Synthesis, Characterization and Antiproliferative Activity Assessment of a Novel 1*H*-5-mercapto-1,2,4 Triazole Derivative

MARIUS MIOC[1,2], SORIN AVRAM[2], VASILE BERCEAN[3], MIHAELA BALAN PORCARASU[4], CODRUTA SOICA[1], RAZVAN SUSAN[1], LUDOVIC KURUNCZI[1,2]*

[1]University of Medicine and Pharmacy Victor Babes Timisoara, Faculty of Pharmacy, 2 Eftimie Murgu Sq., 300041, Timisoara, Romania

[2] Institute of Chemistry Timisoara of Romanian Academy, Department of Computational Chemistry, 24 Mihai Viteazu Av., 300223, Timisoara, Romania

[3] S.C. SINOFIN S.R.L., 41 Tigrului Str., Timisoara, 300544, Romania

[4] Petru Poni Institute of Macromolecular Chemistry, 41A Grigore Ghica Voda Alley, Iasi, 700487, Romania

Angiogenesis plays an important function in tumor proliferation, one of the main angiogenic promoters being the vascular endothelial growth factor (VEGF) which activates specific receptors, particularly VEGFR-2. Thus, VEGFR-2 has become an essential therapeutic target in the development of new antitumor drugs. 1,2,4-triazoles show a wide range of biological activities, including antitumor effect, which was documented by numerous reports. In the current study the selection of 5-mercapto-1,2,4-triazole structure (1H-3-styryl-5-benzylidenehydrazino-carbonyl-methylsulfanil-1,2,4-triazole, Tz3a.7) was conducted based on molecular docking that emphasized it as suitable ligand for VEGFR-2 and EGFR1 receptors. Compound Tz3a.7 was synthesized and physicochemically and biologically evaluated thus revealing a moderate antiproliferative activity against breast cancer cell line MDA-MB-231.

Keywords: 1,2,4-triazole, antiproliferative, angiogenesis, breast cancer, VEGFR-2, EGFR1

Cancer is a life threatening pathology that has the ability to spread to near or distant organs; angiogenesis represents an important process that contributes in tumor growth and proliferation [1]. One of the main angiogenic promoters is the vascular endothelial growth factor (VEGF) which activates the vascular endothelial growth factor receptors (VEGFR), in particular VEGFR-2 [2]. VEGFR-2 represents an intensely studied target in the field of drug discovery for the development of new anticancer treatment [3,4] with the aid of computational chemistry. Numerous triazole derivatives were documented as anticancer agents revealing strong antoproliferative activity through various mechanisms. 5-mercapto-1,2,4-triazole derivatives identified by *in silico* (protein-ligand docking) as DNA topoisomerase II inhibitors and were synthesized [5]; all compounds were *in vitro* assessed against EAC (Ehrlich ascites carcinoma) cells exhibiting good antiproliferative effect while five compounds also revealed significant anti-inflammatory activity. 4-Amino-4,5-disubstituted-[1,2,4] triazole-3-thiols were designed, synthesized and *in vitro* tested revealing a strong bonding to VEGF [6]. Dual VEGFR-2 and tubulin inhibitors were developed in the effort to inhibit simultaneously tumor cells and vasculature [7] leading to the discovery of lead compounds with triazole/oxadiazole moieties with excellent anticancer properties.

In our recent work we built a compound library, containing 1,2,4-triazole derivatives, that was subjected to molecular docking against two targets: VEGFR-2 and epidermal growth factor receptor 1 (EGFR1). As a result of extensive docking simulations, a candidate molecule, 1*H*-3-styryl-5-benzylidenehydrazino-carbonyl-methylsulfanil-1,2,4-triazole (Tz3a.7) emerged as a suitable ligand for both receptors. Some papers were previously published in the field of functionalization of mercapto-triazoles and their complexing abilities, as well their instrumental characterisation and stability [8], by well-known methods, including kinetic studies [9, 10].

