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Determination of biological studies and molecular docking calculations of isatin-thiosemicarbazone hybrid compounds

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ABSTRACT

Design, synthesis, structural elucidation, and investigation of cytotoxic and antimicrobial activity, butyrylcholinesterase (BChE), and acetylcholinesterase (AChE) enzyme inhibition effects of isatin-thiosemicarbazone hybrid compounds (**1**–**15**) are reported in this study. Hybrid compounds (**14** and **15**) were synthesized, isolated, and characterized for the first time. FT-IR, ^{1}H NMR, and ^{13}C NMR spectroscopic methods and elemental analysis were used to characterize the structures of the compounds. In the enzymatic evaluation, hybrid compound **13** was observed as the most potent inhibitor of AChE with a K_i value of 0.94 ± 0.13 µM (all compound K_i values between 0.94 ± 0.13 and 4.47 ± 0.92), also this compound was observed as the most potent inhibitor of BChE with a K_i value of 0.82 ± 0.11 µM (all compounds had K_i values between of 0.82 ± 0.11 and 3.48 ± 0.92). Almost all compounds were shown better inhibition profile than standard compound. In the theoretical calculations, the comparison of the biological activities of isatin-thiosemicarbazone hybrid derivatives against enzymes was studied. The enzymes studied in docking calculations are AChE and BChE. Then, ADME/T analysis was conducted to examine the drug properties of these derivatives. Besides, the antimicrobial activity of these molecules was investigated by the microdilution method according to Clinical Laboratory Standards Institute (CLSI) criteria in the study. Cytotoxic activity of isatin-thiosemicarbazone hybrids was determined by the XTT cell viability assay on human breast cancer cell lines MCF-7 and MDA-MB-231. Among the hybrid compounds, compound **8** exhibited the most potent cytotoxic activity with IC_{50} values of 23.42 ± 0.21 µg/mL and 19.68 ± 0.23 µg/mL on MCF-7 and MDA-MB-231 cell lines, respectively. Overall, the hybridization of isatin and thiosemicarbazone skeleton has played an essential role in the inhibition of enzymes and cytotoxic activity.

1. Introduction

Alzheimer's disease (AD) significantly affects people's quality of life. It is very difficult to treat, especially for the aging population. The disease can be identified by cognitive decline with major problems in executive functions. AD is a progressive brain disorder that gradually destroys thinking skills, memory, and the ability to perform simple tasks [1,2]. Although it is still unknown what exactly triggers this disease, many factors such as neurotransmitter acetylcholine (ACh) deficiency, excessive production of amyloid-β peptide, the formation of neurofibrillary nodes, disruption of metal homeostasis, and formation of reactive oxygen species play a role in brain disruption [3]. On the other hand, cancer, one of the most dangerous diseases in the world, is causing the death of more and more people. Many of the current anticancer agents are toxic and have side effects, so the synthesis of innovative, safe, and selective anticancer molecules has become a crucial target for medical chemistry researchers [4].

Acetylcholinesterase (AChE, E.C.3.1.1.7) is found in central and peripheral, cholinergic and adrenergic, nerve and muscle tissue, erythrocytes, and placental tissue in the body. The AChE enzyme

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inactivates ACh by breaking down ACh into acetate and choline in synaptic spaces. Butyrylcholinesterase (BChE, E.C.3.1.1.8) is another type of cholinesterase. It is synthesized in the liver and released into the blood [5]. The main physiological function of AChE is the destruction of ACh that mediates cholinergic synapses during the transmission of nerve impulses. Although the role of BChE is not yet fully known, it is thought that its task is to remove ACh that cannot be cleared by AChE from tissues. Large amounts of ACh cause inhibition of AChE causes and increase BChE activity. Therefore, when the amount of ACh in the tissue is high, it has been observed that BChE activity is effective in the degradation of Ach [6].

Isatin compounds have gained a reputation as a considerable nucleus and attracted enhancement attention in medicinal chemistry and drug discovery last years. Also, some isatin derivatives have been shown by researchers for developing new cholinesterase inhibitors [7]. It is known that the carbonic anhydrase (CA) isozyme family plays a significant role in many physiological and pathological metabolic pathways. These enzymes catalyze the simple reaction of reversible carbon dioxide (CO_2) hydration to bicarbonate and protons, which is required for the regulation of different $CO_2$$^-$bound chemical types in the body and their transport through biological membranes such as intracellular and extracellular spaces [8–10]. An organosulfide compound with the formula $H_2NC(S)NHN{=}CR_2$, thiosemicarbazones are usually produced by condensing a thiosemicarbazide with an aldehyde or ketone. Thiosemicarbazones are also widely used as ligands in coordination chemistry. Also, they are well known class of compounds, possess numerous activities like antioxidant [11–16], antibacterial [17], antimicrobial [18], anticancer [19], antituberculosis [20], anti-inflammatory [21], and enzyme inhibition effects [22].

Molecular hybridization is considered a method that is used to design new drug molecules based on the recognition of various subunits in the molecular skeleton of two or more biologically active derivatives. In recent years, interest in the discovery of hybrids, which can be simultaneously connected to more than one biological target, has been rising day after day [23–25] utilising hybrid molecules for the cure of illnesses is significant in terms of reducing the risk of drug-drug interaction and minimizing drug resistance.

