Exploring the Pharmacokinetic Properties of $(NH_4)_4[Fe(idadtc)_2]$: *In Silico* Biological screening and ADMET analysis

Marko Antonijević[1], Milica Kosović Perutović[2], Zorica Leka[2*], Zoran Marković[1]

[1] University of Kragujevac, Institute for Information Technologies, Department of Science, Jovana Cvijića bb, 34000 Kragujevac, Serbia; e-mail: mantonijevic@uni.kg.ac.rs, zmarkovic@uni.kg.ac.rs
[2] University of Montenegro, Faculty of Metallurgy and Technology, Džordža Vašingtona bb, 81000 Podgorica, Montenegro, e-mail: zorica@ucg.ac.me, mkosovic@ucg.ac.me

* *Corresponding author*

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Abstract: Carbonic anhydrases (CA) have been identified in the early '50s as potential targets for the treatment of numerous diseases, including cancer, glaucoma, epilepsy, etc. Current inhibitors, i.e., treatment options, offer high efficacy coupled with a high probability of various side-effects. On the other hand, dithiocarbamates and their metal complexes are known for being good CA inhibitors. In this paper, a novel Fe (II) dithiocarbamato complex was investigated for its biological and pharmacological properties using a combination of different *in silico* methodologies. It was found that this water soluble, almost non-toxic (LD_{50} values around 4860 mg/kg), druglike compound shows high inhibitory potential towards CA II. However, it also shows slow gastro-intestinal absorption, which means that, if ever used as a pharmacological agent, in its present form cannot be orally administrated. Binding energies with the value of -7.8 kcal mol^{-1} indicate reversible binding to human serum albumin, which can serve as a delivery system for the investigated compound. Overall, the obtained results indicate a high potential of $(NH_4)_4[Fe(idadtc)_2]$ to be an effective CA II inhibitor.

Keywords: Dithiocarbamates, Carbonic anhydrases, Albumin, ADMET, Acetazolamide

1. Introduction

Carbonic anhydrase II (CA II) is an enzyme from the family of carbonic anhydrases, which catalyses the reversible hydration of carbon dioxide. It is a zinc-containing metalloenzyme found in high concentrations in red blood cells, where it serves a crucial role in the transport of carbon dioxide from tissues to the lungs. In addition, this enzyme has been linked to several other physiological processes, including acid-base regulation, bone resorption, and fluid secretion. Additionally, CA II has been identified as a potential drug target for a variety of diseases, including glaucoma, epilepsy, and cancer [1-2]. Since the 1950s, CA inhibitors have been the subject of extensive research for the treatment of epilepsy, heart disease, cancer, and altitude illness. The medication known as acetazolamide was one of the first and most important carbonic anhydrase inhibitors. It is commonly used to treat, among other conditions, altitude sickness, glaucoma,

epilepsy, edema, etc. Additionally, it is used to treat metabolic alkalosis, a condition characterized by excess concentrations of bicarbonate ions in the organism. This medication reduces the production of cerebrospinal fluid, thereby reducing intracranial pressure, and providing relief to patients with hydrocephalus and pseudotumor cerebri. As with all medications, acetazolamide can cause adverse effects including nausea, dizziness, tingling in the extremities, etc. [2-3].

Dithiocarbamates are a class of chemical compounds which consist of a carbon atom bonded to two sulphur atoms and an additional nitrogen atom. Due to their diverse properties and prospective applications, these customizable compounds have proven to be of great use in different fields. They have shown promising antifungal, antibacterial, and antiviral activities, making them valuable candidates for the development of novel antimicrobial agents. In addition, their potential function as anticancer agents has been investigated due to their ability to induce apoptosis and inhibit tumour cell proliferation via a variety of cellular mechanisms. As chelating compounds that form stable complexes with various metal ions, dithiocarbamates have been utilized in medicinal chemistry for metalloenzyme inhibition [4-6]. Despite their pharmacological potential, the adverse effects and toxicity profiles of dithiocarbamates must be thoroughly evaluated to ensure their safe and effective use in the pharmaceutical industry [3-6].

In this paper, a comprehensive biological screening, including ADMET (*Absorption, Distribution, Metabolism, Excretion and Toxicity*) analysis of $(NH_4)_4[Fe(idadtc)_2]$ was performed. Since this compound in our previous endeavours was found to be highly soluble in water, and dithiocarbamate derivatives are known for being bioactive compounds, it was reasonable to perform a scan of its biological properties and investigate its potential pharmacological application [7].