The aim of the current study was the synthesis, physicochemical characterization and assessment of the antiproliferative activity of compound Tz3a.7.

Materials and methods

Chemistry

Materials and methods

Thiosemicarbazide, cinnamic acid, and solvents (ethanol, N,N-dimethylformamide, pyridine) were purchased (Acros, Aldrich) and used as received. Cinnamoyl chloride was synthsized by treating cynnamic acid with thionyl chloride, and used without further purification. Synthesis of N-(benzylideneamino)-2-chloro-acetamide was carried out by a previously published procedure [11].

Synthesis route of compound Tz3a.7 is depicted in figure 1.

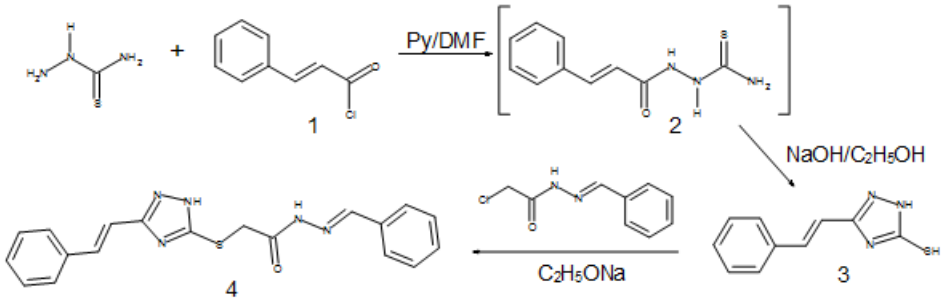


Fig. 1. Synthesis route of 1H-3-styryl-5-benzylidenehydrazino-carbonyl-methylsulfanil-1,2,4-triazole (Tz3a.7)

* *Phone: (+40)256204476 / 483*

The synthesis of 1*H*-3-styryl-5-mercapto-1,2,4-triazole, (3), was carried out by acylation of thiosemicarbazide with the cinnamoyl chloride and pyridine in N,N-dimethylformamide, followed by the cyclisation reaction of the resulted 1-cynammoyl-thiosemicarbazides (2), in ethanolic NaOH, at reflux, according to the previously published procedure [12]. The final compound, Tz3a.7 (4) was synthesized by alkylation of the obtained 1*H*-3-styryl-5-mercapto-1,2,4-triazole (3), with N-(benzylideneamino)-2-chloro-acetamide, following the reaction conditions previously published [13,14].

Determination of the melting point of the analyzed substance was performed on a Boetius PHMK (Veb Analytik Dresden) instrument. The IR spectrum was recorded on a Jasco FT/IR-410 spectrophotometer in KBr pellet. ^{1}H-NMR and ^{13}C-NMR spectrum was recorded on a Bruker Avance DRX 400 spectrometer in DMSO-d6 at room temperature. The chemical shifts are reported as ä values (ppm) and were referenced to the solvent residual peak (2.51 ppm for ^{1}H and 39.47 ppm for ^{13}C). For the elemental analysis we used a Vario EL Elementar Analysensysteme GmbH.

In vitro antiproliferative assay

Cell culture

All cell lines were purchased from ATCC (Manassas, Virginia, USA) and cell culture mediums were purchased from Sigma Aldrich. Cells (HaCaT-human keratinocytes, A375-human melanoma, B164A5-murine melanoma, MDA-MB-231-breast carcinoma, A549-lung carcinoma) were cultured in a Dulbecco's modified Eagle Medium (DMEM) containing 4.5 g·L^{-1} glucose, 15 mM Hepes, and 2 mM L-glutamine, supplemented with 100 U· mL^{-1} penicillin, 100 µg·mL^{-1} streptomycin, and 10% fetal bovine serum (FBS). The cells were kept under humidified atmosphere, 5% CO_2, 37°C and passaged every other day. Cell number was determined by using the cell counting chamber (Neubauer) in the presence of Trypan blue.