Taking these explanations into consideration, we have focused on novel compounds as a strategy to explain isatins in conjunction with the thiosemicarbazone group to study anticancer, enzyme inhibition, and theoretical properties. In the literature, there are a few studies about isatin compounds, whichused as anticancer agents and carbonic anhydrase enzyme inhibitors [26,27]. When the literature scanned there is no information about anticancer, antimicrobial, and enzyme inhibition properties of isatin-thiosemicarbazone hybrids together. This is the first study of these hybrid series as potential enzyme inhibitors. Therefore, we have designed, synthesized isatin-thiosemicarbazone hybrids, investigated their inhibition effects of BChE, AChE and evaluated antimicrobial potentials. Also, the anti-proliferative and anticancer activities of isatin-thiosemicarbazone hybrids were evaluated using XTT cell viability assay on human breast cancer cell lines MCF-7 and MDA-MB-231. Besides, we investigate the activity with the mentioned metabolic enzymes of studied hybrid compounds by molecular docking studies. This study will contribute to elucidating the biological structure of isatin-thiosemicarbazone derivatives.

2. Experimental

2.1. Chemicals and materials

The used equipment, materials, and chemicals were supplied as supporting information.

2.2. Synthesis of the hybrid compounds (1–15)

To a solution of various isothiocyanates (6.0 mmol) and hydrazine monohydrate (6.0 mmol) in ethanol (20 mL) was added dropwise with vigorous stirring and cooling in an ice bath. The reaction mixture was kept in a refrigerator overnight. The resulting precipitate was filtered, dried, and purified with ethanol to afford thiosemicarbazides. Then, formed thiosemicarbazides (2.5 mmol), 5-methoxyisatin (2.5 mmol), and one drop of HCl were added to aqueous EtOH (20 mL) and the mixture was refluxed at 78 °C for 5 h. The resultant solid was filtered, washed, and dried in air. All compounds were successfully obtained with high yields (61–97%) as shown in Scheme 1. The compounds (**1–13**) were prepared with minor modifications according to the reported procedure [16,28]. Compounds **14** and **15** are synthesized for the first time in this study.

Synthesis of (Z)−2-(5−methoxy-2-oxoindolin-3-ylidene)-N-(p-tolyl) hydrazine-1-carbothioamide (**14**)

Red solid, 61% yield, Mp; 235–236 °C. IR (cm^{-1}): 3335 (NH(ist)), 3131 (NH(tsc)), 1660 (C=O), 1527 (C=N), 1432 (C=S), 1272 (C-N), 1132 (C-O). ^{1}H NMR (400 MHz, DMSO) δ 12.78 (s, 1H, NH^1), 11.06 (s, 1H, NH^2), 10.73 (s, 1H, NH^3), 7.48–7.46 (d, Ar H1,H5, 2H), 7.25–7.23 (d, Ar H2,H4, 2H), 7.25–7.23 (d, Ar H4, 1H), 7.42 (s, Ar H6, 1H), 6.96–6.94 (d, Ar H7, 1H), 6.87–6.84 (d, Ar H8, 1H), 3.77 (s, 3H, OCH_3), 2.34 (s, 3H, CH_3). ^{13}C NMR (101 MHz, DMSO) δ 176.9 (C=S), 163.3 (C=O), 136.6 (C=N), 126.2 (C1), 132.9 (C2), 129.3 (C3), 135.9 (C4), 129.3 (C5), 132.9 (C6), 136.3 (C7), 117.9 (C8), 107.1 (C9), 155.8 (C10), 112.3 (C11), 121.2 (C12), 56.1 (OCH_3), 21.1 (CH_3). Elemental Analysis Calcd. for $C_{17}H_{16}N_4O_2S$: C, 59.98; H, 4.74; N, 16.46. Found: C, 60.54; H, 4.85; N, 16.18. LC-MS/MS *m/z* : 339.05

*Synthesis of (Z)−2-(5−methoxy-2-oxoindolin-3-ylidene)-N-(2-chlorophenyl) hydrazine-1-carbothioamide (**15**)*

Red solid, 65% yield, Mp; 245–246 °C. IR (cm^{-1}): 3365 (NH(ist)), 3173 (NH(tsc)), 1692 (C=O), 1534 (C=N), 1436 (C=S), 1269 (C–N), 1155 (C–O). ^{1}H NMR (400 MHz, DMSO) δ 12.78 (s, 1H, NH^1), 11.08 (s, 1H, NH^2), 10.80 (s, 1H, NH^3), 7.47–7.38 (m, Ar H2, 1H), 7.62–7.60 (dd, Ar H3, 1H), 7.57–7.55 (dd, Ar H4, 1H), 7.47- 7.38 (m, Ar H5, 1H), 7.36 (s, Ar H6, 1H), 6.97–6.95 (dd, Ar H7, 1H), 6.88–6.86 (d, Ar H8, 1H), 3.77 (s, 3H, OCH_3). ^{13}C NMR (101 MHz, DMSO) δ 178.1 (C=S), 163.2 (C=O), 136.8 (C=N), 129.4 (C1), 130.1 (C2), 131.1 (C3), 133.3 (C4), 128.1 (C5), 132.1 (C6), 136.6 (C7), 118.2 (C8), 106.8 (C9), 155.8 (C10), 112.4 (C11), 121.1 (C12), 56.1 (OCH_3). Elemental Analysis Calcd. for $C_{16}H_{13}ClN_4O_2S$: C, 53.26; H, 3.63; N, 15.53. Found: C, 52.96; H, 3.70; N, 15.26. LC-MS/MS *m/z* : 359.00

