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Propoxazepam conformation and its orientation in the $GABA_A$-receptor binding site

Introduction. One of 1.4-benzodiazepine 3-alcoxy derivatives – propoxazepam, possessing high analgetic action, also effectively suppressed different experimental seizures types. Unexpected combination of pharmacological spectrum components suggests its different binding sites of $GABA_A$ receptor.

The aim of the work was to determine the geometry of the ligand-receptor complexes of GABA-RC using experimental data of the propoxazepam conformation and calculated data for the three-dimensional structure of the ligand-binding site and subsequent docking to characterize its binding to this receptor.

Materials and methods. X-ray diffraction studies of the compound were performed using Xcalibur 3 single crystal X-ray diffractometer. Calculation of the molecular docking parameters was performed using the iGEMDOCK v2.1 program for the GABA receptor (GABA (A) R-beta3 homopentamer, 4COF), the molecular structures of propoxazepam conformers were prepared using ChemAxon (MarvinSketch 17.11.0).

Results and discussion. Based on the X-ray diffraction analysis, the coordinates of the atoms, bond lengths and valence angles in the propoxazepam molecule were calculated, it is found that it form crystallographic twins as racemate. The molecular docking method showed that propoxazepam several binding sites with the energy of complex formation from -78.64 to -85.29 kcal/mol exist on the isolated site of the GABA-receptor.

Conclusions. The highest contribution to the formation of the bond of the complex is carried out by residues of polar amino acids (serine, asparagine, methionine and arginine in polar binding sub-center). However, also for individual conformers, aromatic amino acids, predominantly phenylalanine (Phe-31, Ala-135 – hydrophobic binding sub-center) make a significant contribution.

***Key words**: propoxazepam; X-ray diffraction; docking; $GABA_A$-recepto*

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Конформація пропоксазепаму та його орієнтація у центрі зв'язування $ГАМК_A$-рецептора

Актуальність. Одне з 3-алкоксипохідних 1,4-бенздіазепіну – пропоксазепам, який продемонстрував високу аналгетичну активність, а також ефективно блокував різні типи експериментальних судом. Нестандартна комбінація компонентів фармакологічного спектра передбачає різні місця його зв'язування на $ГАМК_A$-рецепторі.

Мета дослідження полягала у визначенні геометрії ліганд-рецепторних комплексів ГАМК-РК на підставі використання експериментальних даних про конформацію пропоксазепаму та розрахункових даних тривимірної будови лігандзв'язуючого центра та подальше проведення докінгу для характеристики його зв'язування з даним рецептором.

Матеріали та методи. Рентгеноструктурне дослідження сполуки було виконано на монокристальному рентгенівському дифрактометрі Xcalibur 3, розрахунок параметрів молекулярного докінгу буз здійснений у програмі GEMDOCK v2.1, для ГАМК-рецептора (GABA(A) R-beta3 пентамер); молекулярні структури конформерів пропоксазепаму були підготовлені у програмі ChemAxon (MarvinSketch 17.11.0).

Результати та їх обговорення. На підставі даних рентгеноструктурного аналізу розраховані координати атомів, довжини зв'язків та валентні кути у молекулі пропоксазепаму, встановлено, що він існує у вигляді кристалографічного двійника як рацемат.

Висновки. Методом молекулярного докінгу показано, що на виділеній частині $ГАМК_A$-рецептора існує декілька місць зв'язування пропоксазепаму з енергією утворення комплексів від -78,64 до -85,29 ккал/моль. Найбільший внесок у формування зв'язку комплексу здійснюють залишки полярних амінокислот (серин, аспарагін, метіонін та аргінін – полярний підцентр зв'язування). Однак також для окремих конформерів значний внесок мають ароматичні амінокислоти, переважно фенілаланін (Phe-31, Ala-135 – гідрофобний підцентр зв'язування).

***Ключові слова**: пропоксазепам; рентгенівська дифракція; докінг; $ГАМК_A$-рецептор*

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Конформация пропоксазепама и его ориентация в центре связывания $ГАМК_A$-рецептора

Актуальность. Одно из 3-алкоксипроизводных 1,4-бенздиазепина – пропоксазепам – продемонстрировал высокую анальгетическую активность, а также эффективно блокировал разные типы экспериментальных судорог. Нестандартная комбинация компонентов фармакологического спектра предусматривает разные места его связывания на $ГАМК_A$-рецепторе.

