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ORIGINAL ARTICLE

Medicine Science International Medical Journal

Medicine Science 2020;9(2):305-13

Synthesis and computer - aided drug design studies of novel thiosemicarbazide derivatives as potent and target - oriented anti - cancer agents

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Received 20 September 2019; Accepted 12 December 2019
Available online 08.04.2020 with doi: 10.5455/medscience.2019.08.9188

Abstract

A series of novel thiosemicarbazide derivatives **(2a-d)** were synthesized from 3,5-bis(trifluoromethyl) benzohydrazide **(1)** and various substituted isothiocyanates. The structures of novel compounds were determined by analytical and spectral (IR, ^{1}H-NMR, and elemental analysis) methods. *In silico* studies were conducted to determine and evaluate the potential anticancer activity of the compounds. Target-oriented drug design is crucial for cancer therapy for increasing the selectivity and consequently decreasing the adverse effects of anticancer agents. Computer-aided drug design technology enables us to design and develop target-oriented and hence, selective therapeutic agents. We benefited that technology during our drug design process and selected our targets as ATP-dependent enzyme topoisomerase II (Topo II), epidermal growth factor receptor (EGFR) tyrosine kinase domain, carbonic anhydrase IX and tubulin-colchicine: stathmin-like domain complex, which has significant roles in the cancer development process by their biochemical and physiological activities. In the light of the results obtained from *in silico* studies, the title compounds displayed significant potential activity about possessing the qualification of being multi-target drugs by effecting and hitting a few of the main targets of cancer chemotherapy together and at the same time.

Keywords: Thiosemicarbazide, synthesis, computer-aided drug design

Introduction

Lately, much interest has been focused on the chemistry, and biological activity of the compounds bearing thiosemicarbazide moiety because of their attractive biological activities and consequently, a variety of novel compounds is being added to the science world every year [1,2]. Since their diverse pharmacological activities, compounds bearing thiosemicarbazide moiety possess specific importance. The thiosemicarbazide derivatives have been reported in the literature as antibacterial [3], antifungal [4], antitubercular [5], anticonvulsant [6], antitumor [7], antioxidant [8], antimalarial [9] agents (Figure 1).

Thiocetazone (Antitubercular drug), triapine (Anticancer drug), and methisazone (Anticancer drug) are drug molecules that are used in treatment currently and bearing thiosemicarbazide/ thiosemicarbazone moiety. The chemical structures of the mentioned drugs are displayed in Figure 2.

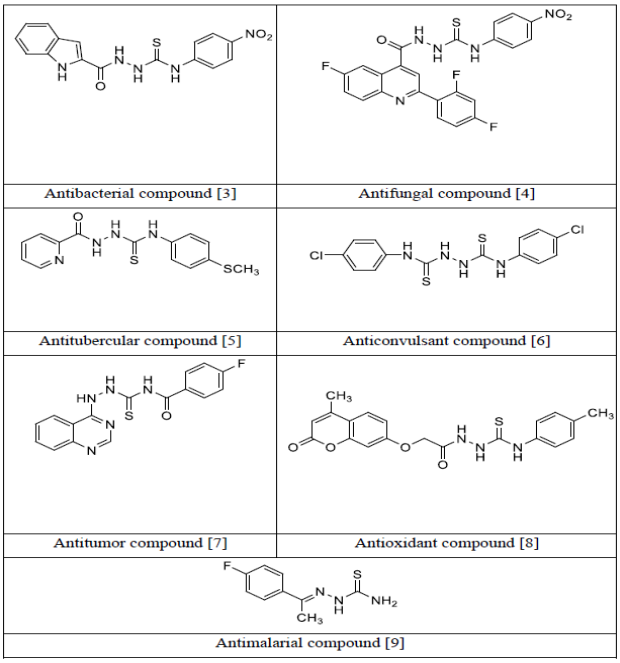

Figure 1. Some biologically active compounds bearing thiosemicarbazide moiety

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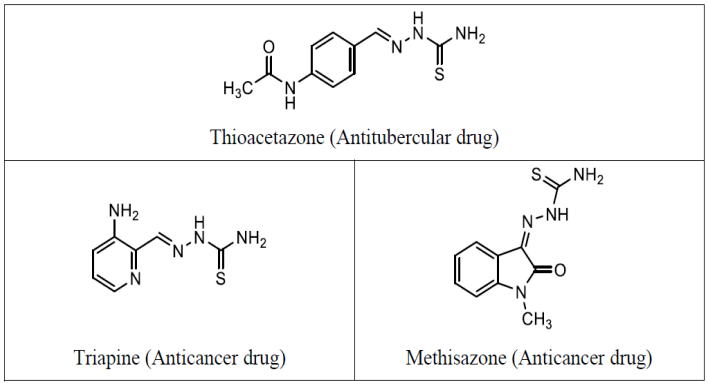

Figure 2. The drug molecules which are currently used in the treatment and bearing thiosemicarbazide/thiosemicarbazone moiety

The addition of diverse isothiocyanates to various hydrazides is one of the convenient methods of the synthesis of substituted thiosemicarbazides [10-12]. The significance of thiosemicarbazides is not only their potential biological activity but also their potential about being used as the starting compounds for the synthesis of diverse compounds like different triazoles and thiadiazoles [13]. All these reasons indicate that the optimization of thiosemicarbazides can eventuate a major discovery of a novel class of therapeutic agents.

