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EXPLORING NON-SPECIFIC INHIBITION OF PHOSPHODIESTERASE 4 BY 1,4-BENZODIAZEPINES USING THE APPROACH OF MOLECULAR DYNAMICS SIMULATION

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Reporter: PhD student Akisheva Alina

The work was performed on the basis of:

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Laboratory of molecular pharmacology and medicine.,
under the supervision of the head of the laboratory
d.b.s. V.B. Larionov
Scientific Supervisor : d.b.s., professor O. A. Makarenko

Actuality of theme

Pain is the most unpleasant symptom of the disease. Treating this unpleasant sensation is a challenge for clinicians.

In addition, addiction, tolerance, and limited efficacy further hinder the successful treatment of chronic pain.

There are a number of medications available to treat pain, such as nonsteroidal anti-inflammatory drugs (NSAIDs), opioids, and their derivatives; however, the treatment of pain remains a challenge because most of the targets for modern analgesics are in the CNS and thus prone to various side effects.

Aim and tasks

The aim of the conducted research is studying the molecular dynamics of complexes of 1,4-benzodiazepines with phosphodiesterase 4 created using AutoDock Vina, to evaluate their dynamic stabilization during the simulation period.

To achieve the set goal, it was necessary to solve the following tasks:

- to conduct an analysis of scientific literary sources of influence of benzodiazepines on phosphodiesterase 4;
- to provide docking analyze and molecular dynamic and of PDE 4;
- to investigate the features of interaction 1,4-benzodiazepins with researched proteins.

Materials and methods

AutoDock Vina was used to calculate the free binding energy and determine the features of their interactions by the molecular docking method.

Molecular docking and molecular dynamics procedure were performed using 3 crystal structures of phosphodiesterase 4 (PDE4) from the Protein Data Bank (http://www.rcsb.org/): PDE4A (3TVX), PDE4B (3W5E), PDE4D (6IM6).

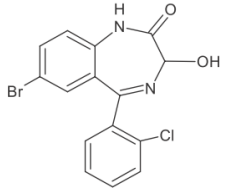
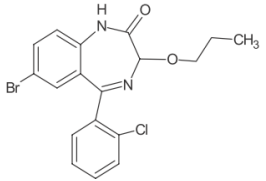
Reference ligands PNX (3TVX), NVW (3W5E) AH3(6IM6) and benzodiazepine ligands, including propoxazepam and its possible metabolite 3-hydroxypropoxazepam, were used for the study.

PlayMolecule software (playmolecule.com) was used to perform molecular dynamic simulations of the ligand-protein complex from the original model of the docking experiments.

Propoxazepam

«Propoxazepam», an innovative drug created by scientist of the O. V. Bogatsky Physical-Chemical Institute of the National Academy of Sciences of Ukraine and SLC «INTERCHIM», has an original pharmacodynamic profile, and can inhibit both acute and chronic pain, as well as have anti-inflammatory and anticonvulsant effects.

Alpha-1 adrenergic receptors, are involved in the mechanisms of propoxazepam analgesic effect.

