Docking Study of 3-mercapto-1,2,4-triazole Derivatives as Inhibitors for VEGFR and EGFR

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Computer-aided drug design plays an important role in modern day drug discovery, because it provides a more specific range for active compound chemical synthesis in detriment of the traditional ways of drug discovery. Relevant studies proved that the epidermal growth factor receptor (EGFR) and vascular endothelial growth factor receptor (VEGFR) are important targets for inhibition, in finding new molecules with potential anticancer activity. The aim of the present study was to create a compound library and submit this set of molecules to a docking-based virtual screening process. Molecular docking was carried out using OEDocking HYBRYD, a software with an improved scoring algorithm, which uses a ligand-based scoring function. The obtained results revealed some molecular structures that showed good predicted binding affinity towards their respective protein targets.

Keywords: EGFR, VEGFR, inhibitor, docking, triazoles

Cancer represents a significant and challenging health issue all over the world, which requires intense scientific efforts in order to clarify the various mechanisms involved in the pathological development and also to provide new alternative therapies [1-4].

Angiogenesis represents an important process in the development of tumors by means of new blood vessel formation in the involved tissue. One key signaling cascade that regulates this process is the vascular endothelial growth factor (VEGF) pathway [5]. In colorectal cancer, VEGF-á is the predominant proangiogenic factor [6] that regulates angiogenesis and tumor cell growth by binding to one of the three tyrosine-kinase receptors, also known as the vascular endothelial growth factor receptors (VEGFR), namely VEGFR-2 [7]. VEGF can be stimulated by the activation of the epidermal growth factor receptor (EGFR) pathway. EGFR is part of a transmembrane receptor family, frequently overexpressed in different cancer cells. The EGFR downstream signaling pathway regulates important processes correlated with cancer pathology such as, cell differentiation, growth, migration and apoptosis.

The inhibition of the two signaling pathways mentioned above, represents a relevant strategy for finding novel molecules with anticancer effects. This fact is sustained by a significant number of currently existent anticancer agents approved for certain types of cancer treatment, that are EGFR and VEGFR inhibitors. In recent years, structure-based drug design (SBDD) and virtual screening, played an important role in aiding the discovery of EGFR and VEGFR inhibitors, by identifying inhibitor molecules for the two receptors, with notable inhibitory activities, as shown by some studies [8-13].

The aim of the current study is to predict compounds with potent anticancer activity, by means of docking-based virtual screening against two active key proteins in the EGFR and VEGFR pathways (VEGFR-2 and EGFR-1), [14,15] using a contrived library of molecules.

Experimental part

Materials and methods

Compound library building

For the aim of this study, we have created a compound library containing 3-mercapto-1,2,4-triazole derivatives by introducing different substituents on the 1,2,4-triazole ring, in the fourth and fifth position (R1, R2) and on the thiol group from the third position. The general structure of these molecules is depicted in Figure 1. The library was directed towards triazole derivatives because we possess a reagent portfolio necessary to obtain a relatively high variety of triazole derivatives. Molecules containing the 1,2,4-triazol scaffold, are known to exhibit multiple biological activities, including anticancer activity, as shown by a search carried out on the WOMBAT database [16]. Furthermore, recent studies have reported anticancer activity of synthesized 3-mercapto-1,2,4-triazole derivatives, on different cancer cell lines [17-19].

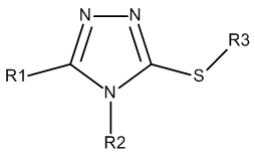

Fig. 1. General structure of the molecules comprising the compound library

Compound library refinement

The compound library (469 molecules) was prepared using OMEGA 2.5.1.4 (OpenEye Scientific Software, Inc.). [20] The library was filtered using OMEGA's BlockBuster filter, applying the default input parameters. After this filtering, for each of the 315 remaining molecules a conformer generation was done (optical and geometric isomers of the same molecule were treated as distinct compounds), resulting in 200 conformers per each

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Table 1

THE SCORING METHOD IN COMPARISON WITH THE BOUNDED LIGAND USED FOR THE DOCKED MOLECULES, WHICH TAKE INTO ACCOUNT THE MOLECULAR INTERACTIONS FORMED IN THE PROTEIN BINDING SITE

Type of ligand-receptor interaction	Interaction formed with the same AA as in the case of the bound ligand	Interaction formed with a different AA as in the case of the bound ligand	Assigned score
Conventional H bond	X	-	4
	-	X	3
Hydrophobic interactions	X	-	2
	-	X	1
Electrostatic interaction	X	-	2
	-	X	1
Other types of interaction	X	-	2
	-	X	1

X -present; - absent

structure. The library created in this way was subjected to molecular docking experiments.

