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Dihydrofolate reductase inhibitors among pteridine and furo[3,2-*g*] pteridine derivatives

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Aim. To the purposeful search for the DHFR-inhibitors among substituted pteridine-2,4,7-triones and 7-aryl-(hetaryl-)furo[3,2-*g*]pteridine-2,4(1*H*,3*H*)-diones for further biological research. **Methods.** *In vitro* methods, molecular docking, SAR-analysis, statistical methods. **Results.** The DHFR-inhibitory activity of substituted 1-methylpteridine-2,4,7-triones (**2**, **3**, **4**) and 7-aryl-(hetaryl-)furo[3,2-*g*]pteridine-2,4(1*H*,3*H*)-diones (**5**, **6**) was studied. It was established that 6-(2-hydroxy-2-aryl-(hetaryl-)ethyl)-1-methylpteridine-2,4,7(1*H*,3*H*,8*H*)-triones (**3**) and butyl 2-(7-aryl- (hetaryl-)-1-methyl-2,4-dioxo-1,4-dihydrofuro[3,2-*g*]pteridine-3(2*H*)-yl)acetates (**6**) inhibited DHFR by 14.59–52.11 %, and were less active comparing to methotrexate. It was found that the introduction of aryl moiety with electron-accepting group, naphthyl substituent or electron-accepting heterocycle (furan, thiophene and benzofuran) caused an increase in the DHFR-inhibitory activity. Additionally, it was shown, that annulation of the furan cycle to the pteridine system was reasonable in the scope of new DHFR-inhibitors synthesis. Thereby it may be concluded that the calculated values of affinity are not reliable predictors for the DHFR-inhibiting activity of studied compound. However, the molecular docking study may be used for evaluation of the interactions between the studied inhibitor and active center of DHFR. **Conclusions.** The conducted primary *in vitro* screening revealed low or moderate DHFR-inhibiting activity of the synthesized compounds. The visualization of molecular docking showed that despite the structural similarity to methotrexate, the obtained compounds form different ligand-enzyme interactions. The calculated values of affinity cannot be used as predictors of DHFR-inhibiting activity because of the absence of correlation between the abovementioned indicators. The obtained compounds may be of interest for further studies aimed at the search for anti-inflammatory, anti-viral, hypoglycemic, hypotensive, anti-ischemic agents due to the expected low-toxicity associated with the slight DHFR-inhibiting activity.

Keywords: DHFR-inhibiting activity, pteridine, furo[3,2-*g*]pteridine, molecular docking, QSAR-analysis.

Introduction

Dihydrofolate reductase (DHFR) is an enzyme required for the regeneration of tetrahydrofolate from dihydrofolate, which is formed under catalysis of thymidylate synthase (TS) [1–7]. The latter uses N^5,N^{10}-methylenetetrahydrofolate (MTHF) as a donor of a single-carbon fragment in the biosynthesis of dTMP [6, 7]. Inhibition of the DHFR-catalytic activity results in the reducing of intensity of abovementioned processes and consequently prevents DNA replication and cell division [8, 9]. Thus, inhibition of DHFR is one of the possible mechanisms of the chemotherapeutic drugs activity [10–13]. Most of "classical" (aminopterin, methotrexate, pemetrexed, lometrexol, raltitrexed, *etc.*) and "non-classical" (trimetrexate, trimethoprim, iclaprim, *etc.*) DHFR inhibitors may be used as antibacterial, antimalarial or antitumor drugs. Moreover, folate metabolism has been reported to play an important role in the development of chronic inflammation, which allows using DHFR inhibitors as antirheumatoid drugs [14].

Despite the existence of drugs with high selectivity and affinity to DHFR, they do not always meet the requirements of modern medicine in terms of toxicity and the possibility of resistance appearance [15, 16]. The search for new drugs, the inhibitors of the main enzymatic pathways of folate formation, which consists in structural modification («bioisosteric» substitutions) of the pteridine cycle and modification or replacement of *p*-aminobenzoyl-glutamate fragment by other functional groups remains an urgent problem [10–13, 17–19]. Moreover, many drugs ("non-classical" antifolates) do not have a *p*-aminobenzoyl-glutamate fragment in the molecule and show high activity [10–13].

Therefore, the aim of this study is the search for the DHFR inhibitors among substituted pteridine-2,4,7-triones and 7-arylfuro[3,2-*g*]pteridine-2,4(1*H*,3*H*)-diones using *in silico* (molecular docking) and *in vitro* methods, selection of promising compounds and choice of direction for further biological research.

Materials and Methods

6-Substituted 1-methylpteridine-2,4,7-triones (**2.1–2.5**, **3.1–3.14**, **4.1–4.2**), 1-methyl-7-arylfuro[3,2-*g*]pteridine-2,4(1*H*,3*H*)-diones **(5.1–5.3**) and butyl 2-(1-methyl-2,4-dioxo-7-aryl-1,4-dihydrofuro[3,2-*g*]pteridine-3(2*H*)-yl)acetates (**6.1–6.8**) were selected for screening the DHFR-inhibitory activity. The synthesis and physicochemical data of the tested compounds were described previously [20–22].

