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Nitrosubstituted analogs of isoxazolines and isoxazolidines: a surprising estimation of their biological activity via molecular docking.

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Abstract: The biological activities in the field of antimicrobial application of trihalomethylated isoxazolines and isoxazolidines were investigated by means of molecular docking. In our work, we compared these two groups of heterocyclic compounds due to their strength of non-covalent binding affinity with several exemplary proteins that are known to partake in various biological processes. The obtained results show that the investigated compounds possess higher binding affinities to selected proteins than many hitherto known and applied compounds.

Keywords: Isoxazolines, isoxazolidines, molecular docking, molecular dynamics, biological activity,

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Introduction

Heterocyclic compounds containing nitrogen and oxygen atoms are a very important group of organic compounds and many natural heterocycles have been widely used as medicine agents for centuries [1]. The interest in application and designing of new heterocyclic products has been steadily growing - they are considered not only to be precursors for synthesis of biologically active molecules, but also exhibit various healing properties and constitute a unique class of pharmacophores in many therapeutic agents

[2-4]. Among such compounds, there are isoxazolines and isoxazolidines, having in their structure oxygen and nitrogen atoms in adjacent positions in the ring, what directly affects their wide spectrum of therapeutic potential and wide spectrum of biological activities [5-7]. There are many organic compounds with isoxazoline, isoxazolidine motif, that are known to exhibit biological activity in various fields such as antifungal, antibacterial, anti-inflammatory, antidepressant or anticancer ones [5-9]. In addition, presence of a trihalomethyl functional group in the heterocyclic ring, increases biocompatibility of the compound [10-12]. The most popular drug containing both isoxazoline ring and trihalomethylated group is Fluranarel (Figure 1), which is a strong antiparasite agent, widely used in veterinary with a broad spectrum of application [13]. There is also well-known drug – Seromycin with an active agent Cycloserine, containing an isoxazolidine ring in the structure (Figure 1), that is used primarily in the treatment of tuberculosis or mycobacteriosis in lungs [14].

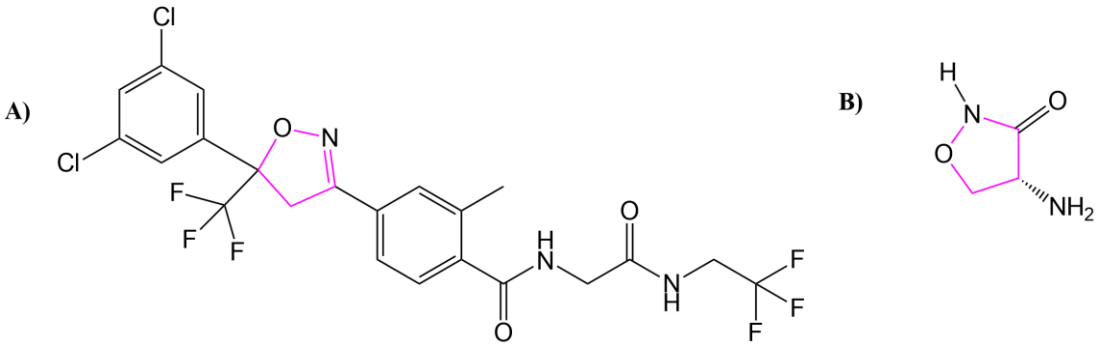


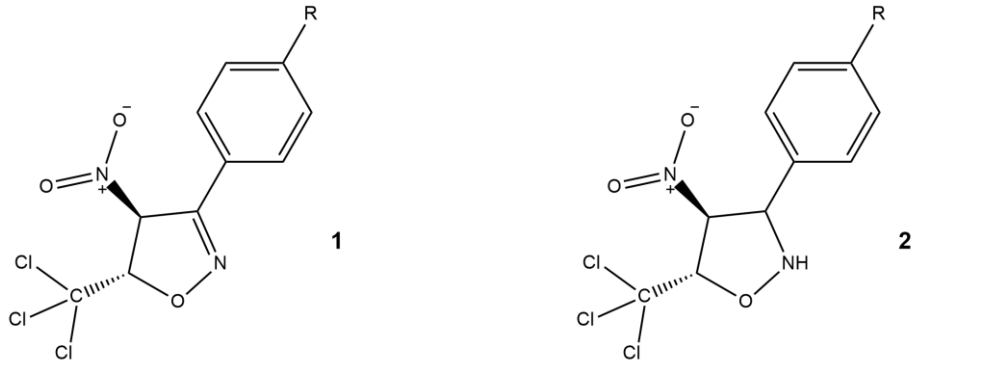
Fig. 1. The chemical structure of A) Fluranaler and B) Cycloserine.

Regarding lethal diseases, one of the greatest problems of global medicine today are infections caused by pathogenic microorganisms [15]. The most problematic issue is the persistence of microbial resistance to medications due to facile mutations of microorganisms [16]. This problem has become increasingly important in the field of human treatment, but also it has lately become a great research interest in treatments of non-human species as well [17]. In the literature, many attempts have been described to find and develop new classes of antimicrobial drugs that would exhibit better properties than present ones [15-17].

Nowadays, computational tools are widely used to provide much information both in chemistry and biological activity of existing and potential drugs [18]. Molecular docking plays a major role in understanding the drug-receptor interactions and is widely used as an important tool in the process of establishing potential applications of a given drug, its affinity

and activity to the target protein [18-20] and has therefore become an indispensable part of modern drug discovery process [21].

In this work, we examined two series of trihalomethylated isoxazolines and isoxazolidines (Figure 2) due to their possible biological activity in various proteins. We carried out molecular docking to estimate and compare the strength of non-covalent bonding between series of compounds under consideration and a series of various proteins already known to exhibit various kinds of activity with similar molecules as described further in a more detailed fashion. This work is sort of a continuation of our recent research of trihalomethylated isoxazole analogs, which were described in our previous papers [10-11].



R: a) -OCH_3; b) -CH_3; c) -F; d) -Cl; e) -NO_2 R: a) -OCH_3; b) -CH_3; c) -F; d) -Cl; e) -NO_2

Fig. 2. The chemical structure of analyzed series of 1) isoxazolines and 2) isoxazolidines.