Docking

Molecular docking was carried out using OEDocking HYBRYD. [21] Unlike its predecessor (FRED), HYBRYD, has an improved scoring algorithm, which uses a ligand-based scoring function. In addition, HYBRYD allows the selection of multiple protein targets that can be used in the docking process.

Three-dimensional crystallographic structures of the target proteins selected for this study, VEGFR-2 and EGFR-1, were obtained from the RCSB ProteinDataBank. [22] For molecular docking, the data base was screened against multiple 3D structures of the same protein, allowed by the docking software. Three-dimensional structures were selected, prior to the docking process, taking into account: i) protein structures with a co-crystallized ligand, as required by the docking program; ii) protein structures that do not exhibit structural mutations; iii) protein structures that have a Cruickshank DPI (diffraction precision index) [23] under 0.5. Finally the following 3D structures were used for each protein in the docking process EGFR-1: 1M17, 2J5E, 2RGP, 3BEL, 3POZ, 3W2S, 3W32, 3W33, 4G5P, 4JQ8, 4JRV, 4LI5, 4RJ4, 4JRJ5, 4RJ6, 4RJ7, 4RJ8, 4WKQ; VEGFR-2: 1Y6A, 1Y6B, 1YWN, 2OH4, 2P2H, 2QU5, 2RL5, 2XIR, 3C7Q, 3CJG, 3CJF, 3EWH, 3U6J, 3VHE, 3VHK, 3VID, 3VNT, 3VO3, 4AG8, 4AGC, 4AGD, 4ASD, 4ASE. The 3D structures were prepared as receptors suitable for docking, using OEDocking's MakeReceptor, a program with a guided user interface. [20] The co-crystallized ligand was saved as part of the receptor file, as it is required by the docking software.

The library was docked in both sets of 3D protein structures, corresponding to VEGFR-2 and EGFR-1. The docking program scores only one (the best) conformational pose per structure for each target in which it was docked, after which the respective conformer is removed from the docking list.

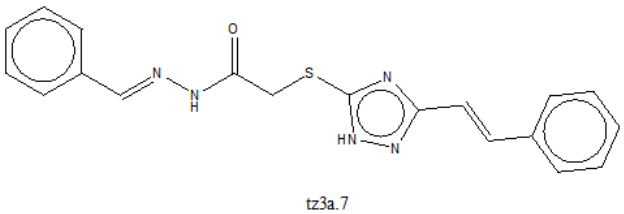
Results and discussions

After the molecular docking process, the compounds were scored using HYBRID's, ligand based scoring function (Chemgauss 4). [20] In analyzing the result from both sets of 3D structures used as targets, one structure was selected, namely that with the highest number of docked molecules. The two proteins were: for VEGFR-2, 3VHK and for EGFR-1, 4RJ8. Because the docking program has a ligand-based scoring function, choosing the structure with the highest number of docked compounds, results that the respective conformation of that protein is the most adequate for later receptor-target binding analysis.

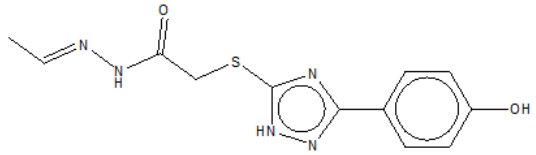
After this selection, for the protein structure in each case, the first 50 ranked compounds (using the Chemgauss 4 score) were scored, using a method conceived by us. The method comprises a similarity evaluation between the docked molecules and the protein's co-crystallized ligand, regarding the number and type of interactions formed in the target's binding site. Briefly: (a) for each protein target and its respective co-crystallized ligand, all interactions formed in the active binding site, were mapped according to the OCA browser-database for protein structure/function [24], (b) afterwards for each of the 50 compounds docked, every interaction formed within the binding site was scored depending on type and similarity between the docked molecules and the co-crystallized ligand. Scores assigned for each interaction present in the binding site between a docked compound and the target protein, are shown in table 1. For each target, the 50 compounds mentioned above were ranked again according to the sum of scores.