AlamarBlue assay

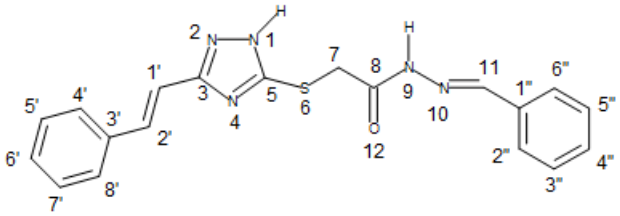
Cell viability was measured by means of AlamarBlue technique; cells (1x10^{4}/200 µL medium/well) were seeded in a 96-well plate, allowed to attach and then stimulated with various concentrations (10 and 50 µM) of the tested compound for a period of 24h. Next, the medium was removed and new medium was added into each well (200 µL/well). At 24h post-stimulation, a volume of 20 µL/well of AlamarBlue solution was added (10% of the medium volume). The plates were incubated at 37°C for 3h. After incubation time ended, spectophotometry of the plates was employed at 570 nm and 600 nm, respectively, by using xMark™ Microplate Spectrophotometer (Biorad). Cell viability was calculated according to the previously published formula [15].

Statistical analysis

The measurements were done in triplicate for each sample and the results appear as mean ± standard error. One-way Anova followed by Bonferroni's post-tests were used to determine the statistical difference between experimental and control groups. $p < 0.05$ was considered statistically significant; *, ** and *** indicate $p<0.05$, $p<0.01$ and <0.001.

Results and discussions

Synthesis of 1H-3-styryl-5-benzylidenehydrazino-carbonyl-methylsulfanil-1,2,4-triazoles (Tz3a.7)



White crystalline powder; **M.p.**= 183-185°C (purification, column chromatography, mobile phase, hexane:ethyl acetate=1:1); **IR** [KBr] (cm-1):3253, 3211, 3061, 3027, 2922, 2853, 1671, 1604, 1543, 1490, 1434, 1396, 1360, 1318, 1228, 1183, 1143, 1065, 1050, 984, 952, 892, 783, 754, 691, 566, 515; **^{1}H-NMR**, 400.13 MHz, ä (ppm), DMSO-d_6: 14.1, 11.7 (NH), 8.24, 8.04 (H11), 7.69-7.42 (H2, H4'-8', H2"-6"), 7.06 (H1'), 4.42, 4.00 (H7). **^{13}C-NMR**, 100.6 MHz, ä (ppm), DMSO-d_6: 169.5, 164.1 (C8), 161.3 (C4'), 159.4, 159.1 (C5), 154.5, 154.3 (C3), 146.9, 143.4 (C11), 135.4, 135.2, 134.0, 132.9, 132.7, 130.2, 130.0, 129.2, 129.0, 128.9, 127.2, 127.1, 126.8 (C1"-C6", C2', C3', C5'-C8'), 118.0, 113.0 (C1') 34.2, 33.2 (C7). **Elemental analysis** calculated for C19H17N5OS: C, 62.79; H, 4.71; N, 19.27; found: C, 62.51; H, 4.98; N, 19.10.

Antiproliferative activity

The antiproliferative activity of compound Tz3a.7 was tested on four tumor cell lines: A375 (human melanoma), A549 (lung carcinoma), MDA-MB-231 (breast carcinoma) and B164A5 (murine melanoma). Toxicity was tested on HaCaT cells (human keratinocytes). The activity on all mentioned cell lines was expressed as inhibition of cell viability and was carried out by means of AlamarBlue assay. Results are shown in figure 2.

