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Steric Complementarity of Some Pyrimidine Derivatives with Active Sites of Mouse Thymidylate Synthase (MUS Musculus)

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Abstract

Molecular docking method was applied to research steric complementarity of 27 uracil derivatives modified into positions N^1, N^3, C^5, C^6 by cyclic and acyclic substituents with the active site of thymidylate synthetase of mice Mus musculus. AutoDock 4.2 scoring function evaluated the thermodynamic characteristics of all compounds' binding with the active site of the given enzyme. 17 uracil derivatives characterized by predominantly unsubstituted position N_3 revealed the capacity of being effective inhibitors of thymidylate synthase and promising as hit compounds for further pre-clinical research and development of new drugs with marked antitumor effect for chemotherapy.

Keywords: thymidylate synthetase inhibitors, molecular docking, uracil derivatives

INTRODUCTION

Cancer is one of the causes of high death rate in modern society. The risk of cancer emergence and development is a global threat to humanity as a whole. In this context, the fight against the emergence and development of tumours of various origins is one of the priorities of modern medicine. This battle is fought in several directions, including preventive measures, surgical treatment and chemotherapy. The growth and development of tumour and normal cells is known to be regulated by a complex of enzymes. One of them is thymidylate synthase (TS) [1-5]. TS (EC 2.1.1.45) is an

enzyme that catalyses the change of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP) and thereby plays an important role in the regulation of normal DNA replication [2-7]. Any change in TS activity has a significant impact on the process of regulation of DNA bio-synthesis and induces various biological and genetic abnormalities in animals and humans. The antineoplastic mechanism of some chemotherapeutic drugs such as 5-fluorouracil and a number of its alternative fluoro-pyrimidines is known to base on the inhibition of the enzyme activity [8-12]. It is proved that the elevated level of TS activity which is observed alongside with tumour cells growth reduces the effectiveness of 5-FU. At the same time, tumours with low TS activity are highly sensitive to the chemotherapeutic drug. The urgency of the problem of reducing the effective dose of 5-fluorouracil is mainly conditioned by a rather high toxicity of the drug. So the search for efficient TS inhibitors is one of the urgent issues of modern biology, biomedical chemistry and biochemistry.