The top compounds docked for the two targets, were once more visually analyzed regarding the positioning in the respective protein binding site and the overall conformational matching with the co-crystallized ligand.

In the case of VEGFR-2, a compound coded tz3a.7 (fig. 2) proved to be very promising. The compound binds well in the protein active site back pocket, as shown in figure 3A, exhibiting key binding features such as, two hydrogen bonds (HB) with ASP1046 and two hydrophobic interactions between the compound's triazole ring and residues VAL898 and LEU1019, in the hydrophobic region of the back pocket. This type of binding could mean that compound tz3a.7 would prove to be a like a kinase back pocket inhibitor as defined in the study of Iwata *et al.* [25] In the Iwata model the kinase inhibitors interact with a hydrophobic back pocket, leaving unoccupied the hinge

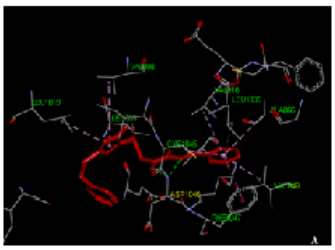


tz3a.7



tz9.6

Fig. 2. Chemical structures of compounds tz3a.7 and tz9.6



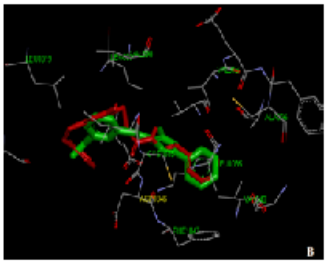
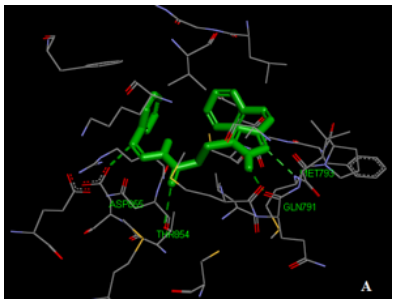


Fig. 3. (A) Binding interactions formed between compound tz3a.7 and the binding site of VEGFR-2 (PDB ID: 3VHK), HB are depicted in green dotted lines and hydrophobic interactions in purple. (B) overlay of compound tz3a.7 (red) and the co-crystallized ligand (green); only the interacting amino acids are labeled

region of the active site, where the ATP molecule would bind. Furthermore tz3a.7 also interacts with the hinge region of the binding site by forming multiple hydrophobic interactions through a phenyl ring. The compound also showed good overall co-planarity with the co-crystallized ligand of the protein structure used as target (fig. 2B).

The same structure yielded a good score in the docking process carried out with EGFR-1. Figure 4 depicts the compound tz3a.7 bound in the active site of the EGFR-1 protein (PDB ID: 4RJ8) and the interactions formed within the binding site. The compound forms HBs with key amino acids, present also in other cases of EGFR-1 inhibition, [9] such as THR854, ASP855, GLN791 and an important HB with MET793 (fig. 4A), interaction present in the case of



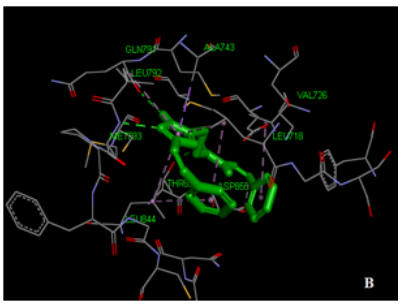
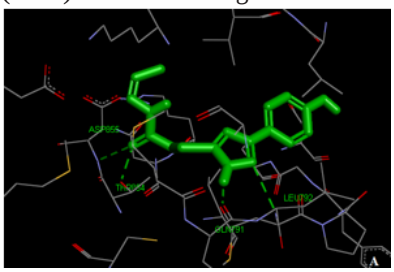


Fig. 4. (A) HB formed (green dotted lines) between compound tz3a.7 and the binding site of EGFR-1 (PDB ID: 4RJ8); (B) HB (green dotted lines) and hydrophobic (purple dotted lines) interactions formed between compound tz3a.7 and the binding site of EGFR-1 (PDB ID: 4RJ8); only the interacting amino acids are labeled

the EGFR inhibitor erlotinib [26]. The molecule is also well stabilized by hydrophobic interactions formed with LEU718, VAL726, LEU792 and ASP844 (fig. 4B).