On the other hand, the design and synthesis of drug or drug candidate compounds containing fluorine atoms have been another topic that has attracted the attention of researchers in recent years [14-16]. The different properties of fluorine atom arising from its natural structure, and different physicochemical properties it brings to the compound to which it belongs, constitute the reason for this interest [14-16]. Studies have shown that the substitution of a fluorine atom to a bioactive molecule causes minimal steric changes, thereby facilitating the interaction between the compound and the active sites of enzymes and other biological systems. On the other hand, the high electronegativity of fluorine atom significantly changes the physical and chemical properties of the molecule in which it is substituted [14-16]. The incorporation of a fluorine atom into a molecule is generally considered chemical and biological. Table 1 summarizes the properties of organic compounds bearing fluorine atoms.

Table 1. The chemical and biological effect of the incorporation of a fluorine atom into a molecule [14-16].

Relatively small atomic diameter	Electronegative effect on neighboring functional groups
Lipophilicity	Strong C-F bond resistant to metabolic processes
High electronegativity	Increasing lipid solubility, biocompatibility
Tightly bound, three non-bonding electron pairs	Synthesis of isosteric derivatives of drugs
Very low polarizability	The excellent overlap between F 2s and 2p with corresponding orbitals of other second period elements

Today, it is clear that, within all serious disease's cancer has specific importance because of its complex pathophysiology. There has been enormous progress in the treatment of cancer but unfortunately, still novel and more efficient drugs are urgently needed to deal with this disease [17]. One of the most important biochemical characteristics of thiosemicarbazides is their potential about being used in cancer treatment. In cancer treatment, the key role of thiosemicarbazide derivatives is usually associated with their capability to suppress the enzyme ribonucleotide reductase. Triapine and methisazone, are the exemplary drugs displaying a similar effect mechanism [18]. Also, recently, diverse enzymes have been reported in the literature as potential points that can be targeted and affected by thiosemicarbazide derivatives in the treatment of cancer.

Computer-aided drug design technology minimizes the wasted time and financial burden used in the drug discovery process [19,20]. Research in the pharmaceutical industry shows a great increase and progress with modern computational medicinal chemistry [21]. 2D QSAR, 3D QSAR, and molecular docking studies can be carried out with the help of that technology [22-24]. Also, pharmacodynamic (potency, affinity, efficacy, and selectivity), pharmacokinetic (ADME: absorption, distribution, metabolism, and excretion) and toxicity data of molecules can be evaluated and analyzed by that technology [25]. As a result, all of these methods contribute to an efficient and selective drug discovery process.

Potential anticancer activity of the compounds bearing thiosemicarbazide moiety, and the biological and physicochemical advantages of the organofluorine molecules mentioned above, have led us to identify our target molecules as compounds containing trifluoromethyl group and bearing thiosemicarbazide moiety. From this point forward, in this study, a series of novel thiosemicarbazide derivatives bearing trifluoromethyl moiety were synthesized, and their chemical structures were illuminated by IR, ^{1}H NMR and elemental analysis. *In silico* studies were conducted to determine and evaluate the potential biological activity of the compounds.

Materials and Methods

Materials

IR spectra were recorded on KBr discs, using a Shimadzu IR Affinity-1 FT-IR instrument. ^{1}H NMR (500MHz) spectra were recorded on Varian UNITY INOVA spectrometer in DMSO-d_6 solution. Chemical shifts (δ) were reported in ppm; coupling constants (*J*) were recorded in hertz (Hz). The reactions were monitored by TLC aluminum plates with silica gel Kieselgel 60 F254 thickness 0.25mm (Merck), using UV light as a visualizing agent. All reagents and solvents were purchased from Merck, Fluka, and Sigma-Aldrich and were used without further purification. Schrödinger Suite of Molecular Modeling Software (Schrödinger Release 2018-3: Schrödinger, LLC, New York, NY, 2018) was used for *in silico* studies.

Chemical Synthesis

General procedure for the synthesis of 2- [3,5-bis (trifluoromethyl) benzoyl] -*N*- arylhydrazinecarbothioamides (2a-d)

0.005 moles of various substituted isothiocyanates are added to a solution of 0.005 moles of 3,5-bis (trifluoromethyl) benzohydrazide (1) in 20 ml of absolute ethanol and heated in a water bath for 3 hours under reflux. After cooling the mixture to room temperature, it is filtered and purified by crystallization with an appropriate solvent.

2- (3,5-bis (trifluoromethyl) benzoyl) -*N*- phenylhydrazine -1- carbothioamide (2a)

The compound is obtained from 1.36 g (0.005 moles) of 3,5-bis (trifluoromethyl) benzohydrazide (**1**) with 0.59 ml (0.005 moles) of phenyl isothiocyanate according to the general synthesis method and purified by crystallization with ethanol.

Mp 168-169 °C, yield: 75.67%. Anal. Calcd. For $C_{16}H_{11}F_6N_3OS$ (M.W. 407.33): C: 47.18 H: 2.72 N: 10.32. Found: C: 47.25 H: 2.78 N: 10.37. IR υmax (KBr, cm^{-1}): 3329, 3190 (N-H stretching), 3061 (Aromatic C-H stretching), 1672 (Amide I C=O stretching), 1552, 1496, 1454 (Aromatic C=C stretching and amide II N-H bending vibrations combined with C-N stretching), 1280, 1230 (Amide III N-H bending vibrations combined with C-N stretching), 1178 (C=S stretching), 906, 682 (Aromatic 1,3,5-trisubstitution), 758, 698 (aromatic monosubstitution). ^{1}H-NMR (500 MHz) (DMSO-d6/ TMS) δ (ppm): 11.07 (s, 1H, NH), 9.85 (s, 2H, NH), 8.57 (s, 2H, ar.), 8.40 (s, 1H, ar.), 7.40-7.33 (m, 4H, ar.), 7.20-7.16 (m, 1H, ar.).

