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ORIGINAL ARTICLE / ÖZGÜN MAKALE

SYNTHESIS AND CHARACTERIZATION OF NOVEL 5-FLOUROINDOLYLMETHYLEN HYDRAZON DERIVATIVES AS SCHIFF BASE COMPONDS: *IN VITRO* AND *IN SILICO* EVALUATION AS POTENTIAL CARBONIC ANHYDRASES (CAI-CAII) INHIBITORS

YENİ 5-FLOROİNDOLİLMETİLEN HİDRAZON TÜREVLERİNİN SCHİFF BAZ BİLEŞİKLERİ OLARAK SENTEZİ VE KARAKTERİZASYONU: POTANSİYEL KARBONİK ANHİDRAZ (CAI-CAII) İNHİBİTÖRLERİ OLARAK İN VİTRO VE İN SİLİCO DEĞERLENDİRMESİ

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ABSTRACT

Objective: *In this study, novel 5-fluoroindolylmethylene hydrazone derivatives were synthesized and assessed for their potential inhibitory effects on human carbonic anhydrase I and II (hCA I and II) enzymes through both in vitro and in silico approaches.*
Material and Method: *Accordingly, starting from 5-fluoroindole, 5-fluoroindole-3-carboxaldehyde was synthesized. The target final compounds were then obtained by condensing substituted phenylhydrazine with 5-fluoroindole-3-carboxaldehyde, resulting in three Schiff base derivatives (2a, 2b, and 2c).*
Result and Discussion: *The synthesized compounds demonstrated effective inhibition of cytosolic carbonic anhydrase isoforms hCA I and II, with Ki values ranging from 32.28±7.09 to 74.86±10.90 nM for hCA I and 8.79±1.92 to 60.40±14.64 nM for hCA II. Among them, compound 2a exhibited the most potent inhibitory effect on both isoenzymes. In vitro results were verified with the results obtained by docking studies and interactions with enzymes were demonstrated. These novel 5-fluoroindolylmethylene hydrazone derivatives show promise as potent inhibitors of cytosolic CA isoenzymes.*
Keywords: *5-Flouroindole, carbonic anhydrase, glaucoma, inhibition*

ÖZ

Amaç: *Bu çalışmada, 5-floroindolilmetilen hidrazonun yeni türevleri sentezlendi ve bunların insan karbonik anhidraz I ve II (hCA I ve II) enzimleri üzerindeki potansiyel inhibitör etkileri in vitro ve in silico olarak değerlendirilmesi amaçlandı.*
Gereç ve Yöntem: *Buna göre, 5-floroindolden başlayarak, 5-floroindol-3-karboksaldehit sentezlendi. Hedef nihai bileşikler daha sonra ikame edilmiş fenilhidrazinin 5-floroindol-3-karboksaldehit ile yoğunlaştırılmasıyla elde edildi ve üç Schiff baz türevi (2a, 2b ve 2c) elde edildi.*

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Sonuç ve Tartışma: *Sentezlenen bileşikler, hCA I için 32.28±7.09 ila 74.86±10.90 nM ve hCA II için 8.79±1.92 ila 60.40±14.64 nM aralığında değişen Ki değerleri ile sitozolik karbonik anhidraz izoformları hCA I ve II'nin etkili inhibisyonunu göstermiştir. Bunlar arasında, bileşik 2a her iki izoenzim üzerinde en güçlü inhibitör etkiyi göstermiştir. İn vitro sonuçlar, yerleştirme çalışmalarıyla elde edilen sonuçlarla doğrulanmış ve enzimlerle etkileşimler gösterilmiştir. Bu yeni 5-floroindolilmetilen hidrazon türevleri, sitozolik CA izoenzimlerinin güçlü inhibitörleri olarak ümit verici görünmektedir.*
Anahtar Kelimeler: *5-Floroindol, glokom, inhibisyon, karbonik anhidraz*