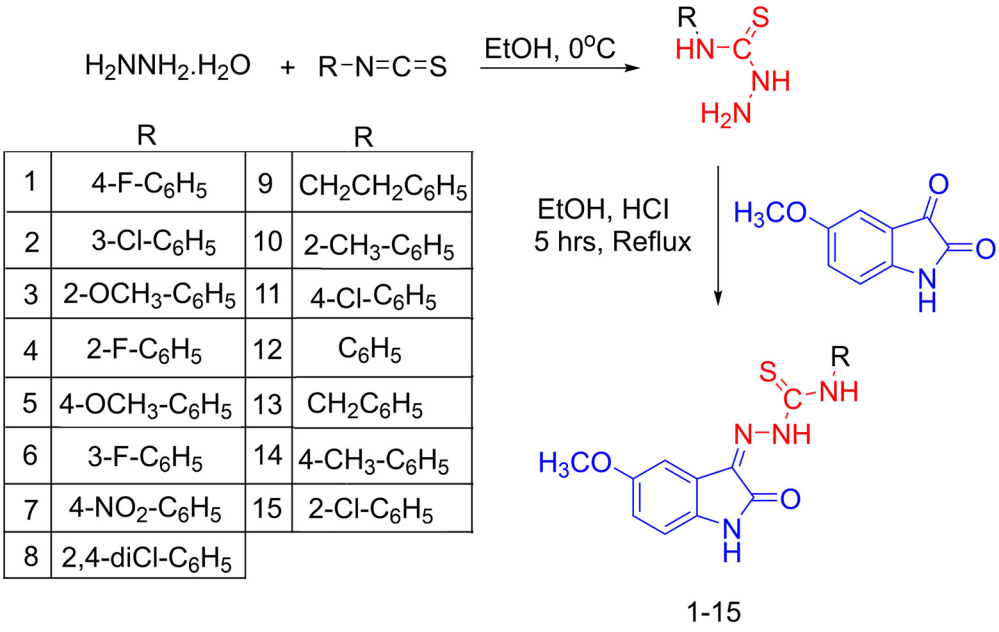
2.3. Biological investigation

2.3.1. Acetylcholinesterase and butyrylcholinesterase inhibition assays

AChE and BChE enzyme inhibitory effects of the isatin-thiosemicarbazone hybrids (**1–15**) were determined according to Ellman's procedure [29]. AChE and BChE activities were spectrophotometrically recorded at 412 nm. Acetylthiocholine iodide and Butyrylcholine iodide were used as the substrate for the enzymatic reaction according to previous studies for AChE and BChE enzymes respectively [30–34].

2.3.2. Antimicrobial activity

Antimicrobial activity of the hybrid compounds against two Gram-positive bacteria (*Staphylococcus aureus* ATCC 29213, *Bacillus cereus* ATCC 11778), two Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853), and two yeasts (*Candida albicans* ATCC 10231, *Candida tropicalis* DSM 11953) were evaluated by broth microdilution method according to Clinical Laboratory Standards Institute (CLSI) criteria, with some modifications [35]. Hybrid compounds were dissolved in DMSO to 100 mg/mL. In this study, Mueller-Hinton Broth



	R		R
1	4-F-C_6H_5	9	$CH_2CH_2C_6H_5$
2	3-Cl-C_6H_5	10	2-CH_3-C_6H_5
3	2-OCH_3-C_6H_5	11	4-Cl-C_6H_5
4	2-F-C_6H_5	12	C_6H_5
5	4-OCH_3-C_6H_5	13	$CH_2C_6H_5$
6	3-F-C_6H_5	14	4-CH_3-C_6H_5
7	4-NO_2-C_6H_5	15	2-Cl-C_6H_5
8	2,4-diCl-C_6H_5		

Scheme 1. The synthetic route of isatin-thiosemicarbazone compounds (**1**–**15**).

(Accumix®AM1072) and Sabouraud Dextrose Broth (Himedia ME033) were used as a medium for bacteria and yeasts, respectively. In the experiment carried out in a 96-well microplate, 90 µL of the medium to the 1st column and 50 µL of the medium to the 2nd −10th columns were added. 10 µL of hybrid compounds were added to column 1st and perform two-fold serial dilutions. 50 µL of bacteria and yeasts were added so that the final concentration was 5.0 × 10^5 CFU/mL for bacteria and 0.5–2.5 × 10^3 CFU/mL for yeasts in each well. Only 100 µL of the medium was added to the 11th column for sterilization control. 50 µL of microorganism and 50 µL of broth were added to the 12th column for reproductive control. The concentration of the hybrid compounds in the wells was ranging from 5.0 to 0.097 mg/mL. The microplates were incubated at 35 °C for 48 h for *Candida spp.* and at 37 °C for 24 h for the bacteria. After incubation, the lowest concentration that inhibits the growth of bacteria and yeast was accepted as the Minimum Inhibitory Concentration (MIC) value [36–38].

2.3.3. Cell culture and cytotoxicity assays

Human breast cancer cell lines MCF-7 (HTB-22) and MDA-MB-231 (HTB-26) were obtained from the American Type Culture Collection (ATCC). Cytotoxicity of isatin-thiosemicarbazone hybrid compounds was measured using the XTT cell viability assay, using MCF-7 and MDA-MB-231 cell lines. Cell culture studies were performed using the study of Taskin et al. [39]. Cell lines were cultured in medium glucose DMEM with 10% FBS, 100 IU/mL penicillin, 1% L-glutamine, and 10 mg/mL streptomycin in 25 cm^2 polystyrene flasks. The cells were kept at 37 °C within a 5% CO_2 humidified atmosphere. Cells were seeded at 1 × 10^4 cells/well in 96-well plates with 100 µL DMEM including 10% FBS and incubated overnight. At the end of the incubation, compounds were dissolved in DMSO. Afterward, dissolved compounds with a concentration of 20 µg/ml per well were put in the 96-well plates and the cells were incubated for 24 h. After the incubation, DMEM was removed and wells were washed two times with phosphate-buffered saline (PBS). Following these periods, for the determination of living cells, 100 µL of transparent DMEM and 50 µL of XTT labeling solution were added to each well and the plates were incubated for 4 h. The absorbance values of XTT-formazan were measured using a microplate (ELISA) reader at 450 nm against the control. The cell viability of isatin-thiosemicarbazone hybrid compounds was calculated compared to control (100% of viability) and all experiments were performed three times. According to the XTT results four hybrid compounds, showing the highest cytotoxicity in MCF-7 and MDA-MB-231 cell lines were chosen. Afterward, XTT cell viability assay was performed to calculate the IC_{50} value of compounds at concentrations of 5, 10, 25, 50, and 100 µg/mL. In this study, cisplatin was used as the positive control.