2- (3,5-bis (trifluoromethyl) benzoyl) -*N*- (4-methylphenyl) hydrazine -1- carbothioamide (2b)

The compound is obtained from 1.36 g (0.005 moles) of 3,5-bis(trifluoromethyl)benzohydrazide (**1**) with 0.74 g (0.005 moles) of 4-methylphenylisothiocyanate (p-tolylisothiocyanate) according to the general synthesis method and purified by crystallization with ethanol.

Mp 165-166°C, yield 86.19%. Anal. Calcd. For $C_{17}H_{13}F_6N_3OS$ (M.W. 421.36): C: 48.46 H: 3.11 N: 9.97. Found: C: 48.45 H: 3.14 N: 10.06. IR υmax (KBr, cm^{-1}): 3323, 3223 (N-H stretching), 3091 (Aromatic C-H stretching), 3005, 2924, 2864 (aliphatic C-H asymmetric and symmetric stretching), 1678 (Amide I C=O stretching), 1554, 1517, 1454 (Aromatic C=C stretching and amide II N-H bending vibrations combined with C-N stretching), 1371, 1332 (Aliphatic C-H asymmetric and symmetrical bending band), 1284, 1199 (Amide III N-H bending vibrations combined with C-N stretching), 1170 (C=S stretching), 906, 682 (Aromatic 1,3,5-trisubstitution), 846, 813 (Aromatic 1,4-disubstitution). 1H-NMR (500 MHz) (DMSO-d_6/TMS) δ (ppm): 11.06 (s, ^{1}H, NH), 9.80 (s, 2H, NH), 8.57 (s, 2H, ar.), 8.38 (s, 1H, ar.), 7.33 (s, 2H, ar.), 7.15 (s, 2H, ar.), 2.28 (s, 3H, CH_3).

2-(3,5-bis(trifluoromethyl)benzoyl)-*N*-(4-fluorophenyl) hydrazine-1-carbothioamide (2c)

The compound is obtained from 1.36 g (0.005 moles) of 3,5-bis(trifluoromethyl)benzohydrazide (**1**) with 0.63 ml (0.005 moles) of 4-fluorophenyl isothiocyanate according to the general synthesis method and purified by crystallization with ethanol.

Mp 172-174 °C, yield 72.16 %. Anal. Calcd. For $C_{16}H_{10}F7N_3OS$ (M.W. 425.32): C: 45.18 H: 2.37 N: 9.88. Found: C: 44.89 H: 2.37 N: 9.95. IR υmax (KBr, cm^{-1}): 3311, 3242 (N-H stretching), 3072 (Aromatic C-H stretching), 1674 (Amide I C=O stretching), 1562, 1510, 1456 (Aromatic C=C stretching and amide II N-H bending vibrations combined with C-N stretching), 1284, 1226 (Amide III N-H bending vibrations combined with C-N stretching), 1178 (C=S stretching), 912, 682 (Aromatic 1,3,5-trisubstitution), 833, 819 (Aromatic 1,4-disubstitution). ^{1}H-NMR (500 MHz) (DMSO-d_6/ TMS) δ (ppm): 11.08 (s, 1H, NH), 9.90 (s, 1H, NH), 9.88 (s, 1H, NH), 8.56 (s, 2H, ar.), 8.40 (s, 1H, ar.), 7.39 (s, 2H, ar.), 7.20-7.15 (m, 2H, ar.).

2- (3,5-bis (trifluoromethyl) benzoyl) -*N*- (4-bromophenyl) hydrazine -1- carbothioamide (2d)

The compound is obtained from 1.36 g (0.005 moles) of 3,5-bis (trifluoromethyl) benzohydrazide (**1**) with 1.07 g (0.005 moles) of 4-bromophenyl isothiocyanate according to the general synthesis method. And purified by crystallization with ethanol.

Mp 173-174 °C, yield 98.76%. Anal. Calcd. For $C_{16}H_{10}BrF_6N_3OS$ (M.W. 486.23): C: 39.52 H: 2.07 N: 8.64. Found: C: 39.84 H: 2.68 N: 8.30. IR υmax (KBr, cm^{-1}): 3213 (N-H stretching), 3055 (aromatic C-H stretching), 1689 (Amide I C=O stretching), 1541, 1533, 1489 (Aromatic C=C stretching and amide II N-H bending vibrations combined with C-N stretching), 1278, 1246 (Amide III N-H bending vibrations combined with C-N stretching), 1188 (C=S stretching), 912, 682 (aromatic 1,3,5-trisubstitution), 846, 810 (Aromatic 1,4-trisubstitution). ^{1}H-NMR (500 MHz) (DMSO-d_6/TMS) δ (ppm): 11.10 (s, 1H, NH), 9.98 (s, 1H, NH), 9.89 (s, 1H, NH), 8.57 (s, 2H, ar.), 8.38 (s, 1H, ar.), 7.54-7.51 (m, 2H, ar.), 7.42 (s, 2H, ar.).