INTRODUCTION

Metalloenzymes consist of one or more metal ions that are tightly bound within a protein. Using the enzyme as a structural framework for transition metal complexes, these systems enable selective chemical transformations. It is well known which metal ions strongly bind to proteins. Identifying these bound metal ions is crucial for advancing enzymatic analysis [1,2]. These enzymes play crucial roles in protein degradation, detoxification, nucleic acid modification and other vital processes [2,3]. The participation of metalloenzymes in these functions also connects them to the development of various diseases, highlighting their potential as therapeutic targets [3]. Human carbonic anhydrase (hCA; carbonate hydrolases, EC 4.2.1.1) belongs to the metalloenzyme family, which includes sixteen isoenzymes (CA I–XVI) in mammals. Because they contain zinc ions, most carbonic anhydrases are categorized as metalloenzymes. Human carbonic anhydrases facilitate the reversible reaction of carbon dioxide (CO_2) with water, yielding a proton (H^+) and a bicarbonate ion (HCO_3^-). This reaction plays a crucial role in pH regulation across various tissues, particularly in erythrocytes [4-9]. Several CA isozymes play essential roles in physiological functions, making them important therapeutic targets. Modulating their activity, either through inhibition or activation, holds therapeutic potential for conditions like edema, cancer, epilepsy, osteoporosis, glaucoma and obesity [5-11]. Carbonic anhydrase inhibitors (CAIs) such as zonisamide (ZNS), acetazolamide (AZA) and methazolamide (MZA) are commonly used in clinical settings as diuretics and to manage intraocular pressure in glaucoma patients [12,13]. The inhibitory effects of several compounds, including anions, metal ions, phenols [14-16], Schiff bases, sulfonamides and their amines, amides and 1H-indazole derivatives [17-20], chalcones [21], pyrimidine-thiones [22], and certain amino methyl derivatives [23], have been investigated against numerous CA isozymes to date. CA II inhibitors have a number of uses, including the treatment of epilepsy and glaucoma, as well as their use as antitumor agents, diagnostic tools, and diuretics [6,8,24,25]. Currently, there is significant scientific interest in the discovery of novel CA inhibitors [6,8,14,24-27]. Recent studies have shown that various phenol derivatives [8,14], salicylic acid derivatives [28], and different benzene and bisphenol compounds with antioxidant properties, along with their derivatives [29,30], are potential inhibitors of hCA I-II isozymes. Recently, researchers in this field have focused on evaluating the pharmacological activity of indole and its derivatives. Numerous studies have demonstrated the diverse therapeutic potential of indole derivatives, including antioxidant [31], antimicrobial [32], and anticancer [33], properties, as well as their potential in Alzheimer's disease [34,35].

This study is primarily focused on the synthesis of three novel indole-derived Schiff base compounds and the evaluation of their inhibitory potential against the hCA I-II enzymes. In this context, firstly, the compound 5-fluoroindole-3-carboxaldehyde it resulted from the reaction of 5-fluoroindole with DMF and $POCl_3$ [36]. Then, 5-fluoroindole-3-carboxaldehyde was reacted with substituted phenylhydrazine derivatives or hydrazine to synthesis the three Schiff bases compounds. The synthesized compounds were evaluated using a variety of characterization techniques like ^1H and ^{13}C NMR and mass spectra analyses. The CA inhibitory effects of the newly synthesized compounds targeting the CA I and II isoenzymes, isolated from human erythrocytes, were assessed based on two parameters: IC_{50} and Ki. IC_{50} values were determined by measuring the percentage of enzyme activity at various inhibitor concentrations, while keeping the substrate concentration fixed, followed by graphical determination of the inhibitor concentration required for 50% inhibition. The results were subsequently compared to acetazolamide.

MATERIAL AND METHOD

General

A melting point for Stuart The SMP30 instrument was used to determine uncorrected melting points. A Varian 500 MHz and 125 MHz spectrometer (Palo Alto, CA) The ^{1}H and ^{13}C NMR spectra were recorded using TMS for internal standard, with DMSO-d6 and $CDCl_3$ serving as the solvents. The ESI mass spectra were obtained using a Waters Micromass ZQ apparatus. Every spectral analysis was carried out at Ankara University's Instrumental Laboratory of the Faculty of Pharmacy. Using Merck silica gel 60 (230–400 mesh ASTM), chromatography was performed. Sepharose-4B, protein assay reagents, 4-nitrophenylacetate and other chemicals used for purification were purchased from Sigma–Aldrich Co. All other chemicals used for kinetic analysis were analytical grade and obtained from Merck. The chemical reagents and other compounds that were utilized in the synthesis.