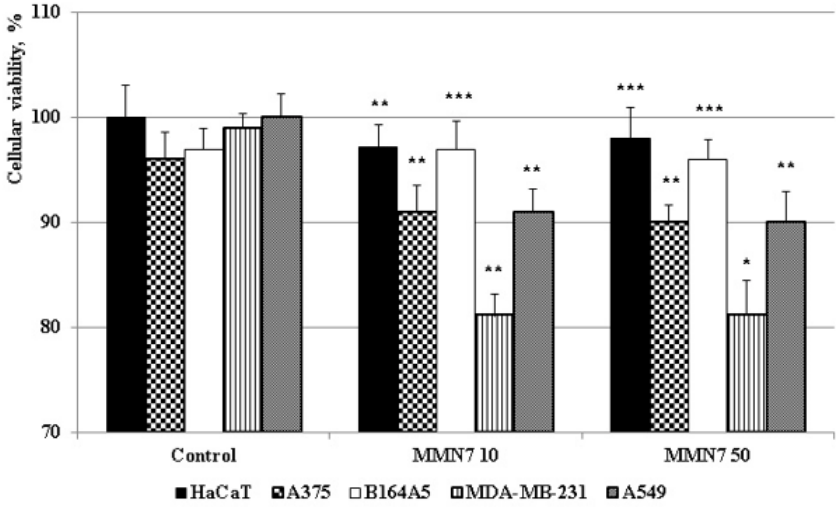


Fig. 2. Cellular viability after stimulation of normal (HaCaT) and cancer (A375, B164A5, MDA-MB-231, A549) cell lines with Tz3a.7 in concentration of 10µM and 50µM, respectively. $p < 0.05$ was considered statistically significant; *, ** and *** indicate $p<0.05$, $p<0.01$ and <0.001

The AlamarBlue test is a simple method by which proliferation of various cell lines can be quantified. The assay contains a cellular growth indicator (AlamarBlue), that is reduced, subsequently to cell growth, to a red fluorescent form that can be spectrophotometrically evaluated [15]. According to the graph representation of AlamarBlue results (fig. 2) a rather poor antiproliferative activity of compound Tz3a.7 was recorded on A375, A549 and B164A5 cell lines (human melanoma, lung carcinoma and murine melanoma, respectively). A stronger inhibition of cell viability was noticed on the MDA-MB-231 cell line (breast carcinoma). Tumor cell progression in the MDA-MB-231 cell line is strongly correlated with angiogenesis as reported by Chen et al. [16]; therefore, we can presume that the higher inhibition reported against the MDA-MB-231 cell line is based on the theoretically predicted antiangiogenic activity by taking into account that: 1) VEGF is a key regulator in angiogenesis by its coupling to the VEGFR-2 receptor [17], and 2) compound Tz3a.7 was predicted by means of molecular docking to be a VEGFR-2 inhibitor. Nevertheless, breast cancers are complex tumors in which growth, cell proliferation and metastasis depend on a variety of inducing factors and, while antiangiogenic therapy, such as VEGFR inhibitors, suppresses tumor growth, a more complex targeted combined treatment is required [18].

Conclusions

As a result of molecular docking simulation, of a series of 5-mercapto-1,2,4-triazole derivatives, conducted in previous work, carried out on two important protein targets, active in cancer development, vascular endothelial growth factor receptor 2 (VEGFR-2) and epidermal growth factor receptor 1 (EGFR1), one particular compound was selected as suitable ligand for both receptors, 1*H*-3-styryl-5-benzylidenehydrazino-carbonyl-methylsulfanil-1,2,4-triazole (Tz3a.7). Compound Tz3a.7 was synthesized and physicochemically characterized as described herein; the *in vitro* biological evaluation by means of AlamarBlue assay highlighted a poor antiproliferative activity against A375, A549 and B164A5 cell lines (human melanoma, lung carcinoma and murine melanoma, respectively) while a stronger activity was reported on MDA-MB-231 (triple negative breast carcinoma) cell line. We hypnotize that the anticancer activity of compound Tz3a.7 is based on an antiangiogenic effect that was previously predicted by means of molecular docking. However, due to the complexity of breast cancer, further studies regarding a targeted combined therapy are highly remain to be performed.