Docking studies

Target proteins and protein preparation

ATP-dependent enzyme topoisomerase II (Topo II)

An attractive target in the treatment of cancer is Topo II, which has the ability to arrange the conformational alterations in DNA topology required for transcription, replication, and chromosome condensation and segregation. Today, we can array the currently used Topo II inhibitors as etoposide, teniposide, doxorubicin, daunorubicin, idarubicin, and mitoxantrone, which can be classified as highly anti-neoplastic drugs. In our study, we chose that enzyme as a potential and effective target for developing new and effective anti-cancer drugs and downloaded the 3D structure of the binding site of Topo II complexed with AMP-PNP (PDB ID 1ZXN [26]). The crystal structure was obtained from Homo-sapiens organism, and the resolution value was 2.51 Å. Protein preparation Wizard of Schrödinger was utilized for the protein preparation process. Hydrogen atoms were added, and bond orders were assigned for the preprocessing procedure of the protein structure. The water molecules, that can constitute bridges between ligand and the protein were kept and the other were removed.

Epidermal growth factor receptor (EGFR)

Angiogenesis has been widely investigated as a significant and remarkable cancer therapeutic target during the last decade. Since the qualification of being the limiting step of the spreading ability of tumor cells, angiogenesis became one of the major targets that attracted the interest of researchers for the treatment of cancer disease. In the literature, some significant receptors were reported and described as being in a close relationship with the angiogenesis process. Vascular endothelial growth factor receptor (VEGFR) and EGFR are the most important of them. The mentioned growth factor kinases have vital roles in the progression and development of many mortal tumors like non-small cell carcinoma and glioblastoma. Within these kinases, EGFR (also called as HER-1), which is overexpressed in cancer tissue and cells, has been considered as being vital in cancer. Erlotinib can inhibit EGFR, and currently, it is an approved antitumor agent.

As a result, in our study, we chose that enzyme as a potential and effective target for developing novel anti-cancer drugs and downloaded 3D structure of the binding site of the EGFR tyrosine kinase domain with 4-anilinoquinazoline inhibitor erlotinib (PDB ID 1M17 [27]). The crystal structure was obtained from Homo-sapiens organisms, and the resolution value was 3.58 Å. Protein preparation Wizard of Schrödinger was utilized for the protein preparation process. Hydrogen atoms were added, and bond orders were assigned for the preprocessing procedure of the protein structure. The water molecules, that can constitute bridges between ligand and the protein were kept and the others were removed.

Carbonic anhydrase IX

The last studies displayed that the necrosis shaped around a cancerous tissue depends on the overexpression of CA IX enzymes at the tumor domain. CA IX controls the pH of the tumor domain and consequently affects the formation of necrosis.

Moreover, hCA IX is expressed in an exceedingly restricted range of healthy tissues, whereas it's overexpressed in tumors. The abundance of hCA IX in cancerous tissue causes a pH imbalance of tumors and promoting dramatically to the acidification of cancerous tissue. For these reasons, it is obvious that the CA inhibitors are considerable molecules for the synthesis of new-generation anticancer drugs.

As a result, in our study, we chose that enzyme as a potential and effective target for developing novel anti-cancer drugs and downloaded 3D crystal structure of the catalytic domain of the tumor-associated human carbonic anhydrase IX (PDB ID: 3IAI [28]). The crystal structure was obtained from Homo-sapiens organism and the resolution value was 2.2 Å. Protein preparation Wizard of Schrödinger was utilized for the protein preparation process. Hydrogen atoms were added, and bond orders were assigned for the pre-processing procedure of the protein structure. The water molecules, that can constitute bridges between ligand and the protein were kept and the others were removed.

Tubulin inhibitors

Alfa and beta-tubulin heterodimers constitute cylindrical proteins. These cylindrical proteins are named microtubules and play vital roles in the formation of cell structure. Inhibition of the formation of microtubules results in mitotic arrest, and this causes cell death. Therefore, inhibition of microtubule formation is a remarkable target for various cancer drugs. Vinca alkaloids, taxanes and other antimitotic agents are used for their tubulin inhibitor activity. However, difficulty in synthesis and high cost limited their uses.

So, searching for new antimitotic agents with better activity still is a necessity. As a result, in our study, we chose that enzyme as a potential and effective target for developing novel anti-cancer drugs and downloaded 3D crystal structure of Tubulin-colchicine: Stathmin-like domain complex (PDB ID: 1SA0 [29]). The crystal structure was obtained from Homo-sapiens organism, and the resolution value was 2.2 Å. Protein preparation Wizard of Schrödinger was utilized for the protein preparation process. Hydrogen atoms were added, and bond orders were assigned for the pre-processing procedure of the protein structure. The water molecules, that can constitute bridges between ligand and the protein were kept and the others were removed.

Ligand Preparation

Three-dimensional structures of newly synthesized thiosemicarbazide derivatives were drawn, and the molecules optimized by the help of the LigPrep of Schrödinger Suite of Molecular Modeling Software (Schrödinger Release 2018-3: Schrödinger, LLC, New York, NY, 2018) by applying OPLS3 force field. The specified chiralities were held at target pH, 7.0(±2.0). Tautomers were also produced and considered for further analyses.

Receptor grid generation, molecular docking, and binding energy determination

The scoring grids were positioned around the ligand molecule with the default size. ''Dock ligands similar in size to the workspace ligand'' option was selected. No constraints were applied to form the grid boxes. Glide was used to describe and detect the appropriate interactions between ligand and receptor. The receptor grid was selected, and the prepared ligands were used for the docking process. Extra precision mode (XP) and Standard precision mode (SP) were used during the docking process. Root mean square deviation (RMSD) value was used to validate our docking protocol. RMSD is the measure of the average distance between atoms of superimposed proteins [30-32]. RMSD calculation can be used for non-protein molecules, such as small organic molecules. RMSD has often been used to measure the quality of reproduction of a known (i.e. crystallographic) binding pose by a computational method, such as docking. Validation of docking protocol means performing docking of a crystallographic complex protein with a ligand in it and checking for the RMSD values [30-32]. If the docking protocol produces similar docking pose of a ligand with respect to the biological configuration of the same ligand in the crystal structure of complex protein, then it means the docking protocol is validated. The lower the value of RMSD, the higher the accuracy of docking procedure [30-32]. Schrödinger's software suite (Schrödinger 2018-3) was used for all processes.