Another compound coded tz9.6 (fig. 2) develops good binding towards EGFR-1. This molecule also forms the important HBs mentioned above, with THR854, ASP855, GLN791 and MET793 (fig. 5A) and a significant number of hydrophobic interactions with amino acids: LEU718, VAL726, ALA743, LYS745, MET793, LEU844 (fig.5B).

Compound tz9.6 received the highest score after our proposed method and shows good overlay with the co-crystallized ligand of the 3D structure of the EGFR-1 protein (4RJ8) as shown in figure 6.



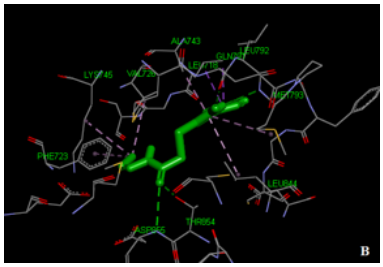


Fig. 5. (A) HB formed (green dotted lines) between compound tz9.6 and the binding site of EGFR-1 (PDB ID: 4RJ8); (B) HB (green dotted lines) and hydrophobic (purple dotted lines) interactions formed between compound tz9.6 and the binding site of EGFR-1 (PDB ID: 4RJ8); only the interacting amino acids are labeled

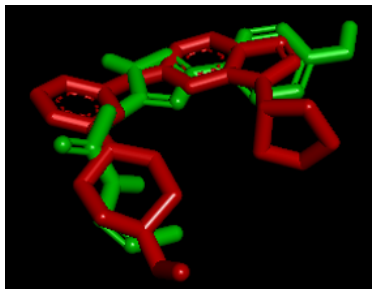


Fig. 6. Superimposition of compound tz9.6 (green) with the co-crystallized ligand of 4RJ8 (red)

Conclusions

A compound library containing 3-mercapto-1,2,4-triazole derivatives, was created for the purpose of predicting molecules with potential anticancer effects active in colorectal cancer, by means of docking-based virtual screening. After screening the library against two protein targets (VEGFR-2 and EGFR-1) and refining the search, we obtained two molecules, tz3a.7 and tz9.6 that showed good binding affinity. As resulted from our study compound tz3a.7 probably will be able to inhibit both protein targets, and would be a very useful dual VEGFR/EGFR inhibitor.

The synthesis and validation of the predicted activity for the two molecules constitute the subject of a forthcoming paper.

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References

1.FALAMAS, A., PINZARU, S.C., DEHELEAN, C.A., et al., J. Raman Spectrosc., **42**, 2011, p. 97.
2.DEHELEAN, C.A., SOICA, C., PEEV, C., et al., Rev. Chim. Farmacia, **59**, 2011, p.51.
3.SOICA, C., PEEV, C., CIURLEA, S., et al., Farmacia, **58**, 2010, p. 611.