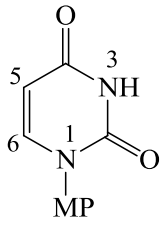
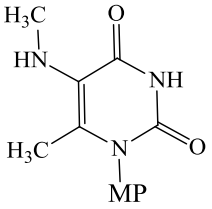
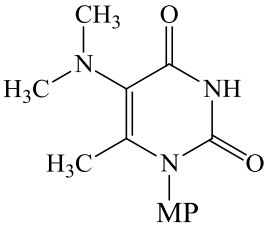
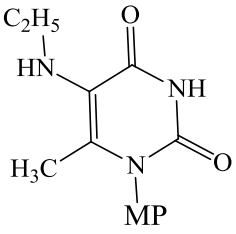
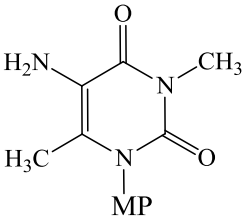
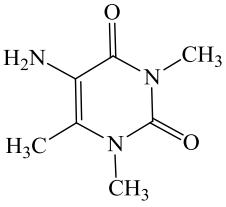
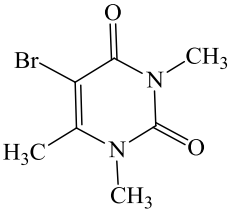
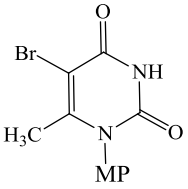
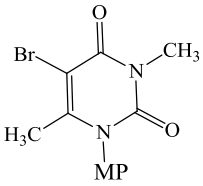
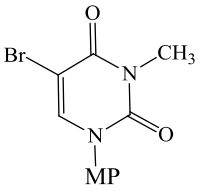
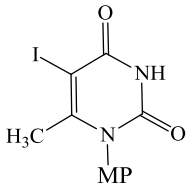
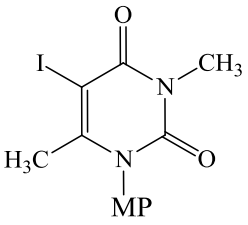
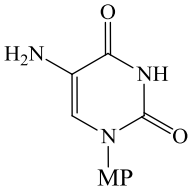
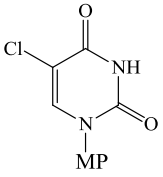
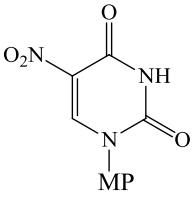
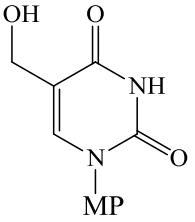
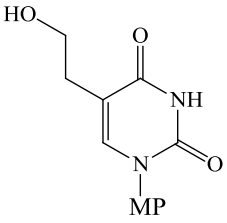
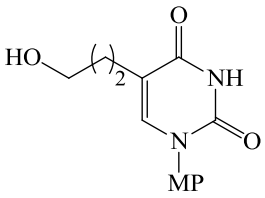
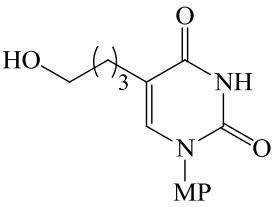
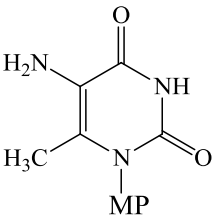
Due to a rather profound level of development of bioinformatics, virtual screening has become highly topical, offering well-targeted monitoring and synthesis of biologically active substances with the desired set of properties. This phase of research precedes organic compounds synthesis and maps out a strategy of their pre-clinical and clinical trials in a variety of model systems and using test animals. The current level of virtual screening allows to use various strategies of hit compounds computer simulation based either separately on one method of (Q)SAR-modelling, of molecular mechanics methods (methods of molecular docking and molecular dynamics), or combine them in a system approach. When selecting hit structures the use of a range of computer methods of biological activity modelling and “structure-property” relation reduces tenfold the time and costs of developing new compounds with the required biological and pharmacological profile [13-15].

The aim of this work was the virtual screening of uracil derivatives modified into positions N^3, C^5 and C^6 by cyclic and acyclic substituents and evaluation of their steric complementarity with the active centre of TS Mus musculus.

EXPERIMENTAL WORK

Subjects of study brief description

As uracil, a natural TS substrate, is located in the active site of the enzyme as a mononucleotide, the structures of the tested uracil derivatives containing a hydrogen atom in N^1 position have modified by replacing the H atom at N^1 with a mononucleotide fragment (see Fig. 1). Stereoisomerism of cyclic and acyclic fragments has been herein rigidly observed. Software Marvin Sketch 15.08.17 [16] was applied to generate and search within a set of solutions for conformers sterically corresponding to the structure of mononucleotide uracil unit. By way of molecular docking modified structures corresponding in their spatial structures to the molecule is a 2'-deoxyuridine-5'-monophosphate were positioned in the active site of TS. Figure 1 shows structural formula of simulated uracil derivatives.

 **1MP**	 **2MP**	 **3MP**	 **4MP**
 5MP	 **6**	 **7**	 **8MP**
 **9MP**	 **10MP**	 **11 MP**	 **12 MP**
 **13 MP**	 **14 MP**	 **15 MP**	 **16 MP**
 **17**	 **18**	 **19**	 **20 MP**

