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COMPUTATIONAL PREDICTIONS OF SITE OF METABOLISM OF A PYRROLE-BASED COMPOUND AS A POTENTIAL ANTITUBERCULAR AGENT

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ABSTRACT

Evaluation of the metabolism profiles of novel molecules is essential for the improvement of their pharmacokinetic characteristics and therapeutic dose. Thus, fast and reliable in silico predictions are being employed in drug design cascade, allowing for screening of large numbers of chemical compounds, and thereafter identifying a small number of promising candidates. The aim of this study was to investigate the potential sites of metabolism (SOMs) of an antitubercular pyrrole-based compound - 13b, and to examine the drug binding to several CYP isoforms through in silico approaches. To achieve this objective, two freely available web servers were used - BioTransormers 3.0 and Regioselectivity-WebPredictor (RS-WebPredictor). Schrödinger suite 2021 was utilized for the docking calculations, including the Induced Fit Docking (IFD) and the free binding recalculations with Molecular Mechanics/Generalized Born Surface Area (MM/GBSA). The BioTransformers 3.0 results indicated that the metabolism of 13b proceeds with reactions catalyzed by CYP 1A2, 2C8, 2C9 and 3A4. The web prediction studies demonstrated 13 possible metabolites mostly produced by hydroxylation and O-dealkylation of the latter compound. In addition, another five CYP isozymes (2A6; 2B6; 2C19; 2D6; 2E1) were identified using RS-WebPredictor as possible biotransforming enzymes. The IFD and MM/GBSA calculations demonstrated that the pyrrole-based structure exerts good binding affinity in the active site of CYP 2C8 and 3A4, however, no active poses were generated in CYP1A2. Further in vitro evaluations should be conducted to validate the in silico results.

Keywords: in silico prediction, site of metabolism, molecular docking, antitubercular hydrazone, CYP enzymes.

INTRODUCTION

The cytochrome P450 is a heme-containing superfamily of enzymes which are accountable for the metabolism of pharmacological active drugs [1]. The latter enzyme system comprises 57 isoforms of which five (CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4) are accountable for the metabolic reactions of more than 90 % of the cases. The most abundant isoform - CYP3A4, is located in the liver's epithelial cells where it catalyzes the metabolism of approximately 50 % of the registered therapeutic agents [2]. The problems associated with the use of CYP450 inhibitors are of great significance. The preliminary assessment of the CYPs activity could avoid potential adverse reactions [3]. Consequently, being able to predict the sites of metabolism (SOMs) of a drug is of great interest to medicinal chemists.

A detailed evaluation of the pharmacokinetic properties of each potential drug is essential considering the risk of unfavorable absorption, distribution, metabolism, excretion and toxicological (ADMET) properties [4]. Two of the main pharmacokinetics - metabolism and toxicity, are responsible for more than 40 % of the occurring pharmacodynamics interactions [5]. Therefore, proper investigations into the metabolic pathway of a pharmacologically active drug is a necessity.

The interest of the application of *in silico* techniques in the early stages of drug discovery is steadily

increasing in interest since the possibility of preliminary simulations is viable. Subsequently, problems such as the use of test animals, the need of soluble compounds, the considerable slow *in vivo* tests and the cost of the experimental results could be solved [6]. Overall, the optimizations of a compound in early development by applying *in silico* tools could shorten the discovery cycle, and increase the late stage drug survival chances.

Consequently, the aim of this work was to investigate the substrate capacity, as well as the metabolic products of synthesized by our research group pyrrole-based compounds towards several CYP isoforms through *in silico* approaches. Initially, two online web servers (BioTransormers 3.0 and RS-WebPredictor) were applied for the detection of SOMs. Subsequent molecular docking simulations demonstrated the possible conformations of the title compound in the active sites of CYP1A2, CYP2C9 and CYP3A4. Furthermore, Induced-fit docking (IFD) and MM/GBSA free energy recalculations were introduced to acquire more reliable *in silico* results.

EXPERIMENTAL

Test compound

In the current research, ethyl 5-(4-bromophenyl)-1-(3-(2-(2-hydroxybenzylidene)hydrazinyl)-3-oxopropyl)-2-methyl-1H-pyrrole-3-carboxylate (**13b**) (Fig. 1) was selected as a model compound with the perspective antitubercular effect, which metabolism could occur in different biotransforming pathways, based on the presence of various functional groups [7].