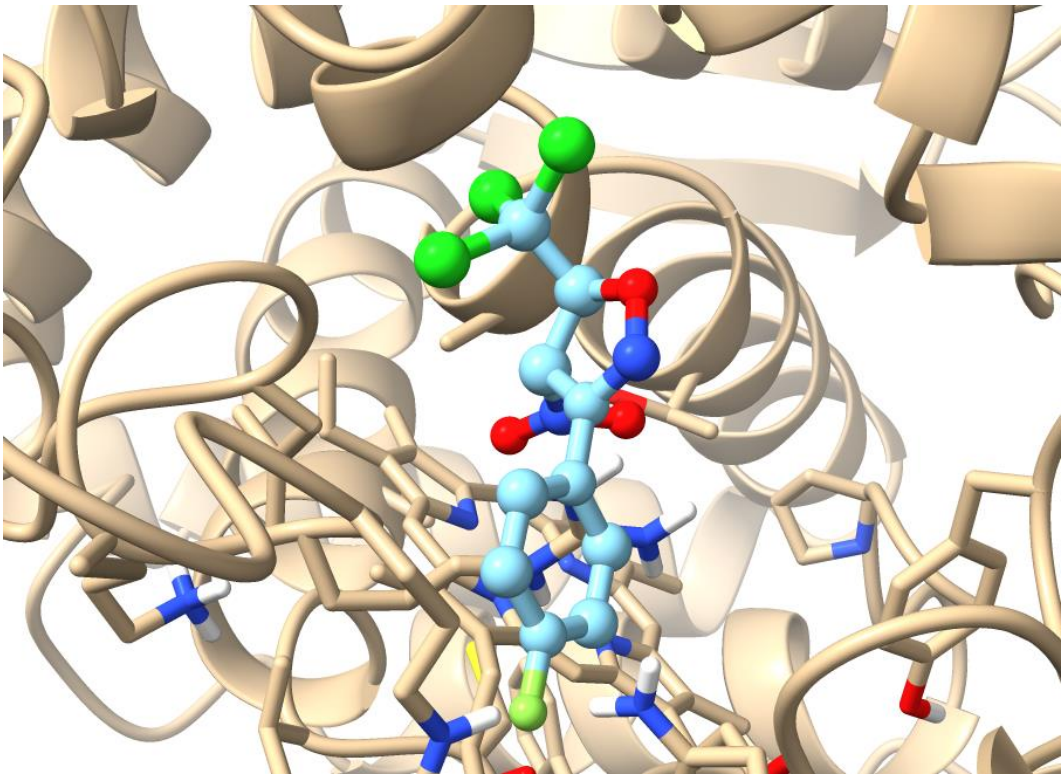
Results and Discussion

Firstly, we evaluated docking to Cytochrome P_{450} 14α-sterol demethylases CYP51 (RSCB Database PDB ID: 1EA1), which is a very important enzyme in sterol biosynthesis in eukaryotes. This feature allows to consider it to be a great target for e.g. antifungal drug design [18,22]. We therefore compared our docking results with several ones already described in the literature. The measured values of ΔG in docking for all tested compound range between -9.15 to -10.58 kcal/mol (Table 1) what supports the notion that the examined isoxazolines **1a-e** and isoxazolidines **2a-e** possess a considerable affinity to CYP51 which may indicate their potential antifungal activity. The series of **2a-e** exhibits somewhat greater affinity. The worst ΔG values in both series show compounds **1d** and **2d.** In molecular docking not only the ΔG value is a factor of significance, but also are the values of pose score (Table 1), which is an indicator of the probability of occurrence of the given compound conformation within the protein active site. After analyzing those scores, it can be said, that **1c** and **2e** (Figure 3) have the highest probability of the occurrence such conformation. Comparing to the results obtained by Bano et al. [18], both our series show better docking scores to CYP51 than the compounds synthesized by them. It is worth

mentioning, that Bano in his work tested a common-use drug for fungal infections (Fluconazole), that resulted in the ΔG equal to -5.63 kcal/mol [18], which also indicates that the compounds investigated by us may bind possibly better and therefore be a more potent receptor agonists than Fluconazole. However, it should be stated clearly, that the isoxazoline and isoxazolidine rings are considered to be less bio-active than the pyrrazole ring [23-24], which is, not supported by our results. Another dubious factor is that the supposedly favorable docking conformations seem not to interact in a significant way with the metal-organic hem framework present in the protein.

Table 1. The best molecular docking scores for CYP51 and series **1a-e**, **2a-e**.

	ΔG (kcal/mol)	CNN pose score	CNN affinity
1a	-9.55	0.6098	6.395
1b	-10.00	0.6516	6.388
1c	-10.57	0.9359	6.754
1d	-9.59	0.7277	6.688
1e	-9.69	0.8245	6.912
2a	-10.27	0.8794	6.859
2b	-10.57	0.8072	6.703
2c	-10.23	0.7664	6.720
2d	-9.57	0.5928	6.606
2e	-10.58	0.8924	7.023



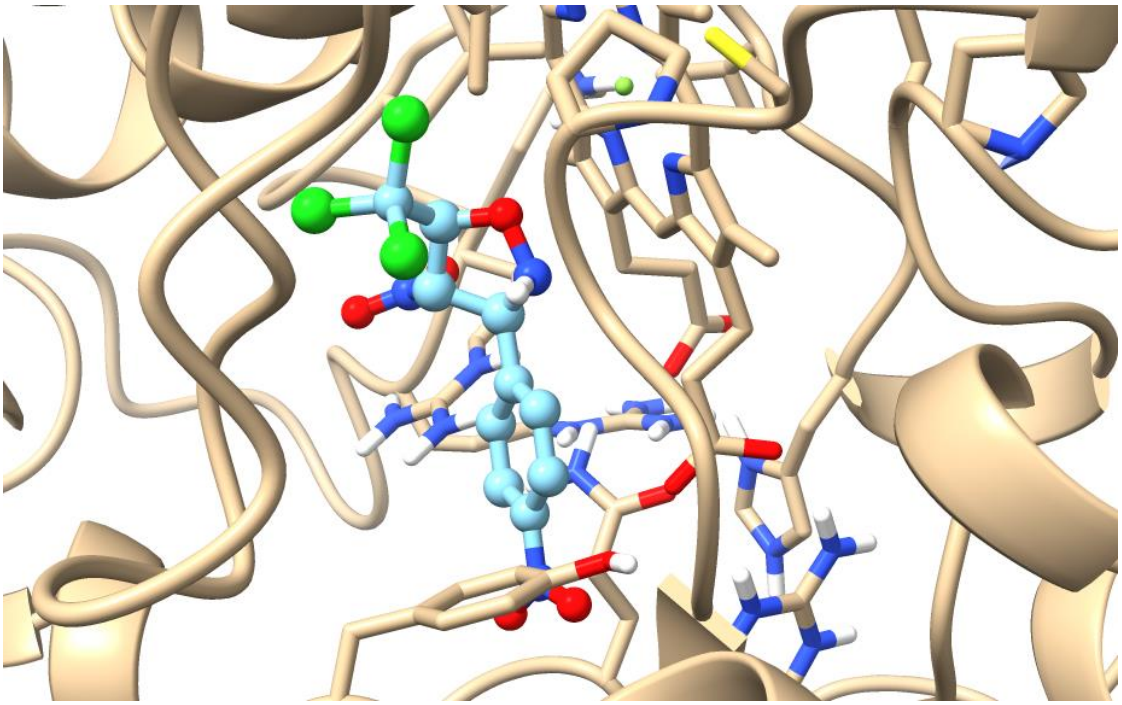


Fig.3. The most favorable non-covalent binding pose of **1c** (upper) and **2e** (lower) to CYP51.

Our analysis showed that the compounds considered create multiple hydrogen bonds with their respective proteins which in turn should provide lower values of their Gibbs free energy of complexation. As the sole docking scores are concerned, we found that the number of hydrogen bonds does not correlate with better scores; this is most probably because Gnina docking software (unlike Vina) does not take them explicitly into account, relying rather on the machine-learning generated scoring functions [25]. The rationalization of the results obtained might thus be done by invoking other favorable (e.g. electrostatic, hydrophobic/-philic) interactions within the protein pocket due to the diverse chemical structure of the ligands with both hydrophobic (e.g. -CH3) and polar - or at least polarizable to some degree - substituents (e.g. -NO2, -F). Figure 4. depicts exemplary hydrogen bonding patterns of **1a** and **2c** and Fig. 5. depicts exemplary docked structures of **1e** and **2e**, where electrostatic potentials and hydrophobic/hydrophilic part of the protein pocket were visualized.

