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SEARCH FOR NEW DRUGS

SYNTHESIS AND *IN SILICO* PREDICTION OF THE MOLECULAR-TARGETING ANTI-EGFR ACTION OF A NOVEL DIHYDROACRIDINONE

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The synthesis and *in silico* prediction of the molecular-targeted anti-EGFR inhibitory activity of a novel dihydroacridinone derivative are reported. 9-Aminium-3,3-dimethyl-3,4-dihydroacridin-1(2*H*)-one *L*-2-hydroxybutanedioate (LHT-17-19) was obtained 99.8% pure by mixing and heating equimolar amounts of 9-amino-3,3-dimethyl-3,4-dihydroacridin-1(2*H*)-one and *L*-2-hydroxybutanedioic acid in 50% EtOH. Virtual molecular screening of the spectrum of effects of the compound revealed inhibitory properties against several intracellular targets, i.e., carcinogenesis drivers, among which the EGFR kinase domain had the highest probability. Docking of LHT-17-19 base to the EGFR kinase domain formed a molecular complex with a high affinity and bonding energy. The results suggested that LHT-17-19 had high antitumor activity against malignant neoplasms expressing EGFR.

Keywords: dihydroacridinone derivative, LHT-17-19, synthesis, validation, molecular docking.

Antitumor chemotherapy remains one of the main interventions for treating malignant neoplasms (MNs), especially of epithelial etiology. Low-molecular-mass molecules continue to play a key role in practically all antitumor treatment protocols, despite the broad clinical application of immunobiological drugs that revolutionized the general approach to treating MNs and heralded a new era in clinical oncology [1, 2]. They are also considered sources of new molecules targeted at carcinogenic intracellular signaling pathways [2].

The epidermal growth factor receptor (EGFR) is a key driver in tumor development and progression [3]. Tyrosine kinase EGFR modulates the growth and differentiation of epithelial cells through phosphorylation of intracellular substrates [4]. This kinase under pathological conditions is involved in oncogenic transformation and acceleration of tumor growth of various neoplasms such as lung, breast, spleen, and head and neck cancers and urothelial carcinoma, glioblastoma, etc. [5]. EGFR inhibitors are a reliable strategy for antitumor chemotherapy [6]. However, the tumor response in many cases is compromised by the formation of early resistance, usually associated with mutations of the EGFR gene [7].

Acridine derivatives are well-known sources of many antitumor drugs [8]. Promising dihydroacridinone derivatives with various amino- and carboxylic-acid substituents

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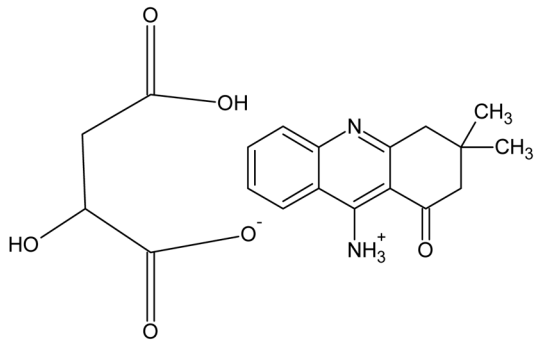


Fig. 1. Chemical Structure of 9-aminium-3,3-dimethyl-3,4-dihydroacridin-1(2*H*)-one *L*-2-hydroxybutanedioate (developer laboratory number LHT-17-19).

were chosen by the scientific group of the All-Union Research Center for Biological Active Compounds Safety during a broad search for new chemical structures with a low molecular mass and antitumor properties. A quantitative structure-activity relationship analysis of chemical structures identified a compound, i.e., LHT-17-19 (Fig. 1), with the highest probability of general antitumor activity. Preliminary results prompted us to begin chemical and pharmacological studies of the new molecule because of the urgent need of modern clinical oncology for effective, safe, and reliable antitumor drugs.

The main goal of the research was to develop the laboratory synthesis technology of the new dihydroacridinone derivative and to predict its molecular-targeted activity against EGFR pro-oncogenic kinase in *in silico* experiments.