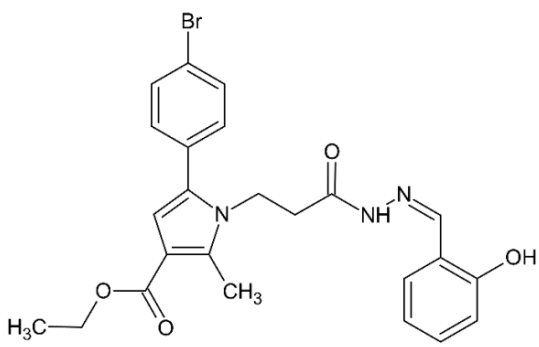


Fig. 1. Chemical structure of the evaluated hydrazide hydrazone **13b**.

Site of metabolism prediction

Two online web servers were applied for the detection of SOM. BioTransormers 3.0 combines machine learning approaches with a rule-based system to predict small-molecule metabolism [8]. Regioselectivity-WebPredictor (RS-WebPredictor; Rensselaer Polytechnic Institute, NY) is a freely accessible server that predicts the regioselectivity of isozyme-specific cytochrome P450 (CYP)-mediated sites of metabolism allowing for high-throughput use in lead optimization projects on drug-like molecules [9].

Molecular Docking

Selection and preparation of proteins

The crystallographic structures of CYP1A2 (PDB ID: **2HI4**), CYP2C8 (PDB ID: **2VN0**) and CYP3A4 (PDB ID: **2V0M**) resolved with the co-crystallized ligands alpha-Naphthoquinone, Troglitaone and Ketoconazole, respectively, were retrieved from the Protein Data Bank (PDB). The Protein Preparation Wizard in Maestro (Schrödinger Release 2021-3: Protein Preparation Wizard; Epik, Schrödinger, LLC, New York, NY, 2021.) was employed for the protein refinements. Hydrogen bonds and het states at pH 7.0 ± 2.0 were generated followed by the removal of waters situated in the active site. Subsequently, the energy of the crystallographic structures was minimized by applying the OPLS2005 force field.

Ligands preparation

The chemical structure of the applied pyrrole-based compound was drawn with the 2D sketcher module in Maestro, and converted to the corresponding 3D structure with the Ligprep module (Schrödinger Release 2021-3: LigPrep, Schrödinger, LLC, New York, NY, 2021). Utilizing the latter module, hydrogen bonds, tautomers, enantiomers, ionization states at pH 7.0 ± 2.0 were generated. Furthermore, the ligand's energy was minimized by applying the OPLS2005 force field.

Docking protocol

The docking module in Maestro - Glide was implemented for the current study considering the reported reliable results when CYP enzymes were simulated with the latter software [10]. Glide utilizes the empirically based GlideScore scoring algorithm which is presented in three forms: High-throughput

screening (HTS), Standard-Precision (SP) and Extra-Precision (XP) modes. The most precise XP docking mode was utilized. In addition, the induced-fit docking (IFD) in Schrödinger was employed to further validate the examined conformations. The IFD examines the protein's side chains as fully flexible, which leads to optimization of the active site conformation. MM/GBSA (Molecular Mechanics-Generalized Born Surface Area) recalculations were also introduced to determine the binding free energies of the obtained complexes. The grid box was generated around a co-crystallized ligand with the help of the Receptor Grid Generation module in Maestro.

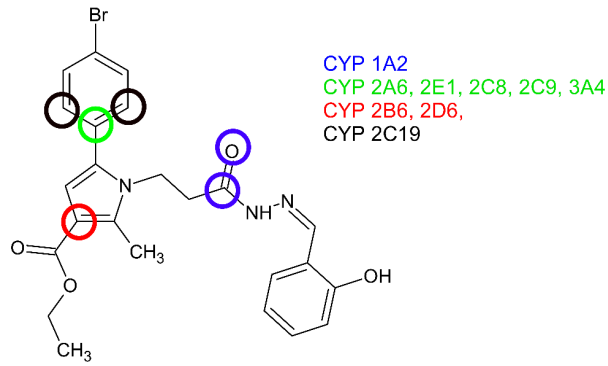


Fig. 2. Predicted CYP isoforms participating in the metabolism of **13b** by RS-WebPredictor.