2.4. Molecular docking calculations

In molecular docking calculations, the Maestro Molecular modeling platform (version 12.2) [40] by Schrödinger was used for the interaction of the novel isatin-thiosemicarbazone hybrids with enzymes. To calculate with this program, the molecular structures of the novel isatin-thiosemicarbazone hybrids were optimized with the Gaussian software program [41]. Using the optimized structures of the novel isatin-thiosemicarbazone hybrids, *.sdf extension files were obtained. Then calculated using the LigPrep module [42] to prepare the studied molecules for calculations. Later, the active sites of the proteins of the enzymes were determined using the protein preparation module [43] to prepare the studied enzymes for calculations. The Glide ligand docking module [44] was used to interact with the novel isatin-thiosemicarbazone hybrids and proteins. Finally, the novel isatin-thiosemicarbazone hybrids were computed using The Qik–prop module [45] of the Schrödinger software for ADME/T analysis.

3. Results and discussion

3.1. Structure characterization

For novel compounds, **14–15**, the current experimental data for physical properties, yields, melting points, and elemental analyses are presented as shown in supporting information Tables S1 and S2.

In the FT-IR spectra, the symmetric and asymmetric amino group (NH_2) stretching frequencies did not observe at 3450–3225 cm^{-1}. Instead, new peaks for the -C=N stretching vibrations of the azomethine group were observed at 1598–1527 cm^{-1}. These data showed a successful reaction as expected. In compounds **14**–**15**, the -NH stretching vibration of isatin and thiosemicarbazide re-

Table 1
The enzyme inhibition results of isatin-thiosemicarbazone hybrids against butyrylcholinesterase (BChE) and acetylcholinesterase (AChE) enzymes.

Compounds	IC_{50} (μM)				K_i (μM)	
	AChE	r^2	BChE	r^2	AChE	BChE
1	3.40	0.9371	2.404	0.9122	3.12 ± 0.34	2.87 ± 0.61
2	2.23	0.9682	1.98	0.9332	2.03 ± 0.26	1.62 ± 0.12
3	4.32	0.9883	2.30	0.9261	3.50 ± 0.58	2.05 ± 0.27
4	1.43	0.9572	1.12	0.9521	1.23 ± 0.26	1.23 ± 0.23
5	4.42	0.9722	2.90	0.9626	3.95 ± 1.01	3.43 ± 0.70
6	5.43	0.9588	3.82	0.9547	4.84 ± 0.82	3.13 ± 0.38
7	5.02	0.9803	3.00	0.9690	5.47 ± 1.14	2.40 ± 0.52
8	4.52	0.9798	3.42	0.9185	4.01 ± 0.35	3.05 ± 0.57
9	1.90	0.9752	1.21	0.9543	2.18 ± 0.38	0.96 ± 0.14
10	1.73	0.9367	1.21	0.9332	1.41 ± 0.41	1.42 ± 0.22
11	3.92	0.9578	2.83	0.9410	3.34 ± 1.47	3.48 ± 0.92
12	4.60	0.9574	3.48	0.9185	4.47 ± 0.92	3.06 ± 0.58
13	1.23	0.9799	7.31	0.9670	0.94 ± 0.13	0.82 ± 0.11
14	1.50	0.9584	1.14	0.9928	1.21 ± 0.27	1.04 ± 0.26
15	1.80	0.9696	1.32	0.9102	1.56 ± 0.46	1.55 ± 0.31
TAC*	11.14	0.9886	9.83	0.9593	9.33 ± 1.45	8.13 ± 2.35

* Tacrine (TAC) was used as a control for AChE and BChE enzymes.

gion was observed at between 3387 and 3322 cm^{-1}; the -C=O, -C=N, -C=S, -C–N, and -C–O stretching vibrations were observed in the range of 1694–1660; 1537–1524; 1438–1432; 1291–1269; and 1155–1127 cm^{-1}, respectively (Figs. S11 and S15). The IR values are presented in Table S3. The ^{1}H and ^{13}C NMR spectra of the obtained products in DMSO–d_6 as a solvent was detected. For compound **14**, while the –NH (N^1) peak of isatin was revealed as a singlet at 12.85 ppm, the –NH (N^2) and –NH (N^3) proton signals of thiosemicarbazone were shown as a singlet at 11.08 and 10.83 ppm, respectively. The methoxy group (–OCH_3) proton signal was observed as a singlet at 3.77 ppm. The proton signals (H1-H5) of the aryl ring were revealed between 7.68 and 7.49 ppm (Fig. S12). The proton signals (H6-H7-H8) of the isatin ring were revealed between 7.42 and 6.84 ppm. Proton chemical shift data of the obtained molecules are shown in Table S4. The ^{13}C NMR spectrum of compound **14** revealed 15 different resonances consistent with the targeted product (Fig. S13). In compound **14**, the -C=S signal of the thiosemicarbazone moiety was observed at 176.9 ppm. The considerable characteristic -C=O and -C=N peaks of the isatin ring were detected at 163.3 and 136.6 ppm, respectively. The aromatic carbons (C1-C6) of the phenyl ring were revealed at between 135.9 and 126.2 ppm. The carbons (C7-C12) of the isatin ring were detected at 136.3, 117.9, 107.1, 155.8, 112.3, and 121.2 ppm, respectively. The methoxy peak (–OCH_3) and methyl group (-CH_3) were revealed at 56.1 and 21.1 ppm, respectively. This spectroscopic evidence showed that our results were consistent with similar compounds previously reported. [13,15,16,46]. The carbon chemical shift data of the obtained molecules are presented in Table S5.