Цель исследования состояла в определении геометрии лигандрецепторных комплексов ГАМК-РК на основе использования экспериментальных данных о конформации пропоксазепама и расчетных данных о трехмерном строении лигандсвязывающего центра и последующее проведение докинга для характеристики его связывания с данным рецептором.

Материалы и методы. Рентгеноструктурное исследование соединения выполнено на монокристальном рентгеновском дифрактометре Xcalibur 3, Расчет параметров молекулярного докинга был осуществлен с использованием программы iGEMDOCK v2.1, для ГАМК-рецептора (GABA(A) R-beta3 гомопентамер, 4COF), молекулярные структуры конформеров пропоксазепама были подготовлены в программе ChemAxon (MarvinSketch 17.11.0).

Результаты и их обсуждение. На основании данных рентгеноструктурного анализа рассчитаны координаты атомов, длины связей и валентные углы в молекуле пропоксазепама, установлено, что он существует в виде кристаллографического двойника в виде рацемата.

Выводы. Методом молекулярного докинга показано, что на выделенном участке ГАМК$_A$-рецептора существует несколько мест связывания пропоксазепама с энергией образования комплекса от -78,64 до -85,29 ккал/моль. Наибольший вклад в формирование связи комплекса осуществляют остатки полярных аминокислот (серин, аспарагин, метионин и аргинин – полярный подцентр связывания). Однако также для отдельных конформеров значительный вклад имеют ароматические аминокислоты, преимущественно фенилаланин (Phe-31, Ala-135 – гидрофобный подцентр связывания).

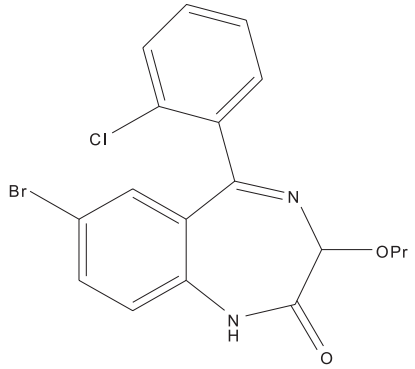
***Ключевые слова*:** *пропоксазепам; рентгеновская дифракция; докинг; ГАМК$_A$-рецептор*

INTRODUCTION

Pharmacological properties of medicinal products are determined with their interactions with therapeutical targets (proteins and nucleic acids). The "drug-target" complex formation is tupical for chemical substances of various structure, which can be either agonists or antagonists (full or partial). In such complexes molecules-ligands are spatially (geometrically) complementary to binding site of the macromolecule surface and are rendered on it by the help of Coulombe`s forces, Van-der Vaal interactions, hydrogen bonds etc.

Among the different biotargets (receptors, enzymes, transporters, ion channels and others) of the drug`s action the main attention is paid to GABA$_A$-receptor complex (GABA$_A$-RC), impairments in which are connected with such disorders as schizophrenia, bipolar disorders, insomnia epilepsy alcoholism and others [1-4]. In addition to GABA-binding site the receptor complex contains allosteric segments, which are capable to bind benzodiazepines, widely used for such disorders treatment [1]. However, for these substances insufficient action selectivity, leading to different side effects, still remains the main problem. In this way, GABA$_A$ receptor and its subtypes selective ligands finding is still actual and this is directed on creating of new medicines for these disorders treatment, as well as for cognitive function stimulation.

Recently our attention was attracted by 3-alcoxy substituted 1.4-benzodiazepine derivatives which, despite others members of this class, on the models of nociceptive pain shared the prominent analgesic activity [5, 6]. One of them, named propoxazepam – 7-bromo-5-(o-clorophenyl)-3-propyloxy-1,2-dihydro-3H-1,4-benzodiazepine-2-one (I) is considered to be the promising analgetic (Scheme).



Scheme. *Chemical structure of propoxazepam (I)*

Paying attention that such antiepileptic drugs as gabapentin and pregabalin successfully used for neuropathic pain treatment [7, 8] we have undertaken the studies of anticonvulsive action of this compound. Earlier we [9] determined propoxazepam the mean effective doses (ЕД$_{50}$) on the models of chemically induced seizures by picrotoxin (1.67 ± 0.09 mg/kg), penthylenetetrazole (0.9 ± 0.04 mg/kg) and strychnine (14.24 ± 0.47 mg/kg), which prove the substance high anticonvulsive activity.

