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Synthesis, Anticancer Activity and Carbonic Anhydrase Inhibitory Activity of new Thiadiazole-hydrazone Derivatives

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ABSTRACT

In five steps, new compounds 5a, 5b of thiadiazole-hydrazone derivatives were synthesized. Various spectral methods, such as ^{1}H NMR, ^{13}C NMR, and elemental analyses, were used to clarify the structures of the compounds. Three cancer cell lines (MCF7, MDA, and HT-29) and one healthy cell line (L929) were tested for the cytotoxicity activity of synthetic compounds, as well as their inhibitory action against carbonic anhydrase I and II isoenzymes (hCA I and hCA II). Among them, the compound 5b exhibited remarkable CA inhibitory activities compared to a standard inhibitor with IC_{50} values at of 27 µM for hCA I and 33,46 µM for hCA II. The compounds have been found to be ineffective against cancer cell lines. Furthermore, the compounds were found to be non-toxic to the healthy cell line.

Keywords: Thiadiazole, Hydrazone, Anticancer, Carbonic anhydrase.

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Introduction

The carbonic anhydrase (CA) enzyme is a group of enzymes that convert carbon dioxide (CO_2) and water (H_2O) into proton (H^+) and bicarbonate anion (HCO_3^-) [1,2]. There are 15 different CA isoforms in humans [3]. These isoforms can be categorized based on where they are found in the body, including cytosolic isoforms (CA I, CA II, CA III, CAVII, CAXIII), mitochondrial isoforms (CA VA, CAVB), transmembrane-bound isoforms (CA IV, CA IX, CA XII, CA XIV), and CA VI, which is found in body fluids like saliva [4,5]. These enzymes are essential for a wide range of physiological and cellular functions, including the transfer of carbon dioxide, control of the acid-base balance, electrolyte secretion, and biosynthetic pathways [6]. Diuretic, anti-Alzheimer, anti-obesity, anti-epileptic, anti-cancer, and anti-infective effects are among the bioactivities of hCA activators and/or inhibitors (hCAIs) [7,8].

Cancer is one of the most dangerous diseases and is one of the leading causes of mortality, despite numerous improvements in drug discovery for its control and treatment. The cancer problem is confined to a specific area due to the disease's metastasis and extensive nature as well as the anticancer medications' high side effect rate due to their lack of selectivity. Additionally, the issue gets worse as a result of ongoing resistance-producing mutations in numerous anti-cancer treatment targets. The development of new anticancer drugs with great selectivity and the ability to overcome drug resistance has recently been the focus of medicinal chemists [9]. Human carbonic anhydrase (hCA) enzymes have recently become the focus of anticancer drugs [10]. CA II, one of the CA isoforms, is frequently linked to several malignancies. In esophageal, melanoma, renal, and lung cancers, CA II is expressed in the endothelium of neovessels, according to a recent study. In other studies, CA II has been connected to the essential target antigen that causes melanoma patients to produce autoantibodies. Furthermore, a number of cancer forms have been found to overexpress CAII, according to several recent investigations [11-13].

Five-membered heterocycle-containing molecules have drawn more and more attention in recent years when it comes to creating anticancer drugs. The 1,3,4-thiadiazole system is regarded as one of the promising structures [14]. The antitumor activity of 1,3,4-thiadiazole derivatives has been extensively investigated. Thiadiazole derivatives can be found in a variety of over-the-counter drugs (Figure 1).

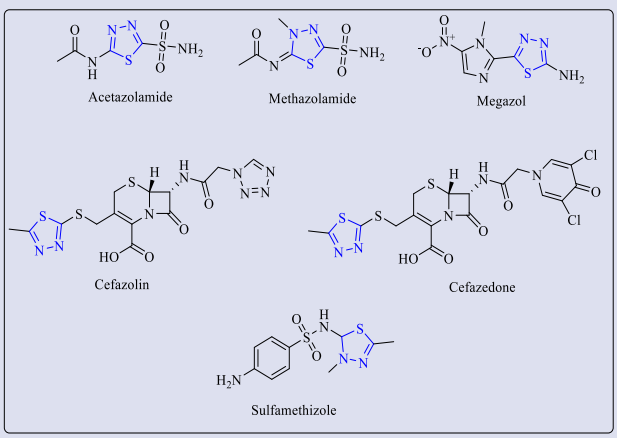

Figure 1. Representative thiadiazole-based drug molecules.

