DOCKING STUDIES AND BIOLOGICAL EVALUATION OF ANTI-CANCER ACTIVITY OF NEW 1,2,4-TRIAZOLE(4*H*) DERIVATIVES

Victoriya Georgiyants[1], Lina Perekhoda[2], Narzullo Saidov[3], Idibeg Kadamov[3]

[1]National University of Pharmacy, Department of Pharmaceutical Chemistry, Ukraine
[2]National University of Pharmacy, Department of Medicinal Chemistry, Ukraine
[3]Tajic National University, Department of Pharmacy, Tajikistan

ABSTRACT

In the present work, 25 of novel 1,2,4-triazole(4*H*) derivatives were evaluated for their anti-tumor activity through docking studies and antitumor screening *in vitro*. The target protein structures of 1YS1, 1FM6, 1BXL were docked with new 1,2,4-triazole(4*H*) derivatives provided excellent results. *In vitro* anticancer activity of the compounds was tested by the National Cancer Institute and some of them have revealed the anticancer activity on leukemia, melanoma, lung, ovarian cancers cell lines. Among tested compounds three samples (3,8,16) showed high antitumor activity level and their in-depth preclinical studies are in progress. *N*-isopropylanilides of 4-benzyl-5-(4-brom)phenoxymethyl-1,2,4-triazolyl-3yl-merkapto-acetic acid *8* was found to be the most active candidate.

Key words: *anti-cancer activity; docking study; 1,2,4-triazole*

INTRODUCTION

Cancer, which is the uncontrolled growth and proliferation of cells due to mutation of genes which accelerate cell division rates and evade the programmed cell death, is the leading cause of death in the world. Over the past decade, a number of chemotherapeutic drugs with different mechanism of actions and targeting various stages of metastatic cell growth have flooded the pharmaceutical market. Based on their mechanism of action, these drugs can be classified into different classes as alkylating agents, antimetabolites, antibiotics, nucleoside analogues, antimitotic agents, etc. However, most of these drugs are associated with severe toxicities and are not effective against all types of cancer (1). Thus, the search for new anticancer drugs and the development of more effective treatment strategies continues to be imperative.

The 1,2,4-triazole(4H) derivatives, which are used in this study, play an important role in medicinal chemistry. Although they have been known from long ago to be biologically active, their varied biological features are still of great scientific interest. Their derivatives have a variety of biological properties, such as anti-microbial and anti-inflammatory (2,3), antidepressant (4), antifungal (5), anticonvulsant (6), and antitumor properties (7-8). Due to their antitumor effect, 1,2,4-triazole(4H) derivatives are attracting more interest. Until now, there have been only a few computational studies on 1,2,4-triazole(4H) derivatives; also, the protein tar-

Address for correspondence:
Lina Alekseevna Perekhoda
Department of Medicinal Chemistry,
National University of Pharmacy
53 Pushkinska St,
Kharkiv, Ukraine 61002
e-mail: perekhodalina@mail.ru

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gets of 1,2,4-triazole(4H) derivatives have not yet received a great deal of attention.

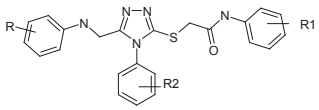
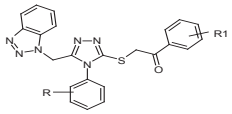
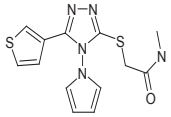
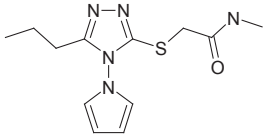
Synthetic strategy of the present study consists in structure modification of 1,2,4-triazole(4*H*) ring scaffolds modifying it in the positions 1, 2 and 5 with the aim of obtaining more potent pharmacologically active compounds. Several key types of the reactions were generally used us that allowed to obtain 25 new 1,2,4-triazole(4*H*) derivatives. The synthetic methods used to prepare the highly functionalized 1,2,4-triazole(4*H*) and related derivatives depicted in Table 1 can be found in previously reported work (9-12).