In vitro DHFR inhibition assay

Reagents

Dihydrofolate Reductase Assay Kit (Sigma-Aldrich, Catalog Number CS0340, Batch Number 067M4065V) was used for evaluation of the DHFR-inhibitory activity of synthesized compounds. The protein content in supplied dihydrofolate reductase was 0.032 mg/ml and the activity of enzyme was 3.75 U/mgP.

The procedure of estimation of the DHFR-inhibitory activity of studied compounds

To the microcentrifuge tube (volume 2 ml) 966 µl of diluted 1:10 assay buffer were added. Then sequentially 13 µl of dihydrofolate reductase and 10 µl of 100 µM solution of studied compound in DMSO were added. The tube was sealed, intensively shacked and the formed mixture was transferred to the 1.4 ml quartz cuvette. To the formed mixture 6 µl of 10 mM solution of the NADPH were added, cuvette was sealed by parafilm and shacked. 5 µl of 10 mM of dihydrofolic acid solution were added the mixture, the cuvette was sealed by parafilm, shacked, and immediately transferred to spectrophotometer ULab 108 UV. The absorption of sample at 340 nm was measured each 15 seconds during 150 seconds.

The activity of enzyme was calculated according to the following formula:

$$Activity\left(Units/_{mg\ P}\right) = = \frac{\Delta OD/_{\min}\,\text{sample} - \Delta OD/_{\min}\,\text{blank}}{12.3 * 0.013 * 0.032}$$

where:

$\Delta OD/min_{blank}$ = ΔOD/min for the blank, from the spectrophotometer readings;

$\Delta OD/min_{sample}$ = ΔOD/min. for the reaction, from the spectrophotometer readings;

12.3 = extinction coefficient (ε, $mM^{-1}*cm^{-1}$) for the DHFR reaction at 340 nm;

0.013 = enzyme volume in ml (the volume of enzyme used in the assay);

0.032 = enzyme concentration of the original sample.

The value of DHFR-inhibitory activity in % was calculated according to the formula:

$$\text{DHFR} - \text{inhibitory activity (\%)} = \frac{3.75 - Activity\left(Units/_{mg\ P}\right)}{3.75} * 100\%$$

Methotrexate was used as a reference compound.

Molecular docking

The κesearch was conducted by flexible molecular docking, as an approach of finding the molecules with affinity to a specific biological target. Macromolecule from Protein Data Bank (PDB) was used as biological targets, namely DHFR (PDB ID - 1RG7) [Protein Data Bank. http://www.rcsb.org/pdb/home/home.-do. Accessed September 6, 2020]. The choice of biological targets was based on the literature data about novel antifolate drugs [10–13].

Ligand preparation. Substances were drawn using MarvinSketch 20.20.0 and saved in mol format [MarvinSketch version 20.20.0, ChemAxon http://www.chemaxon.com]. After that they were optimized by program Chem3D, using molecular mechanical MM2 algorithm and saved as pdb files. Molecular mechanics was used to produce more realistic geometry values for most of organic molecules, owing to the fact of being highly parameterized. Using AutoDockTools-1.5.6, the pdb files were converted into PDBQT, the number of active torsions was set as default [23].

Protein preparation. The PDB files were downloaded from the protein data bank. Discovery

Studio v19.1.0.18287 was used to delete the water molecules and ligands. The structures of proteins were saved as pdb files [Discovery Studio Visualizer v19.1.018287. Accelrys Software Inc., https://www.3dsbiovia.com]. In AutoDockTools-1.5.6 the polar hydrogens were added and saved as PDBQT.Grid box was set as following: center_x = –1.657, center_y = 22.030, center_z = = 23.080, size_x = 22, size_y = 22, size_z = 22 for DHFR. Vina was used to carry docking [23]. For visualization Discovery Studio v19.1.0.18287 was used.

Results

Synthesis of pteridine and furo[3,2-g] pteridine-based derivatives

The general methods for the synthesis of the target 6-substituted 1-methylpteridine-2,4,7-triones (**2.1–2.5**, **3.1–3.14**, **4.1–4.2**), 1-methyl-7-arylfuro[3,2-*g*]pteridine-2,4(1*H*,3*H*)-diones (**5.1–5.3**) and butyl 2-(1-methyl-2,4-dioxo-7-aryl-1,4-dihydrofuro[3,2-*g*]pteridine-3(2*H*)-yl)acetates (**6.1–6.8**) are presented in Fig. 1.