Results

Chemistry

The target compounds **2a-d** were synthesized from 3,5-bis(trifluoromethyl)benzohydrazide (**1**) by a one-step synthesis through the pathway shown in Figure 3. When the chemical structures of the synthesized compounds are examined, it is observed that the C=S group is not present in compound **1**, whereas the same group is present in the thiosemicarbazide moiety of the compounds **2a-d**. Also, according to IR spectroscopy results, the stretching band belonging to the thiocarbonyl group was not visible in compound **1** whereas the mentioned band was able to be observed in the range of 1170-1188 cm^{-1} for compound **2a-d**. In ^{1}H-NMR analysis, the NH_2 protons of the hydrazide moiety of compound **1**, which were observed in the range of 4-4.5 ppm was disappeared after transforming to the compound **2a-d** and also the existence of the three N-H peaks belonging the thiosemicarbazide moiety of compound **2a-d**, which were observed in the range of 11.10-9.80 were distinctive for the structural verification of compound **2a-d**. The compound **1** had just one N-H peak coming from its hydrazide moiety. Also, because of the condensation of aromatic isothiocyanate groups with compound **1**, the novel formed compounds (**2a-d**) had more peaks (Totally 7H for compound **2b-d** and 8H for compound **2a** whereas compound 1 had just 3H arising from aromatic groups) within the aromatic field arising from 2 benzene rings.

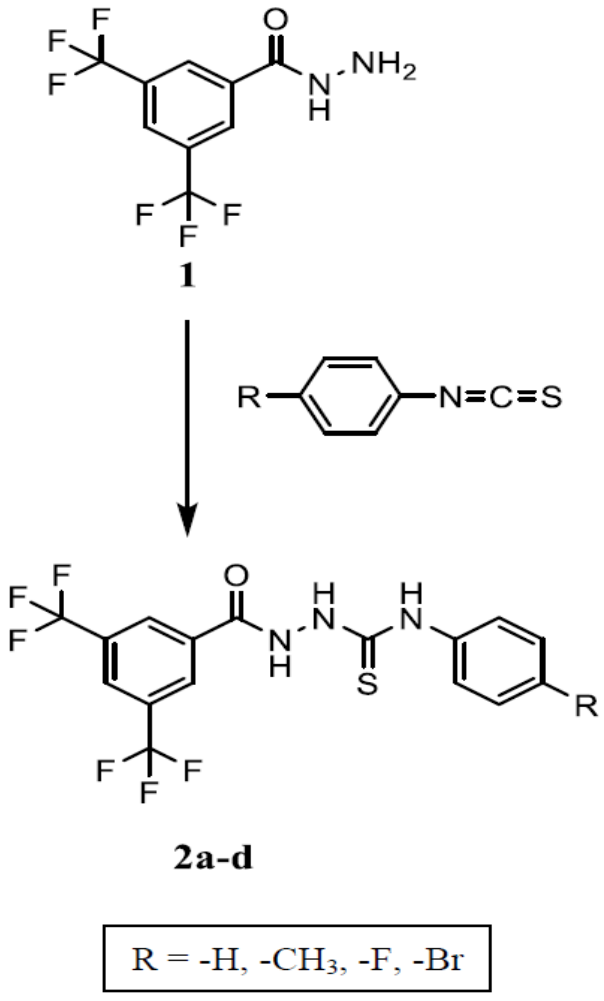

Figure 3. Synthesis of the title compounds (**2a-d**)

Molecular docking

ATP-dependent enzyme topoisomerase II (Topo II)

The validation of the docking model was conducted by the native ligand of the structure, and RMSD value was found as 0.232 which represented a good reproduction of the correct pose. SP (standard precision) was used during the docking process. The interactions that exist between the active site of topoisomerase II enzyme (PDB ID: 1ZXN) and its native ligand Adenosine-5′-diphosphate are shown in Figure 4. As can be seen in figure 4, the native compound fits well into the binding pocket, forming 12 hydrogen bonding interactions with water molecules and ASN91, ASN120, ARG162, GLY164, ASN150, SER148, ALA167. Also, we see the magnesium ion plays an important role with its +2 charge for the existence of interaction that occurs between ligand and the binding pocket. The docking scores of native ligands and our original molecules are given as in Table 2. The compound **2a**, which has the best docking score value within our compounds, and its interactions with the binding pocket of the topoisomerase II enzyme, is given in Figure 5. As can be seen in Figure 5, compound **2a** has two clear hydrogen bond interactions with SER149 and ASN150. Also, we see a pi-cation interaction that exists between ligand and ARG98.

Table 2. The docking scores of native ligand and title compounds.