RESULTS AND DISCUSSION

Site of metabolism prediction

The high degree of variability among the *in silico* programs indicate that applying one single software in isolation might be ambiguous in predicting the correct SOMs which underlines the importance of the utilization of several prediction techniques and programs [4].

Thus two free available servers were applied for prediction of possible CYP isozymes that may metabolize the selected compound. The initial prediction of the SOMs of the title compound was conducted with the Regioselectivity WebPredictor. The results demonstrated that 9 CYP isoforms are involved in the biotransformation of the **13b** (Fig. 2). The most viable group of the structure is the *p*-bromophenyl moiety situated at fifth position of the pyrrole ring.

To obtain better understanding of the metabolic processes against **13b,** additional simulations with the machine learning-based program - BioTransormers 3.0 was carried out. Two major metabolic reactions were recorded - hydroxylation and O-dealkylation. The isoenzymes 2C9 and 3A4 were involved in the hydroxylation of the aromatic 2-hydroxybenzene ring, while 1A2 could catalyze various hydroxylations in the title compound as displayed in Fig. 3. Notably, the BioTransformers 3.0 provides data concerning the major

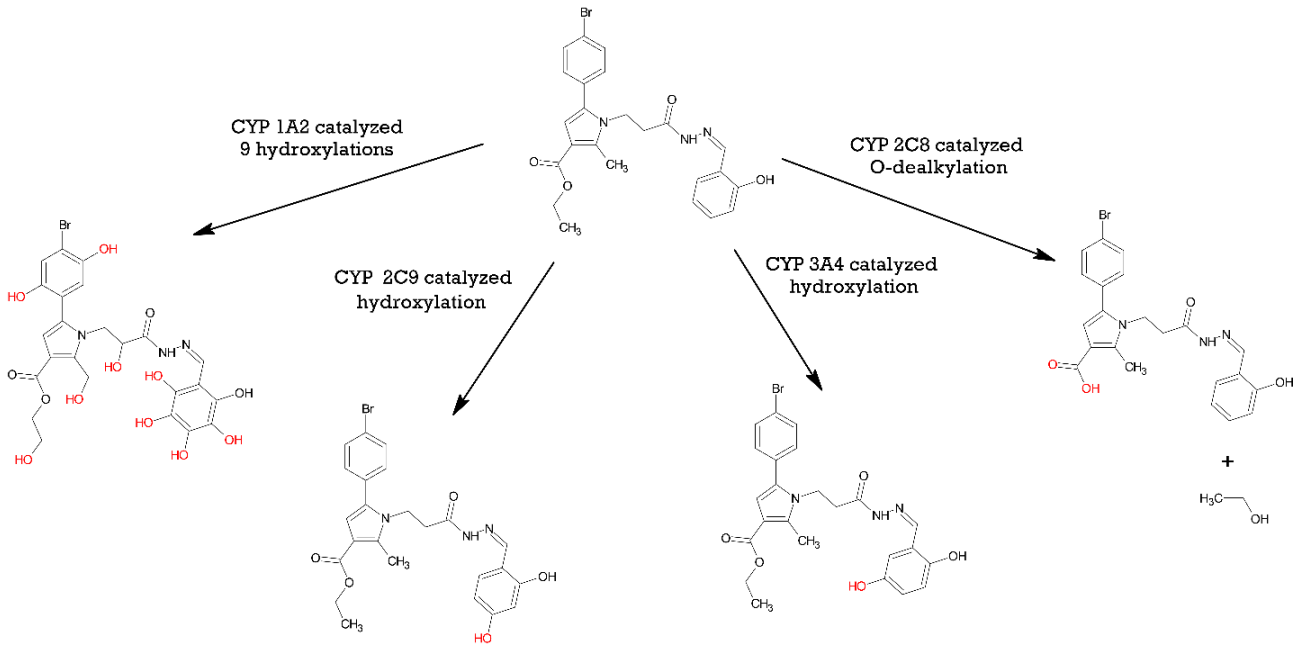


Fig. 3. Predicted CYPs mediated metabolites through BioTransormers 3.0.

isotope mass of the corresponding structures which could be compared to the experimental results.