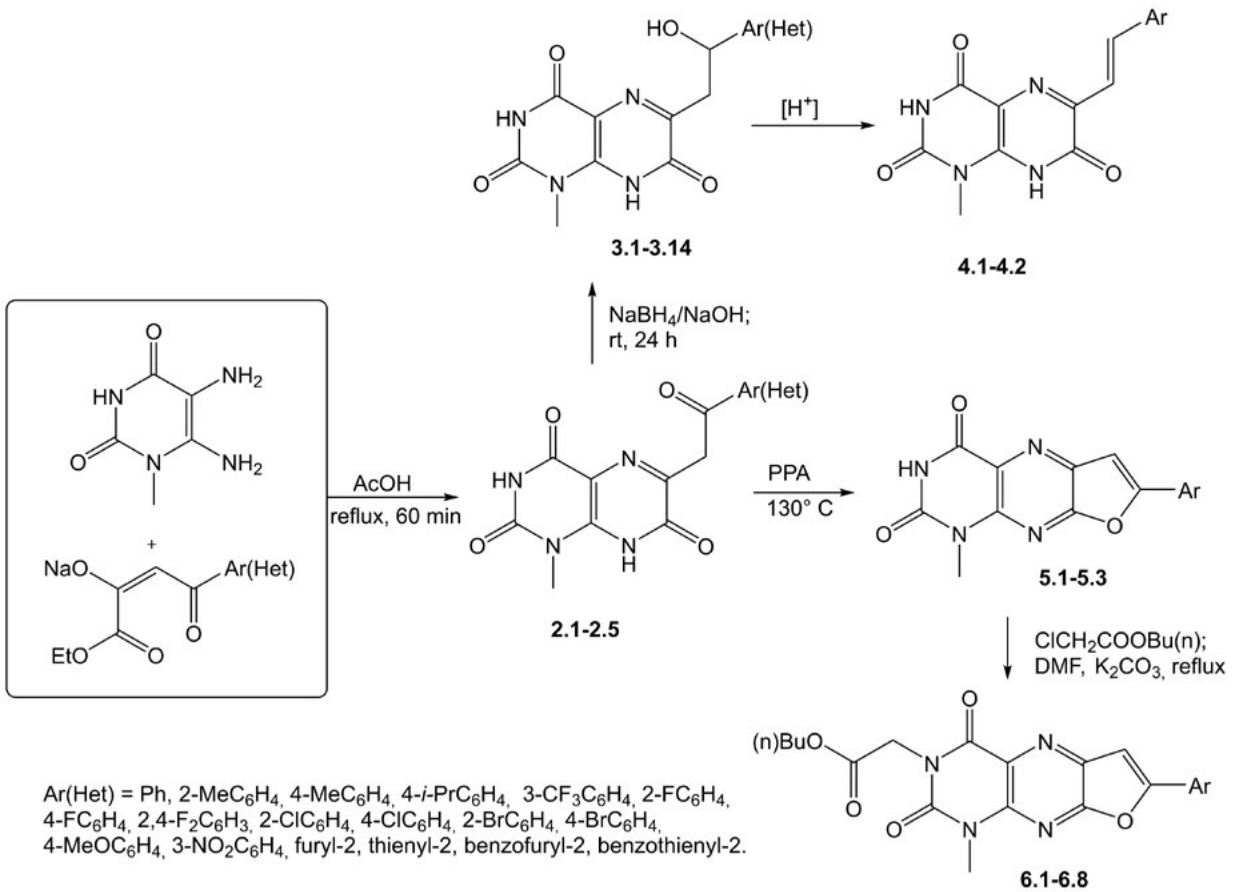

Fig. 1. Synthesis of 6-substituted 1-methylpteridine-2,4,7-triones (**2.1–2.5**, **3.1–3.14**, **4.1–4.2**), 1-methyl-7-arylfuro[3,2-*g*]pteridine-2,4(1*H*,3*H*)-diones (**5.1–5.3**) and butyl 2-(1-methyl-2,4-dioxo-7-aryl-1,4-dihydrofuro[3,2-*g*]pteridine-3(2*H*)-yl)acetates (**6.1–6.8**)

DHFR inhibition assay

It was found that the substituted 1-methylpteridine-2,4,7-triones (**2**, **3**, **4**) inhibited DHFR at the level of 2.08–50.03 % (table 1). 6-(2-Hydroxy-2-aryl)ethyl)-1-methylpteridine-2,4,7(1*H*,3*H*,8*H*)-triones (**3**) were characterized by significant DHFR-inhibitory activity that was higher comparing to the corresponding 1-methyl-6-(2-oxo-2-aryl-(hetaryl-)ethyl)pteridine-2,4,7(1*H*,3*H*,8*H*)-triones (**2**). It was found that the introduction of an electron-accepting substituent to the aryl moiety (compounds **3.4–3.11**) led to the increasing of DHFR-inhibitory activity by 8.34–35.44 % compared with compounds **3.1**–**3.3**. Compounds **3.12–3.14**, which contain an electron-accepting heterocycle (furan, thiophene and benzofuran) in their structure, also showed high DHFR-inhibitory activity. Whereas, 1-methyl-6-styrylpteridine-2,4,7(1*H*,3*H*,8*H*)-triones (**4.1**, **4.2**) show low DHFR-inhibitory activity. The high activity of compounds **3** could be explained by their higher lipophilicity.

