SYNTHESIS, COMPUTATIONAL PHARMACOKINETICS REPORT, ADMET STUDY AND MOLECULAR DOCKING OF NEW THIOSEMICARBAZONES POTENTIAL INHIBITORS OF HER2 -POSITIVE BREAST CANCER

SINTEZĂ, FARMACOCINETICĂ COMPUTAȚIONALĂ, STUDIU ADMET ȘI ANDOAREA MOLECULARĂ (DOCKING) A NOILOR TIOSEMICARBAZONE INHIBITORI POTENȚIALI AI CANCERULUI DE SÂN HER2

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To maintain a high level of healthcare, humanity needs to develop new drugs. Thiosemicarbazones represent a very interesting class of substances from the point of view of their biological activities. Despite this, their use is very limited due to their toxicity. To solve this problem, it is possible to synthesize thiosemicarbazones based on natural substances. This can reduce their toxicity without loss of effectiveness. A number of computational methods can be used for the design and preliminary evaluation of thiosemicarbazones, which will help eliminate many unsuitable candidates. This will help reduce the cost and speed up the pace of development of new effective substances from this class. Such methods include ADMET calculations and molecular docking.

***Keywords**: Thiosemicarbazones, anticancer, HER2, molecular docking, ADMET.*

Pentru a menține un nivel ridicat de asistență medicală, omenirea trebuie să dezvolte în permanență noi medicamente. Tiosemicarbazonele reprezintă o clasă foarte interesantă de substanțe din punct de vedere al gamei de activități biologice. În ciuda acestui fapt, utilizarea lor este foarte limitată din cauza toxicității lor. Pentru a rezolva această problemă, este posibil să se sintetizeze tiosemicarbazone pe bază de substanțe naturale. Acest lucru le poate reduce toxicitatea fără pierderea eficacității. O serie de metode de calcul pot fi utilizate pentru proiectarea și evaluarea preliminară a tiosemicarbazonelor, ceea ce va ajuta la eliminarea unor candidați nepotriviți. Acest lucru va ajuta la reducerea costurilor și la accelerarea ritmului de dezvoltare a noilor substanțe eficiente din această clasă. Astfel de metode includ calcule ADMET și andocare moleculară.

***Cuvinte-cheie:** tiosemicarbazone, anticancer, HER2, andocare moleculară, ADMET.*

INTRODUCTION

In recent years, humanity has faced numerous health challenges. The development of antibiotic resistance is of great concern. New epidemics and global pandemics are forcing greater investment in healthcare and research of methods of combating them. Along with the development of medicine, and as this resulted, increase in average life expectancy, there also followed an increase in the incidence of cancer diagnoses in humans. However, their treatment methods are not at the level of our expectations. Many of these factors indicate an urgent need to develop new drugs.

Unfortunately, the development of a new effective drug is currently very expensive – about 1,9 billion used (**Figure 1**). Most of the substances being tested fail tests, and the synthesis of the substance itself can be really expensive. Based on this, the question arises of optimizing this process, which would help save not only money, but also time.

Optimizing the process of discovering new biologically active molecules may involve computer modeling of some of their properties. This stage is not so expensive and time-consuming. At the same time, it will help to determine a huge number of substances that are obviously unsuitable for medical purposes or simply those that fundamentally cannot exhibit the biological activities we need. In addition, this procedure will help to clarify the directions of real biological testing.

