Design of alkylxanthine derivatives of 3,4-dihydroquinazoline-2-amine as potential serotonin receptor ligands using molecular modelling methods

The research was performed as part of the project “Novel 5-HT_{5A} receptor ligands capable of inhibiting PI3K/Akt/mTOR pathway signaling as a dual approach in the treatment of castration-resistant prostate cancer”, LIDER14/0035/2023, financed by the National Centre for Research and Development under the LIDER XIV program.

Natalia Kozień, Przemysław Zaręba

Politechnika Krakowska
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Serotonin

- A monoamine neurotransmitter that acts through receptors in the central nervous system and peripheral nervous system.
- Biosynthesized in the body from tryptophan.
- Deficiency in the body can cause depression, anxiety disorders and hallucinations
- Regulates sleep quality, appetite, sexual drive and thermoregulation.

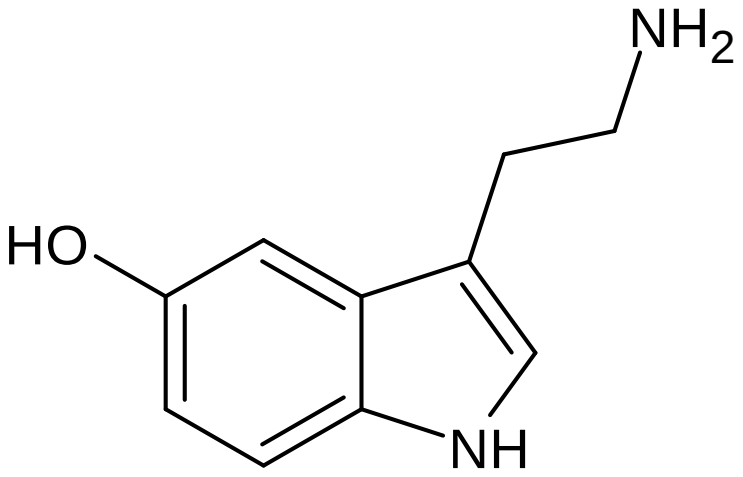

Fig. 1. Serotonin (5-hydroxytryptamine)

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Serotonin receptors

- There are 7 families of serotonin receptors (5-HT_1 – 5-HT_7) divided into 15 subgroups.
- The 5-HT_3 receptor is coupled to an ion channel and the remaining 5-HTRs are coupled to a G protein.
- 5-HTRs play a key role in treating diseases such as depression, schizophrenia, anxiety disorders

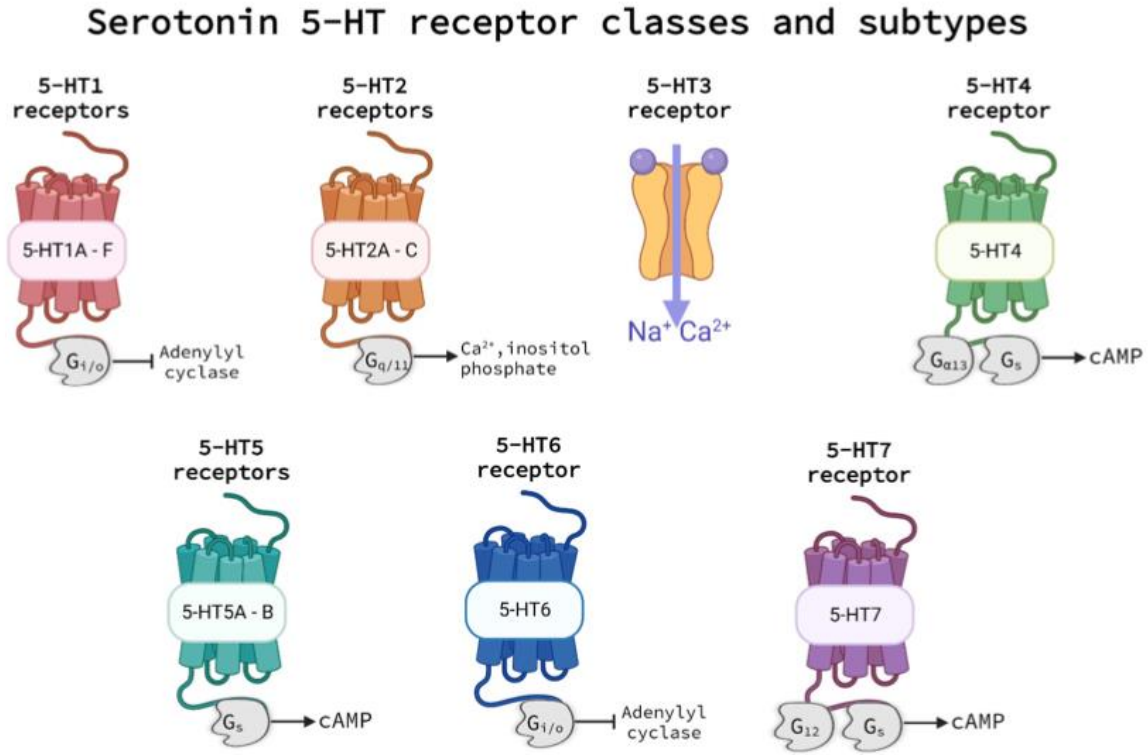
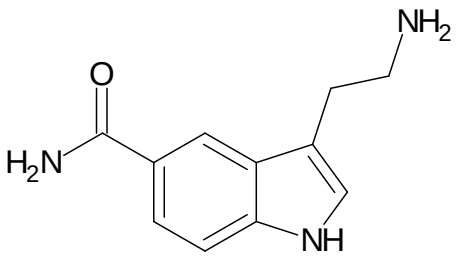


