Molecular Docking Studies of 1,3,4-Thiadiazole Amidoalkyl Derivatives as Potential Inhibitors of Dihydrofolate Reductase.

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Introduction

Dihydrofolate reductase (DHFR) is a widespread enzyme that plays a key role in folic acid metabolism by reducing dihydrofolate to tetrahydrofolate in eukaryotic and prokaryotic cells. Inhibition of DHFR leads to a decrease in the intracellular level of tetrahydrofolate, cessation of RNA and DNA synthesis, and, as a result, a slowdown in their proliferation. This makes DHFR an attractive therapeutic target for the treatment of cancer, bacterial and protozoal infections, and several other diseases [1]. DHFR is a relatively small, water-soluble protein with a molecular weight of 18-25 kDa (Fig 1a). The mechanism of action of some drugs is associated with the inhibition of this enzyme, such as Methotrexate, Raltitrexed, Pemetrexed, Pralatrexate, and many others [1]. A promising class of substances with the ability to inhibit DHFR are 1,3,4-thiadiazole derivatives. These compounds include (*E*)-5-benzylidene-1-(5-(3,5-dinitrophenyl)-1,3,4-thiadiazol-2-yl)-3-phenyl-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione (**1**) [2], *N*-(4-((*Z*)-1-(((*Z*)-5-(4-methoxyphenyl)-3-phenyl-1,3,4-thiadiazol-2(3*H*)-ylidene)hydrazono)ethyl)phenyl)-4-methylbenzenesulfonamide (**2**) [3], 2-((5-amino-1,3,4-thiadiazol-2-yl)thio)-6-methyl-3-(4-phenoxyphenyl)quinazolin-4(3*H*)-one (**3**) [4] and 6-chloro-2-(((5-((4-chlorophenyl)amino)-1,3,4-thiadiazol-2-yl)methyl)thio)-3-(4-methoxyphenyl)quinazolin-4(3*H*)-one (**4**) [5] (Fig. 1b).

In this study, we proposed previously synthesized *N*-amidoalkylated derivatives of 1,3,4-thiadiazole **8a-g** as potential inhibitors of DHFR. These compounds were obtained by the elimination of water from *N*,*N*′-disubstituted hydrazinecarbothioamides **7**, which, in turn, were obtained by the addition of arylcarboxylic acid hydrazides **6** to isothiocyanates **5** [7] (Scheme 1).

The effectiveness of the interaction of compounds **8a-g** with the DHFR active site was evaluated using molecular docking methods. In addition, based on compounds **8a-g**, structures containing an NH group between the 1,3,4-thiadiazole and aromatic rings were simulated virtually. The model structures were also tested *in silico* for their ability to inhibit DHFR.

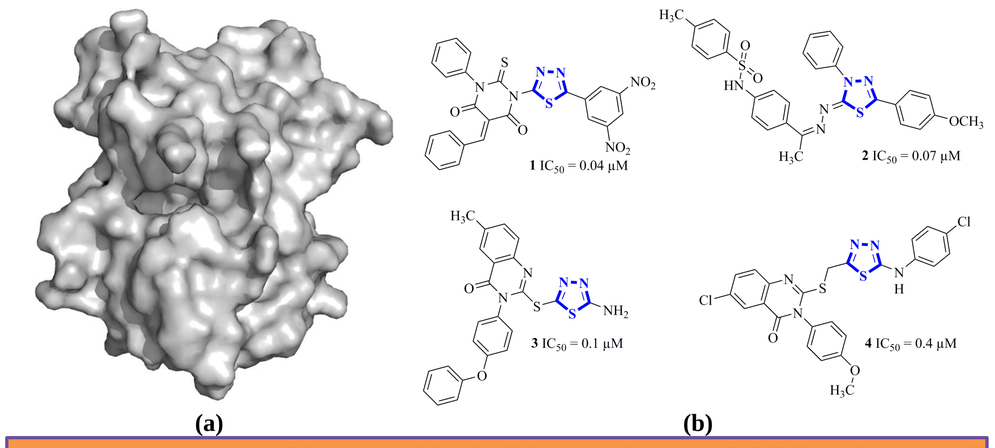
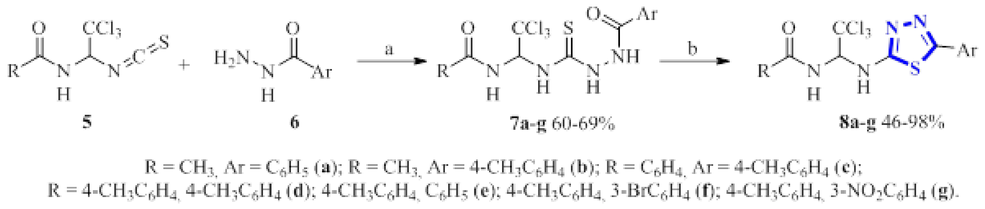


Figure 1. Structure of human dihydrofolate reductase [6] **(a)** and some of its inhibitors containing the 1,3,4-thiadiazole ring **(b)**.