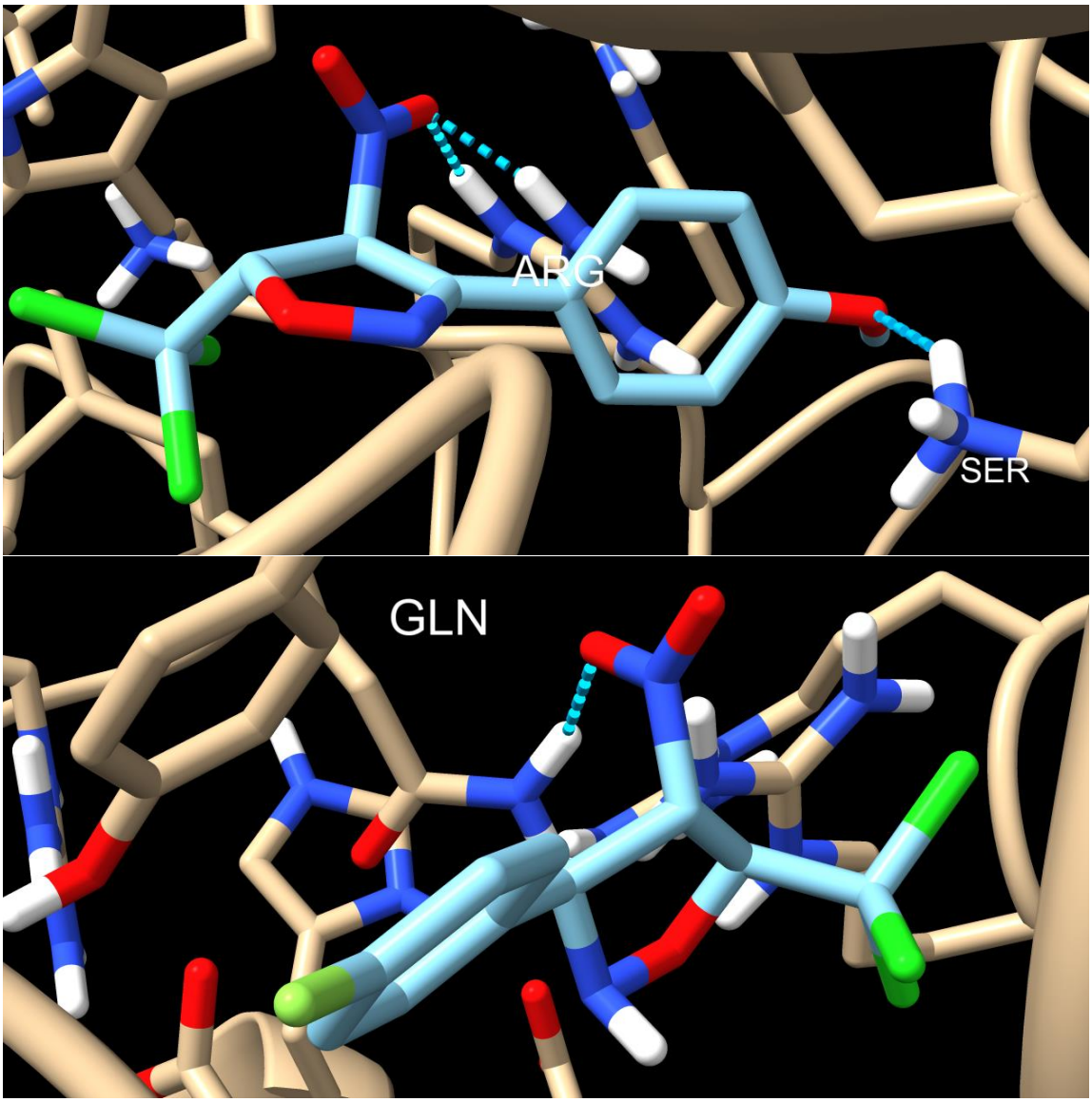


Fig.4. Hydrogen bonds visualization of **1a** (upper) and **2c** (lower) to CYP51.

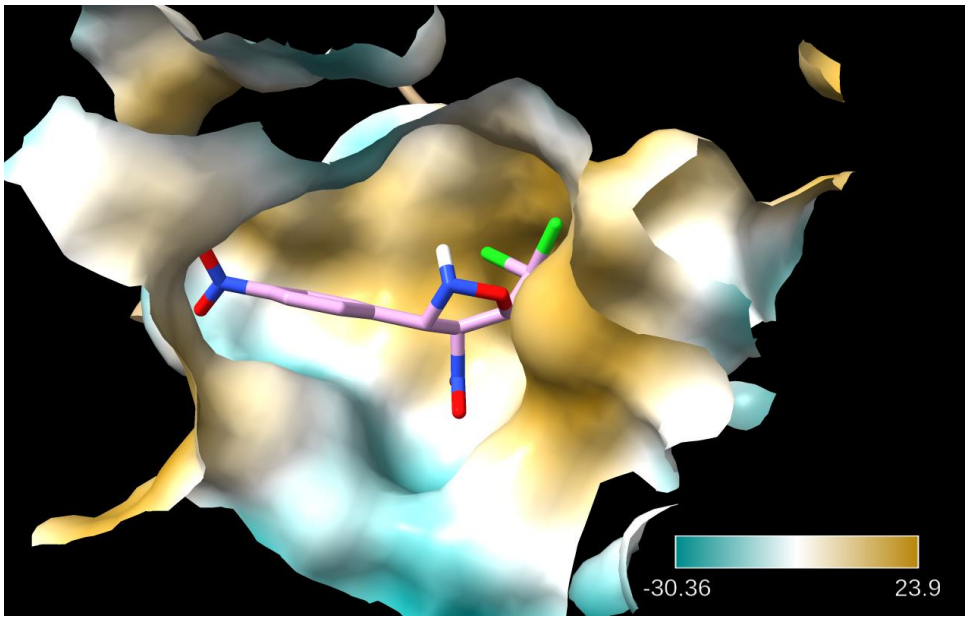


Fig.5. Exemplary hydrophobic (golden) and hydrophilic (cyan) potential for **2e** inside the CYP51 cavity.

Analysis shows that the ligand is located in of favourable size for the given ligand itself and the electrostatic and hydrophobic-hydrophilic potentials are practically in accordance with the spatial arrangements of the hydrophobic and hydrophilic parts of the ligand.

Table 2. The best molecular docking scores for Caspase-7 (PDB: 4ZVT) and series **1a-e**, **2a-e**.

	ΔG (kcal/mol)	CNN pose score	CNN affinity
1a	-7.20	0.9187	5.309
1b	-6.90	0.7414	4.759
1c	-7.17	0.7796	4.879
1d	-6.15	0.7644	4.941
1e	-7.44	0.7603	5.124
2a	-7.00	0.7100	5.117
2b	-6.79	0.7211	5.138
2c	-6.45	0.5554	4.516
2d	-7.17	0.6199	5.099
2e	-7.50	0.5587	4.981

Subsequently, we performed docking to Caspase-7 (PDB ID: 4ZVT) which belongs to a protein family considered to be the strongest apoptosis executioner [26]. It was found that interfering into activation of Caspase-7 can induce therapeutic properties in treating cancer or inflammations. Due to this fact, we perceived that it was worth attempting to dock the investigated compounds to this protein, as many heterocyclic compounds happen to be

strong inflammatory agents [5-9]. Having based on work of Oubella et al. [27], who measured the influence of different isoxazole-isoxazoline hybrids on the activity of Caspase-3 and Caspase-7, we compared of the docking scores of the analyzed series of **1a-e** and **2a-e** and the results indicate that the compounds investigated by us are stronger binders yet again. The ΔG values of Oubella range between -3.00 to -4.40 kcal/mol [27], while the most unfavorable value obtained by us is for **1b** and equal -6.9 kcal/mol (Table 2). In their work, the authors also mentions the ΔG for common used anticancer agent Doxorubicin which is -7.19 kcal/mol [27]. Comparing this with our results, we infer that **1a**, **1e**, **2d** and **2e** (Figure 5.) are worthy of further investigation as potential anticancer or anti-inflammatory agents. The same logic of rationalizing results by favorable interactions with hydrophobic and -philic sites within the pockets as for Bano et al. can be applied here.