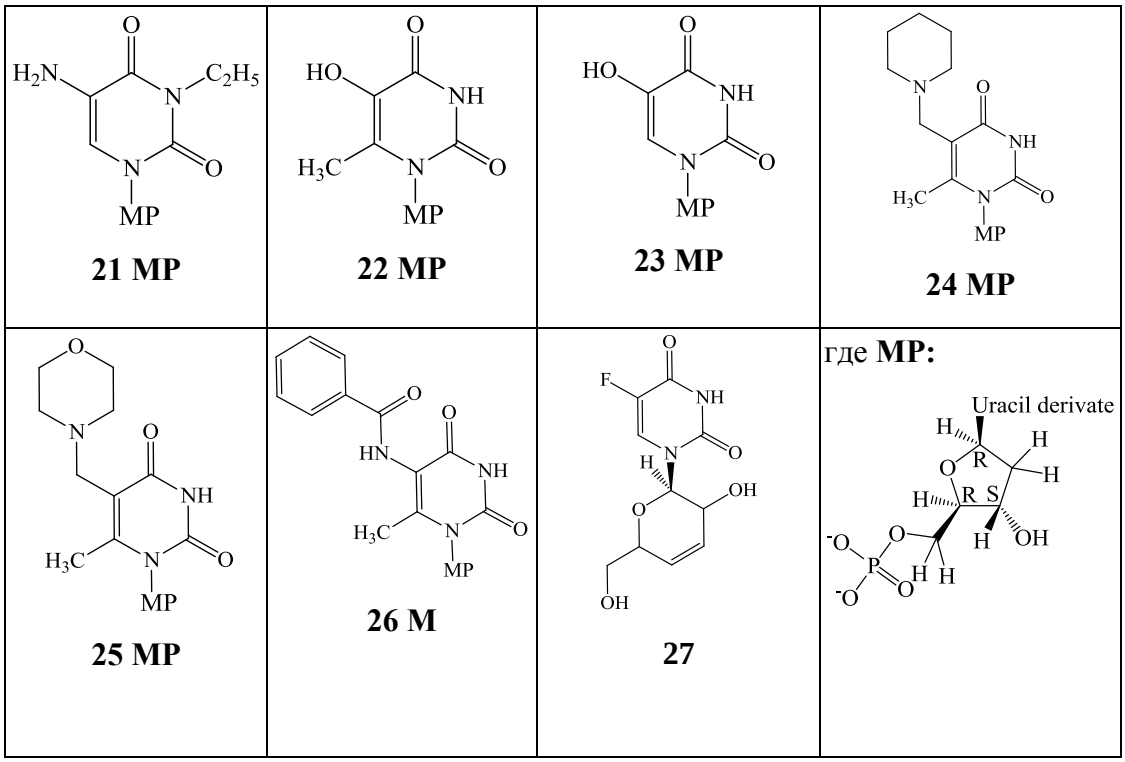


Figure 1: Structural formula of compounds under study.

Methods of molecular docking

Molecular docking of uracil derivatives' structures in the active centre of TS was conducted with AutoDock 4.2 and AutoDock Vina software [17]. Chain A homodimer of AB macromolecules coded 4EB4 in proteins data bank [18] was selected as a protein model. When calculating the protein molecule was rigid, while the ligand molecules were moving. The size of the three-dimensional box, which held the molecular docking of the ligands, was 16.5 Å in all simulations. The position of the natural TS substrate - deoxyuridine-5'-monophosphate coded **1 MP** in Fig. 1 was taken as the box centre. Docking in AutoDock Vina was carried out with the default settings following the Broyden-Fletcher-Goldfarb-Shanno algorithm. AutoDock 4.2 searched for bioactive conformations following the Lamarckian genetic algorithm with default settings except for the angle of rotation around ordinary bonds and rotational motion of the molecules equal to 30^0. The docking solutions were clustered drawing on the value RMSD = 2, 0 Å. The efficiency of binding of the ligands to protein was evaluated following semi-empirical and empirical scoring functions embedded in AutoDock 4.2 [17] and AutoDock Vina [17], respectively, AMBER force field applied [20]. When searching for TS inhibitors of substrate type the reference substance was selected to be 2'-5-fluoro-2'-deoxyuridine-5'-monophosphate structure, which is a metabolite of the active substance of such antineoplastic drugs as "Ftorafur", "Fluorouracil-Ebewe" [20].