Studies showed, that 1-methyl-7-arylfuro[3,2-*g*]pteridine-2,4(1*H*,3*H*)-diones (**5**) inhibited DHFR at the level of 14.59–39.46 % (Table 1). So, annulation of the furan cycle to the pteridine system was reasonable as an approach to the search for DHFR inhibitors. However, subsequent introduction of the butoxycarbonylmethyl moiety at 3rd position of the furo[3,2-*g*]pteridine system alloweding highly active agents only in some cases. In particular, it was found, that butyl 2-(7-phenyl-1-methyl-2,4-dioxo-1,4-dihydrofuro[3,2-g]pteridine-3(2*H*)-yl) acetate (**6.1**) inhibited DHFR by 6.25 %. Additional modification of the phenyl moiety at position 7 (compounds **6)** *via* introduction of methyl group to the *para*-position (**6.3**), of *iso*-propyl group to the *para*-position (**6.4**) and of nitro group to the *meta*-position (**6.8**) resulted [in the] increasing of activity by 12.5–35.4 %. Introduction of the fluorine (**6.5**), chlorine (**6.6**) and bromine (**6.7**) to the *para*-position of the phenyl substituent had a positive effect on the level of DHFR-inhibitory activity as well. Replacement of the phenyl moiety by naphthyl increased the DHFR-inhibitory activity up to 35.44 %.

The conducted studies showed that the most significant DHFR-inhibitory activity was characteristic for 6-(2-hydroxy-2-aryl-(hetaryl-)ethyl)-1-methylpteridine-2,4,7(1*H*,3*H*,8*H*)-triones (**3**), 1-methyl-7-arylfuro[3,2-*g*]pteridine-2,4(1*H*,3*H*)-diones (**5**) and butyl 2-(7-aryl-1-methyl-2,4-dioxo-1,4-dihydrofuro[3,2-*g*]pteridine-3(2*H*)-yl)acetates (**6**). However, the activity of the above-mentioned substances was lower comparing to the reference compound methotrexate (89.57 %).

Molecular docking

Molecular docking was used to elucidate the main types of interactions of substituted pteridine-2,4,7-triones (**2**–**4**) and 7-arylfuro[3,2-*g*]pteridine-2,4(1*H*,3*H*)-diones (**5**, **6**) with an active center of DHFR as well as a tool for possible future prediction of enzyme-inhibitory activity of these compounds. The results of molecular docking showed, that in most of cases the tested compounds revealed higher calculated affinity comparing to methotrexate (Table 1).

Visualization of the X-ray diffraction study by Discovery Studio showed [Protein Data Bank. http://www.rcsb.org/pdb/home/home.do. Accessed September 6, 2020] a significant number of conventional hydrogen bonds between methotrexate and the active center of

Table 1. **Data of molecular docking and inhibition of DHFR for synthesized compounds**

№	Comp.	Ar(Het)	Affinity (kcal/mol) to DHFR (1RG7)	Inhibition of DHFR,%
	2.1	4-FC_6H_4	–9.2	2.08
	2.2	2,4-$F_2C_6H_3$	–9.5	16.67
	2.3	4-BrC_6H_4	–9.1	14.59
	2.4	furyl-2	–8.3	8.34
	2.5	benzothienyl-2	–10.0	8.34
	3.1	Ph	–8.9	14.59
	3.2	2-MeC_6H_4	–8.9	18.76
	3.3	4-MeC_6H_4	–9.1	18.76
	3.4	2-FC_6H_4	–8.8	29.76
	3.5	4-FC_6H_4	–8.9	25.01
	3.6	2,4-$F_2C_6H_3$	–9.2	22.93
	3.7	2-ClC_6H_4	–9.0	27.09
	3.8	4-ClC_6H_4	–8.9	50.03
	3.9	2-BrC_6H_4	–9.2	22.93
	3.10	4-$MeOC_6H_4$	–8.6	39.61
	3.11	3-$NO_2C_6H_4$	–9.0	18.76
	3.12	furyl-2	–7.9	25.01
	3.13	thienyl-2	–8.1	29.18
	3.14	benzofuryl-2	–9.5	22.93
	4.1	Ph	–9.1	4.17
	4.2	3-$CF_3C_6H_4$	–9.7	14.22
	5.1	Ph	–10.4	39.46
	5.2	4-*i*-PrC_6H_4	–10.0	14.59
	5.3	2,4-$F_2C_6H_3$	–10.4	28.62
	6.1	Ph	–8.6	6.25
	6.2	naphthyl-1	–8.6	35.44
	6.3	4-MeC_6H_3	–8.4	31.25
	6.4	4-*i*-PrC_6H_4	–8.4	18.76
	6.5	4-FC_6H_4	–8.5	41.69
	6.6	4-ClC_6H_4	–8.2	52.11
	6.7	4-BrC_6H_4	–8.2	12.3
	6.8	3-$NO_2C_6H_4$	–8.5	41.69
	MTX*	–	–8.7	89.57