Overall, the most probable biotransformations of **13b** were aryl-, alkyl- hydroxylation and O-dealkylation processes carried out in three CYP isoforms 1A2, 2C8 and 3A4.

For subsequent understanding of the active conformations of the title compound, as well as the calculations of the binding scores in the latter enzymes, molecular docking studies were carried out.

Molecular docking simulations

Applying molecular docking in the search of proper SOMs of investigated drugs has increased in interest considering the high correlation with the experimental results [4]. In the current study, the molecular docking simulations were carried out with the docking software Glide (Schrödinger), considering the prominent results of the latter program in similar studies [10]. However, diverse conformations of the active sites of the aforementioned CYP isoforms has been previously described [11]. Moreover, recent work discussed an 89 % success rate after the implementation of IFD against various CYPs [10]. Thus, the application of more precise and hardware demanding simulations, such as IFD and MM/GBSA were incorporated in the current work.

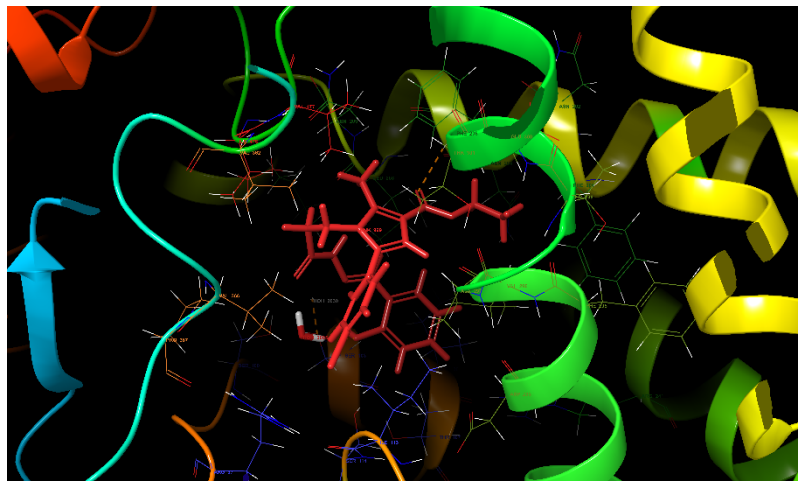
Initial re-docking simulations in **2HI4, 2VN0** and **5VC0** were carried out to validate the docking protocol. Root-mean-square-deviations (RMSD) under 2 Å were observed between the co-crystallized ligand and the obtained poses which demonstrated that docking with Glide is reliable in the former proteins. Subsequently, the IFD scores and the free binding energy recalculations with MM/GBSA of the co-crystallized ligands - alpha-Naphthoquinone, Ritonavir and Troglitazone, were employed as reference values (Table 1). The results demonstrated that the pyrrole-based compound exerts good binding affinities of -8.755 and -7.643 in CYP2C8 and CYP3A4, respectively. The free binding energy value of **13b** in CYP2C8 was calculated to be -41.86 which could determine its potential blocking capacity against the former enzyme. Interestingly, the title compound did not fit into the active site of CYP1A2, which could be associated with the unfeasible participation of the former enzyme in the metabolism of **13b**. The overall data demonstrated that the title compound establishes the most stable complex with CYP2C8. After the IFD and MM/GBSA recalculations it was found that the binding energy of **13b** is close to the native inhibitor Troglitazone. Thus, low inhibition towards the former enzyme could be expected.

The major intermolecular interactions between the active pyrrole-based derivative and the actives site of CYP2C8 are provided in Fig. 3. The IFD demonstrated the importance of the active waters in the docking simulations. Here one water molecule (HOH2005) participated in hydrogen bond with a carbonyl group from the title compound. Additional conventional hydrogen bond was formed between the hydroxyl moiety and the active residue Ser103. Numerous active amino acids - Ile106, Ile113, Val296, Ala297, Val366 and Val477, were involved in weak hydrophobic interactions with the pyrrole-based hydrazide-hydrazone. The hem group from the CYP isoenzyme participated in p-p T-shaped stabilization forces with the *p*-bromophenyl structure.