Scheme 1. Synthesis of 1,3,4-thiadiazole amidoalkyl derivatives (**8**). Reagents and conditions: **a)** EtOH, reflux 1-3 min, r.t. 24 h; **b)** H_2SO_4 conc., r.t. 24 h.

Results and discussion

When docking, we used compounds **1**-**4** as comparators (Fig. 1b). According to the results obtained, these compounds effectively interacted with the DHFR active site. In this case, the calculated data on ΔG correlated well with the experimental values of IC_{50}. As expected, compound **1** bound most strongly to DHFR with IC_{50} = 0.04 μM, while compound **4** formed the least stable complex with IC_{50} = 0.4 μM. The molecule of compound **1** formed six hydrogen bonds with amino acids of the active site, two of which were formed with Leu 28 (length 2.7 and 3.2 Å), two more - with Ser 59 (length 2.9 and 3.3 Å), and one hydrogen bond each - with Asp 31 and Gln 35, 3.1 and 2.7 Å long, respectively (Fig. 2a). The energy of the complex was -8.4 kcal/mol. The inhibitor **2** molecule was efficiently fixed in the active site of DHFR due to four intermolecular hydrogen bonds (Fig. 2b): one formed by the methoxy group and Gln 35 (length 3.0 Å) and three more -by the sulfamide group and the amino acid Glu 30 (length 3.1, 3.2 and 3.6 Å). The energy of the complex was -8.2 kcal/mol. The compound **3** molecule in the DHFR active site was fixed due to the formation of only one hydrogen bond 3.1 Å long, which was formed between the amino group and Pro 66 (Fig. 2c). The ΔG value was -7.0 kcal/mol. The compound **4** molecule in the DHFR active site was fixed by two intermolecular hydrogen bonds with Lys 68 (Fig. 2d), the bond lengths were 3.1 and 3.3 Å. The energy of the formed complex was -6.8 kcal/mol.

Among the compounds we analyzed, most surpassed the standards in terms of the strength of the complex formed with DHFR. The introduction of an NH group between the thiadiazole and aromatic rings mainly led to stronger binding to the enzyme, except for **MSa** and **MSc**, which formed complexes of the same strength with **8a** and **8c**.

Among the tested compounds **8a-g**, 4-methyl-*N*-(2,2,2-trichloro-1-((5-(*p*-tolyl)-1,3,4-thiadiazol-2-yl)amino)ethyl)benzamide (**8d**) and 4-methyl-*N*-(2,2,2-trichloro-1-((5-phenyl-1,3,4-thiadiazol-2-yl)amino)ethyl)benzamide (**8e**) interacted the most effectively with DHFR. Model structures created on their basis, 4-methyl-*N*-(2,2,2-trichloro-1-((5-(*p*-tolylamino)-1,3,4-thiadiazol-2-yl)amino)ethyl)benzamide (**MSd**) and 4-methyl-*N*-(2,2,2-trichloro-1-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)amino)ethyl)benzamide (**MSe**), were also leaders in their series. In addition, **MSd** and **MSe** outperformed the original compounds **8d** and **8e** in terms of the strength of the complex formed with the enzyme and were hit compounds. It is noteworthy that both molecules of compounds **8d** and **8e**, as well as molecules of **MSd** and **MSe**, were fixed in the DHFR active site exclusively due to lipophilic interactions and π-π contacts, without forming intermolecular hydrogen bonds (Fig. 3).

According to the results obtained, **MSd** and **MSe** bind most effectively to the DHFR active site. These compounds are superior to the reference compounds in terms of the strength of the complex formed, and they can be recommended for *in vitro* testing.

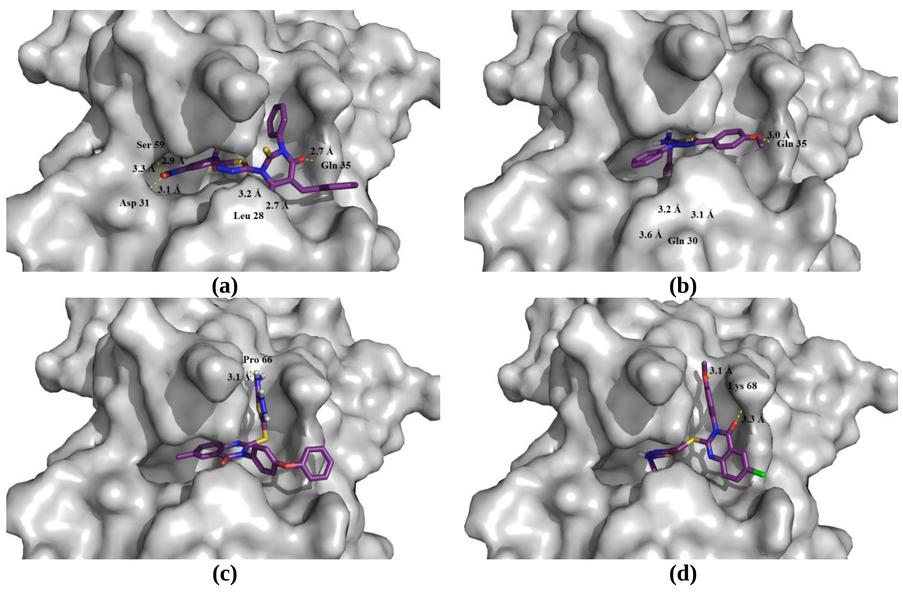


Figure 2. Position of molecules of compounds **1-4** in the DHFR active site according to molecular docking data.