Note: – * Methotrexate

DHFR. They are formed by electron-donor interactions of the amino groups of 2nd and 4th positions in the pteridine cycle with amino acids A:ASP27 (2.64Å), A:ILE5 (2.77Å), A:ILE94 (2.90Å) and A:TYR100 (3.23Å) respectively. The pteridine cycle of the molecule was characterized by Pi-sigma interactions with A:ALA19 (3.65Å), A:ILE5 (5.23Å), A:ASN18 (3.36Å) and Pi-alkyl interactions with A:ALA19 (4.15Å) and A:ALA7 (4.49Å). Attractive bonds, conventional and carbon hydrogen bonds of methotrexate were formed with the enzyme by interactions of the carboxyl and methylamine groups of the *p*-methylaminobenzoyl-glutamate moiety with A:ARG57 (2.96Å), A:LYS32 (4.40Å), A:ARG52 (4.20Å), A:ARG57 (2.56Å) and A:ASN18 (3.36 Å). Additionally, the methotrexate molecule formed hydrogen bonds with water in the active center of the enzyme (Fig. 2).

Visualization of the interaction of compound **3.8**, **5.1** and **6.6** with DHFR, showed that these structures were characterized by other types of interactions with amino acids residues in the active site (Fig. 2). Thus, 6-(2-hydroxy-2-(4-chlorophenyl)ethyl)-1-methylpteridine-2,4,7(1*H*,3*H*,8*H*)-trione (**3.8**) had two conventional hydrogen bonds between Oxygen atoms of 2nd position of the pteridine ring with A:ALA7 (3.40Å), A:TYR100 (2.79Å) and carbon hydrogen bond with A:ILE94 (3.24Å) and A:ALA6 (3.28Å). Whereas, 1-methyl-7-phenylfuro[3,2-*g*]pteridine-2,4(1*H*,3*H*)-dione (**5.1**) unlike compound **3.8** had a methotrxate-like location in the active center of the enzyme and provided three conventional hydrogen bonds of the Oxygen