Methazolamide and acetazolamide, for instance, are potent inhibitors of carbonic anhydrase. Sulfamethizole has antibacterial properties, while megazol has antitrypanosomal properties. Members of the first generation of the cephalosporin family include cefazolin and cefazedone [15-17].

N-Acylhydrazones with the -CO-NH-N= unit have drawn attention due to their intriguing characteristics for a long time and have been used in medicine [18]. It is believed that the capacity of molecules with the hydrazone moiety to form hydrogen bonds with molecular targets is a factor in their bioactivity, which enables them to attach to a variety of enzymes and receptors [19]. Examples of drugs containing the hydrazone structure are shown in Figure 2.

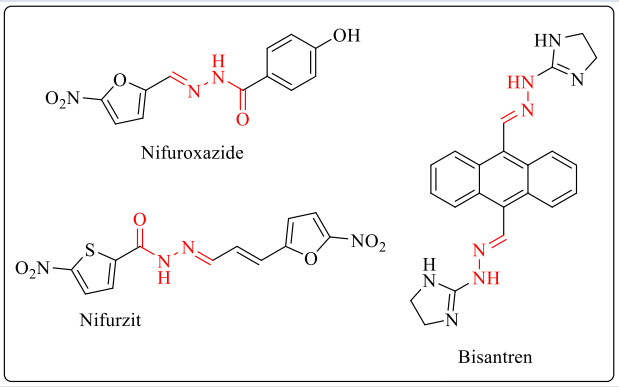

Figure 2. Chemical structure of nifuroxazide, nifurzide and bisanthrene drugs

The facts cited above inspired us to create hybrid molecules that combine the two crucial moieties, hydrazone and thiadiazole, into a single molecule. In order to create novel medication candidates with less cytotoxic effects, this was done. The synthetic substances were tested against three cell lines and a healthy cell line. In addition, the inhibitory activities of the compounds against hCAI and hCAII enzymes were evaluated.

Materials and Methods

Chemistry

Synthesis of *N*-(4-chlorophenyl) hydrazinecarbothioamide (1):

The isothiocyanate (0.02 mol) derivatives are dissolved in ethanol and placed in an ice bath. Hydrazine hydrate (0.024 mol) in ethanol is added dropwise to the reaction content. After the dripping process is finished, the precipitated product is filtered off.,

Synthesis of 5-(4-chlorophenylamino)-1,3,4-thiadiazole-thiol (2):

The compound N-(4-substitutedphenyl) hydrazinecarbothioamide (2) is dissolved in ethanol and carbon disulfide and NaOH are added. The reaction is stirred under reflux for 3-4 hours. Reaction end is controlled by TLC. At the end of the reaction, the product content is poured into ice water and the product is precipitated with HCl acid and filtered.

Synthesis of 2-((5-((4-chlorophenyl)amino)-1,3,4-thiadiazol-2-yl)thio)acetate (3): Acetone is used to dissolve compound 2, potassium carbonate and chloroethylacetate. The mixture is stirred for two hours at 40 °C while in reflux. Filtered off, cleaned with water, and crystallized from ethanol is the precipitated product.

Synthesis of 2-((5-((4-chlorophenyl)amino)-1,3,4-thiadiazol-2-yl)thio)acetohydrazide (4): Hydrazine hydrate was added after compound 3 had been dissolved in ethanol. The mixture was heated for two hours at reflux, and the precipitated portion was removed by filtering.

Synthesis of 2-((5-((4-chlorophenyl)amino)-1,3,4-thiadiazol-2-yl)thio)-N'-(substituted benzylidene) acetohydrazide (5a-5b): A few drops of acetic acid and an aldehyde derivative (0.001 mol) were added after compound 4 had been dissolved in ethanol. Two hours of reflux heating the mixture resulted in the precipitate, which was filtered out and crystallized from butanol.

