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SYNTHESIS OF NEW bis-THIOSEMICARBAZONES BASED ON 2,2'-[PROPANE-1,3-DIIL-bis-(OXI)]DIBENZALDEHYDE WITH BIOLOGICAL POTENTIAL

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Cancer is the second most common cause of death. No known drug meets 100% the needs of the medical industry. For these reasons, many researchers are trying to synthesize new molecules with anti-cancer properties. A class of organic compounds called thiosemicarbazones exhibit a broad spectrum of useful biological activities including anticancer and antimicrobial. One of the possible mechanisms of the anticancer action of thiosemicarbazones is the inhibition of topoisomerase IIα. In this article the synthetic procedure of some novel *bis*-thiosemicarbazones is described. Their topoisomerase IIα inhibition potential was estimated by Molecular Docking method.

Keywords*: bis-thiosemicarbazones, topoisomerase II inhibitors, anticancer activity, molecular docking.*

SINTEZA UNOR bis-TIOSEMICARBAZONE NOI ÎN BAZA 2,2'-[PROPANE-1,3-DIIL-bis-(OXI)]DIBENZALDEHIDEI CU POTENȚIAL BIOLOGIC

Cancerul este a doua cea mai frecventă cauză de deces. Nici un medicament cunoscut nu satisface 100% necesitățile industriei medicinale. Din aceste considerente mulți cercetători încearcă să sintetizeze molecule noi cu proprietăți anticancer. O clasă de compuși organici numită tiosemicarbazone manifestă un spectru larg de activități biologice utile, printre care cele anticancer și antimicrobiene. Unul din mecanismele posibile de acțiune anticancer a tiosemicarbazonelor este inhibarea topoizomerazei IIα. În acest articol este descrisă procedura de sinteză a unor *bis*-tiosemicarbazone noi. Potențialul lor de inhibiare a topoizomerazei IIα a fost estimat cu ajutorul metodei de andocare moleculară.

Cuvinte-cheie: *bis-thiosemicarbazone, inhibitori de topoizomeraza II, activitate anticanceroasă, andocare moleculară*.