Fig 2. Receptor 5-HT types and subtypes

Serotonin 5-HT_{5A} receptor

- The least known of the 5-HT receptors
- Two subtypes : 5-HT_{5A} i 5-HT_{5B}
- Agonists: 5-carboxamidotryptamine, valerenic acid
- Antagonists: ansepamine, dimebolin



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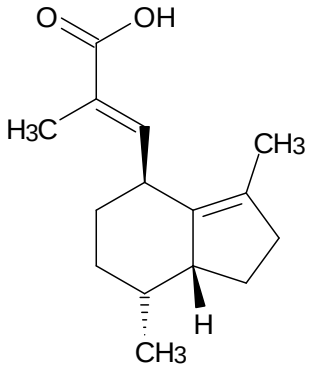

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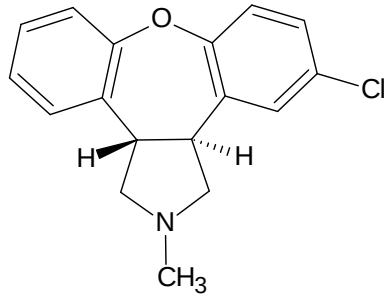
Fig 3. Examples of 5-HT5a receptor agonists: 5-carboxamidotryptamine (1), valerenic acid (2)



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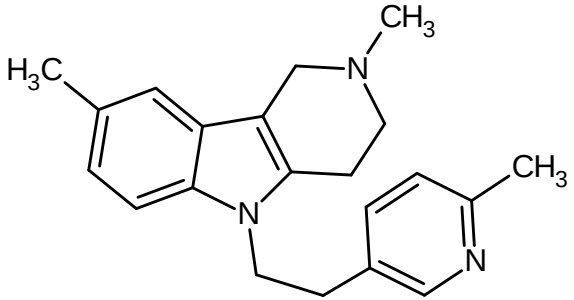

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Fig 4. Examples of 5-HT5a receptor antagonists: asenapine (3), dimebolin (4)

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Available structures of 5-HT$_{5A}$ receptor

