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SEARCH FOR NEW TYROSINE KINASE INHIBITORS AMONG 2-(3-R-1*H*-1,2,4-TRIAZOL-5-YL)ANILINES AS POTENTIAL ANTITUMOR AGENTS USING MOLECULAR DOCKING

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Abstract

The present work is devoted to the *in silico* study of 2-(3-R-1H-1,2,4-triazol-5-yl)anilines as potential inhibitors of EGFR (epidermal growth factor receptor) and RET (rearranged during transfection)-, which play a significant role in regulating the physiological cycle of non-small cell lung cancer. The well-known docking software AutoDock Vina was used for the study. The seven studied compounds and two standard drugs (vandetanib and gefitinib) were docked to the crystal structures of EGFR and RET proteins. It was found that among the newly investigated substances, 2-(3-(indolyl-2)-1H-1,2,4-triazol-5-yl)aniline (a5) has the highest affinity towards EGFR and RET with the binding energy of –9.7 and –8.7 kcal/mol, respectively. Visualization of the molecular docking results of this compound using the Discovery Studio software showed that it is characterized by similar to standard ligands location in the active sites of the enzymes, stable hydrogen bonds and π-stacking interactions, which are provided by the presence of indole and aniline fragments in the molecule. Thus, we have identified a new effective ligand that can be used as a "base" molecule for further fragment-oriented design using molecular hybridization methodology (fragment fusion, coupling or extension methods) or structural modification by introducing "pharmacophore" groups into the molecule. Summarizing the above screening results, we can say that 2-(3-R-1H-1,2,4-triazol-5-yl)anilines require further careful consideration as effective tyrosine kinase inhibitors for the search for promising anticancer agents for the treatment of non-small cell lung cancer.

Keywords: 2-(3-R-1,2,4-triazol-5-yl)anilines; tyrosine kinase inhibitors; molecular docking; non-small cell lung cancer; antitumor agents.

ПОШУК НОВИХ ІНГІБІТОРІВ ТИРОЗИНКІНАЗИ СЕРЕД 2-(3-R-1*H*-1,2,4-ТРИАЗОЛ-5-ІЛ)АНІЛІНІВ ЯК ПОТЕНЦІЙНИХ ПРОТИПУХЛИННИХ АГЕНТІВ З ВИКОРИСТАННЯМ МОЛЕКУЛЯРНОГО ДОКІНГУ

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Анотація

Дана робота присвячена *in silico* дослідженню 2-(3-R-1H-1,2,4-триазол-5-іл)анілінів як потенційних інгібіторів EGFR (рецептор епідермального фактора росту) та RET (перебудований під час трансфекції), які відіграють значну роль у регуляції фізіологічного циклу недрібноклітинного раку легень. Для дослідження було використано загальноприйняту програму для проведення молекулярного докінгу AutoDock Vina. Сім досліджуваних сполук та два стандартних препарати (вандетаніб та гефітиніб) були зістиковані з кристалографічними структурами білків EGFR та RET. Встановлено, що серед нових досліджуваних речовин найбільшу аффініість до EGFR та RET має 2-(3-(індоліл-2)-1H-1,2,4-триазол-5-іл)анілін (a5) з найвищою енергією зв'язування –9.7 та –8.7 ккал/моль, відповідно. Візуалізація молекулярного докінгу зазначеної сполуки з використанням програми Discovery Studio показала, що для неї характерне подібне до стандартних лігандів розміщення у активних центрах ензимів, стабільні водневі зв'язки та π-стекінгові взаємодії, які забезпечуються наявністю у молекулі індольного та анілінового фрагментів. Отже, нами виявлено новий ефективний ліганд, який можна використовувати як «базову» молекулу для подальшого фрагмент-орієнтованого дизайну з використанням методології молекулярної гібридизації (методи злиття,

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з'єднання або нарощування фрагментів) або структурної модифікації шляхом введення «фармакофорних» груп до молекули. Підсумовуючи наведені вище результати скринінгу, можна сказати, що 2-(3-R-1H-1,2,4-триазол-5-іл)аніліни потребують подальшого детального вивчення з точки зору ефективних інгібіторів тирозинкіназ для пошуку перспективних протипухлинних агентів для лікування недрібноклітинного раку легень.

Ключові слова: 2-(3-R-1,2,4-1*H*-триазол-5-іл)аніліни; інгібітори тирозинкінази; молекулярний докінг; недрібноклітинний рак легень; протипухлинні агенти.

Introduction.

Lung cancer is the most common cancer among oncological diseases. Different countries of the world describe mortality statistics from this disease at the level of 20–30 % of the total number of deaths from cancer [1–4]. Non-small cell lung cancer (NSCLC) accounts for 85 % of all types of cancer of this organ [5]. It develops with multiple changes in the DNA of epithelial cells, which lead to their uncontrolled division and the occurrence of malignant tumors. To date, it has been established that tyrosine kinase receptors play a significant role in regulating the physiological cycle of tumors. It has been proven that receptors belonging to the ErbB family provoke the development of NSCLC [6; 7]. One of these receptors is epidermal growth factor receptor (EGFR), which is recognized as one of the main targets for the treatment of malignant tumors, as it plays a role in controlling the growth, proliferation, and differentiation of cancer cells [8; 9]. Another member of this family is the transmembrane receptor (RET), which differs from other tyrosine kinases in that it requires a co-receptor to recognize and activate the ligand [10]. RET activates the kinase domain independently of the ligand by autophosphorylation or homodimerization, which leads to carcinogenesis [11]. This most often leads to the development of tumors such as medullary thyroid cancer (MTC) and NSCLC [12; 13].