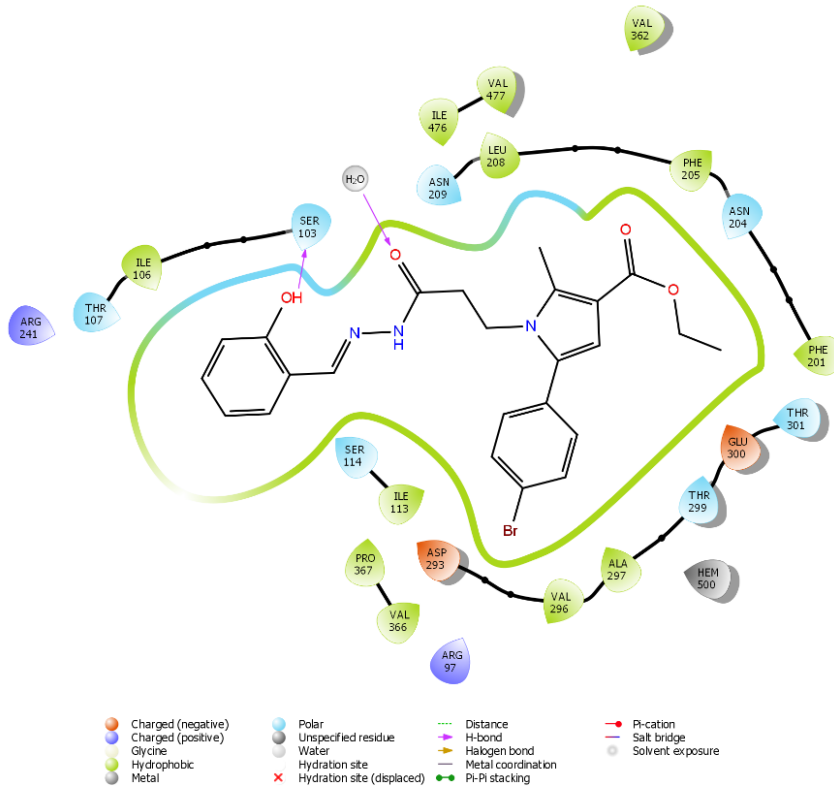
Table 1. XP and MM/GBSA scores of **13b**, Ritonavir and Troglitazone simulated in CYP2C8 and CYP3A4.

Compound	CYP1A2 (PDB: **2HI4**)		CYP2C8 (PDB: **2VN0**)		CYP3A4 (PDB: **5VC0**)	
	IFD	**MM/GBSA**	**IFD**	**MM/GBSA**	**IFD**	**MM/GBSA**
13b	n.a	n.a	-8.755	-41.86	-7.643	-26.51
ritonavir	n.d	n.d	n.d	n.d	-9.194	-45.54
troglitazone	n.d	n.d	-9.317	-58.83	n.d	n.d
alpha-naphthoquinone	-11.241	-89.435	n.d	n.d	n.d	n.d

n.d - not determined; n.a - not available



A



B

Fig. 3. Major intermolecular interactions of **13b** with the active site of CYP2C8 (A, B) after employing IFD and MM/GBSA recalculations. The interactions are provided in 2D and 3D forms. The enzyme structure is given in ribbons, and the pyrrole-based compound is depicted in red.

CONCLUSIONS

The high degree of variability among the *in silico* programs indicate that applying one single software in isolation might be ambiguous in predicting the correct site of metabolism which underlines the importance of the utilization of several prediction softwares. Therefore, initial SOMs prediction with two softwares - BioTransormers 3.0 and Regioselectivity-WebPredictor, was conducted. Subsequently, molecular docking in the most likely CYP enzymes was carried out to observe the scores and the orientation of the title ligand.

Overall, the most probable biotransformations of **13b** were aryl-, alkyl- hydroxylation and O-dealkylation processes carried out in three CYP isoforms: 1A2, 2C8 and 3A4. The molecular docking simulations in the latter isoenzymes demonstrated that the pyrrole-based structure exerts good binding affinity, however no docking poses were generated in the active site of CYP1A2, which could be associated with the unfeasible participation of the CYP1A2 enzyme in the metabolism of **13b**. Further *in vitro* evaluations should be conducted to validate the *in silico* results.

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