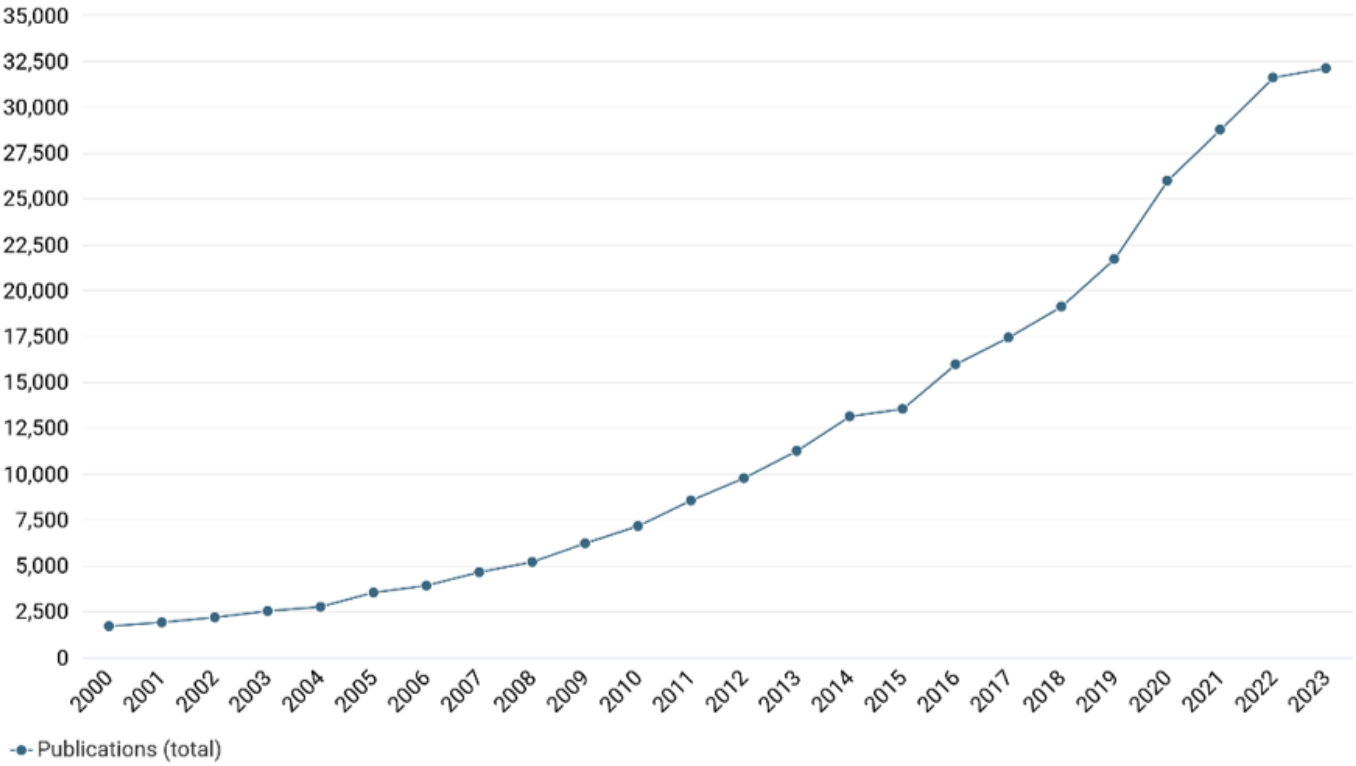
Introduction

Cancer remains one of the most serious problems in contemporary medicine. It is the second most common cause of death after cardiovascular disease. Known and implemented drugs, for example *cis*-platin, have a number of crucial disadvantages, such as very high toxicity [1]. Due to these considerations many research groups aim to synthesize new molecules with better anticancer effects and lower toxicity. This can be observed in Figure 1, in the year 2000 there were published 1684 articles which had in its title the word „anticancer", and in the year 2023 already 32104 articles [2]. Despite these, at the moment there is no drug that would meet the needs of the medicinal industry on 100% and in several cases so far is used drug introduced in 1979 (*cis*-platin) [3].

For these reasons, the development and synthesis of new substances with a potential anticancer effect is an important and promising field. One class of compounds of great interest are thiosemicarbazones. These can be considered as Schiff base's derivatives. Through series of investigations it has been shown that these substances possess a number of versatile biological properties, specifically: anticancer [4], antimicrobial [5], antioxidative [6], antiviral [7], antifungal [8], etc. Besides this, they are good ligands for coordination with 3*d* metals, among which the vitally important ones - Cu and Fe.

In general, the biological activity of thiosemicarbazones strongly depends on their structure, and for this reason *bis*-thiosemicarbazones are of special interest. Having two reactive centres, they may lead to improved biological properties [9-10].

Figure 1. The number of articles published between 2000 and 2023 that have the word „anticancer” in their title [2].



One of the mechanisms of anticancer activity of thiosemicarbazones is the inhibition of topoisomerases [11]. The expression of these enzymes is known to be one of the differences between cancer cells and normal cells [12]. They participate in processes that promote cell growth and division. High levels of topoisomerases are associated with the majority of cancer cells [13-15]. All cells have two major forms of topoisomerases - topoisomerase I and topoisomerase II [16]. In turn, in mammalian cells, there are two isoforms of topoisomerase II - α and β [17-18]. These isoforms are differentially expressed throughout the cell cycle: topoisomerase IIα is preferentially expressed in proliferating cells during S-phase [19], while topoisomerase II_{β} is expressed at all points of the cell cycle, with no significant differences between proliferating and non-proliferating cells [20]. In previous studies it has been shown that inhibition of topoisomerase II_{β} is associated with the development of secondary malignant tumours, these were based on the study of cancer treatment with etoposide [11]. From these considerations a prospective target for the development of new chemotherapeutic preparations with lower risks is namely topoisomerase IIα. In source [21] the authors demonstrated correlation between the theoretical topoisomerase II inhibition study (Molecular Docking) and real anticancer assays, where the substance with the best theoretical score also had the best IC_{50} (Half-maximal inhibitory concentration) in real assays demonstrating also a quite good selectivity.

In this article the methodology for the synthesis of novel *bis*-thiosemicarbazones is described. To assess their potential to inhibit topoisomerase IIα the *in silico* method - Molecular Docking, was used.