2-((5-((4-chlorophenyl)amino)-1,3,4-thiadiazol-2-yl)thio)-*N*'-(2,4-dichlorobenzylidene) acetohydrazide (5a): Yield: 82 %, M.P.= 256 °C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ: 4.43 (2H, s, -S-CH_2), 7.34-7.38 (2H, m, -Aromatic CH), 7.44-7.47 (1H, m, -Aromatic CH), 7.55-7.62 (3H, m, Aromatic CH), 7.92-7.94 (1H, m, Aromatic CH), 8.32 (1H, s, -CH), 10.45 (1H, s, NH), 11.86 (1H, s, NH). ^{13}C-NMR (75 MHz, DMSO-d_6): δ: 36.22, 119.34, 125.87, 128.48, 129.32, 129.75, 130.70, 134.11, 135.43, 139.25, 139.64, 142.57, 153.08, 165.28, 169.42. For $C_{17}H_{12}Cl_3N_5OS_2$, C, 43.19; H, 2.56; N, 14.81. Found: C, 43.25; H, 2.56; N, 14.83.

2-((5-((4-chlorophenyl)amino)-1,3,4-thiadiazol-2-yl)thio)-*N*'-(2,4-dimethoxybenzylidene) acetohydrazide (5b): Yield: 83 %, M.P.= 217.4 °C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ: 3.78-3.82 (6H, m, -OCH_3),4.39 (2H, s, -S-CH_2), 6.56-6.62 (2H, m, -Aromatic CH), 7.35-7.39 (2H, m, -Aromatic CH), 7.57-7.62 (2H, m, Aromatic CH), 7.68-7.71 (1H, m, Aromatic CH), 8.25 (1H, s, -CH), 10.46 (1H, s, NH), 11.47 (1H, s, NH). ^{13}C-NMR (75 MHz, DMSO-d_6): δ: 36.41, 55.86, 56.23, 98.76, 106.91, 115.24, 119.35, 125.82, 127.03, 129.34, 139.71, 140.04, 143.29, 159.49, 162.79, 165.12, 168.75. For $C_{19}H_{18}ClN_5O_3S_2$, C, 49.19; H, 3.91; N, 15.09. Found: C, 49.27; H, 3.90; N, 15.11.

Anticancer Activity

The anticancer activities of compounds **5a** and **5b** were determined by the absorbance values obtained from MTT assays. The MTT method was performed as previously described [20]. The anticancer activities of the compounds were evaluated against 3 cancer cell lines (MCF-7, MDA and HT-29). L929 healthy mouse fibroblast cells were used to evaluate the selectivity of the compounds. In this section, cisplatin was used as a reference drug in cell lines.

Carbonic Anhyrase I/II Inhibition Assay

The esterase activity method is the method used for the determination of the carbonic anhydrase enzyme. With this method, esterase activity of carbonic anhydrase enzyme can be determined. Esterases responsible for the hydrolysis of carboxylic acids are capable of hydrolyzing many substrates. p-Nitrophenyl acetate is a substrate used in esterase and lipase activity assays. Hydrolysis of 1,4 p-nitrophenyl acetate yields p-nitrophenol or p-nitrophenolate, which gives maximum absorbance at 405 nm. The measurement is not affected because the two formed structures peak at the same absorbance value [21, 22].

Carbonic anhydrase inhibitors are currently used in the treatment of many diseases. Edema, epilepsy, ocular hypertension and glaucoma are examples of these disorders. Today, it is tried to create pharmaceutically effective drugs especially for epilepsy and Alzheimer's diseases. Inhibition of this isoenzyme also plays an important role in the treatment of these diseases [23].