Compound	Docking Score
The native ligand of topoisomerase II (PDB ID: 1ZXN), Adenosine-5′-diphosphate	11.971
2a	-4.552
2b	-2.974
2c	-4.103
2d	-3.264

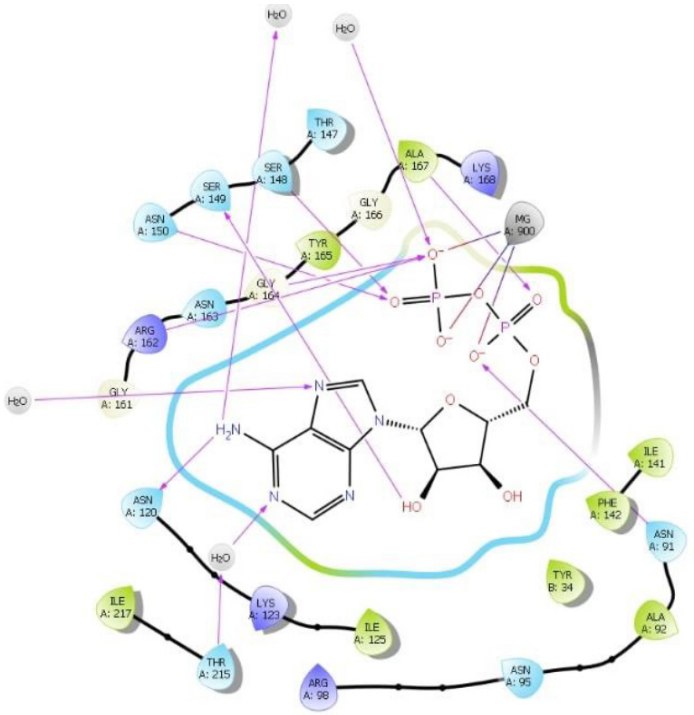

Figure 4. The active site of topoisomerase II enzyme (PDB ID: 1ZXN) in complex with its native ligand Adenosine-5′-diphosphate

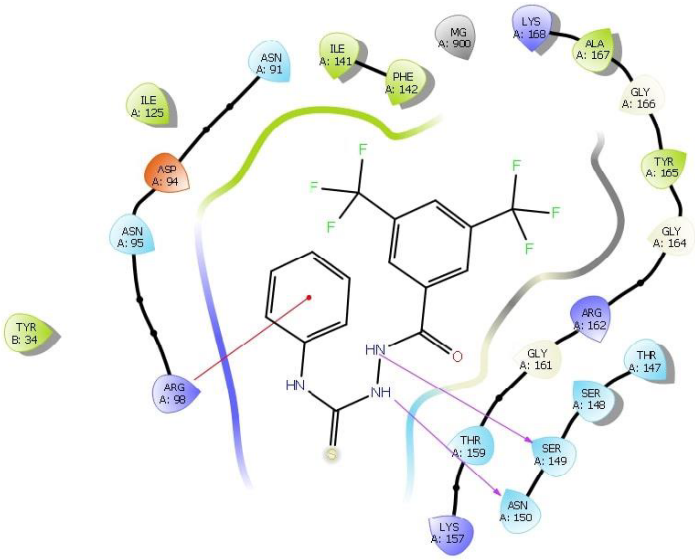

Figure 5. Compound **2a**, which has the best docking score value within our compounds, and its interactions with the binding pocket of the topoisomerase II enzyme (PDB ID: 1ZXN).

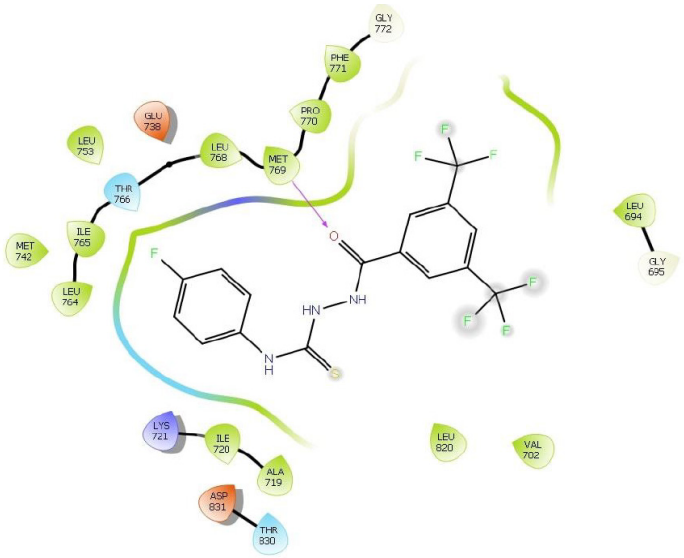

Figure 6. Compound **2c**, which has the best docking score within our compounds, and its interactions with binding pocket of EGFR tyrosine kinase domain (PDB ID: 1M17)

Epidermal growth factor receptor (EGFR)

The validation of the docking model was conducted by the native ligand of the structure, and RMSD value was found as 1.157 which represented a good reproduction of the correct pose. XP (extra precision) was used during the docking process. The interactions that exist between the active site of the EGFR tyrosine kinase domain (PDB ID: 1M17) and its native ligand erlotinib were examined and illuminated. The obtained results displayed that erlotinib fits well into the binding pocket, forming three hydrogen bond interactions with a water molecule and MET769, CYS773 with a docking score of -9.796. The molecular structure of erlotinib is quite favorable for the active site of the receptor. The docking scores of the native ligand erlotinib and our original molecules are given as in Table 3. The compound **2c** which has the best docking score within our compounds and its interactions with the binding pocket of the EGFR tyrosine kinase domain (PDB ID: 1M17), is given in Figure 6. The obtained in silico results displayed that, compound **2a** has one clear hydrogen bond interaction with MET769. Also, the compound **2b** has three hydrogen bond interactions with ARG817 and AS831. Additionally, pi-cation and pi-pi stacking interactions can be observed between **2b** and LYS721, PHE699. In figure 6, it can also be seen that the compound **2c** has one clear hydrogen bond interaction with MET769 and fits quite well in the binding pocket of the receptor. *In silico* studies also displayed the existence of two clear hydrogen bond interactions between compound **2d** and the protein.