General Procedure for Synthesis of Compounds

Compound 5-fluoroindole-3-carboxaldehyde it resulted from the reaction of 5-fluoroindole with DMF and $POCl_3$ [36]. The final three Schiff bases compounds were synthesized by condensation of substituted phenylhydrazine hydrochloride or hydrazine hydrate with 5-fluoroindole-3-carbaldehyde (Figure 1). The novel compounds were synthesized using a Kidwai-adapted technique [37].

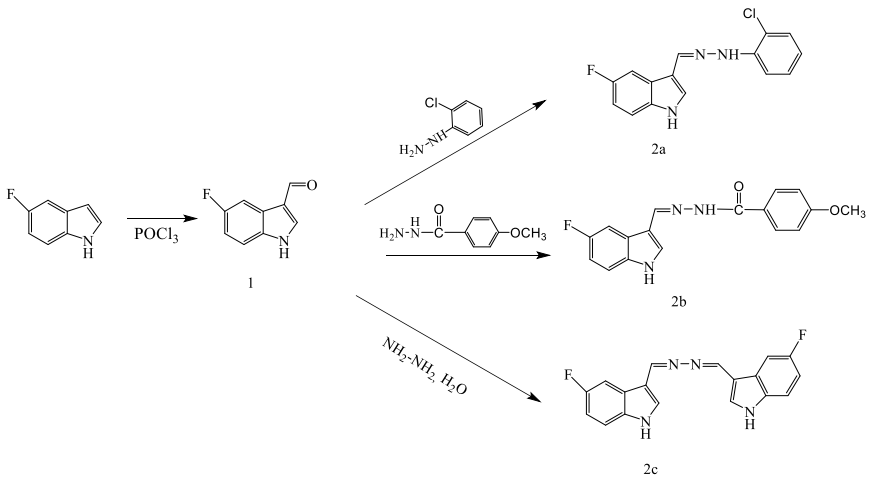

Figure 1. Synthesis of indole hydrazine derivatives

Synthesis of 5-fluoroindole-3-carbaldehyde (1)

5-Fluoroindole (1 mmol) was reacted with $POCl_3$ in DMF. $POCl_3$ was dissolved in DMF at temperatures below 10 ºC, and then 5-fluoroindole, Dissolved in DMF, it was introduced into the mixture, which was then stirred at 35 ºC for 1.5 hours. Afterward, it cooled to 10 ºC, and 1N NaOH was added until the solution became alkaline. The mixture was subsequently heated to 60 ºC and then allowed to cool. Finally, it was filtered, rinsed with water, and left to dry.

Yield: 77% (0.126 g), mp: 163-165°C. ^{1}H-NMR (500 MHz, $CDCl_3$, δ): 8.99 (s, 1H), 8.02 (dd, 1H, J=9.2, J'=2.3), 7.91 (d, 1H, J=3.1), .7.40 (dd, 1H, J=8.8, J'=4.3), 7.28 (s, 1H),7.12-7.08 (m, 1H). ^{13}C-NMR (125 MHz, DMSO, δ): 184.99, 160.74, 158.85, 136.41, 133.03, 119.76, 113.08, 112.87, 112.43, 112.35, 107.58, 107.39. MS (ESI+) m/z: 164.03 (M+H, 55%). 191.29 (M+28, 100%).

Synthesis of Compounds 2a and 2b

A mixture of 5-fluoroindole-3-carbaldehyde (1 mmol), 2-chlorophenyl hydrazine hydrochloride or 4-methoxybenzohydrazide (1.2 mmol) was dissolved in 20 ml of absolute ethanol. Sodium acetate (0.4 g) was added as a catalyst, and the reaction was heated at 80ºC for 2 hours. Upon completion, the

reaction mixture was allowed to cool to room temperature. The resulting precipitate was filtered, washed with cold water, and recrystallized from ethanol to afford the desired compounds.

5-fluoroindole-3-carboxaldehyde (2-chlorophenyl) hydrazone (2a): Yield:51% (0.145 g), mp: 147-148°C. ^{1}H-NMR (500 MHz, DMSO, δ): 11.55 (s, 1H, hydrazon-NH), 9.44 (s, 1H), 8.49 (s, 1H, azomethine-CH), 7.90 (dd, 2H, J = 10, J'=2.6 Hz), 7.76 (d, 1H, J = 2.7 Hz), 7.52 (dd, 1H, J = 8.6, J'=1.4 Hz), 7.47-7.44 (m,1H), 7.34-7.31 (m, 2H), 7.09-7.05 (m, 1H), 6.76-6.73 (m, 1H). ^{13}C-NMR (125 MHz, DMSO, δ): 158.97, 157.12, 142.46, 138.93, 134.15, 130.45, 129.74, 128.63, 124.84, 124.76, 118.93, 116.13, 113.52, 113.41, 113.33. 113.03, 112.99, 111.04, 110.84, 106.64, 106.46. MS (ESI+) m/z: 288.38 (M+H, 100%).