TABLE 1. Molecular-Targeted Docking Parameters for LHT-17-19 with EGFR Kinase (EGFRK) Site

Parameter	Value
Incremental step	0.375 Å
Torsional coefficient of freedom	0.2983
Cluster tolerance	2 Å
External binding energy	1000
Maximum initial energy	0
Maximum number of attempts	10 000
Number of structures in a population	150
Maximum number of calculated energy levels	2 500 000
Maximum number of generations	27 000
Maximum number of structures advancing to the next stage	1
Gene mutation level	0.02
Crossover level	0.8
Crossover method	Arithmetic

EXPERIMENTAL CHEMICAL PART

The synthesis of the target compound was monitored by TLC on Merck F_{254} silica gel plates with elution by H_2O-BuOH-Me_2CO (3:1:1), R_f 0.8. The composition of the compound was confirmed by elemental analysis on a PerkinElmer PE 2400 automated analyzer (USA). The structure of the synthesized compound was confirmed using PMR and IR spectroscopy. The PMR spectrum was recorded in DMSO-d_6 relative to TMS on a Bruker AC-250 spectrometer (USA) at 400 MHz. The IR spectrum was taken from KBr pellets on a Cary FTIR Spectrometer 630 (Agilent, USA). The melting point was determined by the capillary method on a Stuart Scientific SMP20 apparatus. The purity of the synthesized compound was determined by high-efficiency liquid chromatography with mass spectrometry (HPLC-MS) on an LCMS-8030 instrument (Shimadzu, Japan) with a Nanosphere Eco C18(2) column (250 × 4.6 mm, 5 μm).

9-Aminium-3,3-dimethyl-3,4-dihydroacridin-1(2*H*)-one *L*-2-hydroxybutanedioate (LHT-17-19)

A homogenizer was loaded with equimolar amounts of 9-amino-3,3-dimethyl-3,4-dihydroacridin-1(2*H*)-one (the synthetic method is given in RF patent No. 2,567,388 [9]) (9.35 g, 0.25 mol) and *L*-2-hydroxybutanedioic acid (3.35 g, 0.25 mol). The mixture was thoroughly homogenized for 10 – 15 min and treated with EtOH (50%, 70 mL). The mixture was heated and stirred under reflux for 2 h and filtered. The EtOH was distilled in a rotary evaporator at 30 mmHg. The solid was recrystallized from EtOH and dried to constant mass to afford a white crystalline powder with a cream tint (10.93 g) and melting point 193 – 195°C. Found, %: C 60.91; H 5.94; N 7.46. $C_{19}H_{22}N_2O_6$. Calc., %: C 60.95; H5.92; N 7.48; O 25.65. IR spectrum (ν, cm^{-1}): 3160, 3080 (NH), 2980 (CH), 1925 (N^+), 1620, 1630 (C=C), 1560 (COO^-). PMR spectrum (DMSO-d_6, δ, ppm): 1.77 – 1.88 (8H, CH_2); 7.63 (4H, C=C); 7.98 – 8.31 (3H, C-N). The mass spectrum had a peak with *m*/*z* 375.39 [M + 1].

EXPERIMENTAL BIOLOGICAL PART (*IN SILICO*)

The probability of activating (Pa) or inhibitory activity (Pi) against several kinases was determined by a structure-activity analysis using the PASS Online program [10].

Only the base 9-amino-3,3-dimethyl-3,4-dihydroacridin-1(2*H*)-one was considered for molecular docking experiments because LHT-17-19 was a salt containing *L*-2-hydroxybutanedioate. The AutoDock 4.2 suite with an open source code was used for flexible docking directed at the receptors. Ligands were prepared using the MGL Tools 1.5.6 program (The Scripps Research Institute, USA). The ligand was optimized using Avogadro software with an open source code. The starting data for the receptors and ligands were reformatted to the special PDBQT format for calculations in AutoDock 4.2. The crystallographic structures of active sites of the EGFR receptor macromolecule from the protein data-