Results and Discussion

The process used to create the compound 2-((5-((4-chlorophenyl)amino)-1,3,4-thiadiazol-2-yl)thio)-N'-(substituted benzylidene) acetohydrazide (5a-5b) is shown in Figure 3. NMR and elemental analyses were used to identify every chemical that was produced. 4-Chloroisothiocyanate on treatment with 99% hydrazine hydrate in the presence of ethanol yield N-(4-chlorophenyl)hydrazinecarbothioamide (1). The resulting product 1 is then subjected to cyclization in the following step through a reaction with carbon disulfide and NaOH in ethanol, yielding 5-(4-chlorophenylamino)-1,3,4-thiadiazole-thiol (2). Compound 2 and chloroethylacetate were combined with anhydrous potassium carbonate and refluxed for eight hours. To obtain 2-((5-((4-chlorophenyl)amino)-1,3,4-thiadiazol-2-yl)thio)acetohydrazide (4), compound 3 was refluxed with hydrazine hydrate in ethanol. Target compounds were produced by refluxing a combination of hydrazide derivative (4) and the corresponding benzaldehyde derivatives in EtOH (5a-5b).

By using ^{1}H NMR, ^{13}C NMR, and elemental analysis, the target compounds (5a-5b) had their chemical structures verified. The singlet signals observed in all the spectra at δ 8.25-8.32 ppm (N=CH) and δ 11.47 -11.86 ppm (O=C-NH) confirmed the N-acylhydrazone skeleton in the structures of compounds 5a-5b. The compounds 5a and 5b's ^{1}H NMR spectrum analysis revealed that the S-CH_2 (methylene) protons were detected as a singlet between 4.39 and 4.43 ppm. NH protons between thiadiazole and phenyl rings were observed as singlet in the range of 10.45-10.46 ppm.

The anticancer activity results of compounds 5a, 5b against MCF7, MDA, HT-29 and L929 are presented in Table 1. Cisplatin was used as a reference drug. When the effects of the compounds on cancer cell lines were examined, it was observed that the compounds were ineffective against both cancer cells and healthy cells.

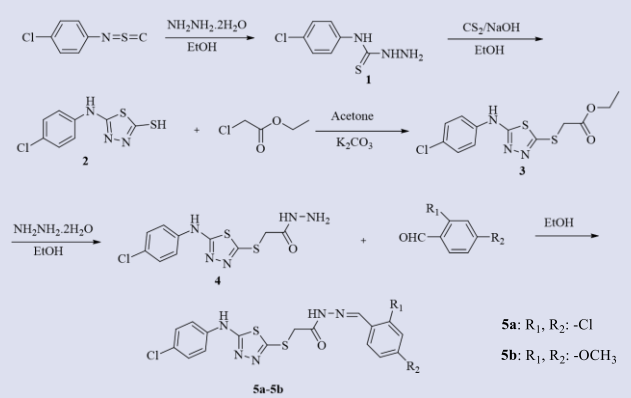

Figure 3. Synthesis pathway of compounds 5a and 5b

Table 1. IC_{50} values (μM) and percent vitality of compounds 5a, 5b and reference drug cisplatin for MCF-7, MDA, HT-29 and L929 cell lines

	IC_{50} values (μM)			
	L929	**MCF7**	**MDA**	**HT-29**
Control	>100	>100	>100	>100
Comp. 5a	>100	>100	>100	>100
Comp. 5b	>100	>100	>100	>100
Cisplatin	4,98	3,145	3,69	7,87

Our synthetic structures were investigated using the esterase assay method with two physiologically relevant isoenzymes carbonic anhydrase. Among these enzymes, carbonic anhydrase-I (hCAI) has a slower cytosolic isoform and carbonic anhydrase-II (hCA II) has a faster inhibition potential. Inhibition data of compounds against hCAI and hCAII isoforms are summarized in Table 2 and Figure 4. While the inhibition effect of the first synthesis was not found, it was determined that our second synthesis had an inhibitory effect on both isoenzymes. The obtained results were compared with the Acetazolamide used as the standard and it was determined that our second synthesis was better than the standard. With these results, it was determined that the compound 5b could be used as an alternative carbonic anhydrase inhibitor. Among them, the compound 5b exhibited remarkable CA inhibitory activities compared to a standard inhibitor with IC_{50} values in the range of 27 μM for hCA I and 33,46 μM for hCA II. Although the compound 5a carrying the chloro substituent on the phenyl ring was ineffective, the compound 5b carrying the methoxy substituent on the phenyl ring was found to be more effective on hCA I and II than the reference drug acetazolamide. It has been determined that the presence of chloro substituent, which is an electron withdrawing group, reduces the effect on hCA, while the methoxy group, which is an electron donating group, increases the activity.