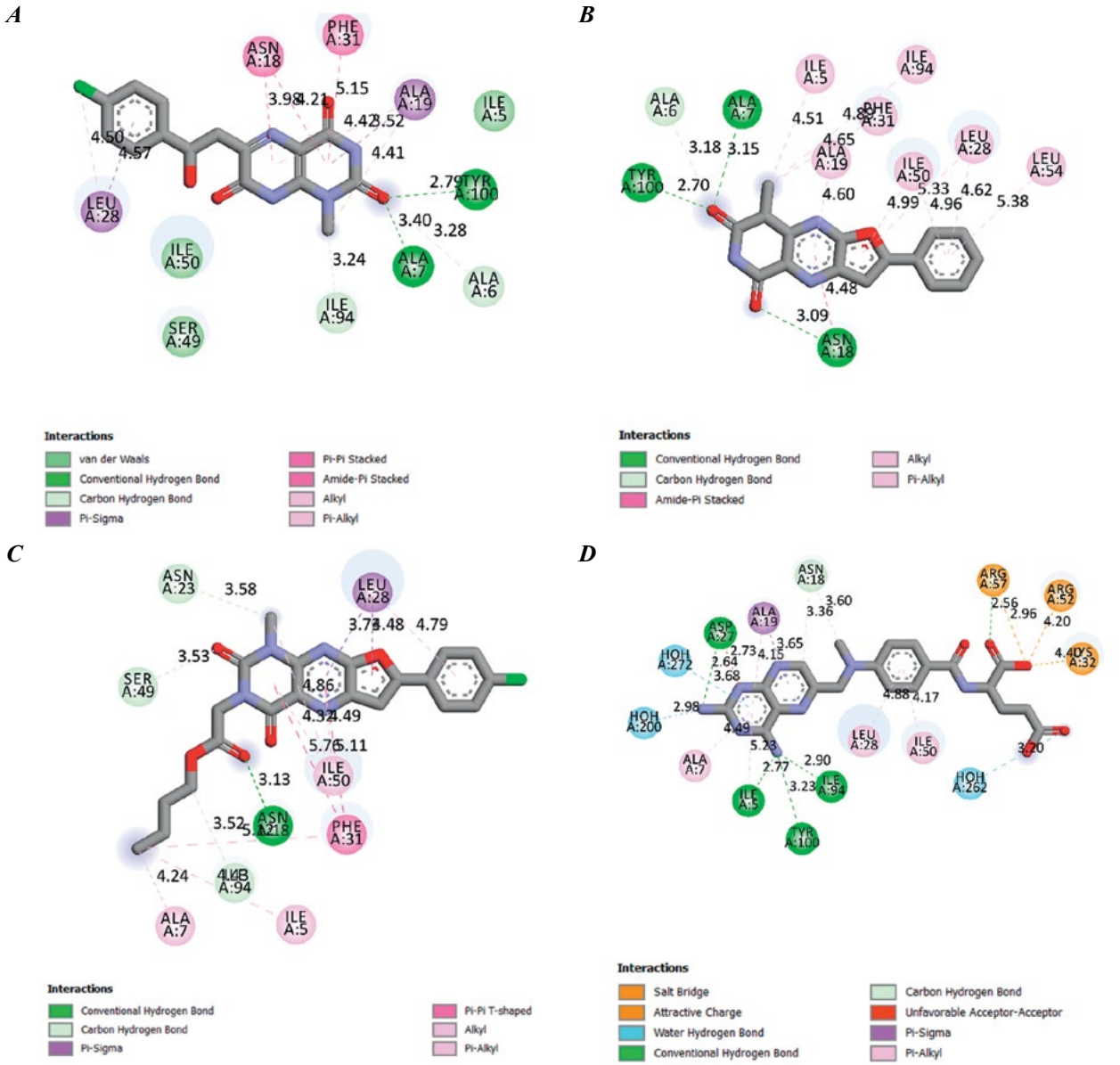

Fig. (2). Visualization of affinity according to the docking study of compounds **3.8** (**A**), **5.1 (B)**, **6.6 (C)** and methotrexate (**D**) with DHFR (1RG7).

atom at the 2nd and 4th positions of the pteridine ring with A:ALA7 (3.15Å), A:TYR100 (2.70Å) and A:ASN18 (3.09Å) and carbon hydrogen bond with A:ALA6 (3.18Å) (Fig. 2, B). Visualization of the interaction between compound **6.6** and DHFR active center (Fig. 2, C) revealed that this structure, like the previous ones (**3.8** and **5.1**), did not have a significant number of interactions similar to methotrexate — enzyme interactions.

Visualization was characterized by conventional hydrogen bonds between the Oxygen atom of the butoxycarbonylmethyl group at the 3rd position of the pteridine ring with A:ASN18 (3.13Å) and the carbon hydrogen bond between the Oxygen atoms of the 2nd position of the pteridine cycle with A:SER49 (3.53Å) and A:ASN23 (3.58Å). Other enzyme binding sites of compounds **3.8**, **5.1** and **6.6** were rather weak and were associated with the presence of Van der Waals, Pi-sigma, Pi-Pi-staked, Pi-alkyl and alkyl interactions.

The analysis of correlation between DHFR-inhibiting activity of synthesized compounds and their calculated affinity to DHFR showed the absence of direct dependency of abovementioned values. Thus, among compounds **2.5**, **5.1**, **5.2** and **5.3** that according to the docking results have affinity values ≥ 10.0 the compounds **2.5** and **5.2** reveal low DHFR-inhibiting activity. Noteworthy, despite the highest enzyme-inhibiting activity, the calculated affinity of reference compound methotrexate to DHFR has value 8.7 kcal/mol, which comparable or lower than the affinity of most of studied compounds. Thereby it may be concluded that for the studied compound the calculated values of affinity are not reliable predictors for DHFR-inhibiting activity. However, the molecular docking study may be used for evaluation of interactions between studied inhibitor and active center of DHFR. Abovementioned information is valuable for the search for promising DHFR-inhibitors among various pteridine derivatives.

Conclusion

The conducted primary *in vitro* screening revealed low or moderate DHFR-inhibiting activity of synthesized compounds. The visualization of molecular docking showed that despite the structural similarity to methotrexate, the obtained compounds form different ligand-enzyme interactions. The calculated values of affinity cannot be used as the predictors of DHFR-inhibiting activity because of the absence of correlation between abovementioned indicators. The obtained compounds may be of interest for further search for anti-inflammatory, anti-viral, hypoglycemic, hypotensive, anti-ischemic agents due to the expected low-toxicity associated with the slight DHFR-inhibiting activity.

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REFERENCES

1. *Avendaño C, Menéndez JC.* Chapter 2 – Antimetabolites. *In: Medicinal Chemistry of Anticancer Drugs.* 2008; 9–52.
2. *Benkovic SJ, Hammes-Schiffer S.* A perspective on enzyme catalysis. *Science.* 2003;**301**(5637):1196–202.
3. *Bailey LB, Caudill MA.* Folate. In Eardman JW Jr, MacDonald IA, Zeisel SH (Eds.). Present Knowledge in Nutrition, Tenth Edition. Ames, IA: ILSI Press/Wiley-Blackwell. 2012: 321–42.
4. *Christensen KE, Mackenzie RE.* Mitochondrial methylenetetrahydrofolate dehydrogenase, methenyltetrahydrofolate cyclohydrolase, and formyltetrahydrofolate synthetases. *Vitam Horm.* 2008;**79**:393–410.