The complexes "enzyme-ligand " were analysed using the online version of PLATINUM software [18]; the software identifies the ligand position in the enzyme active site to evaluate its overall contacting surface S_{total} (including water solvent, in $Å^2$) and S_{buried} (in $Å^2$) (excluding water solvent), ligands' surface forming hydrophilic and hydrophobic contacts S_{HH} and S_{LL} (in $Å^2$) respectively, and the surface fraction of ligand molecules involved in the formation of the above-described contacts of the total area of these molecules $Match^1$ and $Match^2$ (in fractions). The detailed description of the parameters calculated in PLATINUM is given on the website [21].

RESULT AND DISCUSSION

Study of complex formation mechanism of pyrimidine derivatives with active TS site.

The present paper researches the possibility to inhibit the catalytic activity of TS Mus musculus by some uracil derivatives using the method of molecular docking. The modelling part was the calculation of the protein complex configuration with a presumable inhibitor using a Lamarckian genetic algorithm of AutoDock 4.2, as well as the method of iterative local search of the global minimum following Broyden-Fletcher-Goldfarb-Shanno algorithm in AutoDock Vina. As optimal docking solutions AutoDock 4.2 selected those compounds' conformations that occur in the cluster of solutions with a probability of at least 25% and are in the energetically favourable state, coinciding with the global minimum or close to it. As bioactive conformations AutoDock Vina selected the docking solutions, characterized by the minimum value of the scoring function.

It has been found that the deviation in coordination of most ligands except **8 MP, 9 MP, 10 MP, 12 MP, 13 MP, 21 MP, 24 MP**, calculated using the two different types of scoring functions, amounts to RMSD $\leq$ 2.5 Å. This, in turn, shows a rather high credibility and reliability of the calculation results.

Table 1 shows the results of pyrimidine derivatives docking in the active site of TS.

Table 1: Binding free energy and the binding constant of uracil derivatives with the active site of macromolecule 4EB4.

Compound structure	Binding free energy, kcal / mol	Inhibition constant, K_{inh},nmol/L	Amount in cluster, making a total of 20 solutions*
5-fluoro-2'-deoxyuridine-5'-monophosphate (5FUrMP)	-12.33* / -7.10**	1.04* / 6.73•10^{3}**	17
1MP 2'-deoxyuridine-5'-monophosphate (UrMP)	-12.94 / -9.10	0.38 / 2.35•10^{2}	19
2MP	-13.01 / -6.20	0.33 / 3.05•10^{4}	15
3MP	-13.34 / -5.50	0.19 / 9.85•10^{4}	19
4MP	-13.21 / -6.20	0.24 / 3.05•10^{4}	14
5MP	-10.39 / -4.60	27.02 / 4.46•10^{5}	18
6	-4.29 / -2.80	7.50•10^{5} / 9.13•10^{6}	20
7	-4.7/ -2.60	3.77•10^{5} / 1.28•10^{7}	20
8MP	-12.61 / -6.00	0.65 / 4.26•10^{4}	19
9MP	-11.13 / -4.70	7.81 / 3.77•10^{5}	9
10MP	-10.33 / -5.20	26.77 / 1.63•10^{5}	9
11	-12.09 / -5.00	1.56 / 2.28•10^{5}	15
12 MP	-11.02 / -4.20	9.39 / 8.72•10^{5}	15
13 MP	-12.72 / -7.90	0.54 / 1.76•10^{3}	19
14 MP	-13.55 / -8.20	0.13 / 1.06•10^{3}	19
15 MP	-13.50 / -7.40	0.15 / 4.07•10^{3}	19
16 MP	-13.60 / -8.50	0.12 / 6.44•10^{2}	19
17 MP	-13.90 /-8.60	0.07 / 5.44•10^{2}	19
18 MP	-13.71 / -8.10	0.10 / 1.26•10^{3}	12
19 MP	-14.35 / -8.10	0.04 / 1.26•10^{3}	12
20 MP	-13.18 / -7.20	0.25 / 5.70•10^{3}	20
21 MP	-12.28 / -5.30	1.13 / 1.38•10^{5}	9
22 MP	-13.35 / -7.50	0.19 / 3.44•10^{3}	20
23 MP	-13.62 / -8.50	0.12 / 6.43•10^{2}	20
24 MP	-10.68 / -3.40	16.61 / 3.34•10^{6}	11
25 MP	-7.83 / -4.10	1.82•10^{3} / 1.03•10^{6}	12
26 MP	-8.99 / -2.40	282.77 / 1.79•10^{7}	9
27	-6.91 / -4.70	9258.89 / 3.77•10^{5}	20

* - Found in AutoDock 4.2.
** - Found in AutoDock Vina.