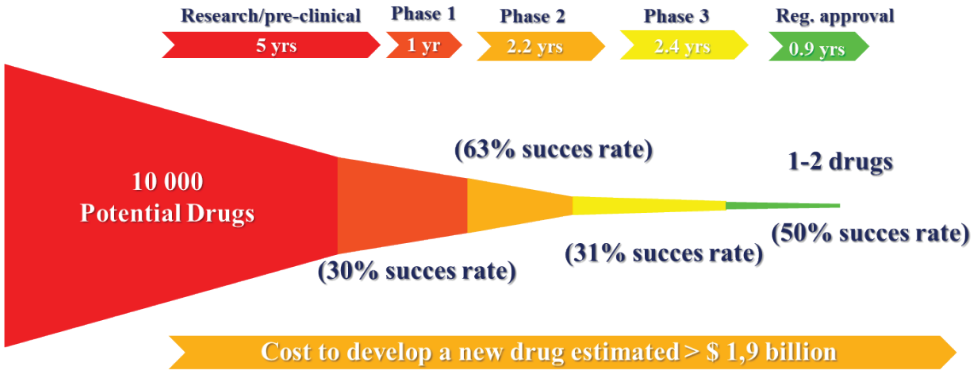


Figure 1. Schematic representation of the process of discovering a new drug [1]

At the moment, there are many directions in the development of new medicines. Among all the diversity, thiosemicarbazones can be distinguished. This class of substances has already proven itself. The substance 3-aminopyridine-2-carboxaldehyde thiosemicarbazone (Triapine®) is used in the treatment of cancer, or the well-known nitrofurazone, although not exactly a thiosemicarbazone, belongs to the class of semicarbazones (the only difference is the replacement of the oxygen atom in semicarbazones with a sulfur atom in thiosemicarbazones), has an antimicrobial effect and is widely used. A major disadvantage of thiosemicarbazones is their toxicity. For the most part, this limits their use. It’s worth noting that their toxicity depends largely on the structure of substi-

tuents in the composition of thiosemicarbazones. In this regard, the development of new thiosemicarbazones based on natural non-toxic substances is of great interest. This may help reduce toxicity, but should not affect effectiveness.

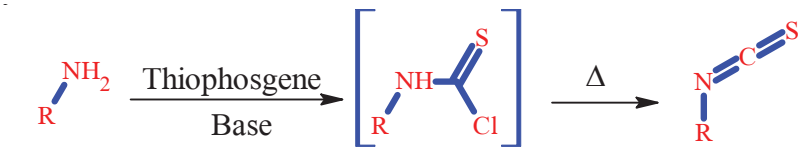
In this work, thiosemicarbazones were synthesized based on α-hexyl cinnamaldehyde. This substance is a derivative of cinnamaldehyde, which is a natural substance that is widely used in the perfume and food industries due to its specific smell and taste. Cinnamaldehyde is non-toxic to humans and has antifungal properties, which also determined its use as a fungicide in the agricultural industry. Various aliphatic radicals were also used to evaluate their effect on the properties of the resulting thiosemicarbazones.

MATERIALS AND METHODS

Research was carried out in the "Advanced Materials in Biopharmaceuticals and Technic" Scientific Research Laboratory of the State University of Moldova. The reagents for synthesis were purchased from "Sigma Aldrich", "Alfa Aesar" and "Acros Organics". No additional purification was performed.

The procedure described in the source [2] was used for the synthesis of the target thiosemicarbazones. Schemes, general procedures, and descriptions of the syntheses performed are presented below.

Synthesis of isothiocyanates



R=*cyclohexyl;ethyl; octyl.*

Figure 2. Scheme of synthesis of isothiocyanates from primary amines

Synthesis of cyclohexyl isothiocyanate

One equivalent of cyclohexylamine was mixed with a solution of $NaHCO_3$ (two equivalents) in water. A solution of thiophosgene (one equivalent) in hexane was carefully added dropwise to the obtained solution, with constant stirring and cooling of the reactant mixture in an ice-salt bath. After the addition of thiophosgene (with the drop, in a hexane - solvent immiscible with water), the mixture was stirred for 3 hours at room temperature. The hexane layer was separated, and washed 3 times with saturated $NaHCO_3$ solution, then washed 3 times with distilled water. The resulting hexane solution of cyclohexyl isothiocyanate was dried with Na_2SO_4. Obtained: colorless oil, yield 85-92%.