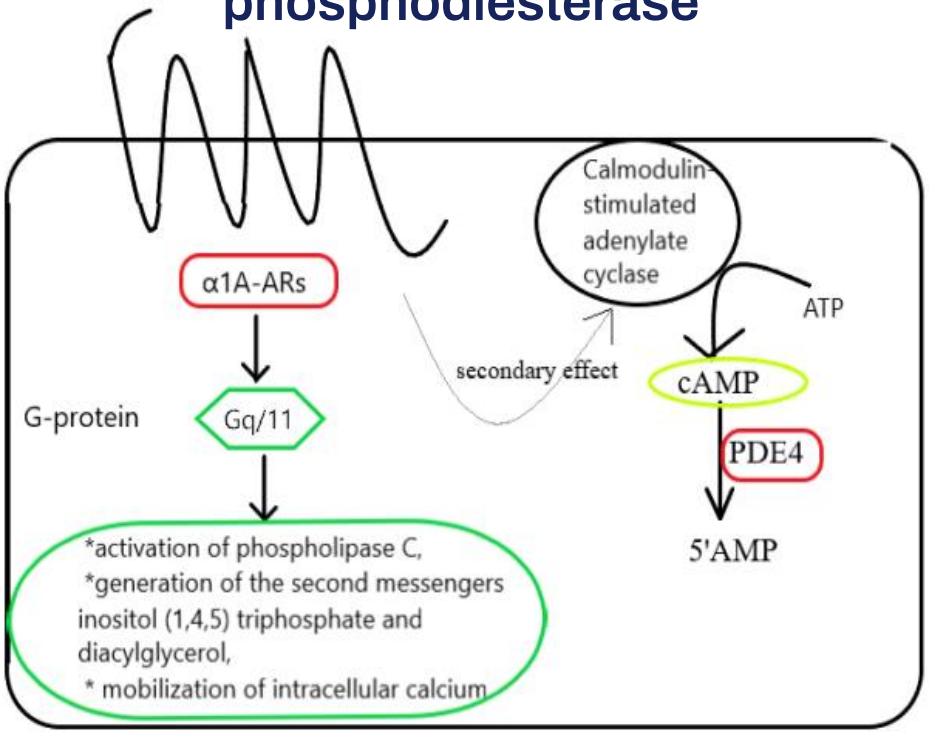
3-hydroxy-propoxazepam	Propoxazepam
	

α1-adrenergic receptors and phosphodiesterase 4

α_1-adrenergic receptors are G-Protein Coupled Receptors that are involved in neurotransmission and regulate the sympathetic nervous system through binding and activating the neurotransmitter, norepinephrine, and the neurohormone, epinephrine.

Phosphodiesterases (PDEs) are enzymes that regulate the intracellular levels of cyclic adenosine monophosphate and cyclic guanosine monophosphate, and, consequently, exhibit a central role in multiple cellular functions.

Cascade of process modulated of a cAMP response element pathway by α1A- and α1B- ARs by off- target inhibition of phosphodiesterase



PDE4 inhibitors prevent pain

There is reason to believe that PDE4 inhibition may reduce neuropathic pain by modulating Cx43 expression in the spinal dorsal horn, as Cx43 is importantly regulated by cAMP signaling.

Inhibition of PDE4

↓

Increase a levels of cAMP

↓

Activation of PKA (protein kinase A)

↓

PKA activation results in the phosphorylation of CREB (cAMP response element-binding protein) and the activation of ATF-1 (activating transcription factor 1).