Undoubtedly, the treatment of these diseases is aimed at inhibiting the catalytic activity of tyrosine kinases [14–16]. This group of drugs is collectively known as tyrosine kinase inhibitors (ITK) and is widely used in the treatment of cancer. One of the first and most widely known are multikinase drugs, such as imatinib, erlotinib, gefitinib, the latter two belonging to the anilinoquinazolines [14]. Patients with NSCLC are treated with EGFR TKIs as first-line treatment. Despite their promising clinical success, many patients remain unresponsive to these targeted drugs. The effectiveness of ITK in NSCLC, depends on the mutational status of the epidermal growth factor receptor (EGFR). Exon 19 deletion and L858R point mutation result in poor sensitivity to ITK such as erlotinib and gefitinib [17]. That is, most patients eventually develop resistance to reversible first-generation tyrosine kinase inhibitors, for example, through the T790M point mutation. Therefore, the development of resistance through various EGFR-dependent and EGFR-independent mechanisms posed a new problem, which is solved by the directed search for new «small» molecules of heterocyclic structure with the ability to affect inhibitor-resistant tyrosine kinases.

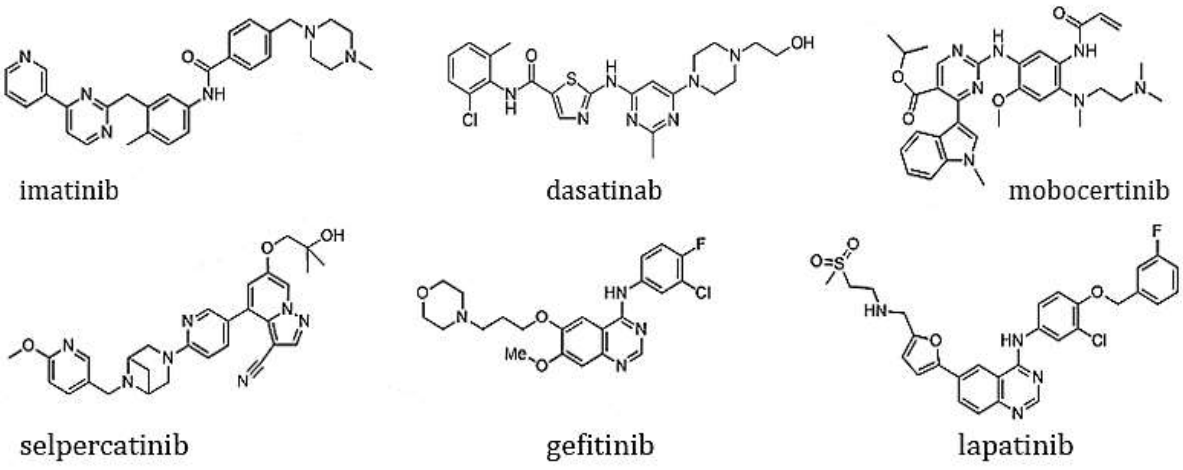

Fig. 1. Examples of medicinal products created using modern fragment-oriented design (methods of merging, connecting or increasing fragments)

The use of anilinoquinazolines as potential hit compounds with «drug-like» properties for enzymatic high-throughput screening (modern fragment-based design) attracted the attention of

medicinal chemists to the search for hybrid molecules or other heterocyclic compounds that bind to the biotarget and remain effective. Thus, new tyrosine kinase inhibitors were created using hybridization methodology (methods of fusion, joining or fragmentation) that combine different molecular fragments in structures (Fig. 1). This approach was used to develop second- and third-generation drugs (irreversible inhibitors) for targeted therapy of NSCLC, such as lapatinib, dasitinib, monocetinib, and others [14; 15; 17–21]

Despite significant advances in the treatment of NSCLC, the problem still remains the occurrence of mutations in kinase receptors and, as a result, a decrease in drug efficacy, as well as severe side effects that can occur in most patients during long-term use (skin rashes, diarrhea, constipation, hypertension, neutropenia, etc.) [18; 22; 23]. Thus, the targeted search for effective ITC drugs does not stop. The present study is devoted to this problem, namely, the search for new effective ligands among 2-(3-*R*-1,2,4-triazol-5-yl)anilines, using *in silico* methodology, which could be further used as «basic» molecules for the design of promising tyrosine kinase inhibitors.

Results and discussion

A series of 2-(3-R-1*H*-1,2,4-triazol-5-yl)anilines (**a1**–**a7**) were selected and studied on biological targets in molecular docking (Fig. 2). The results of the affinity calculations of the compounds were compared with the results of the calculations of the known tyrosine kinase inhibitors vandetanib and gefitinib (Table 1). It was found that the lowest affinity for EGFR- and RET-related proteins was characteristic of compound **a1**. Whereas the introduction of heterocyclic fragments (**a2**–**a7**) to position **3** of the triazole cycle leads to a significant increase in their affinity for the corresponding proteins. This aspect can be explained by the presence of additional donor-acceptor centers in the molecules. Thus, in the case of compounds **a2** and **a5**, the acceptor NH-acid center of pyrrole and indole, and in compounds **a3**, **a4**, a6 and **a7**, the donor center arises from the unshared electron pair of oxygen and sulfur in the corresponding heterocycles (Fig. 2, Table 1).