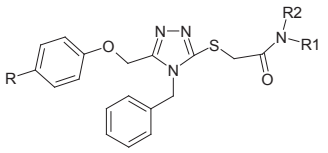
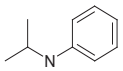
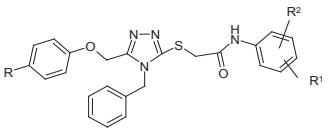
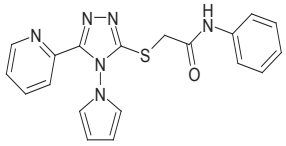
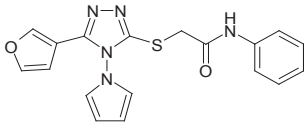
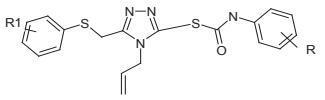
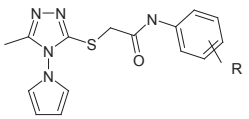
The computer prognosis of biological activity spectrum of all new compound synthesized by program PASS has set that the several substances ***1-25*** are able to show the inhibitor of cell growth activity (13,14). We have selected those substances for further studies. They are shown in Table 1.

MATERIALS AND METHODS

Molecular docking and antitumor screening of 25 new 1,2,4-triazole(4*H*) derivatives has been performed. Molecular docking is a key tool in structural molecular biology and computer assisted drug design. The goal of ligand-protein docking is to predict the predominant binding mode(s) of a ligand with a protein of known three-dimensional structure. A large number of targets are under exploration for chemotherapeutic treatments for cancer. NSAIDs inhibited the expression of the anti-apoptotic protein Bcl-X_L, resulting in an altered ratio of BAX to Bcl-X_L and subsequent mitochondria-mediated cell death. These results establish an unambiguous role for BAX in apoptotic processes in human epithelial cancers and may have implications for cancer chemoprevention strategies. A link between cancer and protein Bcl-X_L has already been ascribed, and there is an interest in developing a specific inhibitor as a new ther-

Table 1. Structure of new compound synthesized

			
№ comp	R	R^1	R^2
1	2-CH_3; 6-CH_3	2-CH_3; 3-Cl	H
2	4-Cl	2-CF_3	H
3	4-Cl	4-F	H
			
4	H	2-Me; 6-Me	H
			
5	3- OCH_3	4- OCH_3	H
			
6	3-OMe	H	H

7	3-CF_3	H	H
			
8	4-Br	H	
9	H	C_6H_5	C_6H_5
			
10	H	4- CH_3	H
11	H	3- CH_3	H
12	H	2- CH_3	H
13	4-Br	2-Cl	4,6-Cl
14	H	H	H
			
15	H	H	H
			
16	H	H	H
			
17	2-Cl	3-Me	H
			
18	2-Me; 4-Cl	H	H
19	4-OMe	H	H

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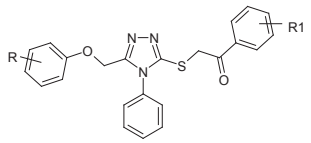
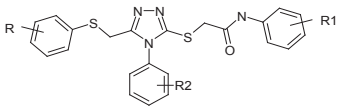
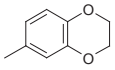
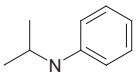
			
20	2-i-Pr; 5-Me	4- CHF_2	H
21	2-i-Pr; 5-Me	4-Cl	H
			
22	4- CH_3		H
23	H		3-CH_3
24	3-CH_3	3-CH_3	H
25	2-OMe	4-OMe	H

apeutic regimen for the cancer (15-16). In the present study, docking studies was carried out by taking proteins Bcl-X_L (code 1YS1) (code 1BXL) (17-22), PPAR$_\gamma$ receptor (code 1FM6) (23) domain as a targets for anticancer activity. The docking simulations were performed by the SCIGRESS program (24). Fast docking method, in which receptor is rigid and ligands are flexible, was adopted and binding energy values were compared with each other.