Materials and methods

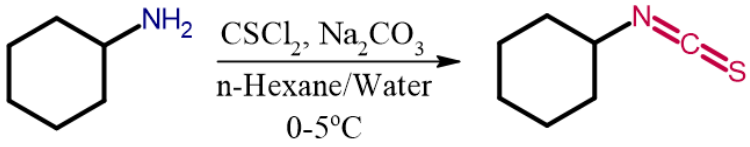
The reagents were purchased from „Sigma-Aldrich” and ”Thermo Fisher Scientific” and used without additional purification. ^{1}H and ^{13}C NMR spectra were recorded on Brucker Ultrashield 400 Plus and Brucker Ultrashield 500 Plus spectrophotometers, chemical shifts are measured in ppm versus tetramethylsilane.

Cyclohexyl isothiocyanate synthesis

Synthesis of cyclohexylisothiocyanate was carried out according to the method described in [22]. Cyclohexyl amine (5 g, 50.4 mmol) was added to the hexane:water mixture (20 mL:50 mL), Na_2CO_3 (5.34 g,

50.4 mmol) was added to the resulting mixture. After its solubilization, the mixture was cooled in an ice-salt bath and thiophosgene (5.8 g or 3.87 mL, 50.4 mmol) was added dropwise. The contents of the flask were stirred for 10-15 min., while cooling, after which the temperature was increased to 45-50 °C and the stirring was continued for another hour. The progress of reaction was investigated by TLC (ethylacetate-hexane, 1:3). The hexane layer is separated and washed with $NaHCO_3$ saturated solution 3x15 mL, dried with Na_2SO_4, the clear solution was concentrated by using rotary evaporator and the crude mixture was purifiedby silica gel (200 mesh) column chromatography using hexane as eluent to obtain cyclohexyl isothiocyanateas an oily liquid.

Figure 2. Scheme of cyclohexoisothiocyanate synthesis.



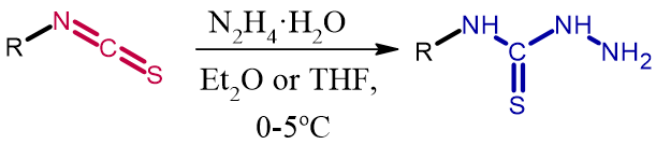
4-Cyclohexyl-3-thiosemicarbazide synthesis

The synthesis was carried out according to the methodology described in the source [23]. Cyclohexyl isothiocyanate (3.0 g, 21.2 mmol) is dissolved in diethyl ether or tetrahydrofuran (10 mL) and dropwise added to hydrazine monohydrate (1.08 g, 21.5 mmol) ethanol solution (10 mL), previously cooled to 0-5 °C. The formation of the white, crystalline solid is observed. After finishing the addition of isothiocyanate the reactant mixture is stirred for another 20-30 minutes, after which the sediment obtained is filtered, washed with distilled water and cold ethanol. A white crystalline solid is obtained, and recrystallized from ethanol. The resulting products were placed in a desiccator ($CaCl_2$ anhydrous) to perform melting point measurements, FTIR, ^{1}H-NMR and ^{13}C-NMR spectra.

4-Allyl-3-thiosemicarbazide and 4-Phenyl-3-thiosemicarbazide synthesis

Synthesis of 4-allylthiosemicarbazide and 4-phenylthiosemicarbazide is carried out according to the similar method described above. All thiosemicarbazides are white crystalline solids.

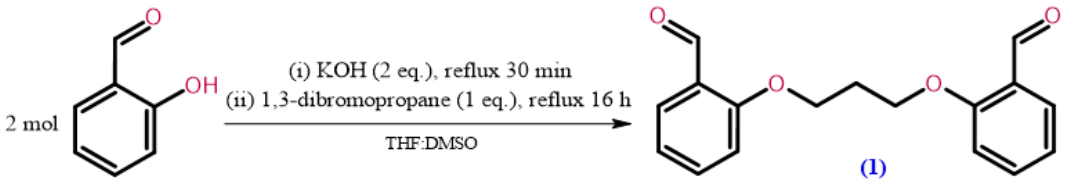
Figure 3. Synthesis scheme of thiosemicarbazides.