↓

A Increase inflammation-inhibiting cytokine production

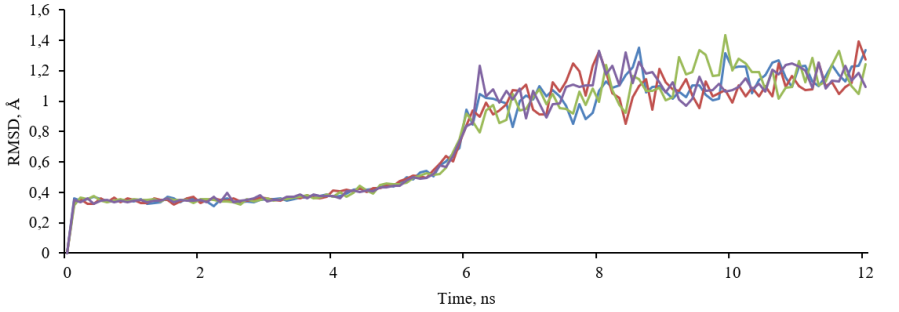
↓

A decrease inflammation-promoting cytokine production

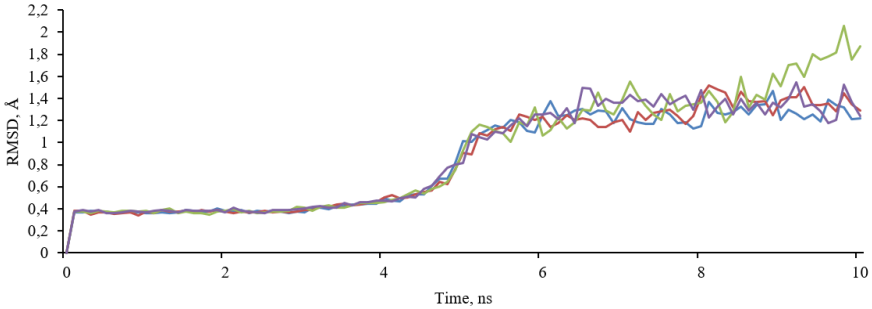
The root mean square deviation (RMSD) of the protein (PDE4) in complex with researched ligands

3TVX	**6 ns**	**12 ns**	**Range**
Without ligand	0.945 Å	1.334 Å	0.389 Å
Reference ligand PNX	0.839 Å	1.273 Å	0.434 Å
Propoxazepam	0.837 Å	1.095 Å	0.258 Å
3-hydroxypropxazepam	0.916 Å	1.244 Å	0.328 Å
3W5E	**5 ns**	**10 ns**	**Range**
Without ligand	1.015 Å	1.216 Å	0.201 Å
Reference ligand NVW	0.907 Å	1.293 Å	0.386 Å
Propoxazepam	0.815 Å	1.244 Å	0.429 Å
3-hydroxypropxazepam	0.927 Å	1.875 Å	0.948 Å
6IM6	**5 ns**	**10 ns**	**Range**
Without ligand	0.868 Å	1.061 Å	0.193 Å
Reference ligand AH3	0.818 Å	1.205 Å	0.387 Å
Propoxazepam	0.836 Å	1.299 Å	0.463 Å
3-hydroxypropxazepam	0.741 Å	1.276 Å	0.535 Å

RMSD values of PDE4 without and in complex with ligands: (*a*)- PDE4 (3TVX), reference ligand - PNX, (*b*)- PDE4B (3W5E), reference ligand - NVW, (*c*)- PDE4D (6IM6), reference ligand – AH3



a



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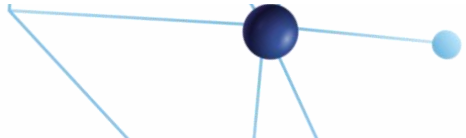
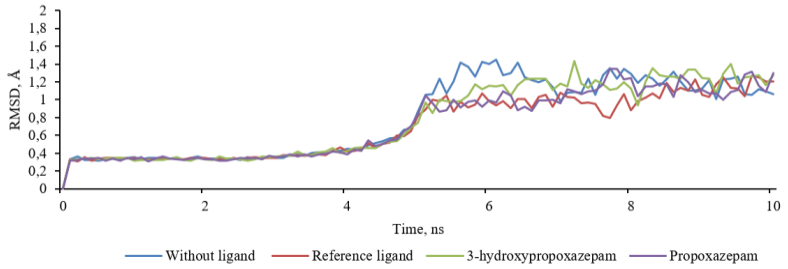
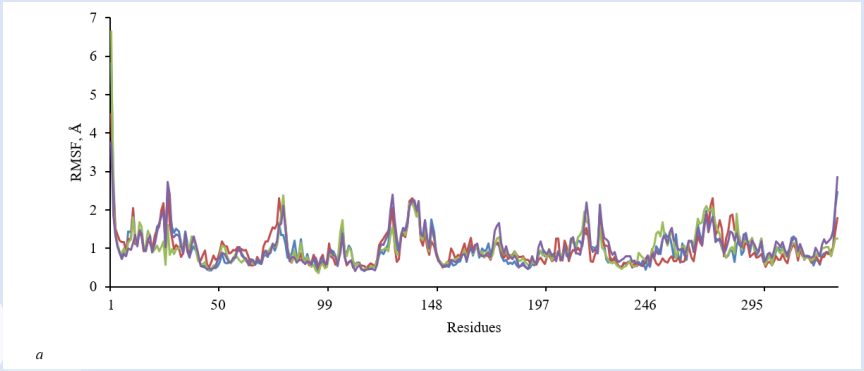
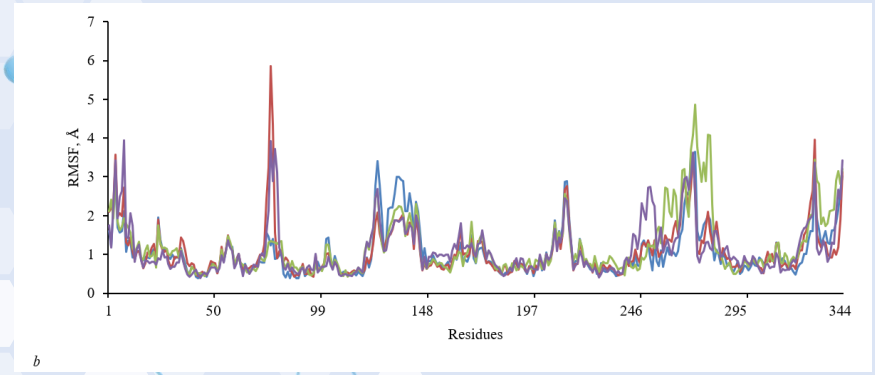