3.2. Biological activity studies

3.2.1. Enzyme inhibition potential

In this study, isatin-thiosemicarbazone hybrids (**1**–**15**) were screened against the AChE and BChE enzymes due to significant reports on AD of phenolic natural or synthetic compounds. IC_{50} and K_i values of the reference drug Tacrine were 11.14 μM (IC_{50}) and 9.33 ± 1.45 μM (K_i) towards AChE and 9.83 μM (IC_{50}) and 8.13 ± 2.35 μM (K_i) towards BChE as shown in Table 1. The isatin-thiosemicarbazone hybrids inhibited the AChE enzyme in micromolar concentration in the range of K_i values of 0.94 ± 0.13 and 5.47 ± 1.14 μM and with IC_{50} values of 1.23 - 5.43 μM. The compounds **13** and **14** were found potent AChE inhibitors with the K_i values of 0.94 ± 0.13 μM and 1.21 ± 0.27 μM, respectively. On the other hand, compound **13** was also considered as one of the potent inhibitors with the lowest IC_{50} value of 1.23 μM against AChE (Fig. 1). The isatin-thiosemicarbazone hybrids inhibited the BChE enzyme with micromolar concentration in the range of K_i values of 0.82 ± 0.11 – 3.48 ± 0.92 μM and with IC_{50} values of 1.12 – 7.31 μM. The compounds **13** and **14** were found potent BChE inhibitors with the K_i values of 0.82 ± 0.11 μM and 1.04 ± 0.26 μM, respectively. On the other hand, compound **4** was also considered as one of the potent inhibitors with the lowest IC_{50} value of 1.12 μM against BChE (Fig. 1).

3.2.2. Antimicrobial activity

The MIC values of hybrid compounds against *E. coli, S. aureus, P. aeruginosa, B. cereus, C. albicans,* and *C. tropicalis* ranged from 0.039 to >5 mg/mL (Table 2). It has been observed that the hybrid compounds are effective only on *S. aureus* range from 0.039 to 0.625 mg/mL. It was determined that the hybrid compounds **11** and **12** have the most effective antimicrobial activity against *S. aureus* (0.039 mg/mL). The antimicrobial activity of other hybrid compounds against *S. aureus* ranges from 0.078 mg/ml to 0.625 mg/mL.

3.2.3. Anti-proliferative activity

The anti-proliferative activities of isatin-thiosemicarbazone hybrid compounds (**1**–**15**) on MCF-7 and MDA-MB-231 cancer cell lines were evaluated and results were shown in Figs. 2 and 3. Results indicated that hybrid compounds **4, 8, 11**, and **13** have more

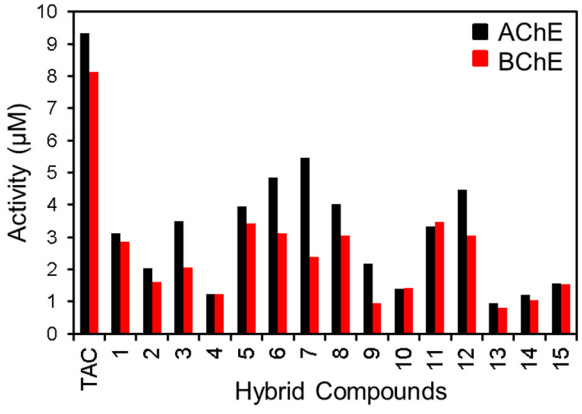


Fig. 1. K_i values of isatin-thiosemicarbazone hybrid compounds (**1**–**15**) on AChE and BChE enzymes.

Table 2
Antimicrobial activity values of hybrid compounds*.

	E.coli	*S.aureus*	*P.aeruginosa*	*B.cereus*	*C.albicans*	*C.tropicalis*
	ATCC 25922	ATCC 29213	ATCC 27853	ATCC 11778	ATCC 10231	DSM 11953
1	>5	0.156	>5	>5	>5	>5
2	>5	0.078	>5	>5	>5	>5
3	>5	0.312	>5	>5	>5	>5
4	>5	0.312	>5	>5	>5	>5
5	>5	0.312	>5	>5	>5	>5
6	>5	0.156	>5	>5	>5	>5
7	>5	0.156	>5	>5	>5	>5
8	>5	0.156	>5	>5	>5	>5
9	>5	0.156	>5	>5	>5	>5
10	>5	0.156	>5	>5	>5	>5
11	>5	0.039	>5	>5	>5	>5
12	>5	0.039	>5	>5	>5	>5
13	>5	0.078	>5	>5	>5	>5
14	>5	0.078	>5	>5	>5	>5
15	>5	0.625	>5	>5	>5	>5

* Microorganisms and MIC values (mg/mL).