The molecules of the tested uracil derivatives when positioning of the active site of TS form a single cluster due to significant similarities in the structure, Fig. 2, Table. 2.

All ligand structures are located in the enzyme's active site polar area formed by such amino acid fragments as: Arg 44 (A chain), Arg 169 (B chain), Arg 170 (B chain), Arg 44 (A chain), Tyr 252 (A chain), His 250 (A chain), Gln 208 (A chain), etc. The position of the tested compounds in the active site of TS is stabilized by large number of hydrogen bonds, caused by interaction of these ligands' mononucleotide fragments with amino acid residues of enzymes, Table. 2, Fig. 2-3. For this reason AutoDock 4.2 assigns fairly high numerical values of scoring functions to almost all mononucleotide derivatives (Tables 1-2. Fig. 2). The orientation of pyrimidine ring is affected by the amino acid residues Asn 220 (A chain), Asp 212 (A chain), Arg 170 (B chain), Fig. 2. They participate in the formation of hydrogen bonds and setting electrostatic interactions between the ligand fragment and the active TS site. Furthermore, the positioning of the ligands in the active site of TS is affected by quinazolin-4-ones cycle characteristic of the structural analogues of the compound with the trivial name Tomudex, Fig. 2. It is known that the cyclic fragment has a high structural similarity to the condensated cyclic frame of tetrahydrofolate (TS co-factor) involved in methylation of 2'-deoxyuridine-5'-monophosphate in C_5 position in the active site of TS. It participates in the formation of lipophilic contacts and π-π-interaction of these substances with the active site of the enzyme under discussion, that is, an additional stabilizing factor (Fig. 2).

Table 2: Configuration characteristics of uracil derivatives binding with the active site of TS analysed with online version of PLATINUM (http://model.nmr.ru/platinum/)

Compound code	$S_{H/H}$, Å^2	S_{buried}, Å^2	S_{total}, Å^2	Match[1]	π-stacking
1MP	120.88	227.72	228.60	0.71	+
5FUr	112.09	222.84	224.23	0.69	+
2 MP	143.29	257.78	257.78	0.56	+
3 MP	165.25	283.03	283.03	0.60	+
4 MP	150.19	274.62	274.61	0.56	+
5 MP	172.28	265.50	265.63	0.76	+
6	65.95	152.72	159.05	0.69	+
7	70.25	164.17	169.29	0.69	+
8 MP	141.20	255.57	255.56	0.56	+
9 MP	146.90	268.60	268.61	0.57	+
10 MP	148.10	265.82	265.95	0.57	+
11 MP	123.48	233.35	233.35	0.73	+
12 MP	113.29	253.35	253.54	0.65	+

13 MP	142.91	235.19	235.38	0.61	+
14 MP	115.38	228.35	228.60	0.70	+
15 MP	123.04	244.62	245.00	0.71	+
16 MP	129.93	248.10	248.09	0.67	+
17 MP	131.33	262.84	262.86	0.69	+
18 MP	133.35	283.92	283.98	0.70	+
19 MP	130.76	309.55	310.37	0.67	+
20 MP	146.64	246.13	246.13	0.60	+
21 MP	181.52	276.77	276.83	0.83	+
22 MP	114.62	243.79	243.79	0.71	+
23 MP	115.57	232.40	232.98	0.70	+
24 MP	165.50	332.78	332.90	0.60	-
25 MP	133.35	283.92	283.98	0.7	+
26 MP	131.45	326.45	327.01	0.68	+
27	75.88	203.29	204.43	0.62	+

The sign "+" implies possible T-stacking interactions of the ligand with nearby aromatic amino acid fragments in the active site of the enzyme