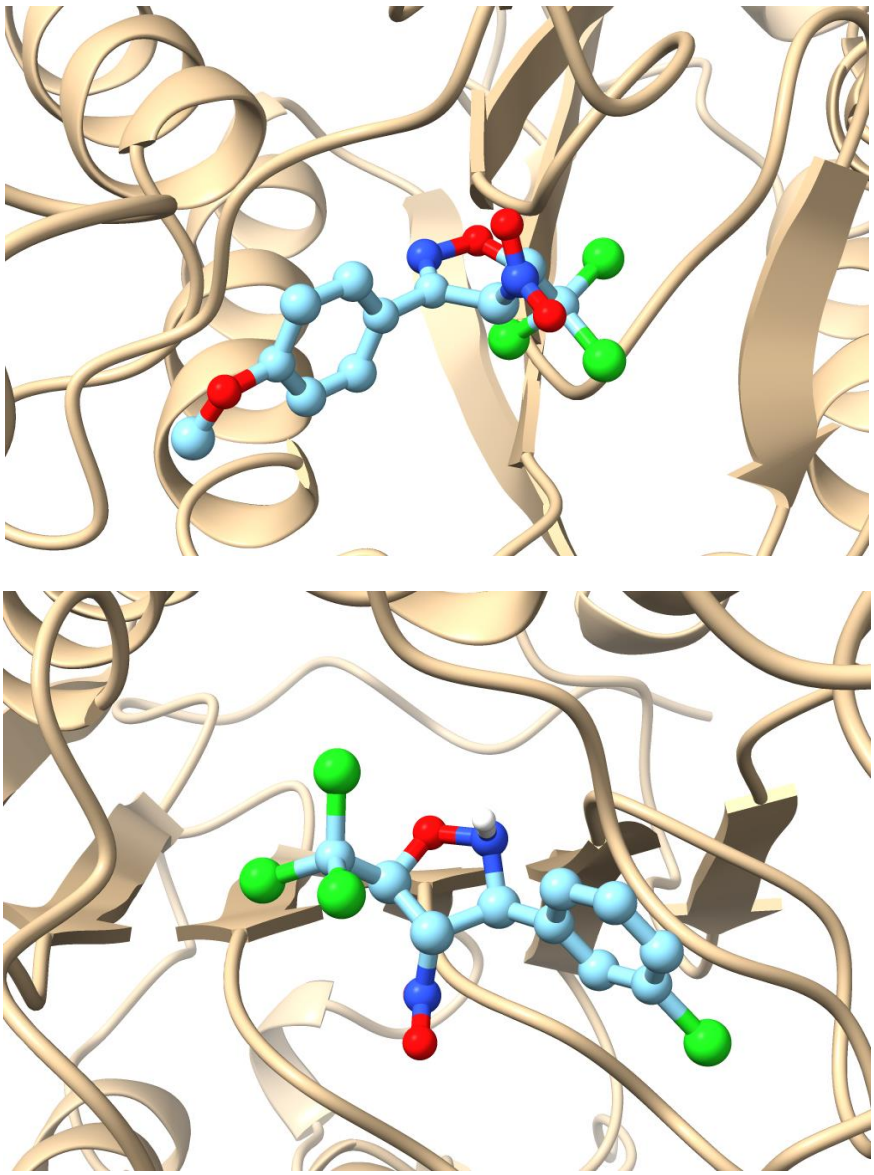


Fig.5. The most favorable non-covalent binding pose of **1a** (upper) and **2d** (lower) to Caspase-7.

The analysis of electrostatic and hydrophobic-hydrophilic potential (Figure 6). shows once more the spatial accordance of the ligand and protein.

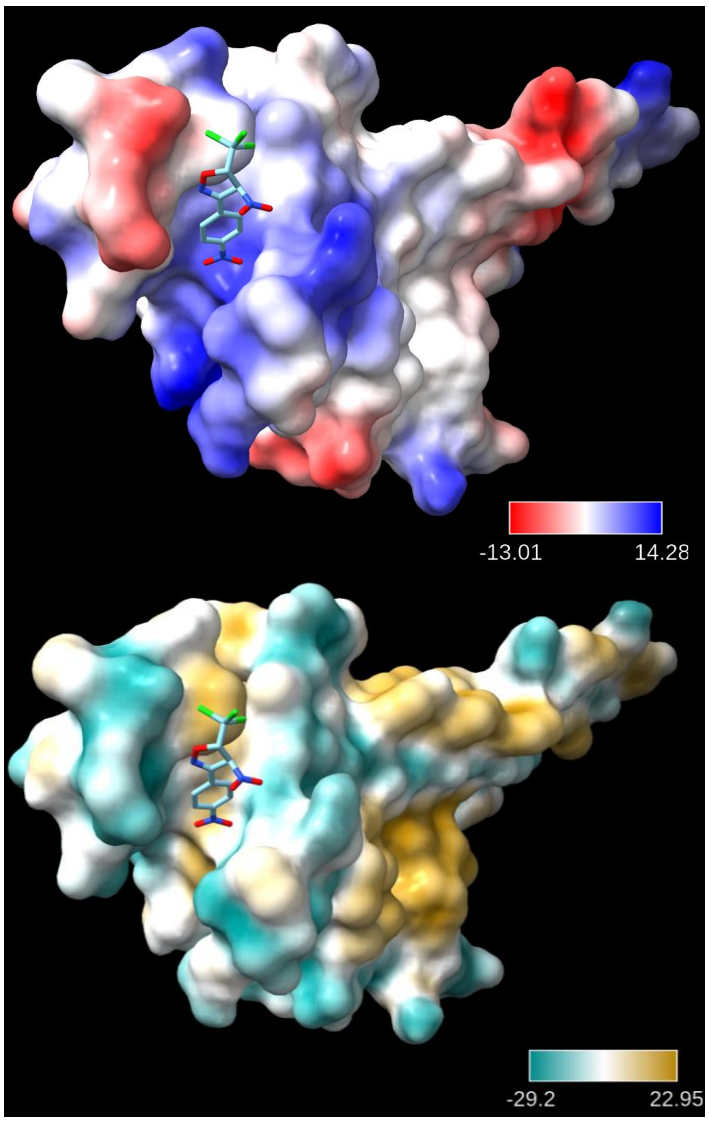
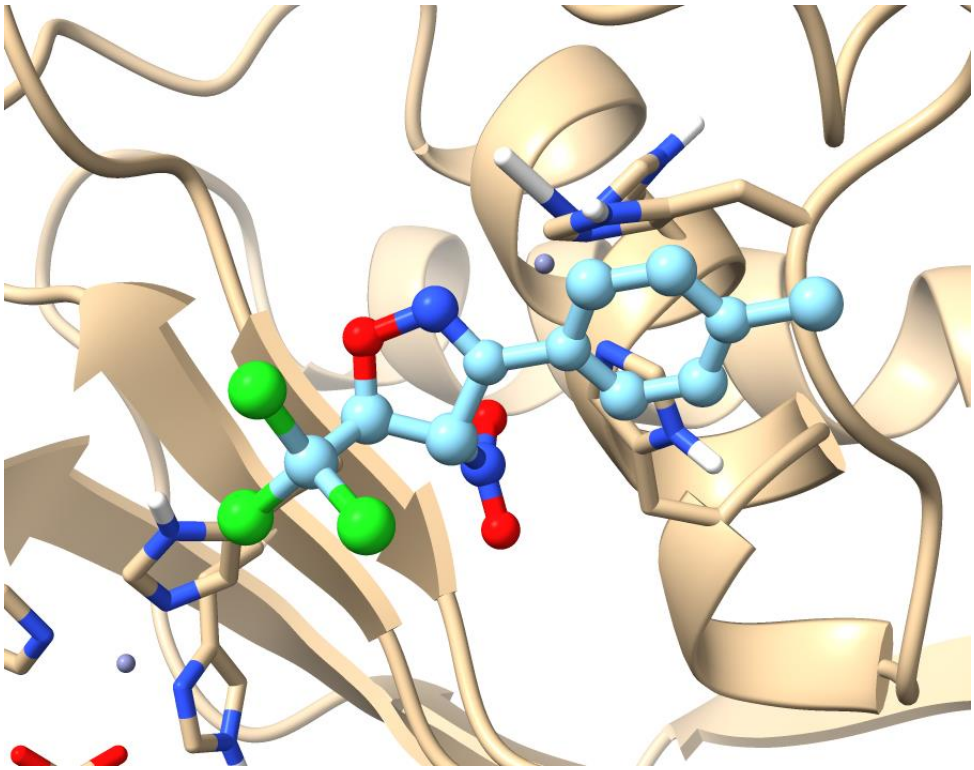