2. Materials and Methods

The SwissTargetPrediction [8] was used to predict the potential protein/enzyme targets for the examined compound. Further, the AutoDock4.2 software package was used to run molecular docking simulations (MD) to acquire more precise parameters and binding affinities of examined complexes towards the selected targets, following the methodology described in detail in our previous work [9]. The protein structure under IDs: 2BDX (*Human Serum Albumin*-**HSA**) and 2EU2 (*Carbonic Anhydrase II* - **CA II**) were obtained from the RCSB Protein Data Bank. BIOVIA Discovery Studio 2020 was used to prepare and visualize the results. Finally, ADMET analysis was performed using ADMETlab 2.0, and toxicological assessment was further confirmed by ProToxII [8, 9].

3. Results and discussions

According to the results obtained through the SwissTargetPrediction website, $(NH_4)_4[Fe(idadtc)_2]$ is a possible inhibitor of CA II. These findings are strongly supported by the literature data [10-12]. To assess the inhibitory potential of the investigated compound towards the CA II, MD techniques were employed. Obtained results

presented in Table 1, show excellent inhibitory activity of the investigated compound towards this enzyme. Moreover, if we compare the results with the data obtained for acetazolamide, as well as ligand (ammonium-amino-diacetato-dithiocarbamate-idadtc), we can conclude that investigated compounds show higher binding potential, indicating better inhibitory activity.

Table 1. Thermodynamic parameters describing inhibitory potential of $(NH_4)_4[Fe(idadtc)_2]$

Compound	acetazolamide	idadtc	$(NH_4)_4[Fe(idadtc)_2]$
Gr_{ind} (kcal mol^{-1})	-6.39	-5.74	-8.67
K_i (μM)	21.08	62.02	0.44

In order for the investigated compound to demonstrate its inhibitory potential, it must possess the appropriate pharmacokinetic and toxicological profiles. Since the first parameter which eliminates the highest percentage of potential pharmacological agents is a toxicological effect, $(NH_4)_4[Fe(idadtc)_2]$ was subjected to the toxicological analysis. Obtained results suggest that the investigated compounds are low in overall toxicity, with LD_{50} values 4860mg/kg, which puts it in Class V (*may be harmful if swallowed*), almost Class VI (*non-toxic*) (LD_{50} > 5000 mg/kg) on the toxicity scale. However, further ADMET analysis showed low gastro-intestinal (GI) absorption, moving the investigated compound towards Class IV: *harmful if swallowed*. The same data was obtained for the idadtc, while acetazolamide had LD_{50} values of 4300 mg/kg. However, ProToxII indicated the potential carcinogen potential of acetazolamide, which was not the case for the other compounds within this study. Other ADMET parameters indicated that $(NH_4)_4[Fe(idadtc)_2]$ is highly water soluble, has no blood-brain barrier permeability, shows no affinity towards P450 enzymes and according to Pfizer and Lipinski rules represents a drug-like structure.

However, because of the low GI absorption, this compound could not be administrated orally. Because of that, HSA binding was investigated, by utilization of the MD once more. According to the obtained results, $(NH_4)_4[Fe(idadtc)_2]$ shows excellent binding to the HSA, with binding energies of -7.8 kcal mol^{-1}. The combination of good inhibitory activity towards the CA II, high water solubility, low toxicity and good HSA binding potential makes $(NH_4)_4[Fe(idadtc)_2]$ a suitable candidate for further pharmacological testing.

4. Conclusions

In conclusion, this paper explored the pharmacological and biological properties of a $(NH_4)_4[Fe(idadtc)_2]$, as a potential inhibitor of CA II. The results revealed that the investigated complex exhibits significant inhibitory activity against CA II, making it a promising candidate for the treatment of various diseases, including cancer, glaucoma, and epilepsy. Despite its high inhibitory potential, the compound displayed slow gastrointestinal absorption, which limits its feasibility for oral administration as a pharmacological agent. However, the compound's water solubility and low toxicity make it an attractive candidate for alternative delivery methods, with human serum

albumin (HSA) identified as a potential delivery system for this compound due to its reversible binding affinity. Further studies and optimizations are necessary to enhance its bioavailability and identify suitable administration routes, thereby unlocking the full therapeutic potential of this dithiocarbamato complex.

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