All chemical structures were generated using ISIS DRAW 4.0 software. The docking study was performed using Scigress Explorer 7.7 installed in a single machine running on a 3.4 GHz Intel Core 2 Duo Processor with 1GB RAM and 160 GB Hard Disk with Windows XP as the Operating System. Ligand structures 1-25 were drawn on Scigress Explorer using standard bond, lengths and angles. The ligands were stored in .csf format. The protein Bcl-XL and PPAR$_\gamma$ receptor modeled using the electron crystallographic structure at 3,5Å resolution was downloaded from the RCSB Protein Data Bank (PDB ID: 1YS1, 1BXL and 1FM6 respectively) (www.pdb.org). Water molecules were removed and hydrogen added to crystal structure of protein before docking. After assigning charge and protonation state final refinement (energy minimization) was done using MM3 force field runs. At the end of the docking study, the minimum Consensus score for the best ligand position for each of ligand was obtained. Energy minimization or complex optimization was done for molecular docking calculations and to optimize geometries within the binding site. Complex optimization gave ligands with minimum energy pose within the active site cavity of the protein.

RESULTS

The target protein structures of 1YS1, 1FM6, 1BXL were docked with new 1,2,4-triazole(4*H*) derivatives which provided excellent results as were seen by the least values of the binding energy in Table 2.

Results of docking studies have shown that utilization of anilide, phuran, pyrrol, benzyl, phenoxymethyl, phenaminomethyl residue for the structure optimization of 1,2,4-triazole(4*H*) scaffolds is effective approach in novel antitumor agents design and may be taken as the variant of hybrid pharmacophore approach. Molecule ***2*** showed better binding energies than the others (highest negative dock score -138.229). It means that it can fit well in the receptor cavity (1YS1) forming energetically most stable drug receptor complex. The best possible binding modes of the ***2*** at targeted protein's 1YS1active sites are displayed in Fig.1 and Fig.2.

Table 2. Compound Dock score

Comp	1BXL	1FM6	1YS1
1	-17.043	-18.836	-77.074
2	-46.471	-51.554	-138.229
3	-50.260	-51.249	-110.468
4	-3.783	-28.531	-83.444
5	-54.238	-76.771	-118.966
6	-15.343	-27.33	-73.57
7	-44.020	-39.93	-110.769
8	-49.847	-64.502	-74.263
9	-37.330	-58.893	-101.541
10	-48.410	-39.861	-100.923
11	-42.09	-44.517	-106.040
12	-44.408	-55.730	-99.36
13	-31.212	-59.468	-113.742
14	positive	positive	positive
15	-28.484	-59.02	-109.123
16	positive	positive	positive
17	-23.371	-32.606	-48.97
18	-10.326	-22.87	-53.951
19	-51.100	-76.548	-111.195
20	31.797	-12.681	-62.831
21	-50.572	-50.369	-106.435
22	-66.029	-64.676	-61.732
23	-60.974	-38.432	-115.087
24	-58.644	-43.738	-102.839
25	positive	positive	positive

Obtained 25 heterocyclic compounds became an object for study concerning anticancer activity identifying according to the standard National Cancer Institute (NCI) procedure (25). The prescreening criteria became strict and the procedure of prescreening consists in testing of compounds activity on 60 tumor cell lines in concentration10^{-5} M, including lines of leukemia, non-small cell, renal, lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, prostate cancer and breast cancer. Using the measurements (time zero, control growth, and test growth) in the presence of drug at the one concentration levels, the percentage growth was calculated. In this assay growth inhibition of 50% – GI50 is obtained. In the screening protocol, each cell line