bis-Aldehyde (1) synthesis

The synthesis of 2,2'-(propane-1,3-diylbis(oxy))dibenzaldehyde (1) was carried out as described in [24]. Salicylic aldehyde (20 mmol, 2.44 g) was dissolved in the mixture consisting of 5 mL of THF and 1 mL of DMSO. The mixture was refluxed for 20-30 minutes. The sudden formation of the porous precipitate with a light-green color is observed. Next, 1,3-dibromopropane (10 mmol, 2.02 g) solubilized in THF and DMSO (3 mL:0.5 mL, correspondingly) is added to the reactant mixture. The reactant mixture is refluxed for 16 hours. After this, it is cooled and poured into cooled distilled water for sedimentation of the product. This results in the formation of a light yellow solid. For purification, it is recrystallized from 10 mL of ethanol. A white, crystalline solid is obtained.

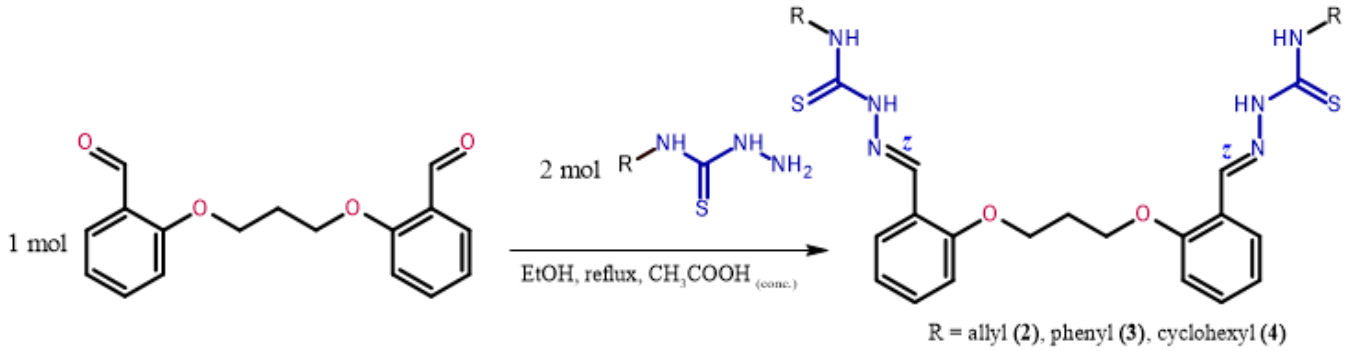
Figure 4. Scheme for the synthesis of bis-aldehyde (1).



bis-Thiosemicarbazones synthesis

To obtain symmetrical *bis*-thiosemicarbazones the mixture consisting of one equivalent of bis-aldehyde 1 (0.5 g, 1.76 mmol) and two equivalents of the respective thiosemicarbazide (3.52 mmol) was dissolved in 15 mL of ethanol and refluxed for 5-8 hours. In catalytic amounts (3-5 drops) glacial acetic acid was added. The precipitate was filtered and recrystallized from ethanol.

Figure 5. Synthesis of bis-thiosemicarbazones scheme.



The in silico study of the interaction of synthesized substrates with the target protein - Molecular docking

Molecular docking is one of the most commonly applied in medicinal chemistry and drug design *in silico* study method. Its use allows the exclusion of molecules with low potential of desired activity. It is known that topoisomerase IIα inhibitors have high potential anticancer action. For these reasons this enzyme was used to evaluate the anticancer potential of synthesized bis-thiosemicarbazones. The coordinates of the active site of topoisomerase IIα (PDB id: 5GWK [25-26]) were determined from the position of the cocrystallized etoposide. After this the etoposide structure was removed from the protein structure, as were cocrystallized water moieties. Similarly, polar hydrogens were added into the protein structure, Kollman and Gasteiger charges were calculated. Molecular docking was performed with AutodockVina [27-28], the results were visualized with BIOVIA Dicovery Studio [29].

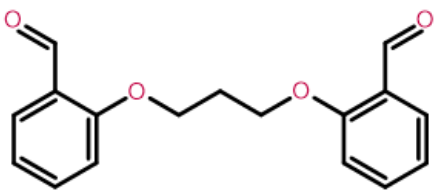
Results and discussions

Three new *bis*-thiosemicarbazones were synthesized. Their structure was confirmed with FTIR and ^{1}H, ^{13}C NMR spectroscopies. Molecular docking method was used to assess the biological potential.