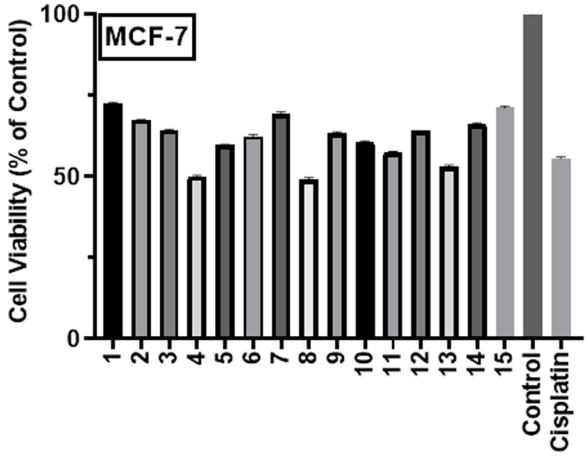


Fig. 2. Cell viability results of isatin-thiosemicarbazone hybrid compounds. Cells were treated with samples at a concentration of 25 µg/mL. Cell viability of the control group was determined as 100%.

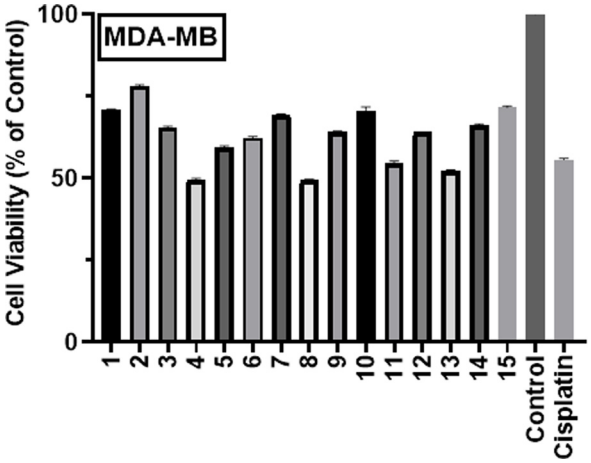


Fig. 3. Cell viability results of isatin-thiosemicarbazone hybrid compounds. Cells were treated with samples at a concentration of 25 µg/mL Cell viability of the control group was determined as 100%.

cytotoxic activity on both MCF-7 and MDA-MB-231 cell lines compared to other compounds. On MCF-7 cell lines ranged between 48.700 ± 0.566% and 72.540 ± 0.637% (Fig. 2). It is clear from the figure that hybrid compounds **4, 8**, and **13** against the MCF-7 cell

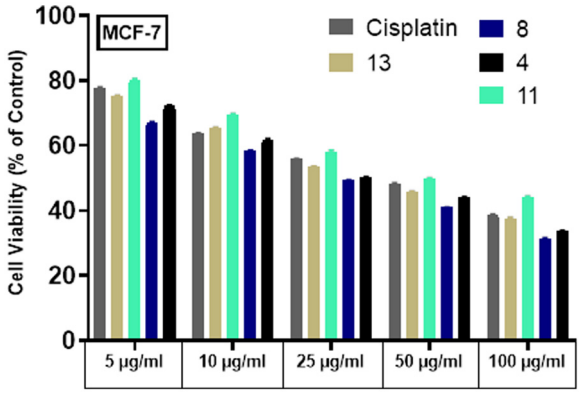


Fig. 4. MCF-7 cell viability and IC_{50} results of hybrid compounds (**4, 8, 11**, and **13**). To determine IC_{50} values five different concentrations of hybrid compounds were used. Cell viability of the control group was determined as 100%.

line have better cytotoxic activity than cisplatin. According to the results, hybrid compounds **4** and **1** showed the highest and lowest anti-proliferative effect on the MCF-7 cell line respectively.

On the MDA-MB-231 cell line, the cell viability ranged between 49.260 ± 0.431 and 78.026 ± 0.050% (Fig. 3). Results showed that **4, 8**, and **13** compounds against the MDA-MB-231 cell line have better cytotoxic and anti-cancer activity than cisplatin. According to the XTT cell viability results, the **4, 8, 11**, and **13** hybrid compounds with the highest anti-proliferative activities were treated with MCF-7 and MDA-MB-231 cells at different concentrations, and IC_{50} values were calculated using XTT cell viability assay.

To determine the IC_{50} values of the isatin-thiosemicarbazone hybrid compounds MCF-7 and MDA-MB-231 cells were exposed to various concentrations of the hybrid compounds ranged between 5 µg/mL and 100 µg/mL. According to the results of Fig. 4, three hybrid compounds **4, 8**, and **13** have better cytotoxic activity (MCF-7/IC_{50} = 25.040 ± 0.160 µg/mL; MCF-7/IC_{50} = 23.420 ± 0.210 µg/mL; MCF-7/IC_{50} = 32.280 ± 0.324 µg/mL, respectively) than the reference substance cisplatin used (MCF-7/IC_{50} = 35.190 ± 0.260 µg/mL). Hybrid compound **11** has lower cytotoxic activity (MCF-7/IC_{50} = 47.860 ± 0.424 µg/mL) than cisplatin.

It is clear from Fig. 5 that isatin-thiosemicarbazone hybrids (**4, 8**, and **13**) have greater anti-proliferative and cytotoxic activity (MDA-MB-231/IC_{50} = 20.120 ± 0.130 µg/mL; MDA-MB-231/IC_{50} = 19.680 ± 0.230 µg/mL; MDA-MB-231/IC_{50} = 34.640 ± 0.280 µg/mL, respectively) than the reference substance cisplatin used (MDA-MB-231/IC_{50} = 39.640 ± 0.380 µg/mL). Hybrid compound **11**