Synthesis of ethyl 2-isothiocyanatoacetate

Ethyl 2-aminoacetate (one equivalent) and $NaHCO_3$ (two equivalents) were dissolved in water. Thiophosgene (one equivalent) dissolved in hexane is added to the obtai-

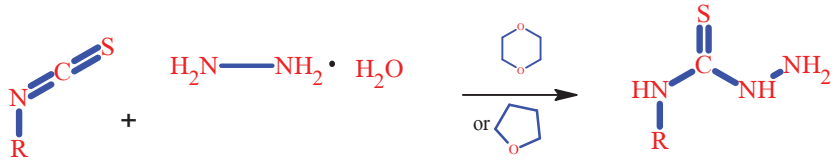
ned solution, with constant stirring, and the reaction mixture is cooled in an ice-salt bath. After the addition of thiophosgene is finished, the reaction mixture is stirred at room temperature for 3 hours. The hexane layer is separated and washed 3 times with saturated $NaHCO_3$ solution and 3 more times with distilled water, after separated and dried with anhydrous Na_2SO_4.The solution is concentrated by distillation of hexane and purified using flash column chromatography with hexane-ethyl acetate (2:1) as eluent. Pink oil is obtained, yield 70-75%.

Synthesis of octyl isothiocyanate

Octylamine (one equivalent) is mixed with a solution of $NaHCO_3$ (two equivalents) in water. A solution of thiophosgene (one equivalent) in hexane is carefully added dropwise to the obtained solution, with constant stirring and cooling of the reactant mixture in an ice-salt bath. After the addition of thiophosgene, the mixture was stirred for 3 hours at room temperature. The hexane layer was separated, and washed 3 times with saturated $NaHCO_3$ solution, then washed 3 times with distilled water. The resulting octyl isothiocyanate solution in hexane was dried by addition of anhydrous Na_2SO_4. Obtained: light yellowish oil, yield 84-95%.

Synthesis of thiosemicarbazides

Hydrazine monohydrate dissolves in tetrahydrofuran/dioxane. An isothiocyanate solution is added dropwise to the resulting solution. The reaction mixture is constantly cooled in an ice-salt bath. After quantitative addition of all the isothiocyanate, the mixture is stirred for about 30 minutes. The precipitate that forms is filtered, washed with cold ethanol and dried.



R=*cyclohexyl;ethyl; octyl.*

Figure 3. Synthesis scheme of thiosemicarbazides from isothiocyanate

Synthesis of N-methylhydrazinecarbothioamide

Alcoholic solution of methyl isothiocyanate is added dropwise to the tetrahydrofuran/dioxane solution of hydrazine monohydrate, with constant stirring. The reaction mixture is cooled in an ice bath. A white precipitate is observed. Then, the mixture is stirred for 30 minutes at room temperature. The obtained solid is filtered, washed with cold tetrahydrofuran/dioxane and air dried. Obtained: white solid, yield 87-89%.

Synthesis of N-ethylhydrazinecarbothioamide

Alcoholic solution of ethyl isothiocyanate is added dropwise to the tetrahydrofuran/dioxane solution of hydrazine monohydrate, with constant stirring. The reaction mixture

is cooled in an ice bath. A white precipitate is observed. Then, the mixture is stirred for 30 minutes at room temperature. The obtained solid is filtered, washed with cold tetrahydrofuran/dioxane and air dried. Obtained: white solid, yield 91-96%.

Synthesis of N-(prop-2-en-1-yl)hydrazinecarbotioamide

Alcoholic solution of allyl isothiocyanate is added dropwise to the tetrahydrofuran/dioxane solution of hydrazine monohydrate, with constant stirring. The reaction mixture is cooled in an ice bath. A white precipitate is observed. Then, the mixture is stirred for 30 minutes at room temperature. The obtained solid is filtered, washed with cold tetrahydrofuran/dioxane and air dried. Obtained: white solid, yield 90-96%.