Fig. 6. Exemplary electrostatic (red-white-blue) and hydrophobic (golden)/hydrophilic (cyan) potential for **1e** inside the Caspase-7 cavity.

It has been also found, that metalloproteinases greatly induce the angiogenesis process of cancer cells. Due to this, they seem indispensable in cancer treatment. Among the proteins of the metalloproteinase family, one of the most interesting and promising is gelatinase B (MMP-9; PDB ID: 4XCT) which poses a great capacity for regulation of cytokine and chemokine activity [28]. In that case, the best molecular docking ΔG values exhibit compounds **2a-e** that range between -7.85 to -9.94 kcal/mol (Table 3). However, **1a-e** possess a bit lower ΔG values, yet with higher pose scores (Figure 7).

Table 3. The best molecular docking scores for MMP-9 (PDB: 4XCT) and series **1a-e**, **2a-e**.

	ΔG (kcal/mol)	CNN pose score	CNN affinity
1a	-9.20	0.8239	5.872
1b	-9.46	0.8223	5.850
1c	-9.17	0.8338	5.872
1d	-9.21	0.6667	5.761
1e	-9.07	0.7599	5.954
2a	-7.85	0.8105	6.069
2b	-9.43	0.7173	5.689
2c	-9.94	0.6655	5.754
2d	-9.81	0.6012	5.773
2e	-9.48	0.6513	5.829



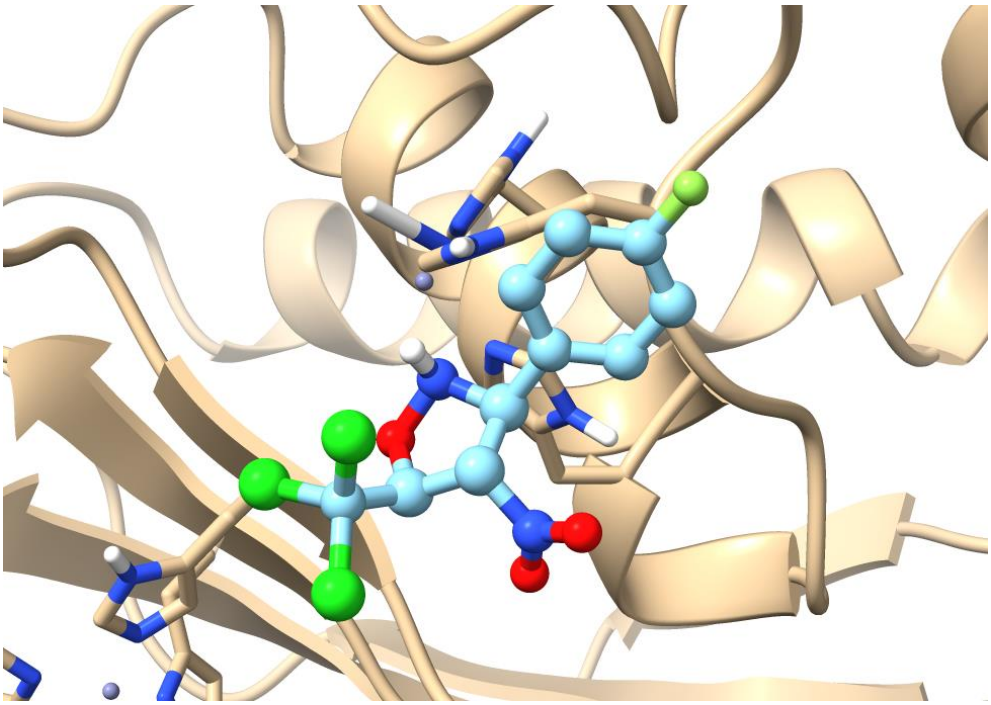


Fig. 7. The most favorable non-covalent binding pose of **1b** (upper) and **2c** (lower) to MMP-9.

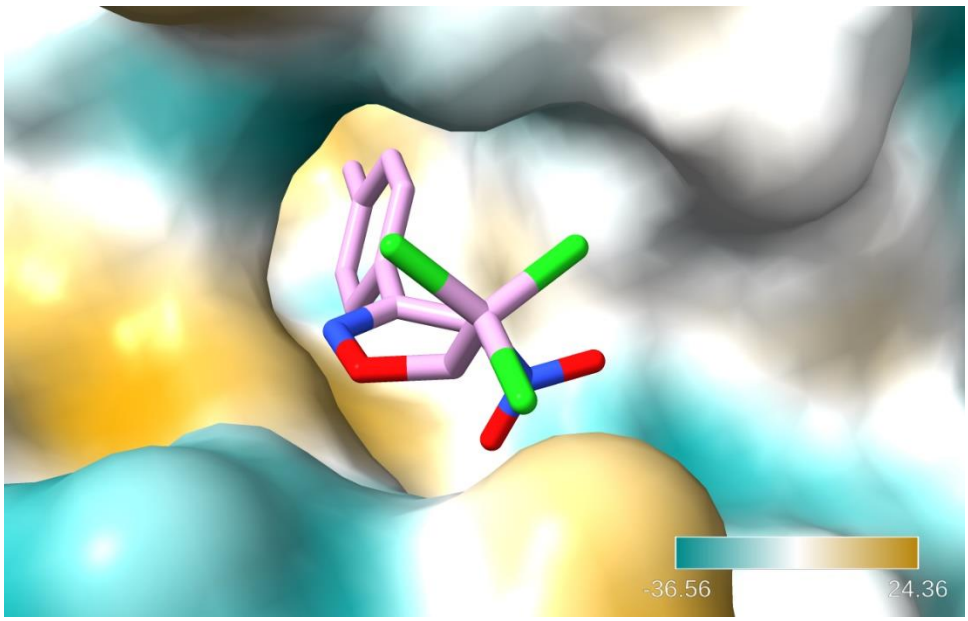


Fig. 8. Exemplary hydrophobic (golden)/hydrophilic (cyan) potential for **1b** inside the MMP-9 cavity.