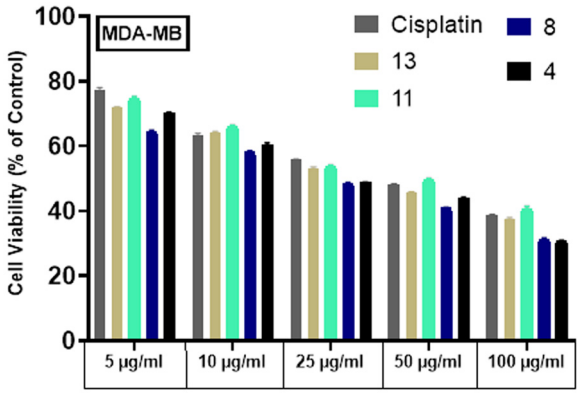


Fig. 5. MDA-MB-231 cell viability and IC_{50} results of hybrid compounds (**4, 8, 11**, and **13**). Cell viability of the control group was determined as 100%.

has lower cytotoxic activity (MDA-MB-231/IC_{50} = 49.510 ± 0.470 µg/mL) than cisplatin. The isatin-thiosemicarbazone hybrid compound **8** was showed the highest anti-cancer activity on both MCF-7 and MDA-MB-231 cell lines. Besides the other hybrid compounds, **4** with 2-F-C_6H_4 substituent to isatin-thiosemicarbazone and **13** with CH_2-C_6H_5 substituent to the isatin-thiosemicarbazone structure have better cytotoxic activity than cisplatin.

3.3. Molecular docking studies

Biological activities of the novel isatin-thiosemicarbazone hybrid derivatives against related enzymes for molecular docking calculations were compared. Some parameters were obtained with this comparison. Among these parameters is the docking score which is a numerical value of the interaction of novel isatin-thiosemicarbazone hybrid derivatives with enzymes. This docking score parameter is the most negative in cases where the novel isatin-thiosemicarbazone hybrids derivatives have the greatest interaction with enzymes. Chemical interactions occur between the novel isatin-thiosemicarbazone hybrid derivatives and enzymes. The greater these chemical interactions. the more negative this docking score parameter becomes [47]. Interactions between enzymes and novel isatin-thiosemicarbazone hybrids derivatives are hydrogen bonds, polar and hydrophobic interactions, π-π, and halogen [48–52]. The interactions of the novel isatin-thiosemicarbazone hybrids derivatives with enzymes are given in Fig. 6.

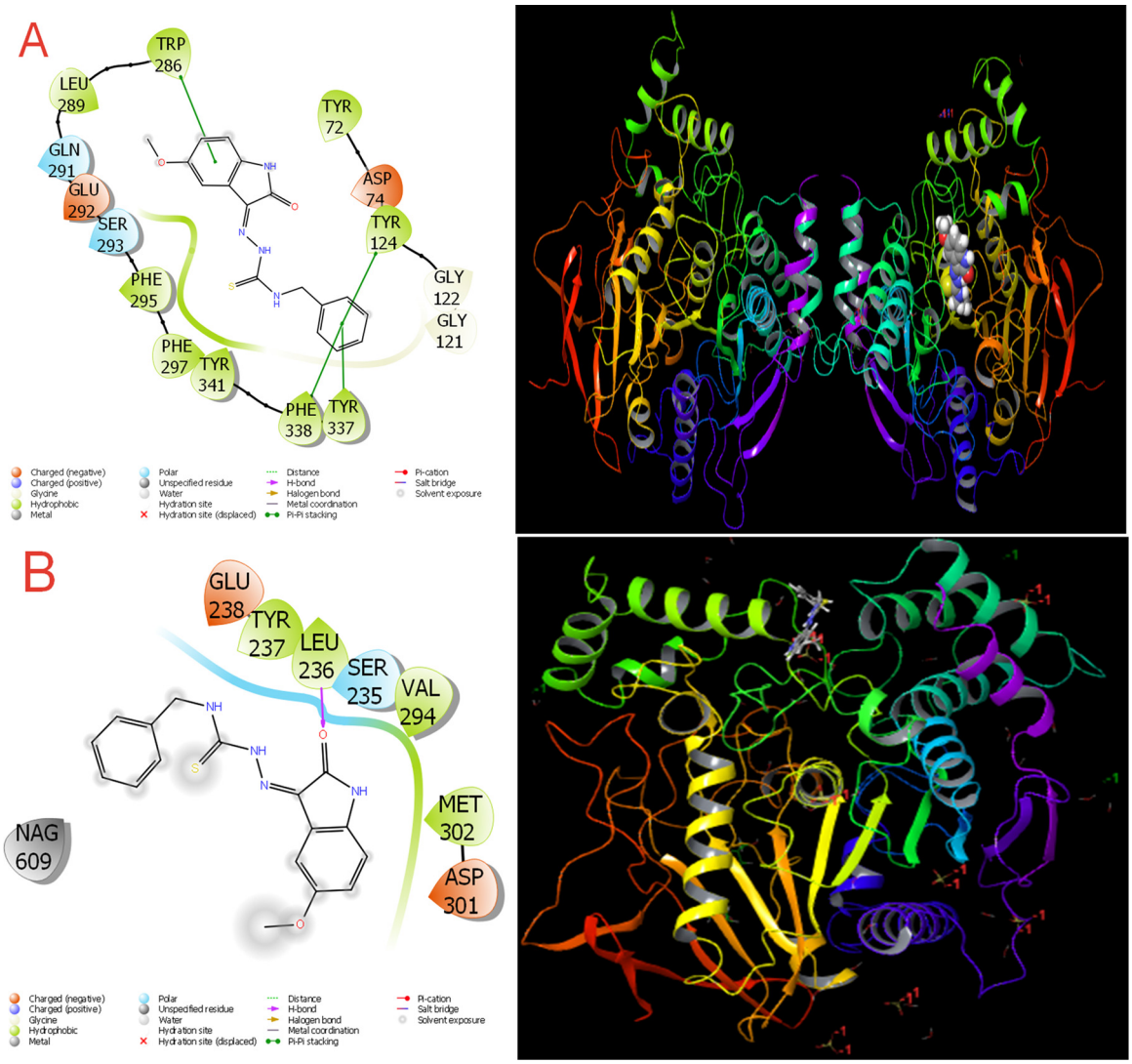


Fig. 6. Interaction of hybrid compound **13** with the AChE enzyme (A) hybrid compound **13** with the BChE enzyme (B).