NMR spectroscopy

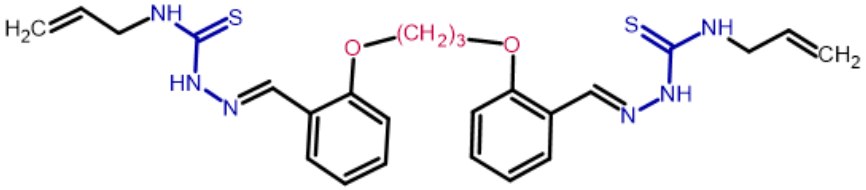
The structure of the synthesized bis-thiothiothiothio meticarbazones was confirmed by ^{1}H and ^{13}C NMR spectroscopy. The data obtained from the spectra are placed below.

Compound 2,2’-(propane-1,3-diylbis(oxy))dibenzaldehyde **(1)**



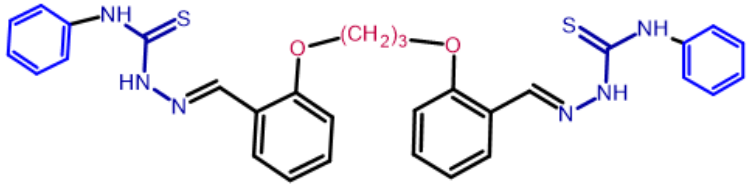
Crystalline white solid. Yield: 70%. **FT-IR (cm^{1}):** 3079, 3039, 3012 ν_s(C–H) (aromatic), 2963, 2940 ν_{sy}(C–H), 2882 ν_{as}(C–H), 2846, 2761 ν_s(C–H, aldehydes), 1678 ν_s(C=O), 1597 and 1451 ν_s(C=C), 751 δ(C–H), 1231 νas(C–O–C), 1052 νs(C–O–C), 756 δ(C–H) (aromatic). **^{1}H RMN (400 MHz, DMSO-d_6) δ(ppm):** 2.33 q 2H; 4.35 t 4H; 7.07 t 2H; 7.26-7.28 d 2H; 7.62-7.70 m 4H; 10.40 s 2H. **^{13}C RMN (100 MHz, DMSO-d_6) δ(ppm):** 28.8 (-CH_2-); 65.5(-CH_2-O); 113.9; 121.1; 124.6; 128.1; 136.8; 161.3; 189.7 (C=O, aldehydes).

*Compound 2,2'-(((propane-1,3-diylbis(oxy))bis(2,1-phenylene))bis(methanylylidene))bis(**N-allyl**hydrazinecarbothioamide)* **(2)**



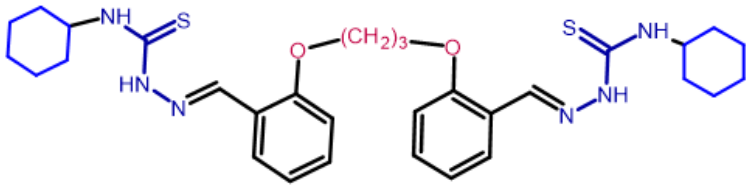
Yellow solid. Yield: 81%. **FT-IR (cm^{1}):** 3370 ν_s(N^2–H), 3173 ν_s(N^4–H), 3083 ν_s(C–H) (aromatic), 2985, 2936 ν_{sy}(C–H), 2878 ν_{as}(C–H), 1647 δ(N-H), 1598 ν_s(C=N), 1534 and 1449 ν_s(C=C), 1249 ν_s(C=S), 1222 ν_{as}(C–O–C), 1046 ν_s(C–O–C), 928 (-CH=CH$_2$), 753 (*ortho*-substitution). **^{1}H RMN (500 MHz, DMSO-*d*$_6$) δ(ppm):** 2.25 m 2H; 4.21-4.27 m 8H; 5.08-5.16 m 4H; 5.90 m 2H; 6.97 t 2H; 7.09-7.11 d 2H; 7.38 t 2H; 8.10-8.11 d 2H; 8.52 s 2H; 11.51 s 2H. **^{13}C RMN (100 MHz, DMSO-*d*$_6$) δ(ppm):** 28.7; 45.7; 64.9; 112.5; 115.4; 120.5; 122.3; 125.9; 131.2; 135.1; 137.9; 157.0; 176.8(C=S).