Synthesis of N-hexylhydrazine carbotioamide

Alcoholic solution of hexyl isothiocyanate is added dropwise to the tetrahydrofuran/dioxane solution of hydrazine monohydrate, with constant stirring. The reaction mixture is cooled in an ice bath. A white precipitate is observed. Then, the mixture is stirred for 30 minutes at room temperature. The obtained solid is filtered, washed with cold tetrahydrofuran/dioxane and air dried. Obtained: white solid, yield 83%.

Synthesis of N-octylhydrazinecarbothioamide

Alcoholic solution of octyl isothiocyanate is added dropwise to the tetrahydrofuran/dioxane solution of hydrazine monohydrate, with constant stirring. The reaction mixture is cooled in an ice bath. A white precipitate is observed. Then, the mixture is stirred for 30 minutes at room temperature. The obtained solid is filtered, washed with cold tetrahydrofuran/dioxane and air dried. Obtained: white solid, yield 95-97%.

Synthesis of N-cyclohexylhydrazinecarbotioamide

Alcoholic solution of cyclohexyl isothiocyanate is added dropwise to the alcoholic solution of hydrazine monohydrate, with constant stirring. The reaction mixture is cooled in an ice bath. A white precipitate is observed. Then, the mixture is stirred for 30 minutes at room temperature. The obtained solid is filtered, washed with cold tetrahydrofuran/dioxane and air dried. Obtained: white solid, yield 94-95%.

Synthesis of N-(bicyclo[2.2.1]heptan-2-yl)hydrazinecarbothioamide

Alcoholic solution of norbornyl isothiocyanate is added dropwise to the tetrahydrofuran/dioxane solution of hydrazine monohydrate, with constant stirring. The reaction mixture is cooled in an ice bath. A white precipitate is observed. Then, the mixture is stirred for 30 minutes at room temperature. The obtained solid is filtered, washed with cold tetrahydrofuran/dioxane and air dried. Obtained: white solid, yield 77-80%.

Synthesis of 2-[(aminocarbamothioyl)amino]ethyl acetate

To an alcoholic solution of hydrazine monohydrate, with constant stirring, an tetrahydrofuran/dioxane solution of ethyl 2-isothiocyanatoacetate is added dropwise. The reaction mixture is cooled in an ice bath. The apparition of the pink precipitate is observed. Then, the mixture is stirred for 30 minutes at room temperature. The obtained solid is filtered, washed with cold tetrahydrofuran/dioxane and air dried. Obtained: pink solid, 90-93% yield.

Synthesis of thiosemicarbazones

All thiosemicarbazones were synthetized using the following general procedure. In a round-bottomed flask equipped with an ascending refrigerant, thiosemicarbazide and carbonyl compound are added in a 1:1 molar ratio. Ethanol is used as a solvent. For catalysis 3-5 drops of glacial acetic acid are added. The mixture is refluxed for 1-8 hours (chromatographic control of the consumption of the initial substances, TLC). After, the flask with the synthesis mixture is cooled. The solid obtained is filtered, washed with ethanol and air dried.

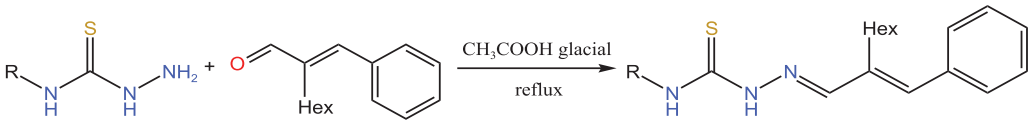


Figure 4. Synthesis scheme of thiosemicarbazones from thiosemicarbazies

Table 1

Summarizes the precursors, synthesis time and yield of the synthesized thiosemicarbazones.