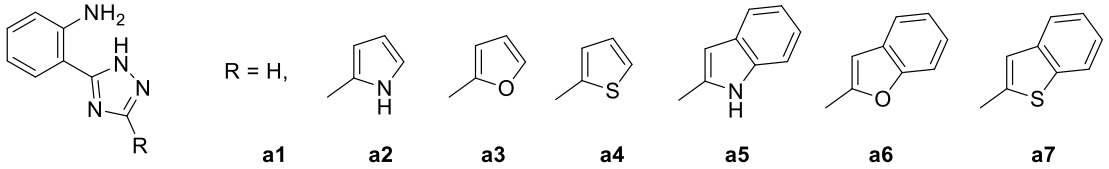

Fig. 2. Structures of 2-(3-R-1*H*-1,2,4-1,2,4-triazol-5-yl)anilines (a1-a7)

In our opinion, the presence of additional donor-acceptor centers will promote additional binding of compounds **a2**–**a7** to amino acids in the active site of the enzymes. In addition, compounds **a5–a7** have higher affinity compared to **a2**–**a4**, which is probably due to the presence of another benzene fragment and, as a result, their higher lipophilicity. Compound **a5** (2-(3-(indolyl-2)-1*H*-1,2,4-triazol-5-yl)aniline) showed the best affinity for biological targets, with values of -9.7 and -8.7 kcal/mol, respectively, for RET and EGFR proteins.

Table 1

Affinity of compounds a1-a7 to binding sites of EGFR-unrelated protein (2ITY) and RET-related protein (2IVU), kcal/mol

№	Compounds	Affinity (kcal/mol) for RET, PDB ID - 2IVU	Affinity (kcal/mol) for EGFR, PDB ID - 2ITY
1	**a1**	–6.6	–6.6
2	**a2**	–8.0	–7.2
3	**a3**	–8.1	–7.0
4	**a4**	–8.0	–7.0
5	**a5**	–9.7	–8.7
6	**a6**	–9.6	–8.7
7	**a7**	–9.7	–8.6
8	vandetanib	–10.0	–
9	gefitinib	–	–8.1

Subsequently, for the synthesized compounds **a1–a7**, the probable mechanism of biological activity was investigated by visualizing molecular docking to enzymes. To find out whether the reproducibility of the placement of the reference ligands (vandetanib and gefitinib) in the active site was observed, as well as the validity of the chosen methodology, a re-docking procedure was performed (Fig. 3).

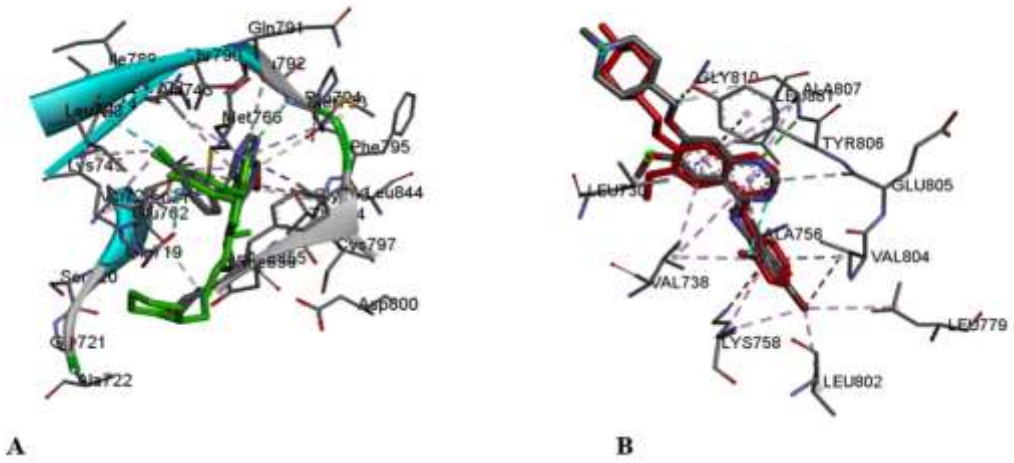

Fig. 3. Visualization of re-docking of reference ligands gefitinib (green color) and vandetanib (red color) relative to the native position (gray color): A for EGFR kinase, B for RET kinase

In this case, we achieved complete reproducibility of the bonds that are recorded in the active sites. Their identification was determined by computer visualization of the structures of 2ITY (gefitinib) and 2IVU (vandetanib), as well as by searching the literature data [24–26].

In addition to evaluating the inhibitory ability of compound **a5** by determining the affinity for reference ligands, the very placement of the compound in the active site was also analyzed: conformational arrangement, interaction with amino acid residues, and types of bonds formed (Table 2, Fig. 4, 5).