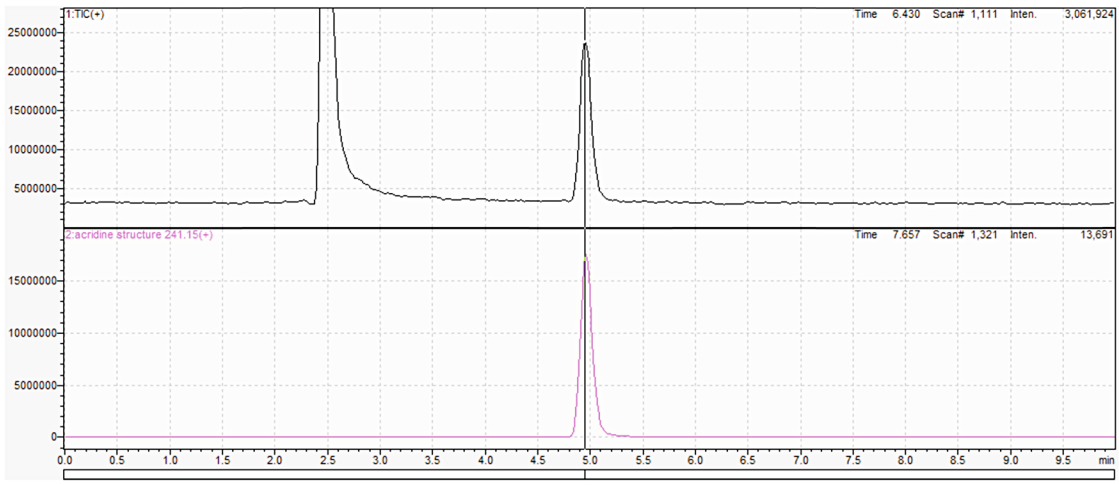


Fig. 2. Chromatogram of LHT-17-19 substance [9-aminium-3,3-dimethyl-3,4-dihydroacridin-1(2*H*)-one *L*-2-hydroxybutanedioate].

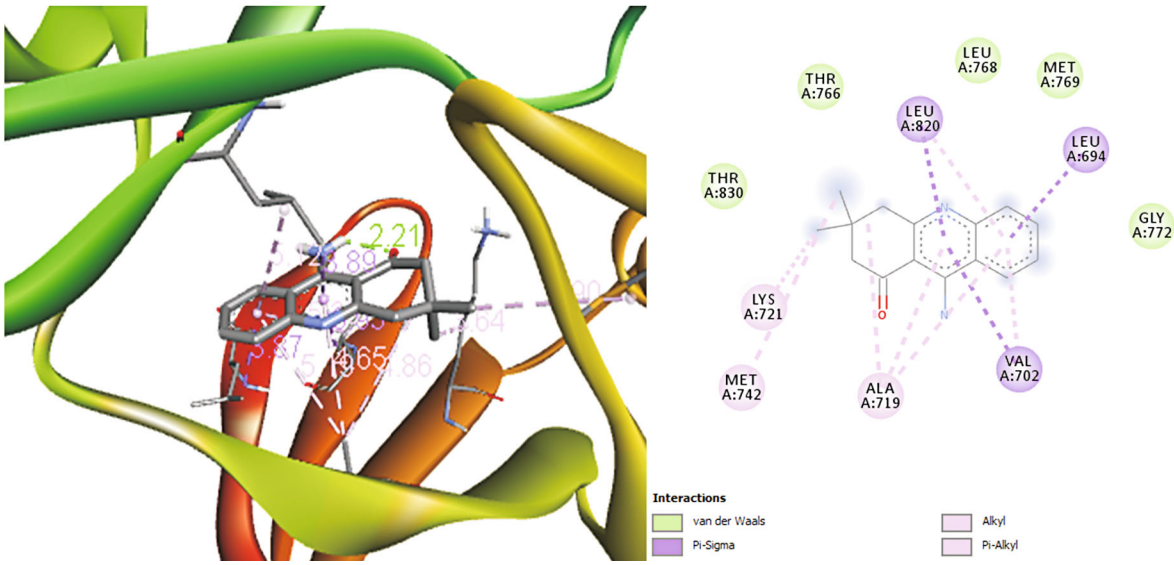
base (PDB ID: 1M17) were used. Receptor maps were prepared using MGL Tools and AutoGrid software. Water molecules, ions, and ligands were removed from PDB ID 1M17. Table 1 lists the parameter set for the molecular docking experiments. We used Discovery Studio Visualizer for visual analysis of ligand-receptor complexes. The binding affinity (evaluation of dG, kcal/mol), binding free energy (EDoc, kcal/mol), and interaction coefficient (Ki, iM) were calculated.