On the base of "dose-effect" curves shapes there were demonstrated different stages of propoxazepam interaction with GABA and glycine receptors *in vivo*. It is assumed that obtained data prove the preferential propoxazepam anticonvulsive effect realization through GABAergic mechanisms. Glycine-ergic components, participating in strychnine-induced seizures suppression, are involved in the process when propoxazepan is administered in doses higher than ED$_{50}$ and obviously serve as additional anticonvulsive protection mechanism.

On the model of thiosemicarbazide-induced GABA-deficient seizures propoxazepam had shown the high activity and on the base of "dose-response" curve shape one can assume the antagonistic interaction with GABA synthesis inhibitor - thiosemicarbazide [10].

The use of mentioned methods of "pharmacological probing" for propoxazepam action let reliably reveal the possible structural-functional sites of GABA-RC, which are responsible for neuronal effects realization on the whole organism level. On the stage of new (original) medicines the different approaches with computation technologies are used. Recently the most effective one is docking procedure, which estimates energetic parameters of molecular matching of ligand (pharmacophore groups separately or the whole structure) to functionally important protein (receptor) sites. Using the docking mechanism one can suggest the molecules interactions, determine spatial structure of complexes and affinity of conformation-dependent interactions. The docking algorithms of low-molecular weights ligands to receptor molecules are essential instrument for rational drug design and are applied on different stages of medicines R&D process (screening, ligand action mechanism clarification, identification of receptor sites, involved in intermolecular interactions). In this case necessary are both the data about spatial ligand structure and three-dimension structure of target protein binding site, obtained by X-ray diffraction methods.

The aim of the work was determination of ligand-GABA-RC complex geometry determination on the base of experimental data of propoxazepam conformations and calculated data of the ligand-binding site three-dimension structure as well as further ligand-receptor docking with its process description.

MATERIALS AND METHODS

The X-ray diffraction study of the substance was performed on monocrystal X-ray difractometer Xcalibur 3 (MoKα radiation, CCD-detector, graphite monochromator, ω-scanning, $2\Theta_{max} = 50$) using SHELXTL-97 software [11] according to the standard method (MoKα-radiation, T 130(2) K, ω-scanning with step 1°). 0.882). The sample was decoded in two spatial groups: three-wedged with cell parameters $a = 10.434(1)$, $b = 10.873(1)$, $c = 17.837(2)$ Å, $\alpha = 74.810(9)°$, $\beta = 77.22(1)°$, $\gamma = 66.06(1)°$, $V = 1769.6$ Å^3, spatial group $P\bar{1}$. Monoclinic with cell parameters $a = 11.695(2)$, $b = 21.507(2)$, $c = 14.506(2)$ Å, $\beta = 92.82(1)°$, $V = 3644.2)$ Å^3, spatial group $P2_1/c$. Minimal divergence factor for triclinic structure was 38 %, while for monoclinic 25 %.

Compound structure war decoded by direct method in isotropic approximation and specified in anisotropic approximation for non-hydrogen atoms. Hydrogen atoms were placed in geometrically calculated sites and included in the refinement on the "rider" model in isotropic approximation with $U_{iso} = nU_{eq}$. Of the non-hydrogen atom, connected with the given hydrogen (n = 1.5 for methyl groups and n = 1.2 for other hydrogen atoms). The structure was refined using F^2 polymatrix LSM in the anisotropic approximation for non-hydrogen atoms to $wR_2 = 0.289$ on the base of 6284 reflections ($R_1 = 0.106$ on the base of 2142 reflections c $F > 4\sigma$ (F), S = 0.882).

Molecular docking parameters calculation was made using iGEMDOCK v2.1 software [12, 13] (freeware, http://gemdock.life.nctu.edu.tw/dock/download.php). As macromolecule the GABA-receptor (crystalline structure, GABA(A) R-beta3 гомопентамер, 4COF) was chosen, being received from biological macromolecules database (http://www.rcsb.org/) as *.pdb file. At the same file extension the ligand structure was also prepared. Propoxazepam conformers molecular structures were prepared using ChemAxon (MarvinSketch 17.11.0) software, conformers internal energy calculations were carried out on Avogadro (v 1.2.0) software, cavities analysis and mutual amino acids residues in active centers – on the base of Mole 2.13.9.6. Docking parameters calculation for ligand and receptor was performed using force field data on 100 generations of flexible ligand conformations (300 states for each population size); from 20 number of solutions the most optimal was chosen. Automatic binding site detection was determined by the referent ligand localization (benzamidine) [14].