While the compounds are expected to have an effect on both the carbonic anhydrase enzyme and cancer cells, as a result of the activity tests, only an effect on the carbonic anhydrase enzyme was obtained. In this study, we found that compound 5b has carbonic anhydrase activity independent of its anticancer effect. When the literature is examined, diseases that occur as a result of different interactions related to carbonic anhydrase I and II have been associated. These interactions include COX, LOX [24], antioxidant [25], α-glucosidase [26], β-glucuronidase [27], antiulcer [28], acetylcholinesterase [29] and antiglaucoma [30]. Furthermore, according to in vitro assay, it is noteworthy that compound 5b showed significant hCA I and hCA II inhibitory activity, although this compound do not carry a sulfonamide group, which is an important pharmacophore for hCA inhibitory activity.

Altintop et al. The hCA I and hCA II enzyme activities of the compounds carrying the thiadiazole-hydrazone structure were investigated and high activity was obtained in some compounds [31]. When the literature was examined, no studies were found in which both cancer and carbonic anhydrase enzyme activities of compounds with thiadiazole-hydrazone structure were studied. In this study, it is thought that examining both activities will contribute to the literature.

Table 2. IC_{50} (μM) values against hCA I and hCA II enzymes

Comp.	IC_{50} (μM)			
	hCA I	r^2	hCA II	r^2
5a	-	-	-	-
5b	27	0.9691	33.46	0.9546
AZA*	28.11	0.9387	35.65	0.9756

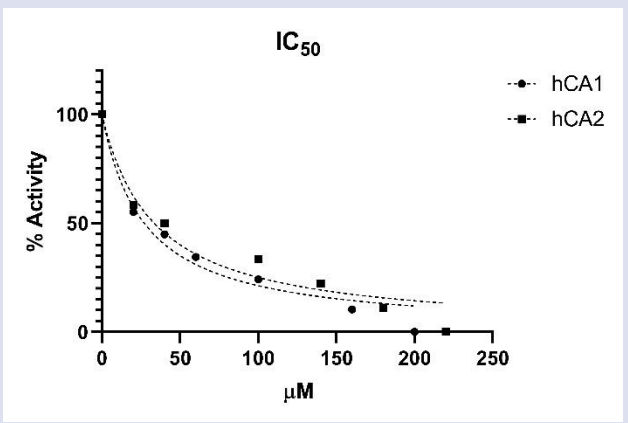

Figure 4. IC_{50} graphs for molecules with the most effective inhibition values against hCA I and II isoenzymes

Conclusion

In this study, two thiadiazole-hydrazone derivatives 5a, 5b were synthesized. The compounds are derivatized via the phenyl ring using the chloro and methoxy substituent. The structures of the compounds were elucidated using spectroscopic methods. The anticancer effects of the compounds were evaluated on the MCF7, MDA, HT-29. In addition, L929 healthy mouse fibroblast cells were used to determine the selectivities of the compounds. In addition, the inhibitory properties of the compounds on hCAI and hCAII enzyme were evaluated. When the anticancer properties of the compounds were examined, it was seen that the compounds were ineffective on cancer cells. When the inhibitory effects of the compounds on the carbonic anhydrase enzyme were evaluated, it was seen that compound 5b was effective on both hCAI and hCAII. The 2,4-dimethoxy groups in the structure of compound 5b seem to be important in both hCAI and hCA II enzyme activity. It was determined that the compounds were not effective on cancer cells, but it was found that the compounds were not toxic on the healthy cell line. In this finding, while the compound 5b is effective against hCA I and hCA II enzymes, it has no toxic effect, which makes this compound more valuable.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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