5. *Ducker GS, Rabinowitz JD.* One-Carbon Metabolism in Health and Disease. *Cell Metab.* 2017;**25**(1):27–42.
6. *Naderi N, House JD.* Recent Developments in Folate Nutrition. *Adv Food Nutr Res.* 2018;**83**:195–213.
7. *Lan X, Field MS, Stover PJ.* Cell cycle regulation of folate-mediated one-carbon metabolism. *Wiley Interdiscip Rev Syst Biol Med.* 2018;**10**(6):e1426.
8. *Jackman AL.* Antifolate drugs in cancer therapy. *Totowa, NJ: Humana Press; 1999, 456 p.*
9. *Johari-Ahar M, Barar J, Alizadeh AM, Davaran S, Omidi Y, Rashidi MR.* Methotrexate-conjugated quantum dots: synthesis, characterisation and cytotoxicity in drug resistant cancer cells. *J Drug Target.* 2016;**24**(2):120–33.
10. *Kompis IM, Islam K, Then RL.* DNA and RNA synthesis: antifolates. *Chem Rev.* 2005;**105**(2):593–620.
11. *Purcell WT, Ettinger DS.* Novel antifolate drugs. *Curr Oncol Rep.* 2003;**5**(2):114–25.
12. *Gonen N, Assaraf YG.* Antifolates in cancer therapy: structure, activity and mechanisms of drug resistance. *Drug Resist Updat.* 2012;**15**(4):183–210.
13. *Raimondi MV, Randazzo O, La Franca M, Barone G, Vignoni E, Rossi D, Collina S.* DHFR Inhibitors: Reading the Past for Discovering Novel Anticancer Agents. *Molecules.* 2019;**24**(6):1140.
14. *Duff MR Jr, Gabel SA, Pedersen LC, DeRose EF, Krahn JM, Howell EE, London RE.* The Structural Basis for Nonsteroidal Anti-Inflammatory Drug Inhibition of Human Dihydrofolate Reductase. *J Med Chem.* 2020;**63**(15):8314–24.
15. *Takemura Y, Kobayashi H, Miyachi H.* Cellular and molecular mechanisms of resistance to antifolate drugs: new analogues and approaches to overcome the resistance. *Int J Hematol.* 1997;**66**(4):459–77.
16. *Zhao R, Goldman ID.* Resistance to antifolates. *Oncogene.* 2003;**22**(47):7431–57.
17. *Yang J, Wang M, Li X, Fan N, Xue L, Li H, Tian C, Wang X, Liu J, Zhang Z.* Syntheses and antiproliferative evaluation of 6-thienyl, 6-polyphenyl aryl and 6-naphthyl derivatives of 2,4-diaminopyrido[3,2-*d*] pyramidine as non-classical antifolate targeting DHFR. *Chem Res Chin Univ.* 2017; **33**: 559–68.
18. *Wang M, Yang J, Yuan M, Xue L, Li H, Tian C, Wang X, Liu J, Zhang Z.* Synthesis and antiproliferative activity of a series of novel 6-substituted pyrido[3,2-d]pyrimidines as potential nonclassical lipophilic antifolates targeting dihydrofolate reductase. *Eur J Med Chem.* 2017;**128**:88–97.
19. *Tian C, Wang M, Han Z, Fang F, Zhang Z, Wang X, Liu J.* Design, synthesis and biological evaluation of novel 6-substituted pyrrolo [3,2-*d*] pyrimidine analogues as antifolate antitumor agents. *Eur J Med Chem.* 2017;**138**:630–43.
20. *Kazunin MS, Voskoboynik OYu, Nosulenko IS, Berest GG, Sergeieva TA, Okovytyy SI, Karpenko OV, Priimenko BO, Kovalenko SI.* Synthesis, Tautomerism, and Antiradical Activity of Novel Pteridinetrione Derivatives. *J Heterocyclic Chem.* 2018; **55**(4): 1033–41.
21. *Kazunin MS, Voskoboynik OYu, Nosulenko IS, Berest GG, Kholodniak SV, Pryymenko BO, Kovalenko SI.* Synthesis, antiradical, and antimicrobial activities of new pteridine-2,4,7-trione derivatives. *J Heterocyclic Chem.* 2019: 57(1):268–80.
22. *Kazunin MS, Voskoboynik OY, Shishkina SV, Antypenko OM, Kovalenko SI.* A Simple and Convenient Method for the Synthesis of 1-Methyl-7-arylfuro[3,2-g]pteridine-2,4(1H,3H)-diones and Their Substituted Derivatives. *Acta Chim Slov.* 2020;**67**(2):586–93.
23. *Trott O, Olson AJ.* AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem.* 2010;**31**(2):455–61.