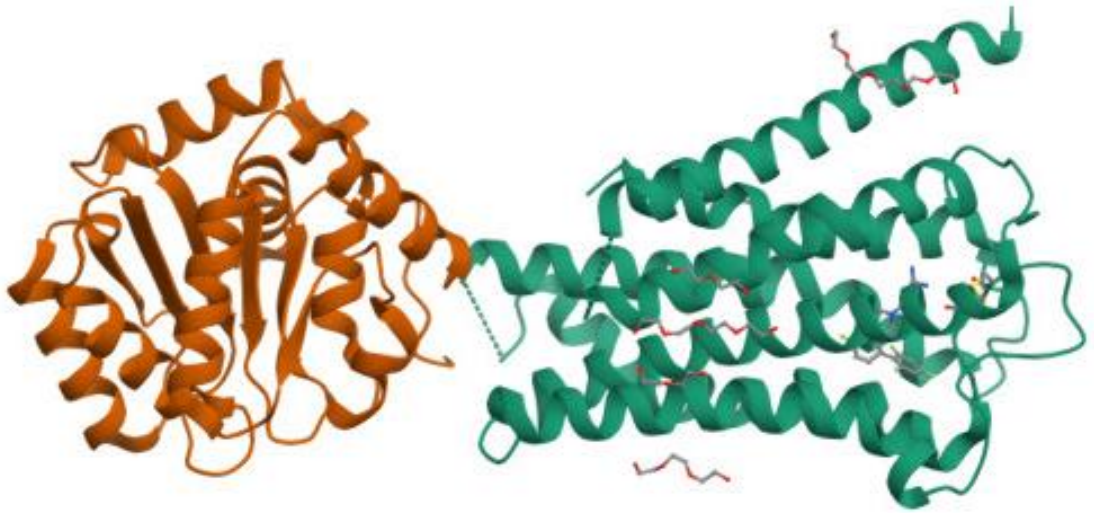

Fig. 7. Crystal structure of inactive 5-HT$_{5A}$R in complex with AS2674723, PDB ID: 7UM4

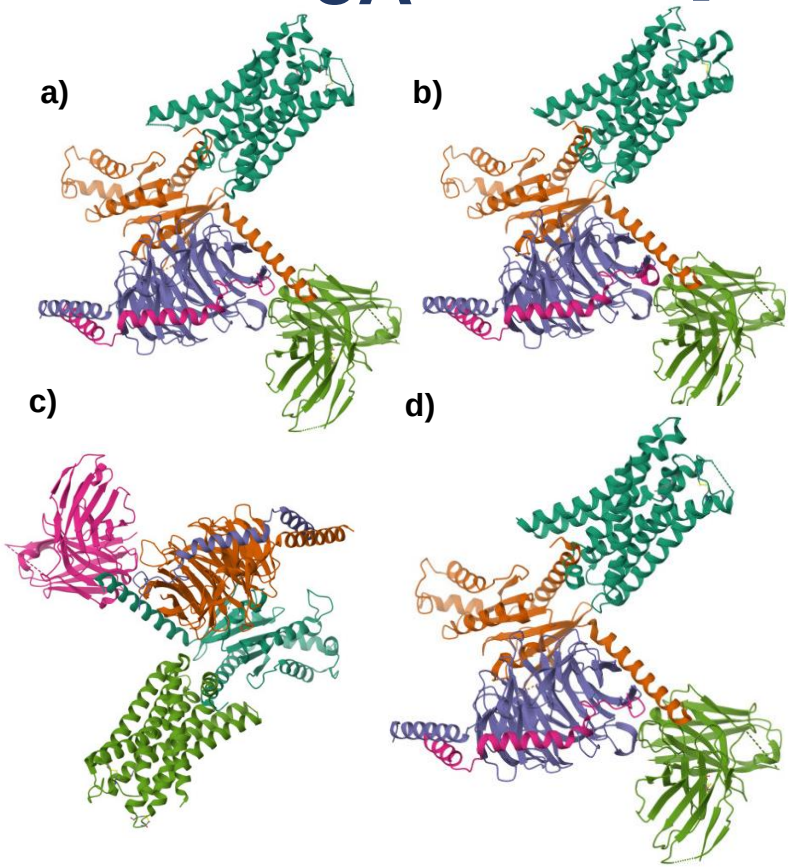

Fig. 7. a) CryoEM structure of Go-coupled 5-HT5$_{AR}$ in complex with Lisuride, PDB ID: 7UM6 b) CryoEM structure of Go-coupled 5-HT$_{5A}$R in complex with 5-CT, PDB ID: 7UM5 c) Serotonin 5A (5-HT$_{5A}$) receptor-Gi protein complex, PDB ID: 7X5H, d) CryoEM structure of Go-coupled 5-HT$_{5A}$R in complex with Methylergometrine, PDB ID: 7UM7