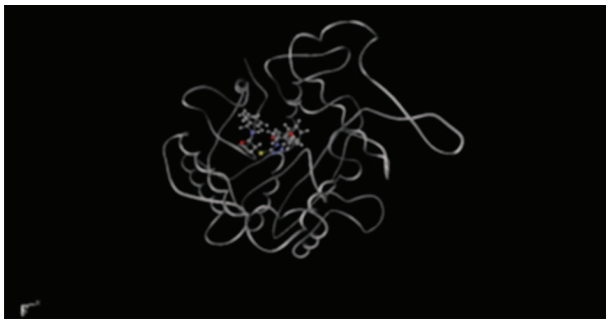


*Fig. 1. Dock a ligand **2** into an active site 1YS1*

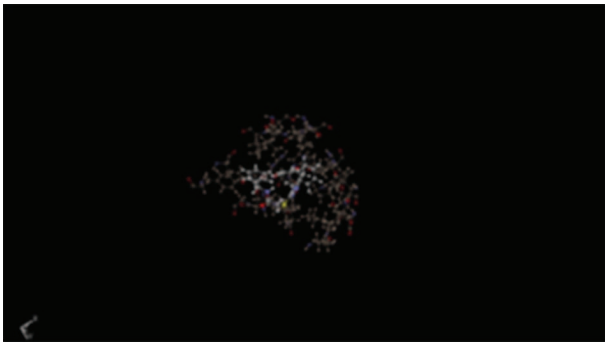


*Fig. 2. Binding interaction of **2** with Bcl-XL protein.*

was inoculated and pre-incubated for 24–48 h on a microtiter plate. Test agents were then added at single concentration and the culture was incubated for an additional 48 h. The end point determinations were made with a protein binding dye, sulforhodamine B (SRB). The results for each test agent were reported as the percent growth of the treated cells compared to the untreated control cells. Anticancer activity preliminary screening results for tested compounds at one dose assay (10^{-5} M) are shown in Table 3.

DISCUSSION

When analyzing the *in vitro* research results it is worth to mention that in the anticancer selectivity rating the most sensitive to 1,2,4-triazole(4*H*) derivatives and related heterocyclic systems was the line of leukemia, non- small cell lung cancer, and prostate cancer PC-3. Level of selectivity on the cell lines of melanoma, renal cancer and breast cancer are approximately the same. Series of cell lines, such as leukemia lines (CCRF-CEM, HL-60(TB), RPMI-8226, SR, K-562, MOLT-4), non-small cell lung cancer line (HOP-92), renal cancer cell lines (UO-31), prostate cancer (PC-3) and breast cancer line (MCF7) have been found to be the most sensitive to testing compounds.

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Table 3. Anticancer activity screening at one dose assay (10^{-5} M)

Comp.	Average growth, %	Range of growth, %	Most sensitive cancer cell lines, (growth %)
1	98.56	57.75	Prostate cancer*PC-3(75.71)
2	85.22	104.59	Leukemia*: CCRF-CEM(37.31) Prostate cancer*PC-3(13.34) Leukemia*:K-562 (43.96);
3	60.19	104.39	Ovarian cancer: OVCAR-4 (–7.63)
4	101. 82	57.68	Non-small cell lung cancer*: HOP-92(74.00)
5	97.32	37.57	Prostate cancer*PC-3(74.84)
6	103.48	50.14	Renal cancer*: UO-31 (71.67)
7	100.75	69.74	Colon cancer*: KM-12 (84.53)
8	56.60	138.59	Leukemia: HL-60(TB) (-16.59); RPMI-8226(-35.98); SR (–2.07); CCRF-CEM (-0.87) Non-small cell lung cancer: HOP-92 (-29.62)
9	98.64	52.12	Leukemia*: SR (66.63)
10	102. 96	46.95	Non-small cell lung cancer: NCL-H522(91.10)
11	98.47	40.32	Renal cancer*: UO-31 (83.52)
12	75.95	77.21	Breast cancer*T47D (30. 56)
13	86.67	80.57	Prostate cancer*PC-3(32.91)
14	90.10	47.75	Prostate cancer*PC-3(64.26) Melanoma*: UACC-62 (68.19) Leukemia*: MOLT-4 (62.11)
15	92.48	93.88	Prostate cancer*PC-3(25.78) Leukemia*: CCRF-CEM(45.92)
16	98.49	46.30	Renal cancer*: UO-31 (71.67)
17	102. 08	44.92	Breast cancer*: T-47D (89.96)
18	106.06	29.56	Renal cancer*: UO-31 (89.72)
19	100.14	40.47	Renal cancer*: UO-31 (76.71)
20	62.13	104.57	Breast cancer MCF7 (29. 03)
21	49.34	87.87	Leukemia: SR (0.25) Melanoma:MDA-MB-43(4.49)
22	91.27	63.69	Melanoma*:SK-MEL-5(47.82)
23	74.28	54.45	Leukemia*: K-562 (43.96)
24	102.00	143.92	Melanoma:MALME-3M(-14.85)
25	101.52	57.65	Prostate cancer*PC-3(76.33)