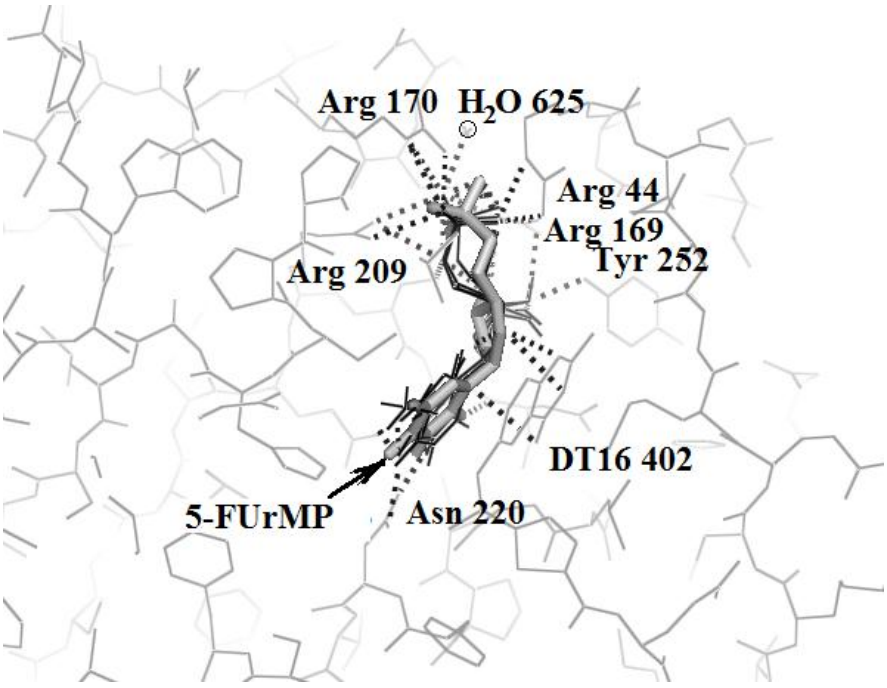


Figure 2: Positioning of some pyrimidine derivatives in the active site of TS.

Table 1 shows that the largest numerical values of scoring functions characterize the structure of pyrimidine mononucleotides **MP 2 - 4 MP, 8 MP, 11 MP, 13 MP - 24MP** with unsubstituted N^3 position. In terms of efficient binding with the active site of TS these structures are comparable with 5-fluoro-2'-deoxyuridine-5'-monophosphate and form from 10 to 14 hydrogen bonds with amino acids of active TS site, Fig. 2. Therefore, it can be expected that they, as well as the compound are capable of inhibiting biosynthesis of deoxythymidine monophosphate, and hence capable of being inhibitors of TS catalytic activity of a competitive type. Lower values of scoring functions characterize the ligands, modified into N^3 position by hydrophobic fragments. These compounds are characterized by a much smaller number of hydrogen bonds with amino acids of TS active site (6-8 hydrogen bonds depending on the nature of the substitute in C_5 position). Ligands with substituted hydrophobic fragments of N^1 positions do not undergo glycosylation and phosphorylation and thereby form a small number of H-bonds and polar contacts with the active TS site. They are characterized by the low binding free energy value of with the enzyme discussed.

Thus, the results of the molecular docking suggest that uracil derivatives **2 MP - 4 MP, 8 MP, 11 MP, 13MP - 24MP** with unsubstituted N^3 position can be effective TS inhibitors and promising hit compounds for further preclinical studies to develop new antitumor drugs for chemotherapy.

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REFERENCES

[1] Orlova, R.V., 2002. New drugs in the treatment of colorectal cancer. Practical oncology. 3 (4), 273-281.

[2] Isanbor, C., O'Hagan, D., 2006. Fluorine in medicinal chemistry: a review of anti-cancer agents. *J. Fluorine Chem*. 127 (3), 303-319.