Comparison of the affinity of compound **a5** for the RET receptor showed that its value is slightly lower than that of the standard ligand, respectively –9.7 and –10.0 kcal/mol (Table 1). Visualization revealed that the amino acid residues of the active site were all involved in binding to the target compound. Only the absence of hydrophobic interaction was observed, which is provided by Leu802 (4.50 Å) and Leu779 (4.96 Å) with vandetanib bromine (Table 2, Fig. 4). This is due to the absence of halogen in the structure of compound **a5**. Instead, additional hydrogen bonds appear between Glu775 (2.20 Å), Asp892 (2.75 and 2.97 Å), and SER891 (2.50 Å) and the amino group of the aniline fragment of the molecule **a5**. Such interactions indicate strong fixation of compound **a5** in the receptor pocket, and hydrogen bonds with the above-mentioned amino acids create additional conformational stability [26]. In addition, compound **a5** is well located in the hydrophobic pocket, with the same size and location as vandetanib bromine (Fig. 6A). In the earlier studies [27; 28] a direct correlation between the formation of hydrogen bonds and the stability of inhibitor molecules was observed. The presence of a π-cationic bond as an electrostatic interaction between the receptor and the ligand, in this case between the LYS758 residue (4.82 Å) and the benzene ring of aniline moiety, contributes to high specificity, selectivity, increases the probability of recognition by the target protein, and improves the bioavailability of the compound [29; 30].

Table 2

Main types of interaction of compounds a1–a7 and pharmacological standards with amino acid residues of enzymes

№	Compound	RET, PDB ID - 2IVU*	EGFR, PDB ID - 2ITY*
1	**a1**	Asp892 (2.59)[a], Asp892 (2.59)[a], Asp892 (4.79)[d], Val738(3.98)[c], Val738 (3.91)[c], Val804 (4.89)[c], Val804 (3.97)[c], Leu881 (3.72)[c], Ala756(4.81)[c], Lys758 (4.88)[c], Ile788*(4.77)[c]	Gln791 (2.18)[a], Thr790 (3.56)[c], Leu844 (3,73)[c], Met766 (5.12)[c], Gys775 (5.98)[d], Val726 (5.18)[c], Ala743 (3.90)
2	**a2**	Asn879 (2.86)[a], Asp874 (3.01)[a] Asp874 (4.34)[d], Gly736(2.36)[a], Asp892(3.41)[d], Phe735 (4.63)[c], Leu895 (4.74)[c], Val738 (4.97)[d]	Met793 (2,55)[a], Leu718 (3.68)[c], Thr790(3.67)[c], Met766 (5.61)[d], Leu844 (3.67)[c], Ala743 (3.60)[c], Ala743 (5.27) [c], Lys745 (5.00)[c]
3	**a3**	Glu805 (2.76)[a], Ala756 (5.05)[c], Ala756 (4.28)[c], Lys758 (4.97)[d], Lys758 (4.76)[c], Asp892 (4.91)[d], Leu730 (3.90)[c], Val804 (3.73)[c], Val804 (3.99)[c], Leu881 (3.59)[c],	Thr854 (2.15)[a], Leu718 (3.87)[c], Leu718 (3.87)[c], Leu718 (3.87)[c], Thr790(3.82)[c], Met766 (5.29)[d], Leu844 (3.60)[c], Ala743 (3.63)[c], Ala743 (5.19)[c], Lys745 (4.95)[c]