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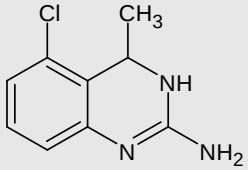
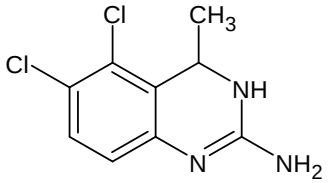
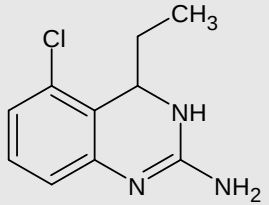
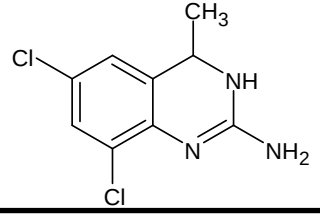
Known active 5-HT_{5A} receptor ligands

Tab. 7. Receptor 5-HT_{5A} ligands

	Structure	5-HT_{5A} K_i [nM]
1		5,1
2		2,0
3		84,4
4		6,5

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Research objective

- There are reports of antitumor activity of 5-HT_{5A}R antagonists, but there are no compounds with the desired pharmacological properties.
- It is important to design a new group of this receptor ligands that could be a candidate for usage as a monotherapy or in combination therapy in the treatment of cancer for example, in combination with compounds targeting adenosine receptors.
- For this purpose, the possibility of expanding the **2-amino-3,4-dihydroquinazoline** chemotype by adding a pharmacophore responsible for binding to adenosine receptors was investigated.

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Research methodology

Molecular modeling with docking:

Protein-ligand system

Protein structure: crystal structure of the inactive 5-HT_{5A} receptor in complex with AS2674723 (PDB ID: 7UM4) retrieved from the PDB RSCP database, the protein was analyzed using the protein preparation wizard in Schödinger Maestro

Receptor grid: centered the grid box on the aspartic acid residue $D^{3.32}$ in Schödinger's Maestro using the "Glide" option

Ligand structures: prepared using the “LigPrep” option and then subjected to virtual screening in Schödinger’s Maestro program, refinement of residues within 5.0 Å of the ligand position using the IFD protocol

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Research methodology

Molecular dynamics:

Membrane and simulation system: created with QwikMD in VMD 1.9.3, membrane: POPC

Method used: program: NAMD, standard all-atom force field (CHARMM), temperature: 27°C, pressure: 1 atm, water model: TIP3, ions: Na^+, Cl^-, time: 100 ns, pH: 7.4

Trajectory analysis, complex stability and representative form: ChimeraMD program

Graphical presentation of results:

PyMOL program

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Docking results

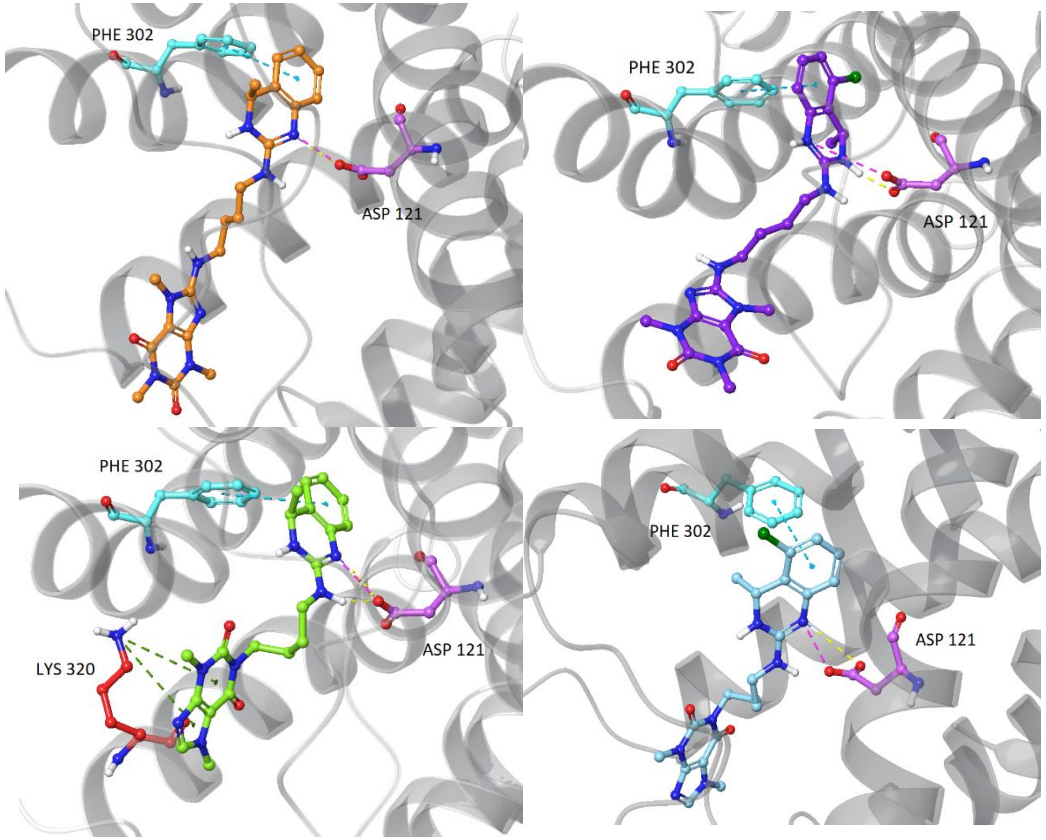

Fig. 7. Compound NK6 (orange), NK13 (purple), NK11 (green) and NK12 (blue) after docking in 5-HT_{5A} protein. Pink lines indicate salt bridge, yellow hydrogen bonds, blue π-π conjugated bonds and green cation-π interaction.

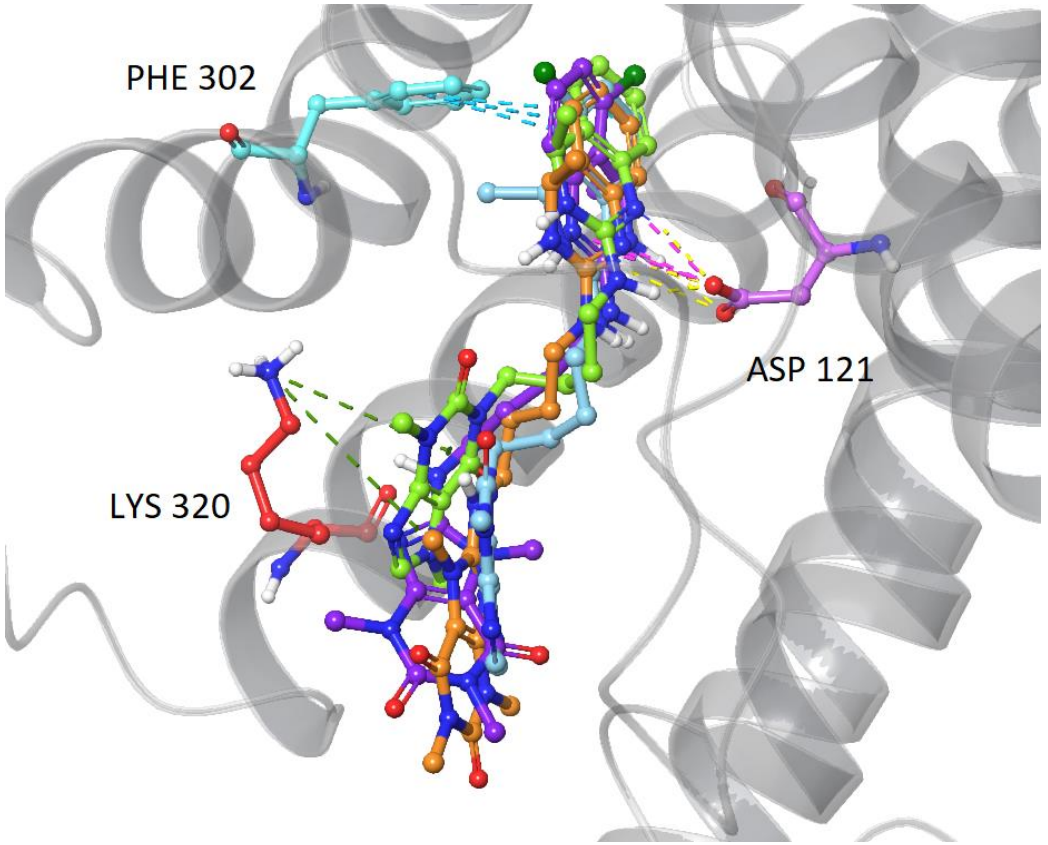

Fig. 8. Comparison of the conformations of active ligands in the binding pocket of the 5-HT_{5A} receptor. Pink lines indicate the salt bridge, yellow hydrogen bonds, blue π-π conjugated bonds and green cation-π interactions.