*Compound 2,2'-(((propane-1,3-diylbis(oxy))bis(2,1-phenylene))bis(methanylylidene))bis(**N-phenyl**hydrazinecarbothioamide)* **(3)**



Yellow solid. Yield: 86%. **FT-IR (cm^{1}):** 3326 ν_s(N^2–H), 3134 ν_s(N^4–H), 3033 ν_s(C–H) (aromatic), 2959 ν_{sy}(C–H), 2877 ν_{as}(C–H), 1674 δ(N-H), 1595 ν_s(C=N), 1531 and 1449 ν_s(C=C), 1247 ν_s(C=S), 1204 ν_{as}(C–O–C), 1064 ν_s(C–O–C), 744 (*ortho*-substitution), 690-730 (monosubstituted). **^{1}H RMN (500 MHz, DMSO-*d*$_6$) δ(ppm):** 2.29 m 2H; 4.31 t 4H; 6.99 t 2H; 7.13-7.14 d 2H; 7.20 m 2H; 7.36 m 6H; 7.57-7.58 d 2H; 8.26-8.28 dd 2H; 8.64 s 2H; 10.08 s 2H. **^{13}C RMN (100 MHz, DMSO-*d*$_6$) δ(ppm):** 28.8; 64.5; 112.5; 120.6; 122.1; 125.2; 125.7; 126.4; 128.0; 131.5; 138.8; 139.0; 157.2; 175.6(C=S).

*Compound 2,2'-(((propane-1,3-diylbis(oxy))bis(2,1-phenylene))bis(methanylylidene))bis(**N-cyclohexyl**hydrazinecarbothioamide)* **(4)**



Yellow solid. Yield: 78%. **FT-IR (cm^{1}):** 3343 ν_s(N^2–H), 3128 ν_s(N^4–H), 3079 ν_s(C–H) (aromatic), 2980, 2927 ν_{sy}(C–H), 2851 ν_{as}(C–H), 1612 δ(N-H)/ ν_s(C=N), 1539 and 1452 ν_s(C=C), 1241 ν_s(C=S), 1231 ν_{as}(C–O–C), 1052 ν_s(C–O–C), 747 (*ortho*-substitution). **^{1}H RMN (500 MHz, DMSO-*d*$_6$) δ(ppm):** 1.29 m 4H; 1.41 m 4H; 1.59 m 2H; 1.70 m 4H; 1.85 m 4H; 2.24 m 2H; 4.18 m 2H; 4.27 t 4H; 6.98 t 2H; 7.10 d 2H; 7.38 t 2H; 7.96 d 2H; 8.05 dd 2H; 8.51 s 2H; 11.39 s 2H. **^{13}C RMN (100 MHz, DMSO-*d*$_6$) δ(ppm):** 24.9; 25.1; 28.7; 31.8; 52.5; 64.5; 112.5; 120.6; 122.2; 126.0; 131.2; 138.0; 157.0; 175.4(C=S).

FTIR spectroscopy

From the FTIR spectra obtained for the synthesized substances can be concluded that in the spectrum of the *bis*-thiosemicarbazones the aldehyde group characteristic band disappears, which means that the condensation process between the *bis*-aldehyde and the thiosemicarbazides has completely occurred. In addition to this, in the spectra of *bis*-thiosemicarbazones the band characteristic for the azomethine group

appears, which once again confirms the structure of the synthesized substances. Also, in the spectra of the substances the characteristic bands of the monosubstituted benzene ring were found. Also in the spectra of the *bis*-thiosemicarbazones the characteristic band for the (N-H) group is present.

Molecular Docking

In order to assess the potential of topoisomerase IIα inhibition, obtained results (presented below in Table 1) are compared with etoposide - the drug used to treat cancer, which is a topoisomerase inhibitor.