Acknowledgments: This work was supported by the intern grant PII – C5 – TC - 2017 offered by the Victor Babes University of Medicine and Pharmacy, Timisoara (M.M). The authors wish to thank Dr. Florin Borcan for his help in the statistical analysis of experimental data.

References

1.NISHIDA, N., YANO, H., NISHIDA, T., KAMURA, T., KOJIRO, M. Vasc Health Risk Manag, **2**(3), 2006, p. 213.
2.RIBATTI, D., DJONOV, V. Cancer Lett, **316**, 2012, p. 126.
3.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.
4.ABDEL AZIZ, Y.M., SAID, M.M., EL SHIHAWY, H.A., ABOUZID, K.A.M., Bioorganic Chemistry, **60**, 2015, p. 1.
5.PHILIP, S., PUROHIT, M.N., KRISHNA, K.L., ESWAR, M.S., RAIZADAY, T., PRUDHVI, S., PUJAR, G.V., International Journal of Pharmacy and Pharmaceutical Sciences, **6**, 2014, p. 185.
6.BASAPPA, SUGAHARA K., THIMMAIAH, K.N., BID, H.K., HOUGHTON, P.J., RANGAPPA, K.S., PLoS ONE, **7**, 2012, e39444. doi:10.1371/journal.pone.0039444
7.CHEKLER, E. L. P., KISELYOV, A. S., OUYANG, X., CHEN, X., PATTAROPONG, V., WANG, Y., TUMA M.C., DOODY, J. F., ACS Medicinal Chemistry Letters, **1**, 2010, p.488
8.FULIAS, A., SOICA, C., LEDETI, I., VLASE, T., VLASE, G., SUTA, L.M., BELU, I., Rev. Chim. (Bucharest), **65**, no. 11, 2014, p. 1281
9.TRANDAFIRESCU, C., SOICA, C., LEDETI, A., BORCAN, F., SUTA, L.M., MURARIU, M., DEHELEAN, C., IONESCU, D., LEDETI, I. Rev. Chim. (Bucharest), **67**, no. 3, 2016, p. 463
10.LEDETI, I., VLASE, G., VLASE, T., MURARIU, M., TRANDAFIRESCU, C., SOICA, C., SUTA, L.M., DEHELEAN, C., LEDETI, A. Rev. Chim. (Bucharest), **67**, no, 3, 2016, p. 549
11.ITO, S., NARUSAWA, T., Bull. Chem. Soc Japan **43**, 1970, p.2257.
12.BERCEAN, V., BADEA, V., SISU, E., BINDILA, L., CSUNDERLIK, C. Rev. Chim.(Bucharest), **54**, 2003, no. 4, p. 368
13.BERCEAN, V.N., LEDETI, I., BADEA, V., BALAN, M., CSUNDERLIK, C. Rev. Chim.(Bucharest), **61**, no. 11, 2010, p. 1028.
14.LEDETI, I., BERCEAN, V. N., BADEA, V., BALAN, M., CSUNDERLIK, C. Rev.Chim.(Bucharest), **6**, no. 9, 2010, p. 833.
15.SOICA, C., OPREAN, C., BORCAN, F., DANCIU, C., TRANDAFIRESCU, C., CORICOVAC, D., et al. Molecules, **19**, 2014, p. 4924.
16.CHEN, M.J., CHIOU, P., LIN, P., LIN, C.M., SIRI, S., PECK, K., CHEN, T. Journal of Cellular Biochemistry **101**, 2007, p.1316.
17.RIZVI, S.U.F, SIDDIQUI, H.L., NISAR, M., KHAN, N., KHAN, I., Bioorg. Med. Chem. Lett., **22**, 2012, p. 942.
18.KRISTENSEN, T.B., KNUTSSON, M.L.T., WEHLAND, M., LAURSEN, B.E., GRIMM, D., WARNKE, E., MAGNUSSON, N.E. Int. J. Mol. Sci., **15**, 2014, p. 23024.

Manuscript received: 28.12.2106