Substance	**Thiosemicarbazide**	**Carbonyl compound**	**Time,** (hours)	**η,** (%)
TSC-1	Hydrazinecarbothioamide	α-hexylcinnamaldehyde	3	95
TSC-2	N-methylhydrazinecarbothioamide			83
TSC-3	N-ethylhydrazinecarbothioamide			85
TSC-4	N-(prop-2-en-1-yl)hydrazinecarbothioamide			96
TSC-5	N-hexylhydrazinecarbothioamide		6	66
TSC-6	N-octylhydrazinecarbothioamide		8	78
TSC-7	N-cyclohexylhydrazinecarbothioamide		6	80
TSC-8	Ethyl [(hydrazinecarbothioyl)amino] acetate		3	65
TSC-9	N-(bicyclo[2.2.1]heptan-2-yl) hydrazinecarbothioamide		6	70

Table 1. Conditions for thiosemicarbazone synthesis

Computational pharmacokinetics report: ADMET parameters calculation

ADMET parameters calculation and studies were performed using SwissDock [3].

Molecular docking

Molecular docking was performed using Autodock Vina [4-5] and visualized using UCSF Chimera [6]. HER2 protein used in docking was obtained from Protein Data Bank [7]. The active site of the protein was determined using the inhibitor complexed with this protein. After it was removed. The coordinates of active site were found to be x=8.78, y= -8.65, z= -14.30 and size 16.5, 17.70 and 13.5 respectively.

Results and discussions

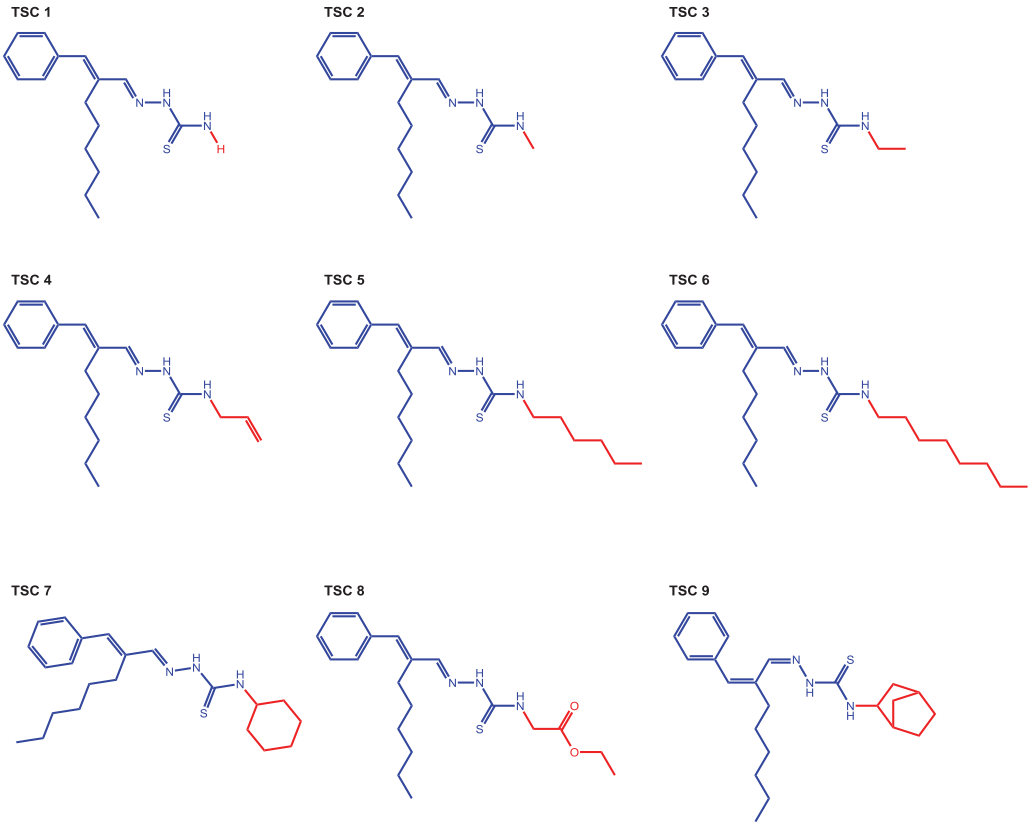


Figure 5. List of synthetized thiosemicarbazones **TSC 1-9**

Nine new thiosemicarbazones were synthesized in good yields (Figure 5.). The functional groups of the synthesized substances were studied using FTIR spectroscopy, which indirectly indicates their structure. The purity of the obtained substances, as well as their precursors, was monitored using thin layer chromatography.