Binding site radius was enlarged to 30 Å for substance binding visualization with simultaneous ligand excluding. Docking results were grouped according to the hierarchic clustering procedure. Clustering was performed using K-means after previous estimation of binding localization topography due to total interaction energy, as well as hierarchic clustering.

RESULTS AND DISCUSSION

Propoxazepam molecular structure

Propoxazepam crystalline sample X-ray analysis (Fig. 1) had shown that it exists as crystallographic twins with different twinning degree, forming during crystallization. The highest precise resolution of twinning was made using Platon software, showing presence of more than two components.

Molecule has asymmetric center at C8 (Fig. 2) and is crystallized in center-symmetric spatial group, forming racemate crystals. In the independent unit cell two molecules were found (A and B) with different conformations and asymmetric atom configurations (A molecule with R-configuration while B molecule with S-configuration).

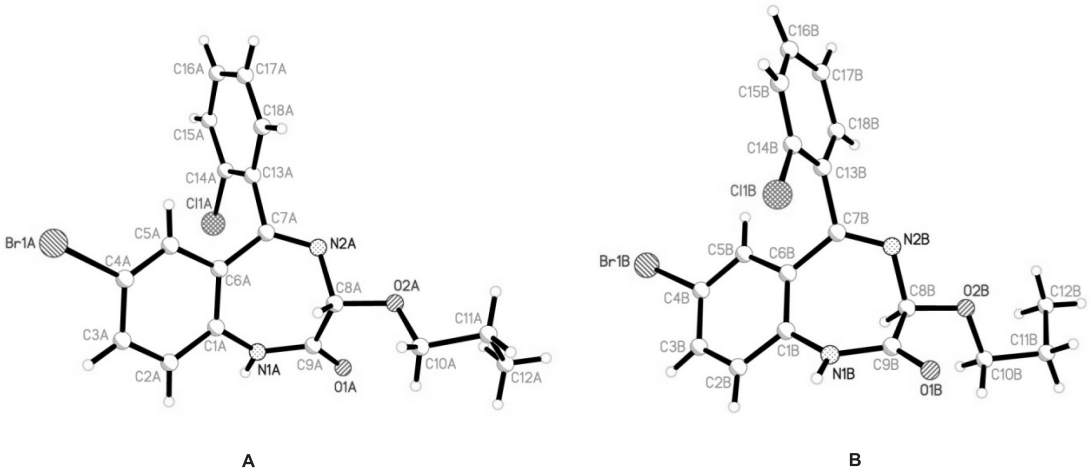


Fig. 1. *Common view of propoxazepam molecule (A and B) as represented by elipsoids of thermal oscillations with 50 % possibility*

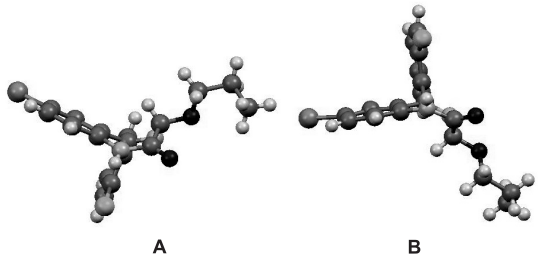


Fig. 2. *Molecules A and B conformations*

Diazepine cycle is in the bath conformation, N1, C9, C7, N2 atoms are coplanar while C1, C6 and C8 atoms are deflected to one side of this plane on 0.70 Å, 0.65 Å and 0.75 Å in the molecule A accordingly and on -0.69 Å, -0.66 Å and -0.74 Å in molecule B accordingly. As a result chlorobenzyl substituent deflects from the plane bicycle fragment in the different sides and is rotated in relation to endocyclic C6-C7 bond on the equal angle, but to different directions (torsion angles C1-C6-C7-C13 and C6-C7C13-C14 are 144(1)o and -73(1)o in the molecule A and -144(1) and 73(1) in the molecule B). The main difference in the unit cell structure, determining the presence of two molecules in it, is in propyl group conformation. The substituent at C8 position has equatorial orientation (torsion angle N1-C9-C8-O2 is 172.1(8)° in the molecule A and -170.4(8) in the molecule Б), propyl group in the molecule A is situated nearly orthogonally to the cycle and has *ap* – *-sc-* conformation. Propyl group in the molecule B is in *+sc-* position in relation to endocyclic C9-C8 bond and has *ap* – *-sc*-conformation (Tab. 1).