One of the methods of relieving pain or inflammations is inhibition of cyclooxygenase COX enzymes, which take part in catalytic synthesis of prostaglandins - hormones that cause the pain and inflammations [29]. Many well-known non-steroidal anti-inflammatory drugs such as aspirin are in fact COX inhibitors [30]. There are three COX variants described repeatedly in the literature, COX-1 (PDB ID: 3KK6) and COX-2 (PDB ID: 5KIR) and COX-3 (not a part of our investigation) which act differently. For example, COX-1 stimulates ovarian cancer, while COX-2 is mainly connected with inflammatory stages and fever. Hawash et al.

[31] evaluated isoxazole-carboxamide derivatives as COX inhibitors. They compared the results of synthesized compounds with the commonly used Ketoprofen and Celecoxib, which bonding energy equals -8.76 kcal/mol for COX1, -8.18 kcal/mol for COX-2 and -11.69 kcal/mol for COX1, -11.53 kcal/mold for COX2 respectively. The compounds investigated by this researcher group show rather moderate activity towards COX 1-2 inhibitors, where in general ΔG ranges between -6.12 to -9.76 kcal/mol [31]. Series **1a-e** and **2a-e**, score even lower in ΔG as their range lies between -10,58 and -9.18 kcal/mol (Table 4 and 5), therefore this compounds could be characterized as probable anti-inflammatory agents once more, a bit better in comparison to Ketoprofen [31]. The series of **2a-e** exhibits better bonding affinity indicates for both COX inhibitors, while **1a-e** exhibits higher pose scores (Figure 9,11).

Table 4. The best molecular docking scores for COX-1 (PDB: 3KK6) and series **1a-e**, **2a-e**.

	ΔG (kcal/mol)	CNN pose score	CNN affinity
1a	-9.18	0.7202	6.414
1b	-9.48	0.8880	6.373
1c	-10.12	0.8198	6.751
1d	-9.44	0.8636	6.666
1e	-9.50	0.8031	6.498
2a	-9.21	0.8304	6.378
2b	-9.81	0.7622	6.422
2c	-9.89	0.8553	6.592
2d	-9.99	0.8645	6.730
2e	-9.68	0.7311	6.457

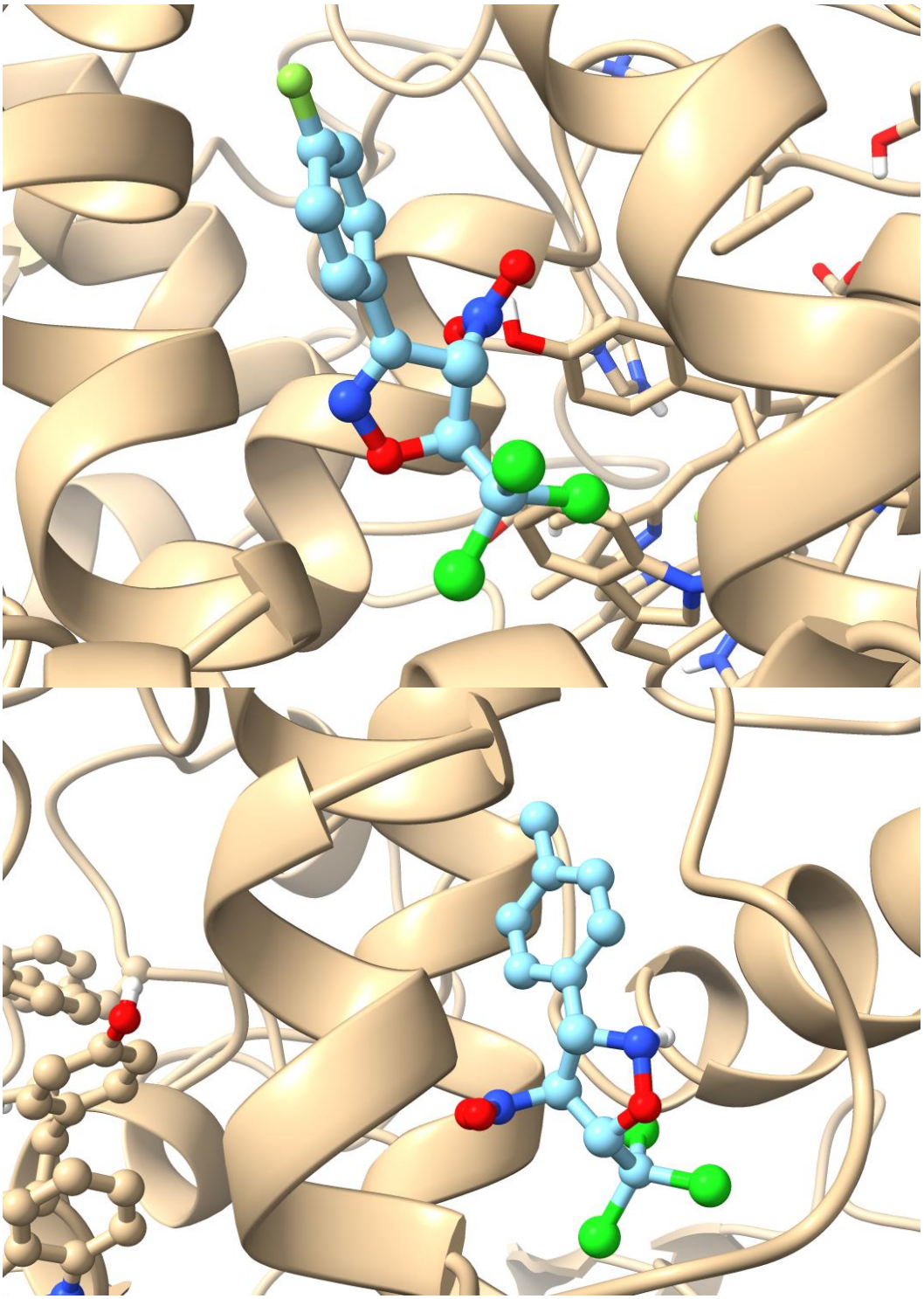


Fig. 9 The most favorable non-covalent binding pose of **1c** (upper) and **2b** (lower) to COX-1.