Continued table 2

		Leu881 (3.91)[c], Tyr806 (5.54)[c], Val738 (4.28)[c], (5.49)[c], Ala756 (5.05)[c], Ala756 (4.28)[c], Ala807 (4.68)[c], Lys758 (4.76)[c]	
4	**a4**	Ser891 (2.22)[a], Asp892 (2.31)[a], Lys758 (4.88)[d], Lys758 (3.68)[c], Val738 (3.71)[c], Val738 (5.35)[c], Leu730 (5.04)[c], Val804 (3.59)[c], Val804 (3.92)[c], Leu881 (3.93)[c], Leu881 (3.73)[c], Ala756 (5.32)[c], Ala756 (4.75)[c], Tyr806(5.80)[b], Ala756 (4.99)[d], Ala756 (4.33)[c], Ala807 (4.75)[c]	Leu718 (3.70)[c], Thr790 (3.76)[c], Leu844 (3.65)[c], Met766 (5.31)[d], Ala743 (3.61)[c], Ala743 (5.34)[c], Lys745 (4.98)[c]
5	**a5**	Ser891 (2.50)[a], Asp892 (2.75)[a] Asp892 (2.97)[a], Glu775* (2.20)[a], Lys758 (4.82)[d], Lys758 (3.70)[c], Leu730 (3.42)[c], Leu730 (4.80)[c], Val738 (3.72)[c], Val738 (4.74)[c], Val804(3.62)[c], Val804 (3.97)[c], Leu881 (3.91)[c], Leu881 (3.65)[c], Ala756 (5.32)[c], Ala756 (4.75)[c]	Met793 (2.79)[a], Thr854 (2.35)[a], Leu718 (3.67)[c], Leu718 (3.87)[c], Leu718 (3.87)[c], Thr790 (3.63)[c], Met766 (5.13)[d], Leu844 (3.63)[c], Ala743 (3.67)[c], Ala743 (5.24)[c], Lys745 (5.01)[c]
6	**a6**	Glu775 (2.31)[a], Ser891 (2.82)[a], Asp892 (2.49)[a], Lys758 (4.84)[d], Lys758 (3.72)[c], Val738 (3.74)[c], Val738 (4.74)[c], Leu730 (3.43)[c], Leu730 (4.85)[c], Val804 (3.57)[c], Val804 (3.97)[c], Leu881 (3.91)[c], Leu881 (3.66)[c], Tyr806 (5.80)[b], Ala756 (4.65)[d], Ala756 (5.21)[c]	Leu718 (3.71)[c], Leu718 (3.70)[c], Leu718 (3,96)[c], Thr790 (3.75)[c], Leu844 (3.62)[c], Met766 (5.18)[d], Ala743 (3.61)[c], Ala743 (5.04)[c], Lys745 (4.94)[c]
7	**a7**	Glu775 (2.21)[a], Ser891 (2.35)[a], Asp892 (2.92)[a], Asp892 (2.88)[a], Lys758 (4.83)[d], Lys758 (4.69)[c], Val738 (3.70)[c], Val738 (4.62)[c], Leu730 (3.42)[c], Leu730 (4.74)[c], Val804 (3.62)[c], Val804 (3.95)[c], Leu881 (3.92)[c], Leu881 (3.65)[c], Ala756 (5.44)[c], Ala756 (5.00)[c]	Thr854 (1.97)[a], Leu718 (3.65)[c], Leu718 (3.80)[c], (3.81)[c], Thr790 (3.63)[c], Leu844 (3.65)[c], Met766 (5.11)[d], Ala743 (3.65)[c], Lys745 (4.99)[c]
8	gefitinib	–	Met793 (2.67)[a], Met793 (5.38)[c], Gly796 (3.42)[b], Leu718 (3.40)[b], Leu718 (5.0)[c], Leu718 (4.08)[c], Pro794 (3.37)[b], Gln791 (3.42)[b], Leu788 (3.18)[d], Glu762 (3.41)[d], Leu844 (3.78)[c], Leu844 (5.24)[c], Lys745 (4.25)[c], Lys745 (4.68)[c], Val726 (5.44)[c], Ala743 (3.79)[c], Ala743 (5.09)[c]
9	vandetanib	Ala807 (3.08)[a], Ala807 (4.57)[c], Ala807 (3.69)[b], Gly810 (3.28)[b], Tyr806 (3.65)[b], Tyr806 (5.98)[c], Tyr806 (5.19)[c] Tyr806 (4.57)[c], Glu805 (3.31)[b], Ala756 (3.65)[d], Ala756 (4.08)[c], Ala756 (3.63)[c], Lys758 (4.88)[d], Lys758 (3.99)[c], Lys758 (4.28)[c], Leu730 (3.77)[c], Leu730 (4.86)[c], Leu881 (3.77)[c], Leu881 (3.81)[c], Leu779 (4.96)[c], Leu802 (4.50)[c], Val804 (4.43)[c], Val804 (4.34)[c], Val738 (5.30)[c], Val738 (4.85)[c]	–

Notes: * – bond length is in Å, a – conventional hydrogen bond; b – carbon-hydrogen bond; c – hydrophobic bond; d – other type of bond.

The docking of compound **a5** to EGFR indicates a higher affinity compared to gefitinib, that is –8.7 and –8.1 kcal/mol, respectively (Table 1). The placement of compound **a5** in the hydrophobic pocket is optimal and fully ensures the fixation of the molecule with amino acid residues of the active site, that are similar to the reference ligand (Fig. 5B). However, compound **a5** has an additional hydrogen bond between Thr854 (2.35 Å) and the amino group of the aniline fragment (Fig. 5). It is important to note that the hydrogen bond of the indole NH group with Met793 (2.79 Å) is the leading stabilizing bond, which is also observed in anilinoquinazoline inhibitors of EGFR [31–33].