In the crystal molecules A and B alternate forming chains along the crystallographic direction [1 0 0] because of intermolecular hydrogen bonds N1a-H…N2b' H…N 2.24 Å N-H…N 162°, N1b-H…N2a' (x-1, y, z) H…N 2.22 Å N-H…N 173°.

Atom coordinates, bonds lengths and valent angles in the propoxazepam molecule were also calculated.

Structure and properties of the $GABA_A$-receptor complex

$GABA_A$-receptor complex ($GABA_A$-RC) belongs to the ligand-depending ionic channels class and is the main therapeutic target, participating in the human physiological processes: education and memory formation, awaking and sleeping. The terms GABA-benzodiazepine-receptor ionophoric complex, GABA-benzodiazepine-ionophore and others are also often appear. In these terms not only complicity but tight junctions between its components are reflected.

$GABA_A$-RC is built with fie subunits, which belong to different classes (α, β, γ, δ, ε, π, θ, ρ), forming the symmetrical ion channel, posing with second transmembrane domain to each other. At present from the mammals nervous system there were cloned and sequined six α-, three β-, three γ-, one δ-, one ε-, one π-, one θ-, and three $GABA_A$-PC ρ-subunits, as well as forms which form as a result of alternative splicing of some of these subunits [1-4]. The most common subunits combination in the CNS

Table 1

THE RELEVANT TORSION ANGLES

	Molecule A	Molecule B
C9-C8-O2-C10	-88(1)	75(1)
C8-C2-C2-C11	-172(1)	175.5(8)
O2-C10-C11-C12	-82(1)	-63(1)

(about 40 % of $GABA_A$-RC) is formed from two α1, two β2 and one γ2s, surrounding the chloride-transporting pore. When binding two GABA molecules complex changes its conformation, opens pore for anions transport and as a result hyperpolarization develops leading the cell to be less sensitive to excitement signals of other neurons – the process accompanied with postsynaptic inhibitory potential development.

The main ligand-binding of the $GABA_A$-RC is that of GABA (agonist) binding site which is situated in the area between α-and ρ- subunits contact. On the surface of α and γ subtypes contacts the benzodiazepine binding site is located. Barbiturates and ethanol binding sites are supposed to be located on the transmembrane domains in the deep of the channel. In the first case, perhaps, the main role is played by β-subunit while ethanol interacts with different subunits, including ρ and δ, however with different sensitivity.

Subunits combination in the pentamer determine ligand pharmacological profile. It was found that benzodiazepines pharmacological action as well as their analogs is primarily determined by α-subunits subtype. Particularly, α1-selective ligands usually possess tranquilizing, anticonvulsive amnesic action; α2 and α3 – anxyolytic hypnotic, anticonvulsive and muscle relaxation, though α5-selective –stimulate education and memory processes [2]. Thus new ligands have to be highly selective to the certain $GABA_A$-RC subtype for sharing unique therapeutic properties simultaneously lucking side effects, inherent to classical benzodiazepines.

The one of main molecular docking aims is new possible binding sites search. Correct screening algorithm have to determine and estimate as much as possible the modes of two molecules interaction. However this process can be too calculation- and time consuming. Due to this there have to be balance between computer process costs and screening space. As a screening algorithm the method of energy interaction estimating, calculated in accordance to ligand and receptor cavity fields, was used.

Despite the other 1.4-benzodiazepine derivatives propoxazepam, as alcoxy derivative, possess mainly analgesic action in its pharmacological spectrum while inhibitory action (muscle relaxation, hypnotic and tranquilizing) is markedly reduced. As the alcoxy radical is not the dramatic change in the substance structure, using molecular docking results there was made the attempt to analyze this flexible substituent influence on the ability to

be bind to the GABA receptor. Detailed docking with this receptor was analyzed on the base of 20 number of solutions (19 conformations and one non-optimized structure, excluded from further analysis) for each the optimal conformation 100-times generated in 500 approaches. The most optimal from the energetic point of view (minimal energy) conformers further were estimated as the most favorable and effective (according to the binding energy).

It was rather unexpected that generated propoxazepam conformers had not the only binding site (Fig. 3). Maximal biding energies difference for all the conformers is 6.65 kcal/mole (from -78.64 to -85.29 kcal/mole), that is not a big value but can be significant in the substance low concentrations in brain *in vivo*. Cluster analysis revealed six binding sites with one only conformer (No. 5) separate binding, which can be explained as non-specific binding with surface hydrophobic regions. This suggestion is supported also with the low complex formation energy (-79.76 kcal/mole, Tab. 2).