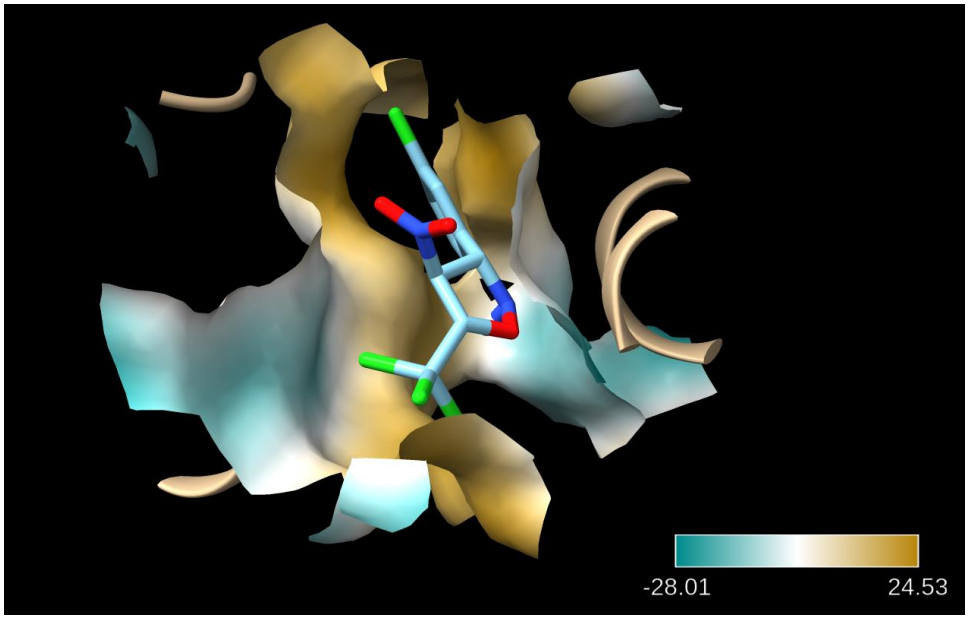


Fig. 10. Exemplary hydrophobic (golden)/hydrophilic (cyan) potential for **2d** inside the COX-1 cavity.

Table 5. The best molecular docking scores for COX-2 (PDB: 5KIR) and series **1a-e**, **2a-e**.

	ΔG (kcal/mol)	CNN pose score	CNN affinity
1a	-9.28	0.8417	6.476
1b	-9.89	0.8667	6.519
1c	-9.84	0.8095	6.399
1d	-9.51	0.8673	6.630
1e	-10.58	0.9731	6.729
2a	-9.66	0.8868	6.661
2b	-10.63	0.8730	6.644
2c	-10.71	0.8863	6.897
2d	-9.62	0.9147	6.826
2e	-10.15	0.9293	6.478

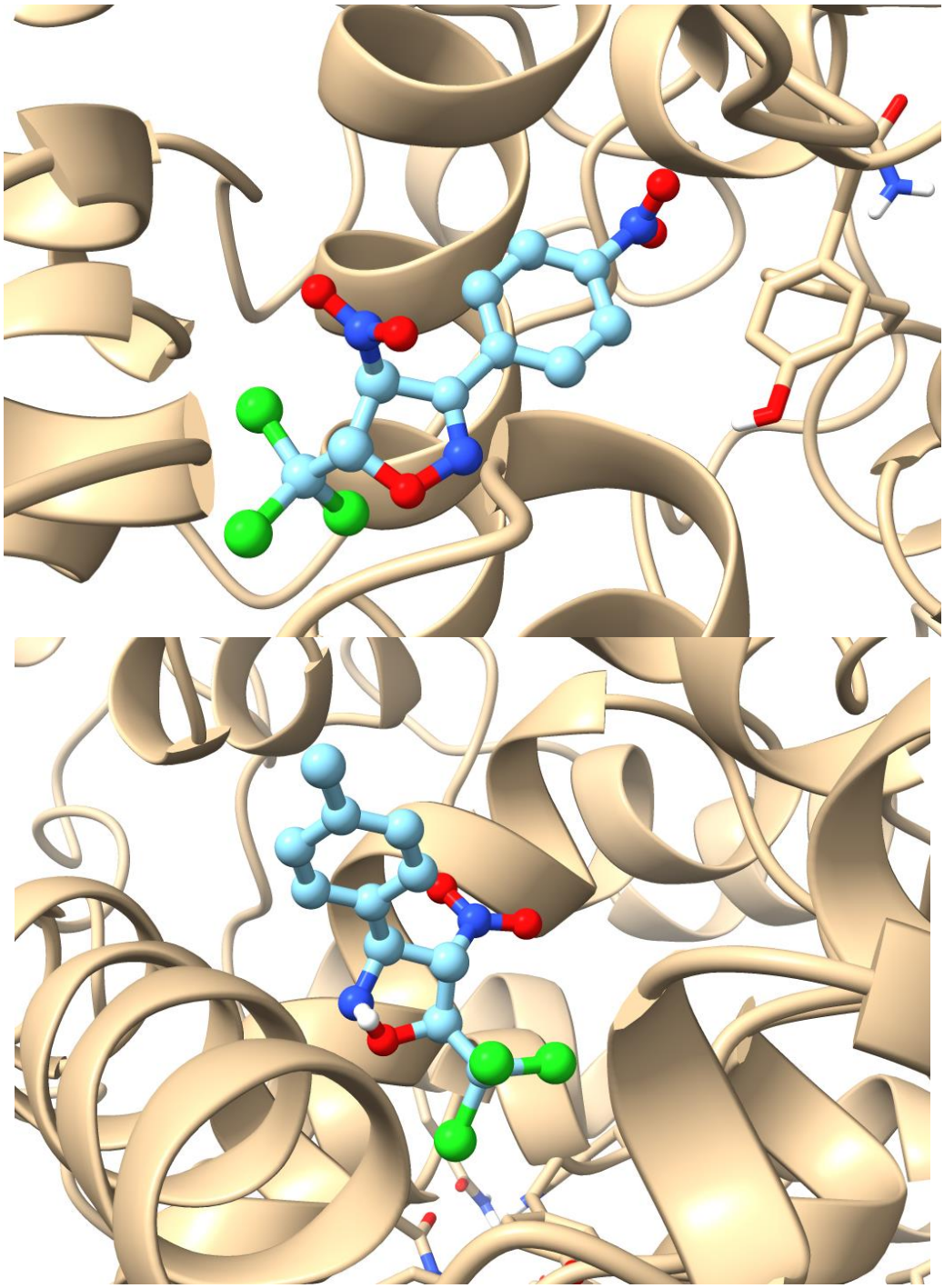


Fig. 11. The most favorable non-covalent binding pose of **1e** (upper) and **2b** (lower) to COX-2.

As mentioned earlier for CYP51, in other cases as well it is not possible to state that the higher number of hydrogen bonds (Figure 12,13) correlates with lower energy.

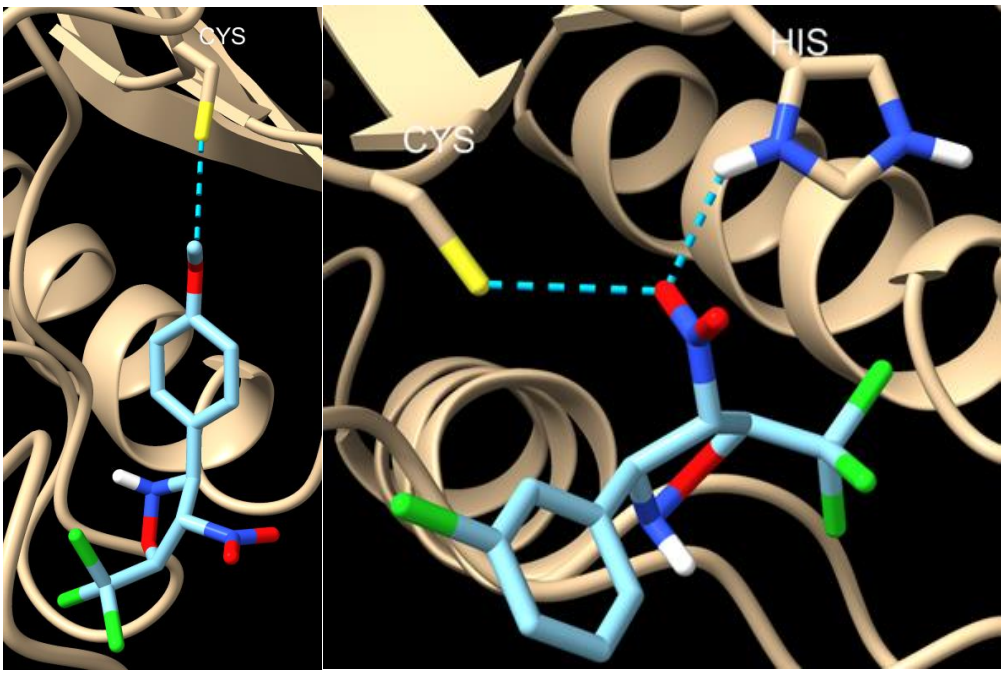


Fig. 12 Hydrogen bond formation visualisation in case of **2a** i **2c** in protein (PDB:4ZVT).