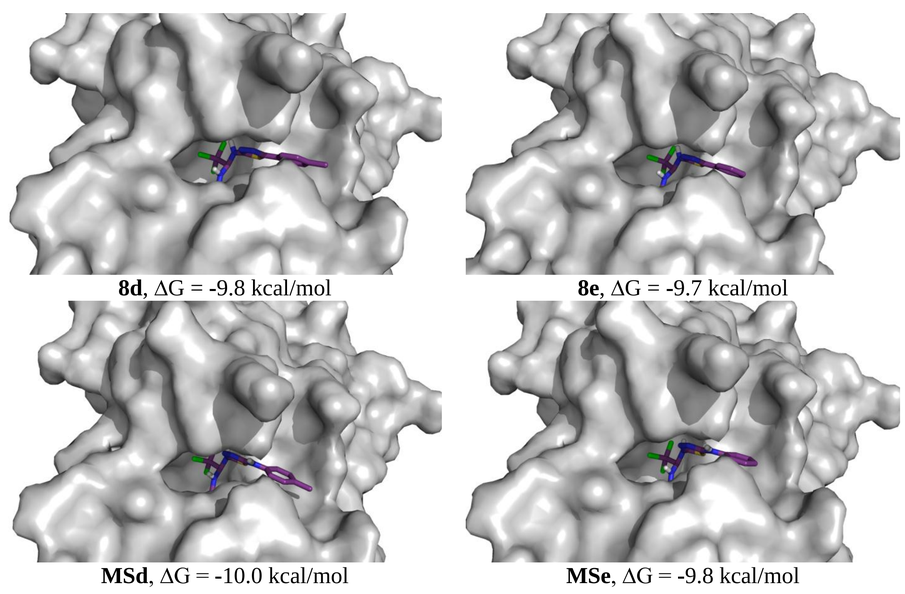


Figure 3. Position of molecules of compounds **8d**, **8e**, and **MSd**, **MSe** in the DHFR active site.

References

1. Raimondi, M.V.; Randazzo, O.; La Franca, M.; et al. DHFR Inhibitors: Reading the Past for Discovering Novel Anticancer Agents. *Molecules* **2019**, *24*, 1140.
2. El-Naggar, M.; Sallam, H.A.; Shaban, S.S.; et al. Design, Synthesis, and Molecular Docking Study of Novel Heterocycles Incorporating 1,3,4-Thiadiazole Moiety as Potential Antimicrobial and Anticancer Agents. *Molecules* **2019**, *24*, 1066.
3. Riyadh, S.M.; El-Motairi, S.A.; Ahmed, H.E.A.; et al. Synthesis, Biological Evaluation, and Molecular Docking of Novel Thiazoles and [1,3,4]Thiadiazoles Incorporating Sulfonamide Group as DHFR Inhibitors. *Chem. Biodivers.* **2018**, *15*, e1800231.
4. Al-Rashood, S.T.; Hassan, G.S.; El-Messery, S.M.; et al. Synthesis, biological evaluation and molecular modeling study of 2-(1,3,4-thiadiazolyl-thio and 4-methyl-thiazolyl-thio)-quinazolin-4-ones as a new class of DHFR inhibitors. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 4557-4567.
5. El-Gazzar, Y.I.; Georgey, H.H.; El-Messery, S.M.; et al. Synthesis, biological evaluation and molecular modeling study of new (1,2,4-triazole or 1,3,4-thiadiazole)-methylthio-derivatives of quinazolin-4(3H)-one as DHFR inhibitors. *Bioorg. Chem.* **2017**, *72*, 282-292.
6. Lewis, W.S.; Cody, V.; Galitsky, N.; et al. Methotrexate-resistant variants of human dihydrofolate reductase with substitutions of leucine 22. Kinetics, crystallography, and potential as selectable markers. *J. Biol. Chem.* **1995**, *270*, 5057-5064.
7. Zadorozhnii, P.V.; Kiselev, V.V.; Kharchenko, A.V. Synthesis of nitrogen-containing heterocycles based on *N*-(isothiocyanatoalkyl)carboxamides. In *Modern Directions in Chemistry, Biology, Pharmacy and Biotechnology*. Novikov, V.P., Ed.; Lviv Polytechnic Publishing House: Ukraine, Lviv, **2015**; pp. 212-219.