The rest conformers form five classes (clusters) second of them is the most numerous (Tab. 2) with similar binding energy values. Though one have to mention that similar values of complex formation total energy consist of Van-der-Vaal interaction energies and hydrogen bond energies with quite different impacts. As the benzodiazepine "bath" conformation is very rigid and changes negligibly, the difference can be due to conformation mobility/flexibility of alcoxy (propyl) radical. In is confirmed by the calculated every conformer intrinsic energy with nearly similar for each representative (Tab. 2).

As the alcoxy radical flexibility plays such a big role for binding site preference, the main amino acids residues, participating this process were also determined.

Fig. 3. *Schematic localization of propoxazepam conformers preffered binding sites*

For data reduction to the most significant in a z-normalization procedure the binding energy values, nearest to the representative mean, for each amino acid residue were selected (which equals to z = 0, Tab. 3). For conformer № 5 with low binding energy value there haven't been reveled amino acid residues with energy, near to mean values of other conformers. As it was found earlier, the main impact in the complex formation residues of polar amino acids which can fix polarization-able parts and groups of ligand (M-Ser-10, M-Asp-30, S-Asn-100, S-Met-137, S-Lys-13, M-Asp-30, S-Arg-71, M – main chain, S – side chain). Though for some conformers large contribution have aromatic amino acids, mainly phenylalanine (M-Phe-31, S-Phe-31, M-Ala-135) and even glycine (M-Gly-32). Be-

Table 2

CALCULATED PROPOXAZEPAM CONFORMERS BINDING ENERGY AND THEIR INTERNAL ENERGY

Cluster number	Ligand conformer (No.)	Total binding energy kcal/mole	Van-der-Vaal interaction	Hydrogen bonds	Internal conformer energy, kcal/mole
1	5	-79.76	-61.50	-18.25	91.48
2	2	-80.01	-76.51	-3.50	91.50
2	3	-78.64	-61.72	-16.92	91.39
2	4	-81.96	-62.99	-18.97	91.41
2	9	-82.00	-63.03	-18.98	91.44
2	11	-79.98	-76.48	-3.50	91.53
2	12	-80.05	-76.55	-3.50	91.42
2	16	-80.04	-76.54	-3.50	91.47
2	19	-81.99	-63.00	-19.00	91.45
3	1	-83.75	-69.59	-14.17	91.45
3	8	-83.76	-69.59	-14.17	91.49
4	13	-83.49	-67.85	-15.63	91.45
4	14	-83.49	-67.75	-15.74	91.47
4	18	-83.49	-67.74	-15.75	91.44
5	6	-85.29	-74.91	-10.38	91.41
5	10	-85.29	-74.83	-10.46	91.51
5	15	-85.28	-74.86	-10.42	91.50
6	7	-82.29	-68.93	-13.36	91.48
6	17	-82.29	-68.95	-13.34	91.47

Table 3

AMINO ACIDS RESIDUES WHICH PARTICIPATE BONDS FORMATION WITH DIFFERENT PROPOXAZEPAM CONFORMATION (BOND ENERGIES NORMALIZATION AT z = 0, M – MAIN CHAIN, S – SIDE CHAIN)