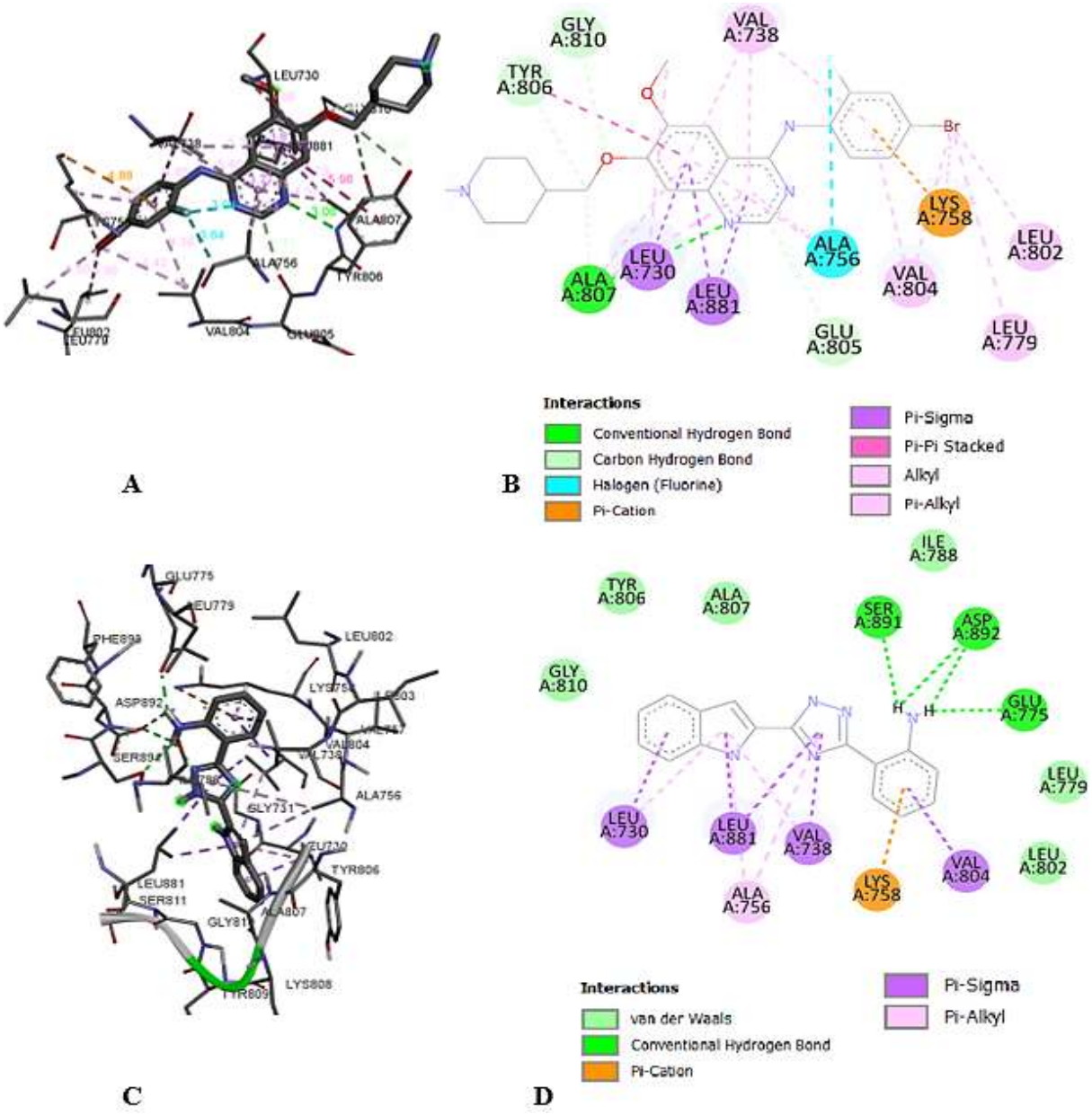

Fig. 4. Interaction of vandetanib (A, B) and compound a5 (C, D) with amino acids in the active site of RET

In addition, there is a π-sulfide bond of Met766 (5.13 Å), a π-sigma bond of Thr790 (3.63 Å), and a π-alkyl interaction of Lys745 (5.01 Å) with the benzene fragment of aniline. Additionally, the π-alkyl interaction between Ala743 (5.24 Å) and the triazole system of the ligand **a5** can be traced. In general, the existing π-stacking in this receptor-ligand interaction has an impact on receptor stabilization and enzyme catalysis [34].

The molecular docking results showed that the presence of an indole fragment in compound **a5** provides a significant number of bonds with enzymes, π-alkyl bonds with RET, π-sigma bonds and hydrogen bonds with EGFR. In addition, in both cases, compound **a5** has more hydrogen bonds with proteins than the standard ligands, that should ensure a more stable structure with the receptor.