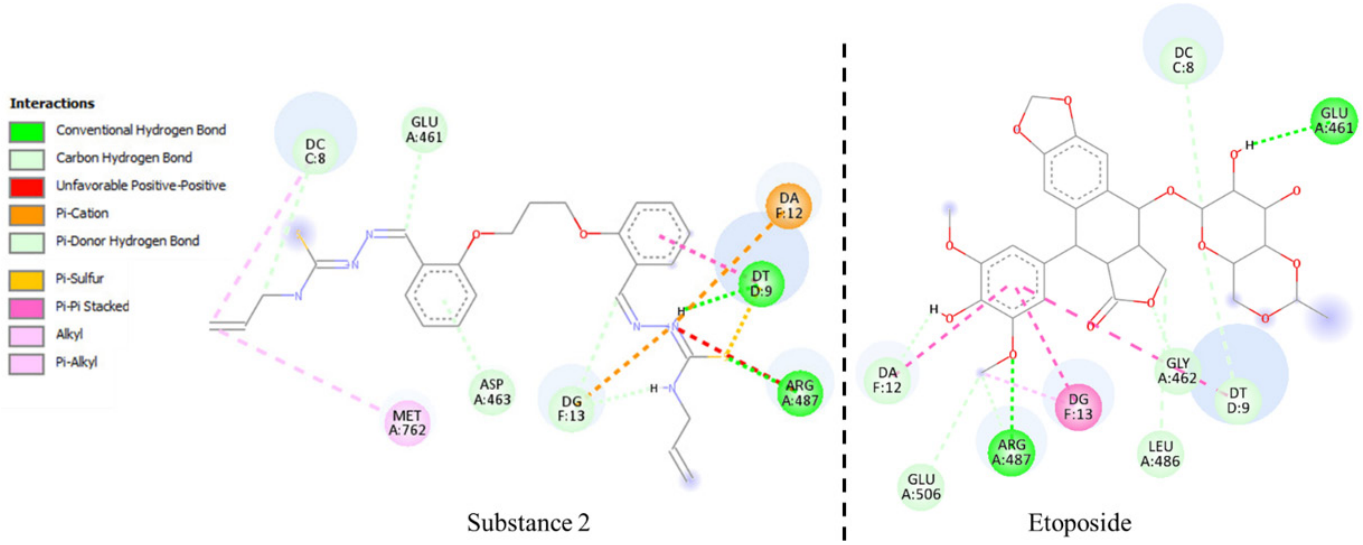
Table 1. Binding affinity of synthesized substances and etoposide to the target protein.

Substance	Etoposide	2	3	4
*K_a, kcal/mol	-12.2	-8.0	-9.5	-8.9

**Binding affinity*

As can be observed from Table 1, the affinity of the known inhibitor (etoposide) is lower than that of the synthesized substances, which indicates that energetically its interaction is more favorable with the target protein compared to the synthesized bis-thiosemicarbazones. The closest to etoposide is the bis-thiosemicarbazone (3) having binding affinity -9.5 kcal/mol results (presented below in Figure 6).

Figure 6. Interactions of substance (2) and etoposide with the topoisomerase IIα active site.



In general the synthesized substances manifest a lower affinity for the target protein compared to etoposide, but they have a fairly similar binding mode to the active site of topoisomerase IIα. This may indicate good potential to manifest the desired activity results (presented below in Table 2).

Table 2. Interaction of etoposide and synthesized substances with the active site amino acid residues of topoisomerase IIα active site.

Substance	Interaction with active site amino acid residues
Etoposide	DC C:8; GLU A:461; DT D:9; GLY A:462; LEU A:486; DG F:13; ARG A:487; GLU A:506; DA F:12
2	DC C:8; GLU A:461; DT D:9; DG F:13; ARG A:487; DA F:12; ASP A:463; MET A:762
3	DC C:8; GLU A:461; DT D:9; DG F:13; ARG A:487; DA F:12; MET A:766; LYS A:614; GLY A:615; ASP A:541; GLY A:488
4	DC C:8; DT D:9; DG F:13; GLY A:760; MET A:766; MET A:762; HIS A:759; LYS A:614

Conclusions

Three new *bis*-thiosemicarbazones were obtained according to the synthesis protocols in very good yields, the structural formulas were confirmed with the help of spectral research methods such as ^{1}HRMN and ^{13}C NMR and FTIR. The anticancer activity potential of the synthesized substances was assessed using molecular docking.

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