RESULTS AND DISCUSSION

Mixing, homogenizing, and heating equimolar amounts of 9-amino-3,3-dimethyl-3,4-dihydroacridin-1(2*H*)-one and *L*-2-hydroxybutanedioic acid in EtOH (50%) afforded 99.8% pure 9-aminium-3,3-dimethyl-3,4-dihydroacridin-1(2*H*)-one *L*-2-hydroxybutanedioate.

The retention time (RT) of LHT-17-19 according to HPLC-MS was 4.95 min. Figure 2 shows chromatograms of the total ion current of the substance (upper) and of the isolated ion with *m*/*z* 241.15 (lower), which corresponded to 9-amino-3,3-dimethyl-3,4-dihydroacridin-1(2*H*)-one.

The probabilities of inhibitory activity of the compound against the following intracellular kinases (in order of decreasing probability) were found by analyzing the structure-activity relationship of LHT-17-19: against EGFR kinase domain, 0.93; CSF1 receptor system, 0.67; and human folate receptor FOLR2, 0.53. Therefore, inhibitory ac-



a) *b*)

Fig. 3. Three-dimensional structure (*a*) and molecular interactions (*b*) of the complex of 9-amino-3,3-dimethyl-3,4-dihydroacridin-1(2*H*)-one with the EGFRK domain (PDB identifier: 1M17).

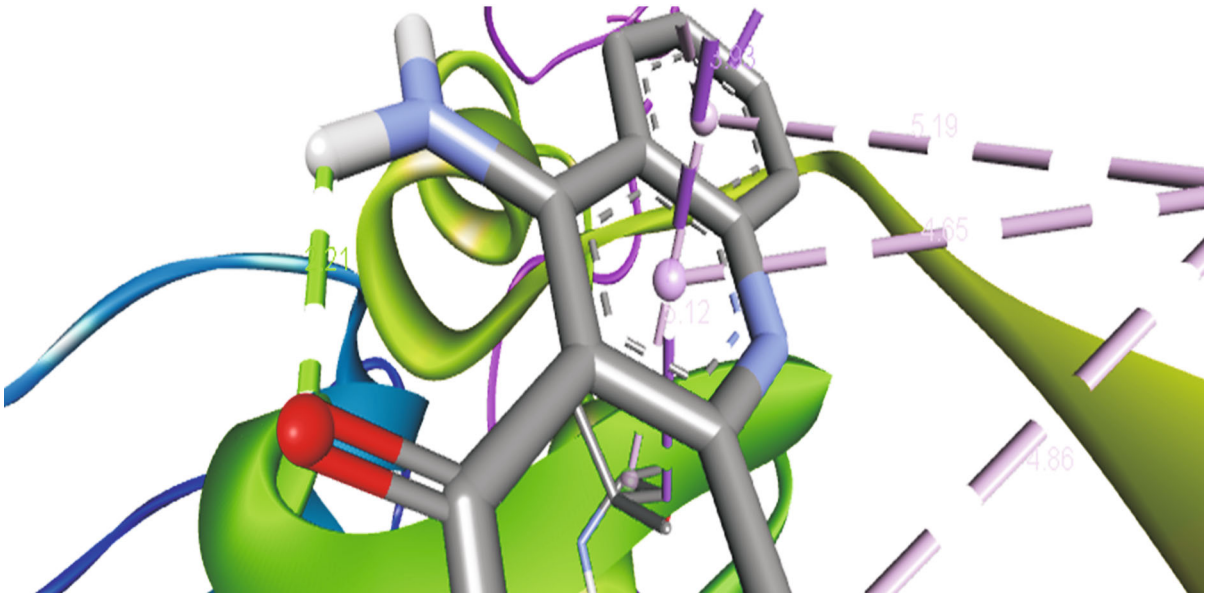


Fig. 4. Three-dimensional structure of the complex of 9-amino-3,3-dimethyl-3,4-dihydroacridin-1(2*H*)-one with the EGFRK domain (PDB identifier: 1M17).

tivity was predicted with the greatest probability for EGFRK. The docking experiment was performed against just this molecular target.