4.HEGHES, A., SOICA, C.M., ARDELEAN, S., AMBRUS, R., MUNTEAN, D., GALUSCAN, A., DRAGOS, D., IONESCU, D., BORCAN, F., Chem Cent J, **7**, 2013, p. 66.
5.SUN, W., Journal of Hematology & Oncology, **5,** 2012, p. 63.
6.ARAUJO JR, R.F., LIRA, G.A., VILACA, J.A., GUEDES, H.G., LEITAO, M.C.A., LUCENA, H.F., RAMOS, C.C.O., Pathology – Research and Practice, **211**, 2015, p. 71.
7.RIZVI, S.U.F, SIDDIQUI, H.L., NISAR, M., KHAN, N., KHAN, I., Bioorg. Med. Chem. Lett., **22**, 2012, p. 942.
8.ABDEL AZIZ, Y.M., SAID, M.M., EL SHIHAWY, H.A., ABOUZID, K.A.M., Bioorganic Chemistry, **60**, 2015, p. 1.
9.BELAL, A., Bioorganic Chemistry, **59**, 2015, p. 124.
10.LI, S., SUN, X., ZHAO, H., TANG, Y., LAN, M., Bioorg. Med. Chem. Lett., **22**, 2012, p. 4004.
11.WU, X.Y., WAN, S.H., WANG, G.F., JIN, H., LI, Z.H., TIAN, Y.X., ZHU, Z.G., ZHANG, J.J., Journal of Molecular Graphics and Modelling, **56**, 2015, p. 103.
12.DANCIU, C., SOICA, C., OLTEAN, M., et al., Int J Mol Sci, **15**, 2014, p. 1962.
13.DEHELEAN, C.A., SOICA, C., PEEV, C., et al., Rev. Chim. (Bucharest), **59**, 2008, p. 887.
14.ZHU, Q., IZUMCHENKO, E., ALIPER, A.M., MAKAREV, E., PAZ, K., BUZDIN, A.A., ZHAVORONKOV, A.A., SIDRANSKY, D., Human Genome Variation, **2**, 2015.
15.SMITH, N.R., Baker, D., James, N.H., Ratcliffe, K., Jenkins, M., Ashton, S.E., Sproat, G., Swann, R., Gray, N., Ryan, A., Jürgensmeier, J.M., Womack, C., Clin Cancer Res, **16**, 2010, p. 3548.
16.OLAH, M., RAD, R., OSTOPOVICI, L., BORA, A., HADARUGA, N., HADARUGA, D., MOLDOVAN, R., FULIAS, A., MRACEC, M., OPREA, T.I., Chemical Biology: from Small Molecules to System Biology and Drug Design, **1-3**, S. L. Schreiber, T. M. Kapoor and G. Wess, Wiley-VCH Verlag GmbH, Weinheim, Germany, 2008.
17.LI, X., LI, X.Q., LIU, H.M., ZHOU, X.Z., SHAO, Z.H., Organic and Medicinal Chemistry Letters, **2**, 2012, p. 26.
18.CHOWRASIA, D., KARTHIKEYAN, C., CHOURE, L., SAHABJADA, GUPTA, M., ARSHAD, M., TRIVEDI, P., Arabian Journal of Chemistry, 2013.
19.CHAND, P., CHESNEY, J.A., CLEM, B.F., TAPOLSKY, G.H., TELANG, S., TRENT, J.O., Patent US 2011/0257211 A1, 2011.
20.HAWKINS, P.C.D., SKILLMAN, A.G., WARREN, G.L., ELLINGSON, B.A., STAHL, M.T., OMEGA 2.5.1.4: OpenEye Scientific Software, Santa Fe, NM, J. Chem. Inf. Model., **50**, 2010, p. 572.
21.MCGANN, M., J Comput Aided Mol Des, **26**, 2012, p. 897.
22.Berman, H. M., Westbrook, J., Feng, Z., Gilliland, g., Bhat, T. N., Weissig, H., Shindyalov, I. N., Bourne, P. E., Nucleic Acids Res. **28**, 2000, p. 235; www.rcsb.org, accesed 05.2016
23.GURUSARAN, M., SHANKAR, M., NAGARAJAN, R.., HELLIWELL, J.R., SEKAR, K., IUCrJ.,**1**, 2014, p. 74.
24.PRILUSKY, J., OCA, a browser-database for protein structure/function., 1996-2013, URL: http://oca.weizmann.ac.il/oca-bin/ocamain, accesed 05.2016.
25.IWATA, H., OKI, H., OKADA, K., TAKAGI, T., TAWADA, M., MIYAZAKI, Y., IMAMURA, S., HORI, A., LAWSON, J.D., HIXON, M.S., KIMURA, H., MIKI, H., ACS Med. Chem. Lett., **3**, 2012, p. 342.
26.YADAV, I.S., NANDEKAR, P.P., SHRIVASTAVA, S., SANGAMWAR, A., CHAUDHURY, A., AGARWA, S.M., Gene **539**, 2014, p. 82.

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