DOCKING ANALYSIS OF 1,4-BENZODIAZEPINES WITH ALPHA-1 ADRENERGIC RECEPTOR AND PHOSPHODIESTERASE 4

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Introduction. «Propoxazepam», an innovative drug created by scientist of the O. V. Bogatsky Physical-Chemical Institute of the National Academy of Sciences of Ukraine and CAR «INTERCHIM», has an original pharmacodynamic profile, and can inhibit both acute and chronic pain, as well as have anti-inflammatory and anti-convulsant effects. Propoxazepam's analgesic effect is mediated by its dopaminergic system, NMDA receptors, and alpha-1 adrenoceptors [1]. Midazolam, diazepam, and lorazepam, which are benzodiazepines, have been proposed to act as positive allosteric modulators (PAMs) for α1- ARs. In contrast, multiple benzodiazepines had a positive impact on phenylephrine's stimulation of a cAMP response element pathway through α1A- and α1B- ARs; from the literature it appears that this was caused by off-target inhibition of phosphodiesterase, which are known targets of diazepam [2]. Activated G proteins is frequently associated with adenylyl cyclase, a membrane-associated enzyme that, when activated by the GTP-bound alpha subunit, synthesizes

cAMP from molecules of ATP. cAMP plays a role in the response to sensory input, hormones, and nerve transmission in humans. It is interesting to note that cAMP is only degraded enzymatically through hydrolysis by phosphodiesterase (PDE) enzymes [3]. PDE4 inhibitors have the ability to prevent nociceptive effects by enhancing Cx43 expression via cAMP-PKA signalling in the spinal dorsal horn. PDE4, among the 11 PDEs, has been demonstrated to be the main PDE family that causes cAMP hydrolysis in nerve and immune cells; and PDE4 inhibition has an antinociceptive and anti-inflammatory effect in the brain [4,5]. **The aim of the study:** the molecular study of interactions of benzodiazepines with the alpha-1-adrenergic receptors and phosphodiesterase 4 by molecular docking, and analysis of components of these interactions.

Main part. *Materials and Methods.* Molecular docking studies of the compounds under investigation were performed with Schrödinger Suite and AutoDock Vina (http://vina.scripps.edu) and the results were compared [6].

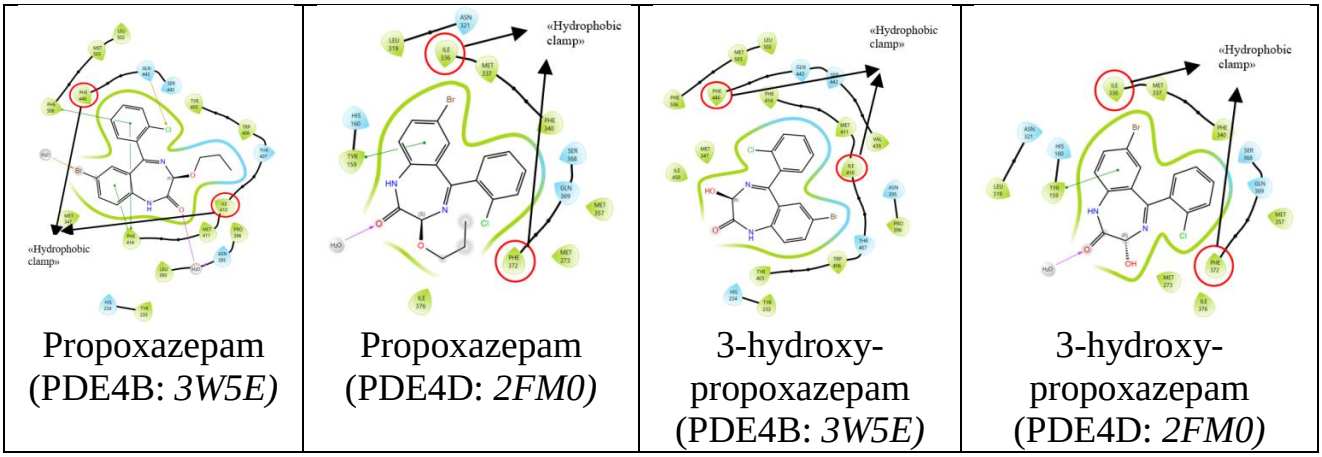
Results. Alpha-1 adrenergic receptor. Among the investigated benzodiazepines, diazepam shows the lowest MMGBSA values for all receptors 7YM8 (-56.9 kcal/mol), 7YMH (-35.7 kcal/mol), 8THL (-28.3 kcal/mol), reflecting a stable ligand-receptor complex. The highest MMGBSA and Glide Score values are found in propoxazepam among all ligands. However, its metabolite, 3-hydroxypropoxazepam, shows better predicted affinity values for the alpha 1A adrenergic receptor.