It is nevertheless worth to point out, that hydrogen bonds prefer to occur in most cases on oxygen atoms belonging to the nitro group in the isoxazoline/isoxazolidine ring (Figure 13). We regard these results as a confirmation of the fact, that introducing the nitro group to the structure of organic compounds could increases affinity to proteins [22-23].

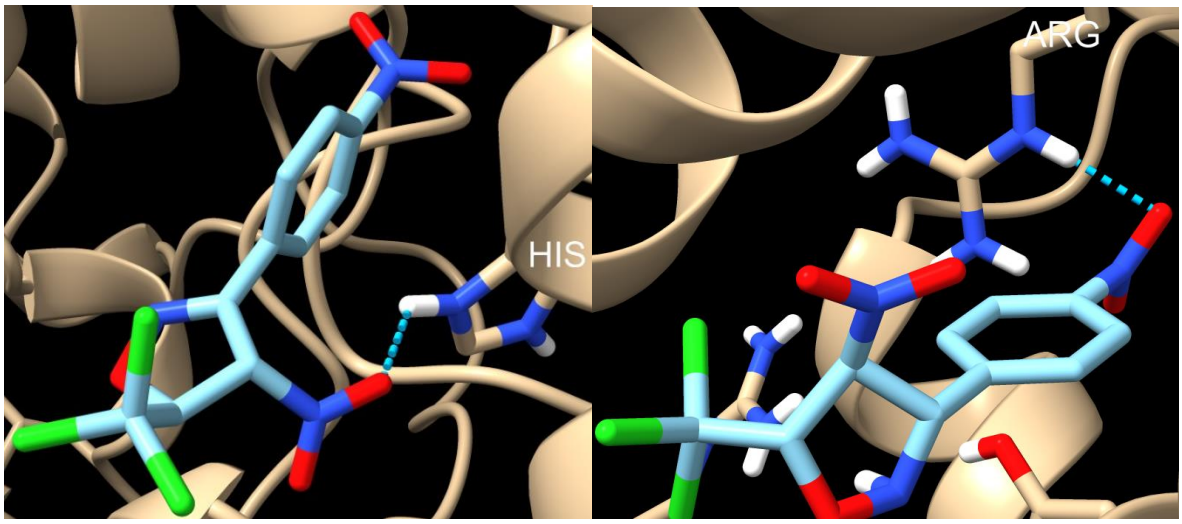


Fig. 13. Hydrogen bond formation visualisation in case of **1e** i **2e** in protein (PDB:5KIR).

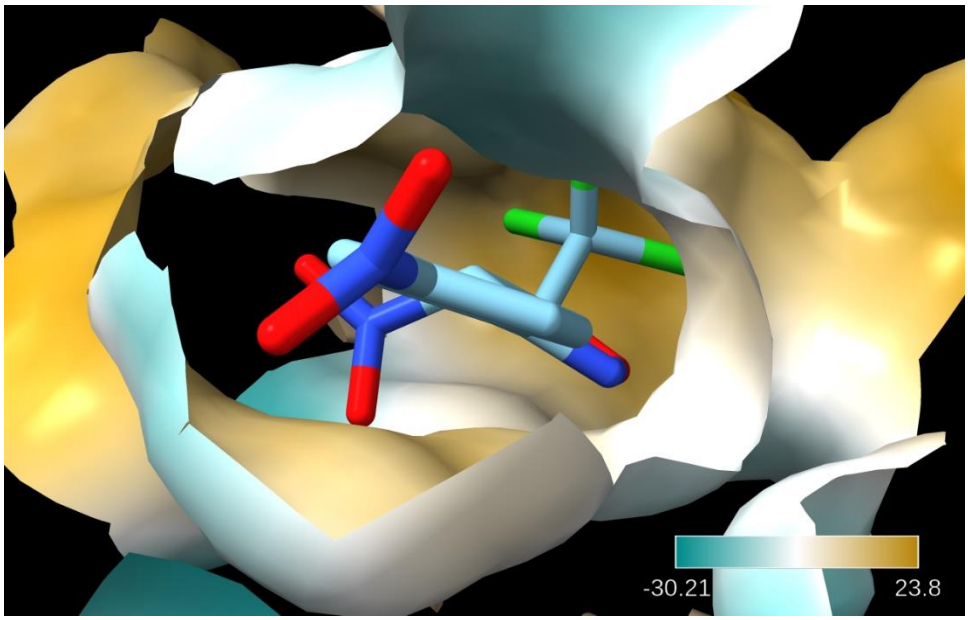


Fig. 14. Exemplary hydrophobic (golden)/hydrophilic (cyan) potential for **2d** inside the COX-2 cavity.

Methods

Geometries of all selected ligands were optimized with Gaussian16 software [32]. Hybrid B3LYP [33] functional was applied together with a Pople's 6-31+G(d) basis set. Vibrational analysis was performed in order to ensure that all structures found correspond to local minima and the scaling factor was chosen to be 0.98. Partial charges were calculated with Merz-Singh-Kollman scheme [34] and all the results (geometry and charges) were saved and subsequently applied as the input files for molecular docking.

The docking was performed by Gnina software [25]. The software is a fork of smina [35] (that itself in turn is a fork of Autodock Vina [36]) and supports scoring with convolutional neural networks. Flexible docking mode was chosen and all residues within the radius of 5 Å from the native ligand position were considered flexible. The ligands studied were chosen to autobox to the coordinates of the native ligand as well. By the „native ligand" the existing ligand from the original RSCB PDB database [37] protein file is meant. The ensemble of neural network scoring models was chosen with the crossdock_default2018 keyword.

Conclusions

Our research showed that the investigated heterocyclic isoxazolines and isoxazolidines posse surprisingly high docking affinity to several selected enzymes and their scores exceed even the ones of the currently applied medications. This particular feature alone renders them possible and promising drug candidates. It remains unclear however,

why the docking scores are so favorable in the broad range of possible protein targets, despite e.g. the fact that the docking poses are sometimes not quite physically sound.

In our opinion, this feature could be traced back to the better pre-docking preparation of the ligands (DFT geometry optimization and electrostatic potential-based assignment of the partial charges) and better scoring software, harnessing the power of machine learning models. We presume that the rather small structure of analysed compounds and the presence of various hydrophilic and hydrophobic groups as e.g. nitro group or heterocyclic rings, could indicate their great potential as bio-active agents due to favouralble interactions wirhin the protieins' active sites as indicated by visualisations of electrostatic and hydrophobic-hydrophilic protein potentials of the docked systems.

As the issue remains inconclusive at the moment, further investigation is needed, especially one making use of more sophisticated computational models, including molecular dynamics. We are in the process of conducting appropriate research.

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