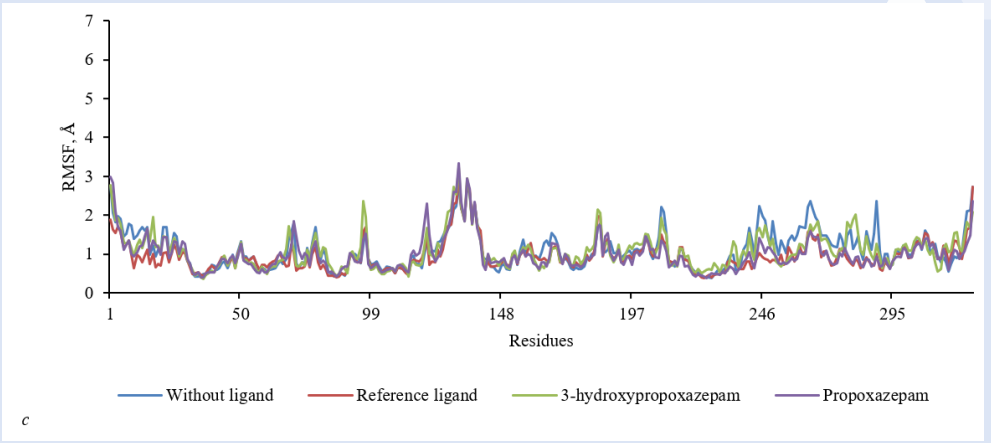


c

RMSF values of PDE4 without and in complex with ligands: (*a*)- PDE4 (3TVX), reference ligand - PNX, (*b*)- PDE4B (3W5E), reference ligand - NVW, (*c*)- PDE4D (6IM6), reference ligand – AH3







Conclusions

1. The root mean square deviation values for the complex of phosphodiesterase 4A with propoxazepam are smaller than for the positive control, indicating the formation of a stable protein-propoxazepam complex and the potential efficacy and reliability of propoxazepam as a PDE4A inhibitor.
2. The range of RMSD values for PDE4B (3W5E) and PDE4D (6IM6) in complexes with 1,4-benzodiazepines are higher than complexes of these proteins with reference ligands or without ligands. It's possible that there are differences in the binding specificity or affinity between propoxazepam and the protein compared to the reference ligand.
3. Binding of propoxazepam and its metabolite 3-hydroxypropoxazepa to PDE4B reduces the fluctuations of M-pocket residues and supports the conclusion that ligand binding stabilizes the protein structure of PDE4B.

Thank you for your attention!