** activity was not significant*

Among tested 1,2,4-triazole(4*H*) derivatives except highly active ***3,8*** and ***16*** selected for the in-depth study, noteworthy is the ***8***, for which there was observed the selective effect on leukemia and non-small cell lung cancer.

Other compounds tested in one-dose primary assay didn't show any impressive anticancer activity and therefore can't be considered as prospective anticancer agents.

In addition, correlation studies between experimental average growth and docking scores with Build QSAR program were conducted (26). The correlation coefficients of q^2=0.38, 0.29, 0.20 between experimental and docking scores was obtained. The predictive capacity of these models is bad, taking into account the commonly accepted values for a satisfactory QSAR model, $q^2 > 0.500$. However, it should be noted that if there is docking function for Bcl-XL-BH3 protein complex in the model, its partial contribution in the PLS model is more essential, than if docking is performed to other biotargets. In consequence of performed studies *in silico* it can be assumed that the most probable mechanism of anticancer activity of 1,2,4-triazole(4*H*) derivatives may be binding with the anti-apoptotic protein complex Bcl-XL-BH3.

CONCLUSIONS

Overall, this work illustrated that potential anti-tumor compounds were found among new derivatives 1,2,4-triazole(4*H*). The anticancer activity showed promising results. Compound ***8*** was substantially more active than others and requires further study. The studies confirmed compound ***8*** as potent lead compound for drug discovery and further optimization. The ***8*** discovered in this study may provide valuable therapeutic intervention for the treatment of cancer disease. The binding pattern can be further used as a tool for the structure-based novel anti- tumor drug design. Molecular docking study is used to clarify the binding mode of the medicinal compound. Taken together in vitro results; our docking results show that there is a positive correlation between the dock scores and the inhibition of protein Bcl-XL receptor.