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Dynamics simulation results

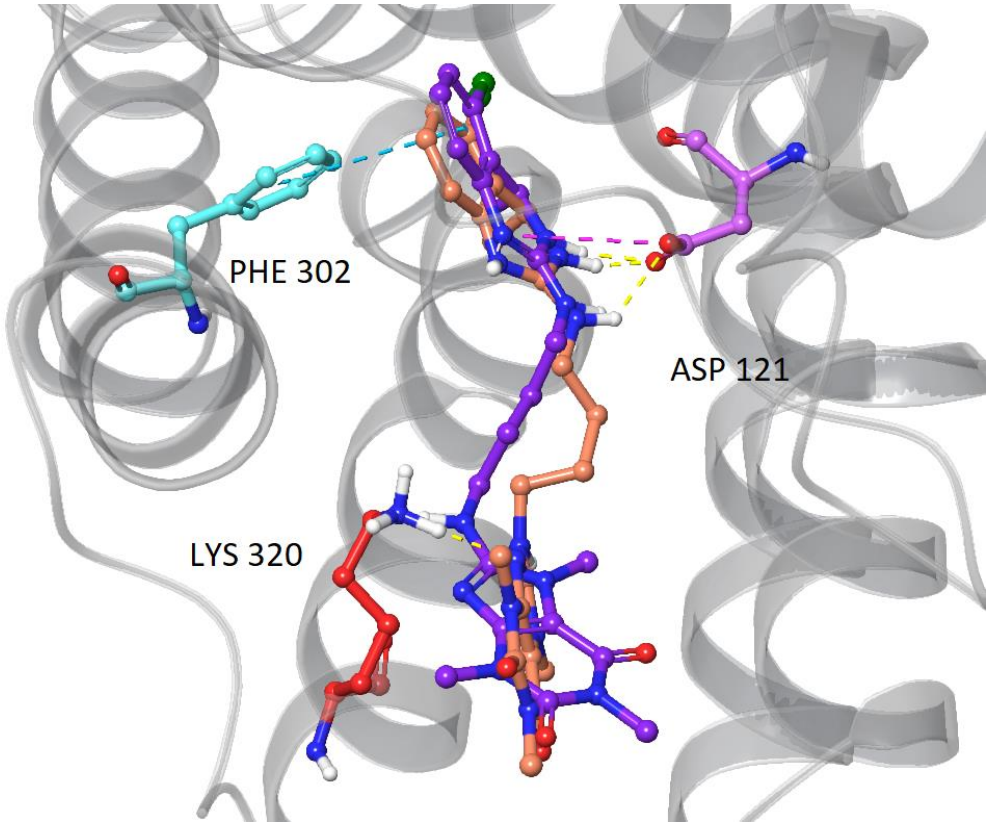

Fig. 9. Active conformation of NK13 – comparison of the complex after docking (purple) with the most representative molecular dynamics pose (faded red-orange)