9-Amino-3,3-dimethyl-3,4-dihydroacridin-1(2*H*)-one showed high affinity for the EGFR kinase domain (PDB identifier: 1M17) with parameter dG -7.9 kcal/mol; EDoc -5.45 kcal/mol, and Ki 101.24 μM. This complex formed through π-σ-bonds between aromatic cores of the 1,2,3,4-tetrahydroacridin-1-one moiety and amino-acid residues Leu820, Leu694, and Val702 (Fig. 3*a*). Also, the alkyl and π-alkyl complex was stabilized by interactions of the 3-methyls and the 1,2,3,4-tetrahydroacridin-1-one moiety with amino-acid residues Lys721, Met742, Ala719, Leu820, and Val702 (Fig. 3*b*, Table 2).

TABLE 2. Molecular Basis of Affinity of LHT-17-19 for EGFR Kinase Domain

Distance, Å	Interaction	Bond type
3.87	Hydrophobic	π-σ
3.93	Hydrophobic	π-σ
3.89	Hydrophobic	π-σ
4.86	Hydrophobic	Alkyl-
3.64	Hydrophobic	Alkyl-
4.90	Hydrophobic	Alkyl-
4.65	Hydrophobic	π-Alkyl
5.36	Hydrophobic	π-Alkyl
5.19	Hydrophobic	π-Alkyl
5.12	Hydrophobic	π-Alkyl

It is noteworthy that an intramolecular H-bond between the carbonyl O atom and the amine proton with an interatomic distance of 2.21 Å (Fig. 4) may have assisted the formation of the complex of 9-amino-3,3-dimethyl-3,4-dihydroacridin-1(2*H*)-one with the tyrosine kinase active site.

Activation of the intracellular signaling pathway of EGF and its receptor (EGF/EGFR) plays one of the key roles in tumor progression and is closely related to expression of the natural EGFR activating ligand, a homolog of ErbB erythroblastic leukemia viral oncogene. In turn, expression of ErbB was observed to increase during formation of the tumor microenvironment and progression of the neoplasm and upon development of antitumor chemoresistance. In this respect, the search for promising ways of controlling carcinogenic transformation of tumor cells and progression of MNs expressing EGFR is one of the most promising and developing directions in modern molecular pharmacology [11, 12]. The new molecule 9-aminium-3,3-dimethyl-3,4-dihydroacridin-1(2*H*)-one *L*-2-hydroxybutanedioate was synthesized by us. Virtual molecular screening found inhibitor properties in its spectrum of effects against several intracellular targets, e.g., carcinogenesis drivers, among which the most probable was shown to be the kinase domain of EGFR. A preliminary analysis of the chemical structure showed that the presence of the N heteroatom and saturation of the O atom of dihydroacridine system could lead to the appearance of intermolecular interactions with amino-acid residues of the biotarget active site. The presence of the amine could lead to the formation of H-bonds; of the methyls, to the generation of hydrophobic interactions. These were important for addi-

tional stabilization of the molecule-target complexes. Molecular docking based on LHT-17-19 with the EGFRK domain was associated with high affinity and binding energy, which may have been assisted by an intramolecular H-bond that stabilized the ligand in the active site. The results suggested that LHT-17-19 had high antitumor activity against MNs expressing EGFR and justified the need for further in-depth investigations in cell culture and laboratory animals.

Conflict of interest

We declare no conflict of interest.

Financing

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Contributions of authors

DSB developed the concept of the work; SYaS, AIO, EAL, and IVF performed the synthesis and analytical control; DSB, AAE, VAP, and KDB performed the structure-activity analysis; EVBo, EAK, OMT, and DNSh performed the molecular docking experiments; EVS, MVT, EVBl, and EVSh analyzed the data; AIO, EAL, IVF, AAE, VAP, KDB, EVBo, EAK, OMT, DNSh, EVS, and MVT wrote the manuscript; and DSB, SYaS, EVBl, and EVSh edited the manuscript.

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