ADMET parameters were calculated for all substances. In general, these parameters establish the conditions under which a substance is likely to be biologically active. The obtained data are displayed in the Figure 6. As can be seen from this figure, substances

TSC-5, TSC-6, TSC-7 and TSC- 9 are too lipophilic. This is explained by the presence of a large number of sp^3 hybridized carbon atoms in the composition. In the case of substances 5 and 6, these are linear radicals hexyl and octyl. In the case of substances 7 and 9, these are aliphatic cyclic systems containing 6 and 7 carbon atoms, respectively. The lipophilicity rule is described by the partition index (logP). Exceeding the optimal range violates Lipinski's rule, which significantly reduces the chances of a substance being biologically active when administered orally. Also, all substances except 1 and 2 exceeded the range of rotatable bonds (Flexibility parameter).

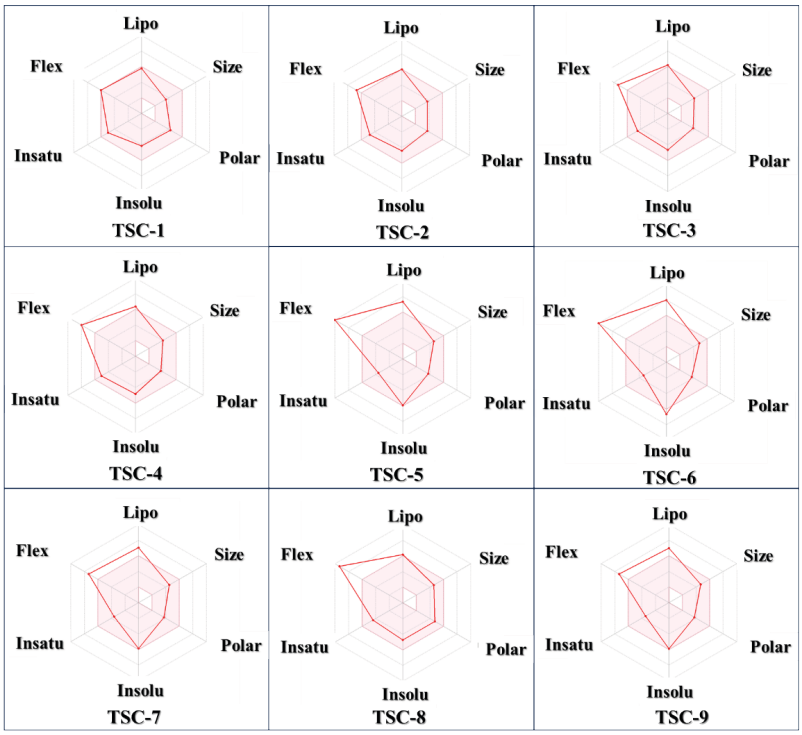


Figure 6. Representation of principal ADMET parameters of synthetized thiosemicarbazones

Otherwise, all substances are in the optimal ranges of values. At the other hand, only substance TSC-1 does not exceed any parameter, and TSC-2 only slightly violates the flexibility parameter. At the Figure 7 can be observed the so-called boiled egg diagram.