Інгібітори дигідрофолатредуктази серед похідних птеридину та фуро[3,2-*g*]птеридину

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Мета. Пошук інгібіторів ДГФР серед заміщених птеридин-2,4,7-трионів та 7-арил-(гетарил-)фуро[3,2-*g*] птеридин-2,4(1*H*,3*H*)-дионів та вибір напрямку подальших біологічних досліджень. **Методи** *in vitro*, молекулярний докінг, SAR-аналіз, статистичні методи. **Результати.** Досліджена здатність нових заміщенних 1-метилптеридин-2,4,7-трионів (**2**, **3**, **4**) та 7-арил-(гетарил-)фуро[3,2-*g*]птеридин-2,4(1*H*,3*H*)-дионів (**5,**

6) щодо інгібування ДГФР. Встановлено, що 6-(2-гідрокси-2-арил-(гетарил-)етил)-1-метилптеридин-2,4,7(1*H*,3*H*,8*H*)-триони (**3**) та бутил 2-(7-арил-(гетерил-)-1-метил-2,4-диоксо-1,4-дигідро[3,2-*g*] птеридин-3(2*H*)-іл)ацетати (**6**) інгібують ДГФР на 14.59–52.11 %, поступаючись метотрексату. Виявлено, що введення електроноакцепторних замісників до арильної субституенти, нафтильного замісника або електроноакцепторного гетероциклу (фуран, тіофен та бензофуран) приводить до посилення активності. Крім того, показано, що анелювання фуранового циклу до птеридинової системи є виправданим у контексті пошуку інгібіторів ДГФР. Проведений молекулярний докінг показав, що основний масив досліджуваних сполук має високу спорідненість до ДГФР, хоч і дещо інше розміщення в активному центрі ферменту. **Висновки.** Проведений первинний скринінг *in vitro* дозволив виявити низьку або помірну ДГФР-інгібуючу активність синтезованих речовин. Візуалізація докінгових досліджень дозволила виявити, що незважаючи на структурну подібність до метотрексату синтезовані сполуки формують відмінні ліганд – ензимні взаємодії. Розраховані значення афінності не можуть бути використані як предиктори ДГФР-інгібуючої активності внаслідок відсутності кореляції між зазначеними показниками. Одержані речовини є цікавими для подальших досліджень спрямованих на пошук протизапальних, противірусних, гіпоглікемічних, гіпотензивних, та анти-ішемічних агентів в наслідок очікуваної низької токсичності асоційованою з невисокою ДГФР-інгібуючою дією.

Ключові слова: ДГФР-інгібуюча активність, птеридини, фуро[3,2-*g*]птеридини, молекулярний докінг, QSAR-аналіз.

Ингибиторы дигидрофолатредуктазы среди производных птеридина и фуро[3,2-*g*]птеридина

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Цель. Поиск ингибиторов ДГФР среди замещенных птеридина-2,4,7-трионов и 7-арил-(гетарил-)фуро[3,2-*g*] птеридина-2,4(1*H*,3*H*)-дионов и выбор направления дальнейших биологических исследований. **Методы** *in vitro*, молекулярный докинг, SAR-анализ, статистические методы. **Результаты.** Исследована способность новых замещенных 1-метилптеридин-2,4,7-трионов (**2**, **3**, **4**) и 7-арил-(гетарил-)фуро[3,2-*g*]птеридина-2,4(1*H*,3*H*)-дионов (**5**, **6**) по ингибированию ДГФР. Установлено, что 6-(2-гидрокси-2-арил-(гетарил-)етил)-1-метилптеридин-2,4,7(1*H*,3*H*,8*H*)-трионы (**3**) и бутил 2-(7-арил-(гетерил-)-1-метил-2,4-диоксо-1,4-дигидро[3,2-*g*]птеридин-3(2*H*)-ил)ацетаты (**6**) ингибируют ДГФР на 14.59–52.11 %, уступая метотрексату. Выявлено, что введение электроноакцепторных заместителей к арильной субституенте, нафтильного заместителя или электроноакцепторного гетероцикла (фуран, тиофен и бензофуран) приводит к усилению активности. Кроме того, показано, что аннелирование фуранового цикла к птеридиновой системе оправдано в контексте поиска ингибиторов ДГФР. Проведенный молекулярный докинг показал, что основной массив исследуемых соединений имеет высокое сродство к ДГФР, хотя и несколько иное размещения в активном центре фермента. **Выводы.** Проведенный первичный скрининг *in vitro* выявил низкую или умеренную ДГФР-ингибирующую активность синтезированных веществ. Визуализация исследований, проведённых методами молекулярного докинга, показала, что несмотря на структурную подобность к метотрексату, синтезированные соединения формируют иные лиганд-ферментные взаимодействия. Рассчитанные значения аффинности не могут быть использованы как предикторы ДГФР-ингибирующей активности вследствие отсутствия корреляции между вышеуказанными показателями. Полученные соединения представляют интерес для дальнейших исследований по поиску противовоспалительных, противовирусных, гипогликемических, гипотензивных, и антиишемических агентов вследствие ожидаемой низкой токсичности, которая ассоциирована с низким ДГФР-ингибирующим действием.

Ключевые слова: ДГФР-ингибирующая активность, птеридины, фуро[3,2-*g*]птеридины, молекулярный докинг, QSAR-анализ.

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