REFERENCES

1. P Callery and P Gannett. Cancer and cancer chemotherapy. In Foye's Principles of Medicinal Chemistry, Lippincott Williams and Wilkins, ed. Philadelphia 2002; 934–935.
2. Meenakshi VA, Sabir HB. Synthesis and Anti-inflammatory Evaluation of 1,2,4-Triazole Derivatives. RRJC. 2014;3(2):24-26.
3. Rakesh K, Shahar M.Y, Birendra S. Synthesis, characterization and biological evaluation of novel 1,2,4-triazole derivatives as potent antibacterial and anti-inflammatory agents. Der Pharma Chemica. 2014;6(1):137-143.
4. Chelamalla R, Venkatesham A, Sarangapani M. Synthesis and Antidepressant Activity of Some Novel 1, 2, 4-Triazole Derivatives. Journal of Pharmacy Research. 2012;5(9):4739.
5. Ram JS, Dharmendra KS. Syntheses of Some 3,5-Diaryl-4H-1,2,4-triazole Derivatives and their Antifungal Activity. E-Journal of Chemistry. 2009;6(1):219-224.
6. Северіна ГІ, Перехода ЛО, Георгіянц В.А.Синтез та протисудомна активність похідних [1,2,4-триазоло(1,5-а]піримідину. Фармацевтичний часопис. 2009;3(9):6-9.
7. Bekircan O, Gьmrьkzьoplu N. Synthesis of some 3,5-diphenyl-4H-1,2,4-triazole derivatives as antitumor agents. Indian Journal of Chemistry. 2005;44(10):2107-2113.
8. Sztanke K., Tuzimski T., Rzymowska J.et al. Synthesis, determination of lipophilicy, anticancer and antimicrobial properties of some fused 1,2,4-triazole derivatives . European Journal of Medicinal Chemistry 2008;43(2):404-419.
9. Georgiyants VA, Perekhoda LO, Saidov NB, Kadamov IM. Synthesis, docking studies, and biological evaluation of anti-ulcer activity of 4-allyl-5-(4-R1)-phenylthiomethyl-1,2,4-triazole-3-ylmercaptoacetic acid derivatives. Eur. Chem. Bull. 2014;3(5):466-471.
10. Saidov NB, Kadamov IM, Georgiyants. The synthesis of new 3-phenacylmetylthio-4-aryl-5-phenylaminomethyl-1,2,4-triazoles(4h) as potential neurotropic agents. Zh. Org. Farm. Khim. 2013;11,1(41):44-48.
11. Saidov NB, Kadamov IM, Georgiyants VA. Zh. Synthesis of the new biological active compounds among derivatives of 3-mercapto-4-benzyl-5-methoxyphenyl-1,2,4(4H)-triazole.Org. Farm 2012;10:25-28.
12. Saidov NB, Kadamov IM, Georgiyants, Taran AV. The synthesis of new 3-phenacylmetylthio-4-aryl-5-phenylaminomethyl-1,2,4-triazoles(4H) as potential neurotropic agents. Khim. Farm. Zh. 2013;47,11:11-15.
13. PASS: Prediction of Activity Spectra for Substances. http:///www.ibmh.msk.su/PASS.
14. Boshkayeva A, Georgiyants VA, Perekhoda LO. Study of acyl substitution on docking properties of substituted dihydrogercetines as anti-inflammato-

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ry agents. Journal of Chemical and Pharmaceutical Research 2014;6(4):749-753.

15. Vogler, Meike, et al. Bcl-2 inhibitors: small molecules with a big impact on cancer therapy. Cell Death & Differentiation 2008;16(3): 360-367.

16. Oltersdorf T. An inhibitor of Bcl-2 family proteins induces regression of solid tumours. Nature 2005;435.70(42):677-681.

17. Kaminskyy DV, Lesyk RB. Structure–anticancer activity relationships among 4- azolidinone-3-carboxylic acids derivatives. Biopolym Cell. 2010; 26: 136–145.

18. Upreti M. Key role for Bak activation and Bak-Bax interaction in the apoptotic response to vinblastine. Molecular Cancer Therapeutics. 2008;7:2224-2232.

19. Zhang L, Park BH. Role of BAX in the apoptotic response to anticancer agents. Science 2000;290:989–996.

20. Mozziconacci J, Arnoult E., Bernard P. Virtual Screening for COX-2 Inhibitors. J. Med. Chem. 2005;48:1055–1068.

21. Richard M., Peek Jr. Prevention of colorectal cancer through the use of COX-2 selective inhibitors. Cancer ChemotherPharmacol. 2004;54:50–56.

22. Harris RE, Beebe JG. Cancer chemoprevention by cyclooxygenase-2 (COX-2) blockade. Inflammation in the Pathogenesis of Chronic Diseases. 2007;193–212.

23. Chang AJ, Song DH, Wolfe MM. Attenuation of peroxisome proliferator-activated receptor γ (PPARγ) mediates gastrin-stimulated colorectal cancer cell proliferation. The Journal of Biological Chemistry. 2006;281(21):14700–14710.

24. Scigress Explorer Ultra 7.7 Bio Applications Getting Started Manual, Fujitsu

25. Boyd MR, Teicher BA, Andrew PA. The NCl Human Tumor Cell Line(60 Cell)Screen: Concept, Implementation, and Application.Anticancer drug development Guide: Preclinical Screening, Clinical Trials, and Approval. Totota, New Jersey: Human Press 2004;41-62

26. De Oliveira DB, Gaudio AC. Build QSAR: A New Computer Program for QSAR Analysis. Quant. Struct . Activ. Relat. 2001; 19(6):599–601.