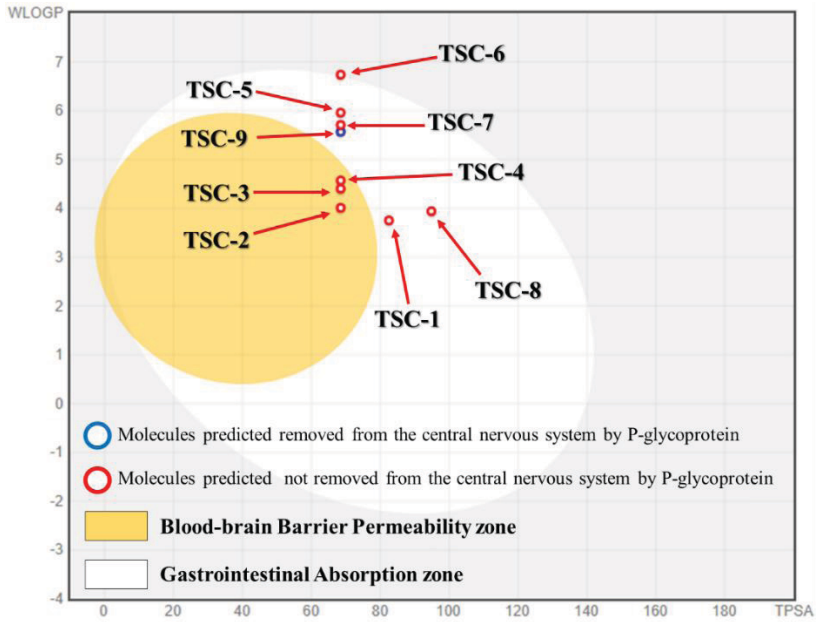


Figure 7. The "Boiled-Egg" diagram for synthetized thiosemicarbazones

This diagram shows which substances can pass the blood-brain barrier. These substances are found in the yolk of diagram. Also, on a white background there are substances that are likely to have high gastrointestinal absorption. And in addition to the above, the dots representing substances are colored blue and red. This indicates whether the substance is a substrate for P-glycoprotein. This fact is very important, since the second name of P-glycoprotein is multidrug resistance protein 1 (**MDR 1**). If a substance is a substrate of this protein, then it will block the cellular uptake of the corresponding substance. This leads to a decrease in the effectiveness of drugs [8]. Of all the synthesized substances, only TSC-9 is substrate for this protein. Therefore, this protein should not affect the mechanism of action of the remaining eight substances.

For all substances with the exception of TSC-6 and TSC-9, a simulation of interaction with the HER2 protein was performed. This protein promotes the growth of breast cancer cells. HER2 positive cancer are usually more aggressive. Research shows that treatment of this type of cancer by targeted inhibition of the HER2 protein is quite successful [9]. These factors determined the choice of this particular protein for screening. From Table 1 can be observed that most of the thiosemicarbazones have quite good results. But TSC-7 shows the best result by a wide margin. Overall, it has binding energy comparable to anti-cancer drugs. [10]

Table 2

Results of protein-tiosemicarbazone interaction simulations

Binding Energy of Interaction with HER2 protein							
Substance	TSC-1	TSC-2	TSC-3	TSC-4	TSC-5	TSC-7	TSC-8
Binding Energy, kcal/mol	-6.4	-6.9	-6.4	-6.8	-7	-7.6	-6.9

Figure 8 shows the interaction of TSC-7 with the active site of the HER2 protein. A strong hydrogen bond has formed between the sulfur atom from the thiosemicarbazone and the hydrogen atom from the amino acid lysine.