Conformer number	6	10	15	1	8	4	9	19	2	11	12	16	3	13	14	18
Energy, kcal/mole	-85.3	-85.3	-85.3	-83.8	-83.8	-82.0	-82.0	-82.0	-8.0	-82.0	-82.0	-82.0	-78.6	-83.5	-83.5	-83.5
M-LEU-99									-3.5	-3.5	-3.5	-3.5				
S-ASN-100						-3.5	-3.5	-3.5					-1.9			
M-ALA-135						-7.0	-7.0	-7.0					-6.6			
M-THR-151						-3.5	-3.5	-3.5					-3.5			
S-THR-151						-5.0	-5.0	-5.0					-5.0			
M-SER-10	-5.5	-5.7	-5.6													
M-LYS-13	-3.2	-3.1	-3.7													
S-ASP-30														-2.5	-2.5	-2.5
S-ASP-69														-5.5	-5.6	-5.6
M-LYS-70														-3.5	-3.5	-3.5
S-LEU-27	-4.2	-4.2	-4.2													
M-ASP-30	-5.9	-5.9	-5.9	-0.5	-0.5											
S-ASP-30	-4.4	-4.4	-4.4													
M-PHE-31	-8.2	-8.2	-8.2	-3.3	-3.3											
S-PHE-31	-6.3	-6.3	-6.3	-5.7	-5.7											
M-GLY-32	-4.7	-4.7	-4.7	-4.7	-4.7											
S-ARG-71	-6.6	-6.5	-6.5													
M-LEU-99						-0.2	-0.2	-0.2	-4.7	-5.0	-4.6	-4.6	-0.3			
S-LEU-99						-0.3	-0.3	-0.3	-5.2	-4.8	-5.0	-5.0	-0.4			
M-ASN-100						-1.7	-1.7	-1.7	-11.8	-11.1	-11.9	-11.9	-2.4			
S-ASN-100						-7.0	-7.0	-6.9	-4.3	-4.4	-4.5	-4.5	-6.6			
M-ASP-101									-5.7	-5.9	-5.6	-5.6	-0.3			
M-CYS-136						-1.2	-1.3	-1.3					-8.8			
M-MET-137						-1.5	-1.5	-1.5					-9.7			
S-MET-137						-5.8	-5.8	-5.8	-0.7	-0.1	-0.7	-0.7	-5.4			
S-GLU-153						-5.5	-5.5	-5.4	-1.7	-1.8	-1.8	-1.8	-5.2			
S-LYS-13														-6.9	-6.9	-6.9
S-LYS-13	-12.6	-12.6	-12.6													
M-PRO-29														-6.5	-6.5	-6.5
M-ASP-30														-8.5	-8.6	-8.6
M-ALA-45						-0.3	-0.4	-0.3	-9.8	-9.7	-9.8	-9.8	-0.6			
S-SER-46						-1.4	-1.5	-1.5	-5.1	-5.9	-5.3	-5.3	-1.3			
M-ARG-71														-4.2	-4.3	-4.3
S-ARG-71														-14.7	-14.6	-14.6

cause propoxazepam conformers are bind with the polar amino acids residues, one can suggest that flexibility of the alcoxy radical can acquire conformations with ether oxygen more available for binding.

Docking results visualization had shown that when interacting with phenylalanine residues propoxazepam conformer is situated in the way of the main influence to be fulfilled through bromine atom (Fig. 4, A).

On the contrary the more polar subcentre carries out the binding not only via phenylalanine residue (through bromine atom), but also with more polar amino acids – asparagine and arginine (Fig. 4, B).

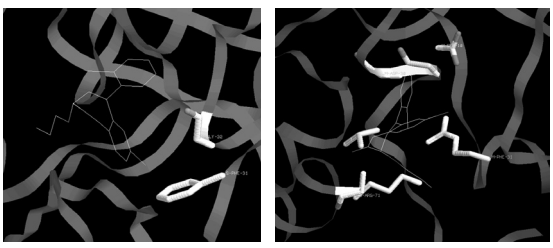


Fig. 4. *Spatial propoxazepam arrangement when binding with esidues of phenilalanine and glycine (A) and phenilalanine, asparagine, arginine and serine (B)*

CONCLUSIONS

1. On the base of X-ray analysis data the propoxazepam crystalline sample structure was described and its existence as crystallographic twins was demonstrated. The twinning appears due to crystallization in centrosymmetric spatial group (racemate crystals formation) as the compound has C8 asymmetric center. The substituent at this position (alcoxy radical) is in equatorial *+sc-* and *-sc*-position. Atoms coordinates, bounds lengths and valence angles in the propoxazepam molecule were determined, that gives the possibility to validate the computer methods of its structure description.
2. Using the molecular docking method on the selected $GABA_A$-receptor part some binding sites were determined with complex formation energy from -78.64 to -85.29 kcal/mole.
3. The total binding energy of the most numerous propoxazepam conformers cluster and $GABA_A$-receptor is similar though the contribution of Van-der-Vaal interactions and hydrogen bonds are not equal for different conformers. The main contribution in the complex formation make polar amino acids residues (serine, asparagine, methionine and arginine – polar binding subcenter). However for some conformers the significant contribution have aromatic amino acids, mainly phenylalanine (Phe-31, Ala-135 – hydrophobic binding subcenter).

Conflict of Interests: authors have no conflict of interests to declare.

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