Phosphodiesterase 4. Scientific research has shown that the beneficial effect of diazepam and other benzodiazepines on the phenylephrine-induced response at the α1-AR CRE is likely due to phosphodiesterase 4 (PDE 4) inhibition. These drugs could increase the signalling pathway that involves cAMP by inhibiting PDEs, which could result in a stronger response to phenylephrine at the α1-AR CRE [2].

Propoxazepam demonstrate the low value of MMGBSA for all subtypes of PDE4 (PDE4A 3TVX -60.7 kcal/mol, PDE4B 3W5E 37.5 kcal/mol, PDE4D 2FM0 -52.6 kcal/mol) and predicted binding energy calculated by AutoDock Vina (PDE4A 3TVX -9.2 kcal/mol, PDE4B 3W5E -10.3 kcal/mol, PDE4D 2FM0 -8.7 kcal/mol). This suggests favourable interactions with the target proteins. Its metabolite 3-hydroxypropoxazepam also shows strong binding energies, ranging from -35.8 to -47.7 kcal/mol (MMGBSA) and from -8.3 to – 9.3 kcal/mol (AutoDockVina).

PDE4A: *3TVX:* The results of docking studies show the formation of a hydrogen bond between propoxazepam, 3-hydroxypropoxazepam and the residue ASP 530 through the hydrogen of the amide group. Propoxazepam have one more hydrogen bond with ASN 533 by oxygen of alkoxy group. Most of the ligands (propoxazepam, diazepam, oxazepam and reference ligand 0MO) establish pi-pi stacking interaction with PHE 584. 3-hydroxypropoxazepam creates the metal coordination bond with

Mg^{+2} through Oxygen of amide group. This type of interaction is also appropriate for 0MO (by Oxygen of carbonyl group).



Propoxazepam (PDE4B: *3W5E)*	Propoxazepam (PDE4D: *2FM0)*	3-hydroxy-propoxazepam (PDE4B: *3W5E)*	3-hydroxy-propoxazepam (PDE4D: *2FM0)*

Fig.1 Visualize the position in specific binding sites PDE4B (3W5E), PDE4D (2FM0) of the investigated ligands (Schödinger Suite)

PDE4B: *3W5E:* All investigated benzodiazepines except 3-hydroxypropoxazepam create pi-pi stacking interaction with PHE 414 as NVW. Only propoxazepam has water-bridged hydrogen bonds with ASN 395 through the oxygen of the amide group. NVW is also involved in the interaction with ASN 395, but by the oxygen of thiopyrandioxyde. Pursuant to figure 1 propoxazepam and its metabolite 3-hydroxypropoxazepam are located in the active site of PDE4B and create interactions with residues of Q pocket, in the hydrophobic clamp (P-clamp) PHE 446 and ILE 410.

PDE4D: *2FM0:* All investigated ligands form pi-pi stacking interaction with TYR 159. Referring to figure 1 propoxazepam and its metabolite 3-hydroxypropoxazepam occupied the P-clamp and interacted with the key residues ILE 336 and PHE 372.

Conclusions

1. Propoxazepam have average value of MMGBA and Glide Score in docking process with α1A - adrenoceptors (7YM8, 7YMH, 8THL), which indicates a moderate affinity of propoxazepam to α1A-AR. Its metabolite, 3-hydroxypropoxazepam shows better predicted affinity values for the α1- AR.
2. Based on AutoDock Vina results propoxazepam tends to exhibit moderate to strong free binding energies across various PDE4 types compared to reference ligands, with values ranging from -8.7 to -10.3 kcal/mol (AutoDock Vina). The lowest free binding energy is demonstrated for PDE4B (3W5E) -10.3 kcal/mol. Propoxazepam has the lowest MMGBSA value among other ligands with PDE4D PDE4D (2FMO) -52.6 kcal/mol.

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