Table 3. The docking scores of erlotinib and title compounds

Compound	Docking Score
Erlotinib, the native ligand of EGFR tyrosine kinase domain (PDB ID: 1M17)	-9.796
2a	-6.387
2b	-4.541
2c	-6.889
2d	-3.769

Carbonic anhydrase IX

The native ligand of the structure conducted the validation of the docking model and RMSD value was found as 1.604 which represented good reproduction of the correct pose. SP (standard precision) was used during the docking process.

The interactions that exist between the active site of the catalytic domain of the tumor-associated hCA IX (PDB ID: 3IAI) and its native ligand 5-acetamido-1,3,4-thiadiazole-2-sulfonamide were examined and illuminated. The obtained results displayed that the native ligand fits well into the binding pocket with a docking score of -5.604, forming three hydrogen bond interactions with water molecules and THR200. The molecular structure of the native ligand is quite favorable for the active site of the receptor. The docking scores of the native ligand 5-acetamido-1,3,4-thiadiazole-2-sulfonamide and our original molecules are given in Table 4.

Table 4. The docking scores of 5-acetamido-1,3,4-thiadiazole-2-sulfonamide and the title compounds

Compound	Docking Score
5-acetamido-1,3,4-thiadiazole-2-sulfonamide, the native ligand of the crystal structure of the catalytic domain of the tumor-associated hCA IX (PDB ID: 3IAI)	-5.604
2a	-5.347
2b	-4.679
2c	-4.587
2d	-2.360

Compound **2a**, which has the best docking score within our compounds and its interactions with the active site of the catalytic domain of the tumor-associated hCA IX (PDB ID: 3IAI), is given in figure 7. The obtained *in silico* results displayed that, compound **2a** has three clear hydrogen bond interactions with water molecules and additionally, one pi-pi stacking interaction with HIS94. Results also indicated that compound **2b** has four clear hydrogen bond interactions with water molecules and GLN92, additionally

one pi-pi stacking interaction with HIS94. It was also illuminated, compound **2c** has two clear hydrogen bond interactions with a water molecule and the obtained results displayed the existence of one clear hydrogen bond interaction between compound **2d** and a water molecule.

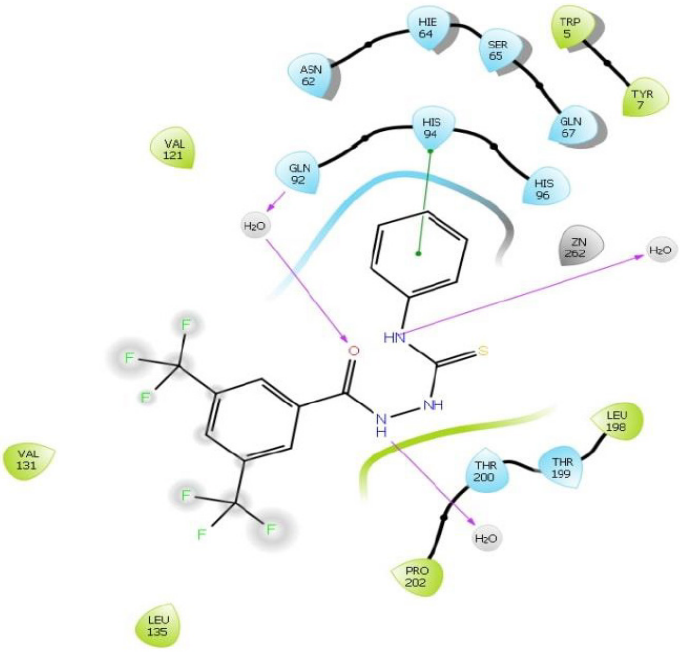

Figure 7. The active site of the catalytic domain of the tumor-associated hCA IX (PDB ID: 3IAI) in complex with **2a**

Tubulin inhibitors

The validation of the docking model was conducted by the native ligand of the structure, and RMSD value was found as 1.304 which represented a good reproduction of the correct pose. SP (standard precision) was used during the docking process. The interactions that exist between the active site of the Tubulin-colchicine: Stathmin-like domain complex (PDB ID: 1SA0) and its native ligand 2-mercapto-*N*-[1,2,3,10-tetramethoxy-9-oxo-5,6,7,9-tetrahydro-benzo[*a*]heptalen-7-yl]acetamide were examined and illuminated.

The obtained results displayed that; the native ligand fits well into the binding pocket with a docking score of -7.288. A pocket created by LEU B:248 surrounds the native ligand, ALA B:250, ASP B:251, LEU B:252, LYS B:254, LEU B:255, ASN B:258, MET B:259, ILE B:378 and also VAL B:318, ALA B:317, ALA B:316, VAL B:315 and VAL A:181, ALA A:180, THR A:179, SER A:178.

The molecular structure of the native ligand is quite favorable for the active site of the receptor. The docking scores of the native ligand 2-mercapto-*N*-[1,2,3,10-tetramethoxy-9-oxo-5,6,7,9-tetrahydro-benzo [*a*] heptalen-7-yl]acetamide and our original molecules are given in Table 5.

Compound **2c**, which has the best docking score within our compounds, and its interactions with the active site of Tubulin-colchicine: Stathmin-like domain complex (PDB ID: 1SAD) are given in Figure 8.