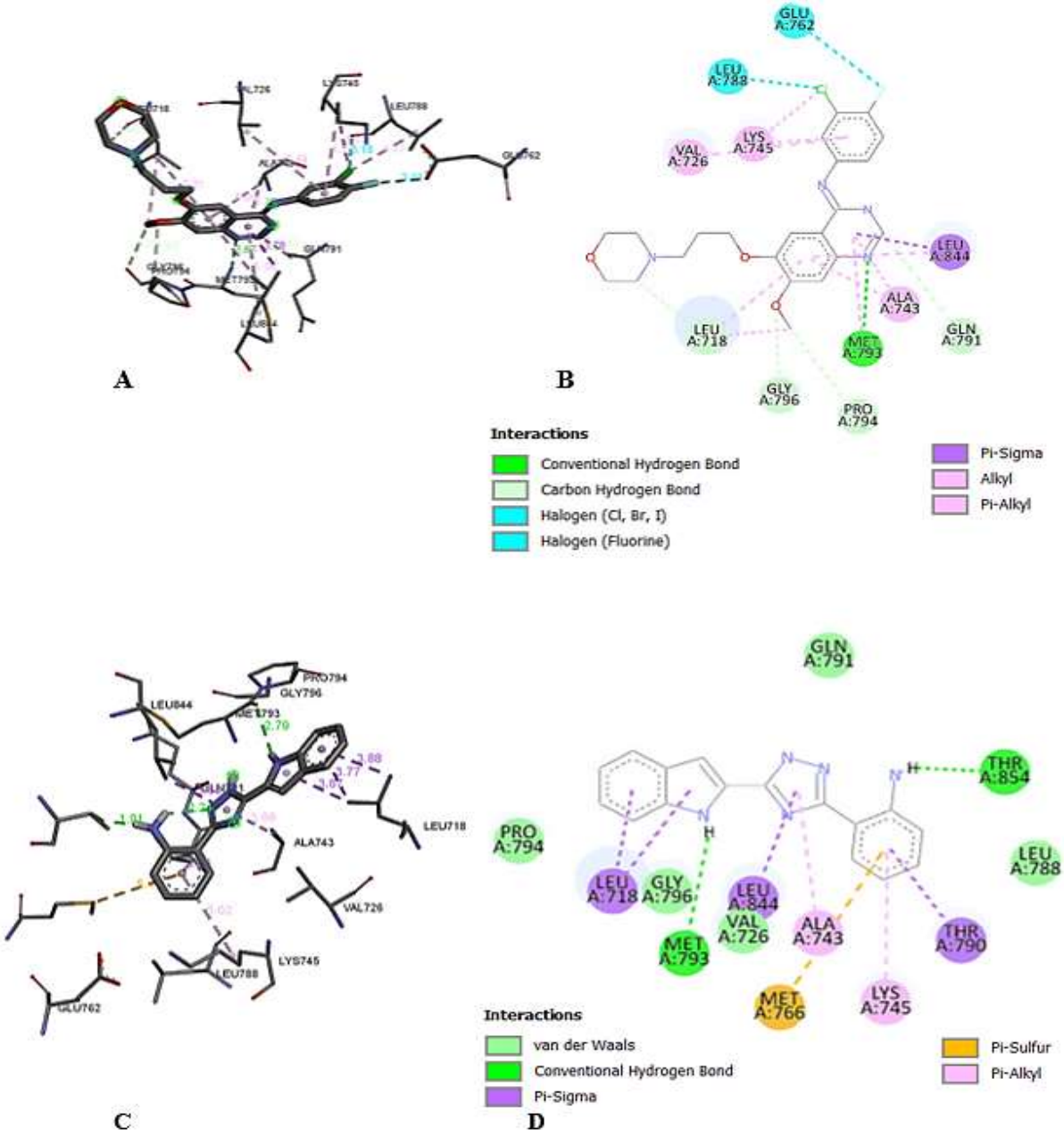

Fig. 5 Interaction of gefitinib (A, B) and compound a5 (C, D) with amino acid residues in the active site of EGFR

In general, as for the aniline fragment in compound **a5**, which is the result of nucleophilic degradation of the quinazoline cycle [35], it stabilizes the ligand-receptor interaction more rigidly due to hydrogen bonds and π-bonds, compared to the standard ligands. In both cases, the triazole fragment is fixed in the pocket by means of π-sigma and π-alkyl interactions, i.e., hydrophobic bonds prevail. All of the above contributes to the similar placement of the structure **a5** in the receptor pocket as in the case of standard ligands (Fig. 6).

According to calculations the 2-(3-R-1,2,4-1*H*-triazol-5-yl)anilines are effective ligands with high affinity for EGFR and RET enzymes, and visualization confirmed the important role of the aniline fragment at position **5** and the donor-acceptor substituents at position **3** of the triazole molecule on the location in the active site of the enzymes and their conformational stability. The structures **a5–a7** can be further used in fragment-oriented design (molecular modeling, virtual screening, ADME analysis) due to the unlimited functionalization of benzene fragments and position **3** of the triazole moiety, that will increase their antitumor activity [36; 37].

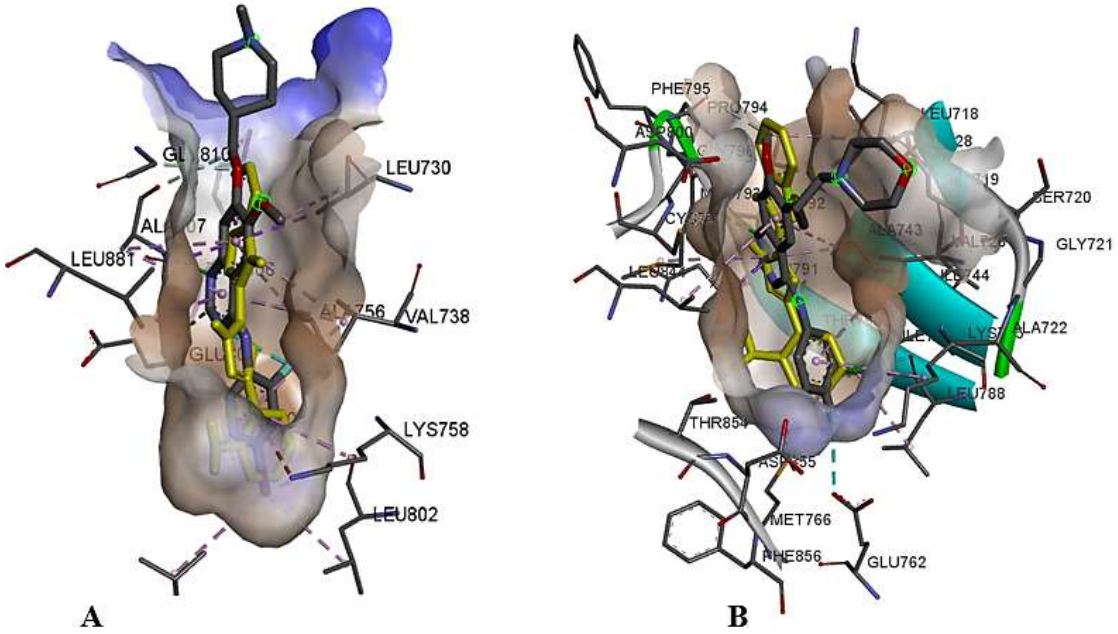

Fig. 6 Compatible conformation and placement in the hydrophobic pocket of compound a5 (yellow) with vandetanib (A) and gefitinib (B)