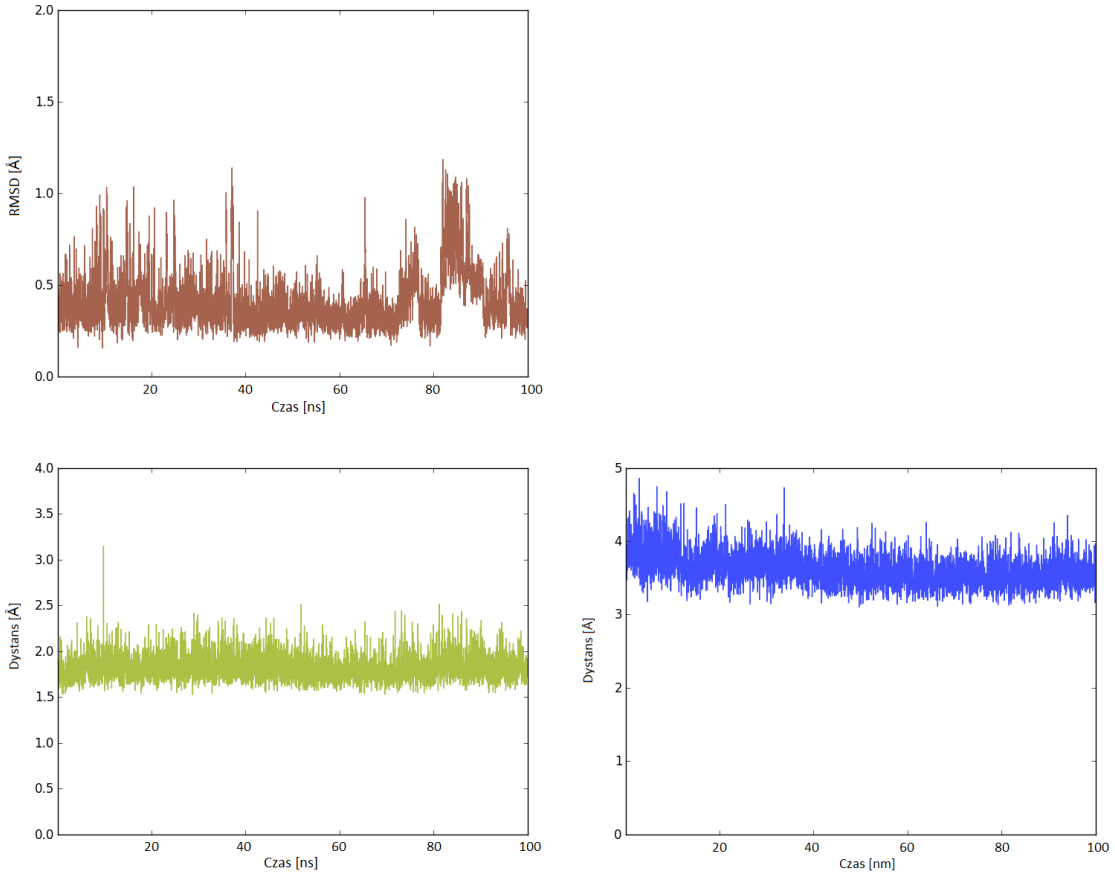

Fig. 10. RMSD measurement (orange) of compound NK13 and bond lengths with ASP 121 (green) and PHE 302 (blue) residues

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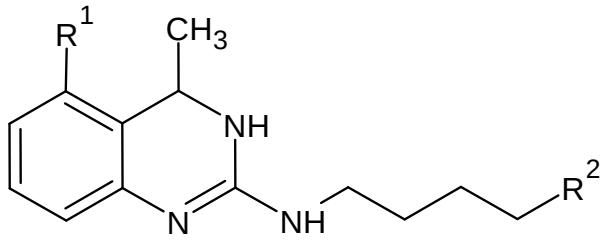
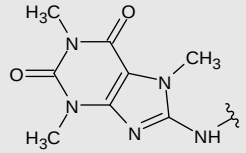
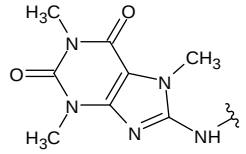
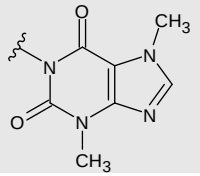
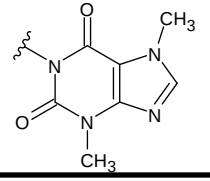
In vitro research



Tab. 3. Summary of 5-HT_{5A} receptor affinity results

	R^1	R^2	5-HT_{5A} K_i [nM]
NK6	H		400
NK13	Cl		267
NK11	H		555
NK12	Cl		203

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Conclusions

After docking, interactions of all designed ligands with aspartic acid residue $D^{3.32}$ were observed

From the obtained results of molecular dynamics simulations, it was concluded that the complex is stable

Higher affinity is exhibited by derivatives that contain a substituted chlorine atom in their structure

The compounds initiated a new, unique chemotype of long-chain 5-HT_{5A} receptor ligands combined with pharmacophore responsible for binding to adenosine receptors which is promising for use in oncological co-therapy

Designing new molecules, the focus should be on modifications towards other halogen substituents and the influence of the length of the carbon chain connecting xanthine substituents with quinazolines on the position of the molecule in the protein.

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