The obtained in silico results displayed that, compound **2c**, which has the best docking score within our compounds, has a clear hydrogen bond interaction with SER A:178. And also, it can easily be seen that other title compounds are surrounded by the pocket of the receptor and fit well into the binding pocket of the receptor pretty well too.

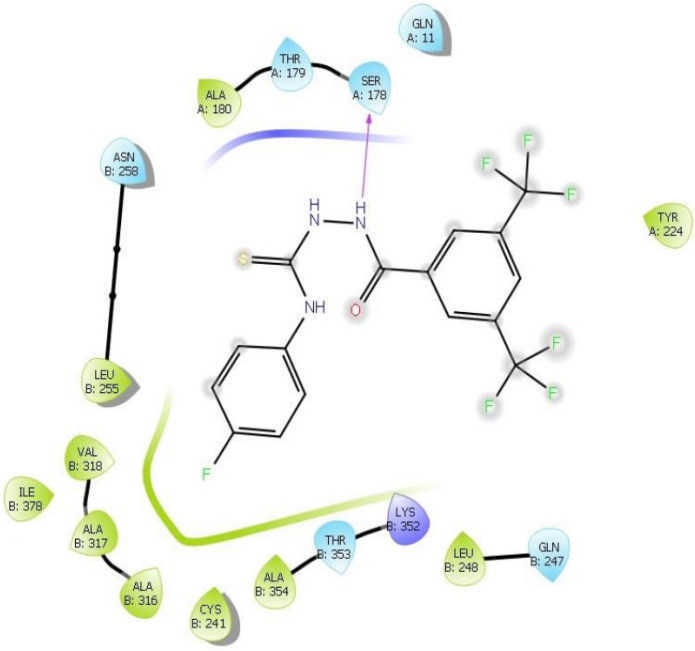

Figure 8. The active site of the Tubulin-colchicine: Stathmin-like domain complex (PDB ID: 1SA0) in complex with compound **2c**

Table 5. The docking scores of 2-mercapto -N- [1,2,3,10-tetramethoxy-9- oxo-5,6,7,9-tetrahydro-benzo [*a*] heptalen-7-yl] acetamide and the title compounds

Compound	Docking Score
2-mercapto-*N*-[1,2,3,10-tetramethoxy-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]acetamide, the native ligand of the crystal structure of of Tubulin-colchicine: Stathmin-like domain complex (PDB ID: 1SA0)	-7.288
2a	-6.837
2b	-6.777
2c	-6.955
2d	-6.184

Discussion

Today, it is obvious that, within all serious disease's cancer has specific importance because of its complex pathophysiology. There has been a great development in the chemotherapy of cancer but unfortunately, still new and more effective drugs are urgently needed to combat this disease.

In the present study, to find novel anticancer agents, novel thiosemicarbazide derivatives were designed and synthesized. The structure of the compounds was verified by spectroscopic methods. *In silico* studies were conducted to analyze the potential anticancer activity of the compounds.

Target-oriented drug design is crucial for cancer chemotherapy for

increasing the selectivity and consequently decreasing the adverse effects of the anticancer agents. Computer-aided drug design technology lets us design and develops target-oriented and, by that way selective therapeutic agents. We benefited technology during our drug design process.

We selected our targets as Topo II, EGFR tyrosine kinase domain, CA IX, and tubulin-colchicine: stathmin-like domain complex, which has significant roles in the cancer development process by their biochemical and physiological activities.

Compound **2a** displayed the best *in silico* activity results against ATP-dependent enzyme topoisomerase II (Topo II) with a docking score of -4.552. It had two clear hydrogen bond interactions with SER149 and ASN150, also a pi-cation interaction that exists between ligand and ARG98. Also, compound **2c** was hope promising with it's in silico activity against Topo II.

Compound **2c** and **2a** displayed significant and meaningful docking score values against the EGFR tyrosine kinase domain. The values (**2c**: -6.889 **2a**:-6.387) were even comparable with the native ligand erlotinib (-9.796).

In the studies conducted by targeting CA IX, the results were very attractive. The docking scores were very close to the native ligand, and there were serious interactions between compounds and the receptor. Compound **2a** had the best docking score with a value of -5.347. Compound **2a** also had three clear hydrogen bond interactions with water molecules and also one pi-pi stacking interaction with HIS94. The docking score of the native ligand was -5.604. Also, compounds **2b** and **2c** displayed meaningful in silico activity results indicating its potent carbonic anhydrase IX inhibitor activity.

All of our compounds displayed significant in silico activity against tubulin-colchicine: stathmin-like domain complex. The docking values were comparable with the native ligand, and all of the title compounds were surrounded by the pocket of the receptor well.

The *in-silico* studies displayed that our compounds have potential about being effective against many crucial targets of cancer therapy.

The results also showed that the title compounds have the potential about possessing the qualification about being multi-target drugs by effecting and hitting a few of the main targets of cancer chemotherapy together and at the same time. Compound **2a** and **2c** were found as more potent when compared to the compound **2b** and **2d**.

Conclusion

ATP-dependent enzyme topoisomerase II (Topo II), epidermal growth factor receptor (EGFR) tyrosine kinase domain, carbonic anhydrase IX, and tubulin-colchicine: stathmin-like domain complex are well known targets possessing significant roles in the development of many kind of cancer types by their biochemical and physiological activities. In the light of the hope promising results obtained from in silico studies against these targets, further in-vitro activity studies against diverse cancer cell lines will be conducted by our team.

Competing interests
The authors declare that they have no competing interest.

Financial Disclosure
This work was supported by Istanbul University Scientific Research Project (Project Number: TSA-2017-26543).

Ethical approval
No ethic approvall is needed to this research.

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