[3] Zob, D., Stanculeanu, D.L., Gruia, L., 2014. Mecanisme moleculare implicate în rezistenţa la chimioterapie în celulele neoplazice din cancerul mamar metastatic. *Romanian J. Oncology & Hematology*. 2 (2), 90-97.

[4] Jackman, A.L., Calvert, A.H., 1995. Folate-based thymidylate synthase inhibitors as anticancer drugs. *Annals of Oncology*. 6 (9), 871-881.

[5] McGuire, J.J. 2003. Anticancer antifolates: current status and future directions. *Current pharmaceutical design*. 9 (31), 2593-2613.

[6] Chu, E., Callender, M.A., Farrell, M.P., Schmitz, J.C., 2003. Thymidylate synthase inhibitors as anticancer agents: from bench to bedside. *Cancer chemotherapy & pharmacology*. 52 (1), 80-89.

[7] Sousa, M.M.L., Krokan, H.E., Slupphaug, G., 2007. DNA-uracil and human pathology. *Molecular aspects of medicine*. 28 (3), 276-306.
[8] Zhang, N., Yin, Y., Xu, S.L., Chen, W.S., 2008. 5-Fluorouracil: mechanisms of resistance and reversal strategies. *Molecules*. 13 (8), 1551-1569.
[9] Noordhuis, P., Holwerda, U., van der Wilt, C.L., van Groeningen, C.J., Smid, K., Meijer, S., Pinedo, H.M., Peters, G.J., 2004. 5-Fluorouracil incorporation into RNA and DNA in relation to thymidylate synthase inhibition of human colorectal cancers. *Annals of oncology*. 15 (7), 1025-1032.
[10] Longley, D.B., Harkin, D.P., Johnston, P.G., 2003. 5-Fluorouracil: mechanisms of action and clinical strategies. *Nature Reviews Cancer*. 3 (5), 330-338.
[11] Fischer, J.A., Muller-Weeks, S., Caradonna S.J., 2006. Fluorodeoxyuridine modulates cellular expression of the DNA base excision repair enzyme uracil-DNA glycosylase. *Cancer research*. 66 (17), 8829-8837.
[12] Salonga, D., Danenberg, K.D., Johnson, M., Metzger, R., Groshen, S., Tsao-Wei, D.D., Lenz, C.G., Leichman, C.G., Leichman, L., Diasio, R.B., Danenberg, P.V., 2000. Colorectal tumors responding to 5-fluorouracil have low gene expression levels of dihydropyrimidine dehydrogenase, thymidylate synthase, and thymidine phosphorylase. *Clinical Cancer Research*. 6 (4), 1322-1327.
[13] Khayrullina, V.R., Gerchikov, A.Ya., Lagunin, A.A., Zarudii, F.S., , 2015. Quantitative analysis of structure-activity relationships of tetrahydro-2H-isoindole cyclooxygenase-2 inhibitors. *Biochemistry (Moscow)*. 80 (1). 74-86.
[14] Gerchikov, A.Ya., Vasil′ev, M.N., Khayrullina, V.R., Tsypysheva, I.P., Zarudii, F.S., 2015. Search for nootropic substances based on molecular docking of methanepyrido[1,2-***a***][1,5]diazocin[(-)-cytisine] derivatives to the active center of the nicotinic acetylcholine receptor. *Pharm. Chem. J*. 49 (9), 582-586.
[15] Khayrullina, V.R., Taipov, I.A., Gerchikov, A.Ya., Vasil′ev, M.N., Zarudii, F.S., Boegel, H., 2014. Structural analysis of leukotriene B4 (LBT_4) receptor (BLT_1 AND BLT_2) antagonists. *Pharm. Chem. J*. 48 (5), 317-322.
[16] www.chemaxon.com/products/marvin/marvinsketch/
[17] http://autodock.scripps.edu/
[18] http://www.rcsb.org.
[19] Wang, J., Wolf, R.M., Caldwell, J.M., Kollman, P.A., Case D.A., 2004. Development and testing of a general amber force field. *J. Comput. Chem*. 25 (9), 1157-1174.
[20] http://medi.ru/doc/7200407.htm
[21] http://model.nmr.ru/platinum/