As a result of molecular docking calculations, only the docking score parameter was not found. All parameters obtained from docking calculations are given in Table S6. Among the other calculated parameters. The Glide ligand efficiency parameter is the numerical value of the activities of the molecules studied. The other parameter is Glide hbond, Glide evdw, and Glide ecoul. On the other hand, parameters such as Glide emodel, Glide energy, Glide einternal, and Glide posenum are parameters related to the interaction exposure of novel isatin-thiosemicarbazone hybrid compound with enzymes. Also, when the biological activities of three enzymes against proteins are compared. The docking score parameter of hybrid compound **13** against the AChE enzyme is −9.21. The docking score parameter of hybrid compound **13** against the BChE enzyme, which is the other enzyme, is –3.92.

ADME/T calculations were made to examine the possibilities of using novel isatin-thiosemicarbazone hybrids derivatives as advanced drugs. As a result of these calculations, many parameters were found are given in Table S7. The first parameter among these parameters is Solute Molecular Weight, which requires the molecule to have a certain molecular weight [47]. Another parameter is PISA, which is also called Solute Total SASA. This parameter is the π (carbon and attached hydrogen) component of the SASA. Another parameter is QP Polarizability, which is predicted polarizability in cubic angstroms [48].

Another important parameter is QPlogHERG, which is the numerical value of the estimated IC_{50} value of HERG K channels when occluded [44]. The next parameter is QPPCaco, which is Caco-2 cell permeability at the gut-blood barrier for inactive transport. Another parameter is QPlogBB, which is the brain-blood barrier coefficient of an orally administered drug [45]. The next parameter is Human Oral Absorption, which is predicted qualitative human oral absorption: 1, 2, or 3 for low, medium, or high.

Among all ADME/T parameters, the two most important are RuleOfFive and RuleOfThree [49]. The RuleOfFive and RuleOfThree parameters are more important than any other parameter. The numerical value of these two parameters is expected to be zero. The RuleOfFive parameter is also Lipinski's Pfizer's fifth rule [53]. The rules are: mol MW<500, QPlogP o/*w*<5, donorHB≤ 5, accptHB≤ 10. However, the RuleOfThree parameter is known as the three of Jorgensen's rules [54]. The three rules are: QPlogS> −5.7, QP PCaco> 22 nm/s, #Primary Metabolites< 7. If the numerical value of the RuleOfThree parameter is zero, this molecule can be used orally as a drug. The last and another important parameter is Jm, which is the predicted maximum transdermal transport rate, $Kp \times MW \times S$ ($\mu g.cm^{-2}.hr^{-1}$). Kp and S are obtained from the aqueous solubility and skin permeability, QPlogKp, and QPlogS. These are the theoretical estimated numerical values obtained by applying the molecules that can be drugs with this parameter to the skin.

4. Conclusions

Herewith, a series of isatin-thiosemicarbazone hybrid compounds have been designed, synthesized, and successfully elucidated by using spectroscopic methods such as ^{1}H NMR, ^{13}C NMR, and FT-IR and elemental analyses. In the enzymatic evaluation, hybrid compound **13** was observed as the most potent inhibitor of AChE with a K_i value of 0.94 ± 0.13 μM (all compound K_i values between 0.94 ± 0.13 and 4.47 ± 0.92). Also, compound **13** was observed as the most potent inhibitor of BChE with a K_i value of 0.82 ± 0.11 μM (all compound K_i values between 0.82 ± 0.11 and 3.48 ± 0.92). Almost all hybrids showed better inhibition than standards. As a result of molecular docking calculations of isatin-thiosemicarbazone hybrid compounds, the best active molecules against AChE and BChE enzymes are compounds **2, 4**, and **13**. After this comparison, ADME/T analysis of novel isatin-thiosemicarbazone hybrid compounds was performed. In the anticancer activity assessment of novel isatin-thiosemicarbazone hybrids, **8** was observed as the most potent cytotoxic and anti-cancer agent both MCF-7 (IC_{50} = 23.420 ± 0.210 μg/mL) and MDA-MB-231 (IC_{50} = 19.680 ± 0.230 μg/ml). Also, isatin-thiosemicarbazone hybrids (compounds **4 8,** and **13**) have higher anti-cancer activity than the standard substance cisplatin. As a result of these analyses, it was seen that the numerical values of the parameters of some compounds were above the upper limit. As all of these calculations are investigated theoretically. Also, it was determined that the hybrid compounds **11** and **12** have the most effective antimicrobial activity against *S. aureus* (0.039 mg/mL). Overall, these isatin-thiosemicarbazone hybrid compounds may be notable for designing novel enzyme inhibitors, which had further potential lead candidates for the design of new drugs to treat some diseases.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

CRediT authorship contribution statement

Ümit M. Koçyiğit: Writing – original draft, Investigation, Supervision. **Murat Doğan:** Writing – original draft, Investigation. **Halit Muğlu:** Writing – original draft, Supervision. **Parham Taslimi:** Writing – original draft, Investigation, Resources. **Burak Tüzün:** Data curation, Methodology, Validation. **Hasan Yakan:** Writing – review & editing, Project administration. **Halil Bal:** Writing – original draft, Investigation. **Emre Güzel:** Writing – original draft, Writing – review & editing, Investigation. **İlham Gülçin:** Writing – review & editing, Conceptualization.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.molstruc.2022.133249.

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