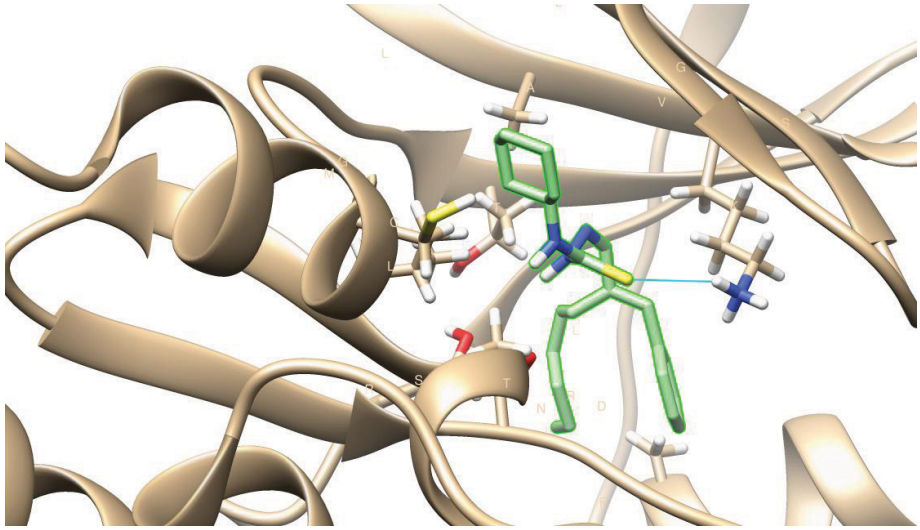


Figure 8. Interaction of TSC-7 with the HER2 active site

In addition to the above, Electrostatic Potential Maps were generated for the synthesized thiosemicarbazones. These are shown in Figure 9. These maps are a useful tool for finding reaction centers within a molecule, as they display the partial charges formed on the atoms.

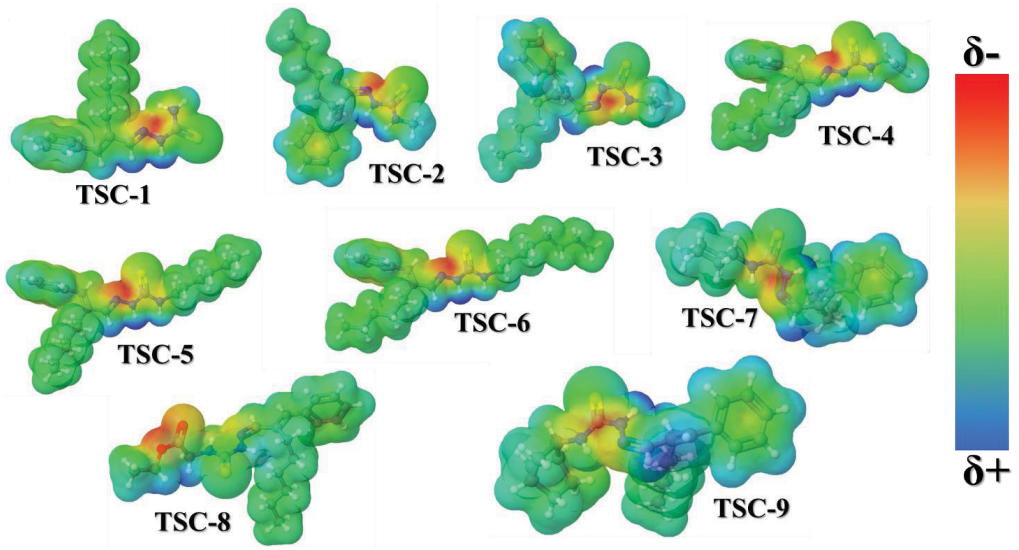


Figure 9. Electrostatic Potential Map for synthetized thiosemicarbazones (visualized with jmol)

In our case, no cardinal changes are observed in most cases. However, in the case of the TSC-8, the reaction center shifts towards the carboxyl group. This may mean the possibility of further functionalization of thiosemicarbazone, because in this case the azomethine group appears to be less reactive and therefore more stable.

CONCLUSIONS

Nine thiosemicarbazones were synthesized in excellent yields and characterized, evaluated *computational pharmacokinetics report:* principal ADMET parameters, interaction of TSC-7 with the HER2 active site, Electrostatic Potential Map for synthetized thiosemicarbazones. For all substances with the exception of TSC-6 and TSC-9, a simulation of interaction with the HER2 protein was performed. This protein promotes the growth of breast cancer cells. HER2 positive cancer are usually more aggressive. Research shows that treatment of this type of cancer by targeted inhibition of the HER2 protein is quite successful. These factors determined the choice of this particular protein for screening.

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