Moreover, the analysis of the results of molecular docking of compound **a5** and its comparison with standard ligands (gefitinib, vandetanib) allows us to unequivocally state that compound **a5** has antitumor activity. This has been confirmed by previous studies [38]. Thus, the dose-dependence on NSCLC cell lines in the concentration range of 100-0.01μM was studied for compound **a5** according to the standard procedure of the National Cancer Institute and it was shown that it exhibits significant anticancer activity against 9 NSCLC cell lines (Table 3).

Table 3

Anticancer activity of compound a5 against NSCLC cell lines in the concentration range of 100-0.01μM according to data [41]

Cell lines *	$logGI_{50}$	logTGI	$logLC_{50}$
A549/ATCC	–5.66	–4.57	>–4.00
EKVX	–5.40	>–4.00	>–4.00
HOP-62	–5.33	–4.71	–4.25
HOP-92	–4.72	–4.21	>–4.00
NCI-H226	–5.30	–4.69	–4.31
NCI-H23	–5.48	–4.69	–4.08
NCI-H322M	–5.37	–4.46	>–4.00
NCI-H460	–5.53	–4.57	>–4.00
NCI-H522	–5.89	–4.92	–4.38

Note: $logGI_{50}$ – inhibition level, logTGI - cytostatic effect, $logLC_{50}$ – cytotoxic effect

Experimental Section

For molecular docking, 2-(3-R-1*H*-1,2,4-triazol-5-yl)anilines (**a1–a7**) were selected, the methods of synthesis of which are known [38].

The study was conducted by the method of flexible molecular docking, as an approach to finding molecules with affinity for a specific biological target. The crystal structures of EGFR (PDB code 2ITY) and RET (PDB code 2IVU) proteins from the Protein Data Bank were used to determine the inhibitory activity of compounds **a1–a7** [39]. The choice of biological targets was based on the literature data on the mechanism of action of anticancer drugs [24; 25].

Preparation of the protein macromolecule

The tyrosine kinase molecules were prepared for docking by removing water molecules and ligands in the structure in the Discovery Studio

program. Using the program AutoDockTool-1.5.7, the lost enzymes atoms were found, and the structure of the enzymes was restored, polar hydrogen atoms were added, and charges were distributed. The structures were saved in pdbqt format. A grid-box was created in the prepared molecule at the defined active site. For RET, the active site corresponds to the following amino acid residues: LEU730, VAL738, ALA756, LYS758, LEU779, LEU802, VAL804, GLU805, TYR806, ALA807, GLY810, LEU 881. The dimensions of the grid-box, according to the described active site, are as follows: center_x = –21.431center_y = 5.522, center_z = –10.612, size_x = 40 size_y = 64 size_z = 50. For EGFR, the active site was determined by the following amino acids: LEU718, VAL726, ALA743, LYS745, GLU762, LEU788, GLN791, MET793, PRO794, GLY796, LEU718. The dimensions of the grid box, according to the described active site, are as follows: center_x = –52.773 center_y = –1.805, center_z = –24.680, size_x = 64 size_y = 60 size_z = 72.

Preparation of ligands

Structures of the studied ligands (**a1**–**a7**) were created using Gauss View5.0 and optimized using Gaussian 09W at the pm3 level of theory. For docking, the compound files were converted to pdb format using OpenBabel. The torsional angles and centers for ligand rotation were determined using AutoDockTool-1.5.7. The substances were saved in the pdbqt format. As a comparison, two compounds were selected - vandetanib and gefitinib, which are contained in the X-ray crystallographic structures of EGFR (PDB code 2ITY) and RET (PDB code 2IVU) proteins. The calculations required for docking were performed using the AutoDock Vina program [40]. To control the accuracy of the results, re-docking of vandetanib and gefitinib inhibitors in the corresponding protein structures was performed. The results were visualized using the Discovery Studio v21.1.20298 program [41].

Conclusions

The *in silico* study of 2-(3-R-1*H*-1,2,4-triazol-5-yl)anilines revealed a new effective ligand, 2-(3-(indolyl-2)-1*H*-1,2,4-triazol-5-yl)aniline (**a5**), which is the potentially good inhibitor of EGFR and RET with the highest binding energy of –9.7 and
–8.7 kcal/mol, respectively. It has been shown that the affinity for receptors directly depends on the aniline fragment at position **5** and the donor-acceptor substituents at position 3 of the triazole moiety. Visualization of the molecular docking of compound **a5** showed that it is characterized by location similar to standard ligands in the active sites of enzymes, stable hydrogen bonds and π-stacking interactions, which are provided by the presence of indole and aniline fragments in the molecule. These structures can be further used as "basic" molecules in the fragment-oriented design due to unlimited functionalization of benzene fragments and the **3**-position of triazole, that will increase their antitumor activity.

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