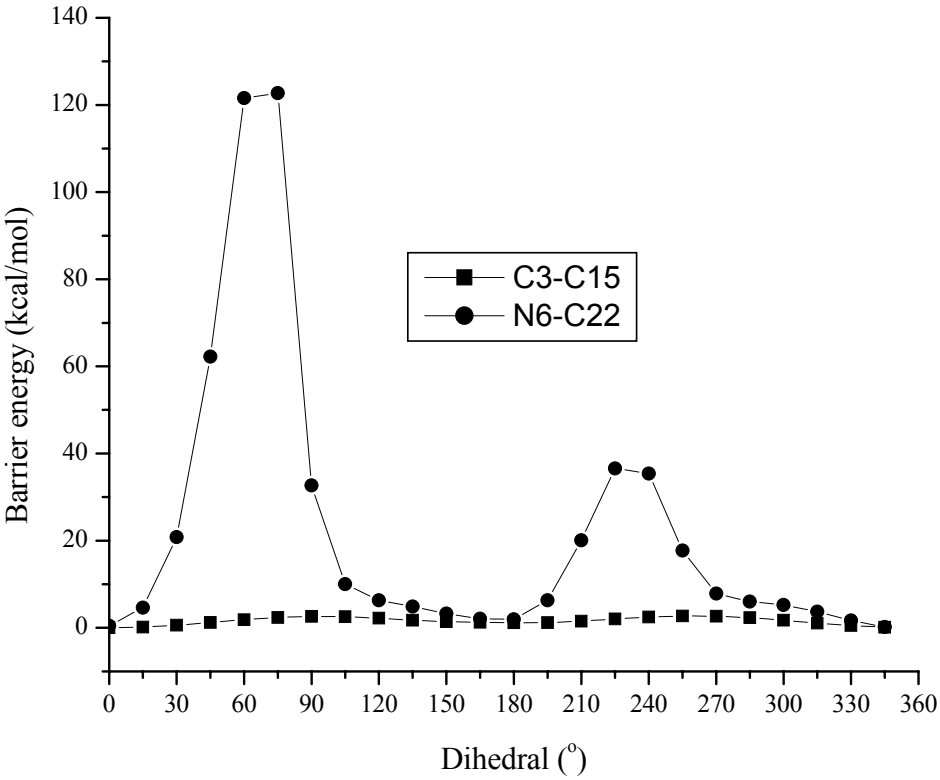

Fig. 3 – Rotation barriers of C_3-C_{15} and N_6-C_{22} bonds resulted from AM1 MO calculations. Dihedrals C_4-C_3-C_{15}-O_{16} and C_7-N_6-C_{22}-N_{23} have been modified with steps of 15°.

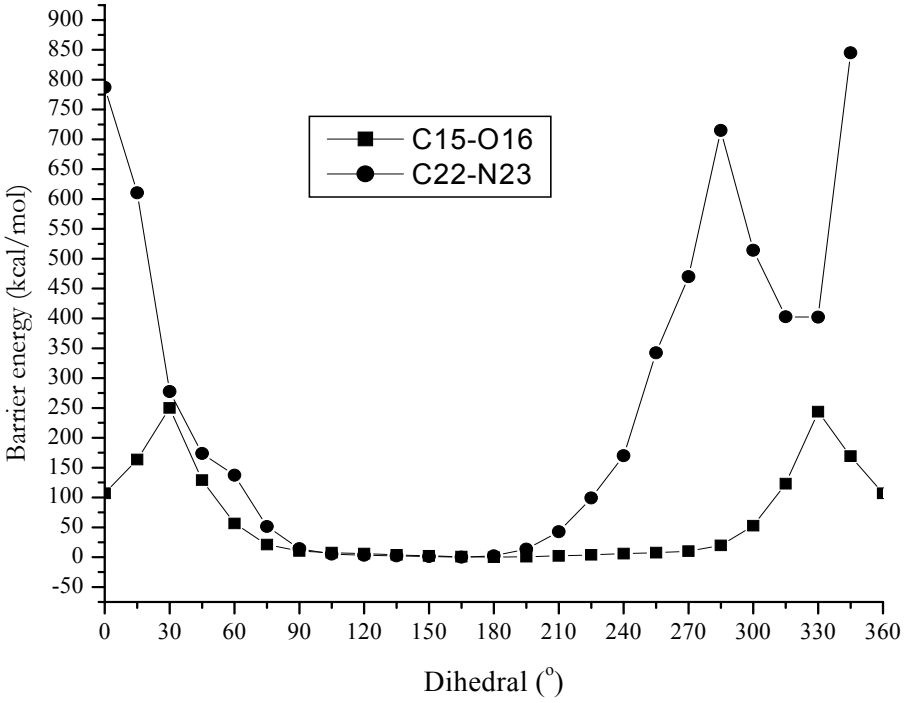

Fig. 4 – Rotation barriers of C_{15}-O_{16} and C_{22}-N_{23} bonds resulted from the AM1 MO calculations. Dihedrals C_3-C_{15}-O_{16}-C_{17} and N_6-C_{22}-N_{23}-C_{24} have been modified with steps of 15°.

From Figures 2 and 3 one can observe that the C_4-C_3-C_{15}-O_{16} dihedral can take values around 0 or 180°, while the C_7-N_6-C_{22}-N_{23} dihedral can have values around 0° and around 165 - 180°. The rotation barriers in figure 4 show that the conformers in low minima on PES can have the C_3-C_{15}-O_{16}-C_{17} and N_6-C_{22}-N_{23}-C_{24} dihedrals in a range between ±90 and 180°.

High values of rotation barriers suggest that for a better interaction with key amino acid residues in the receptor active site only limited interconversions of conformers are possible.

CONCLUSIONS

The AMBER94 force field gave a small number of distinct conformers, even if the energy criterion was set to 100 kcal/mol above the best conformer.

The energy difference between AMBER94 conformers 1 and 100 kept from conformational analysis is of 0.873 kcal/mol, namely with only 0.3 kcal/mol above the thermal energy. Of 100 conformers kept only 24 are distinct. The small energy difference between the best conformer and the last conformer kept from conformational search suggests that many conformers populating low energy levels on the potential energy surface can be obtained through synthesis.

According to rotation barrier values, structures that reach the benzodiazepine site should have a conformation close to the active conformation, because in the active site the bond rotations need high energies.

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