N'-((5-fluoro-1H-indol-3-yl)methylene)-4-methoxybenzohydrazide (2b): Yield: 37%(0.114 g), mp: 220-222°C. ^{1}H-NMR (500 MHz, DMSO, δ): ^{1}H-NMR (500 MHz, DMSO, δ): 11.68 (s, 1H, hydrazon-NH), 11.47 (s, 1H), 8.60 (s, 1H, azomethine-CH), 8.03 (dd, 1H, J=10, J'=2.5 Hz), 7.94-7.90 (m, 3H), 7.47-7.44 (m, 1H), 7.09-7.05 (m, 3H), 3.84 (s, 3H). ^{13}C-NMR (125 MHz, DMSO, δ): 162.49, 162.20, 159.12, 157.27, 144.36, 134.10, 132.20, 129.78, 126.46, 125.18, 125.10, 114.1, 113.38, 113.31, 112.47, 112.43, 111.27, 111.06, 107.33, 107.14, 55.86. MS (ESI+) m/z: 312.46 (M+H, 100%).

Synthesis of 1,2-bis((5-fluoro-1*H*-indol-3-yl)methylene)hydrazine (2c)

5-Fluoroindole-3-carbaldehyde (2 mmol) was combined with hydrazine hydrate (1 mmol) in 20 mL of absolute ethanol and heated on a hot water bath for 2 hours. After completion, the reaction mixture was cooled to room temperature. The resulting precipitate was filtered, washed with cold water, recrystallized from ethanol to obtain the desired compounds.

Yield: 77% (0.248 g), mp: 324-325°C. ^{1}H-NMR (500 MHz, DMSO, δ): 11.84 (s, 2H, hydrazon-NH), 8.92 (s, 2H), 8.05 (dd, 2H, J=10, J'=2.6 Hz), 7.99 (s, 2H, azomethine-CH), 7.51-7.48 (m, 2H), 7.11-7.06 (m, 2H). ^{13}C-NMR (125 MHz, DMSO, δ): 185.44, 159.23, 157.37, 155.60, 134.26, 133.96, 125.60, 125.52, 113.59, 113.51, 112.59, 112.55, 111.30, 111.09, 107.36, 107.17. MS (ESI+) m/z: 323.47 (M+H, 100%).

Molecular Docking Studies

The protein data bank provided hCA I (PDB: 2FOY) [38] and hCA II (PDB: 1IF7) [38], which were downloaded in pdb format for the molecular docking investigation. AutoDockTools 1.5.6 [39,40] was used to remove water, ions and ligands from the downloaded proteins, add polar hydrogens, and add a gaister charge before saving the protein in pdbqt format. Ligands were prepared in the ChemDraw3D 19.0 application. Molecular docking analysis was performed using the most recent AutoDock Vina software [40] following conversion to pdbqt format using AutoDockTools 1.5.6. The results are shown in 2D and 3D the Discovery Studio Client program [41].

Biological Activity Studies

Carbonic Anhydrase Isoenzymes Inhibition Analysis

The enzyme purification process was conducted in accordance with established protocols from previous research [42-46]. The protein concentration in active fractions was measured spectrophotometrically at 595 nm using the Bradford assay [47]. Carbonic anhydrase (CA) isoenzyme activity was determined based on the method outlined by Verpoorte et al., with spectrophotometric absorbance monitored at 348 nm to track changes in the reaction medium [48].

To assess the inhibitory potential of thiophene derivative Schiff base compounds (2a-c) on human CA isoenzymes, a graph representing enzyme activity percentage against inhibitor concentration was generated. The IC_{50} values were extracted from these plots. For Ki determination, three different inhibitor concentrations were tested alongside five varying substrate concentrations. Additionally, inhibition graphs for the most effective compounds were plotted and analyzed. The same approach used for AZA, the reference inhibitor for CA isoenzymes, was used to calculate Ki and IC_{50} values.

RESULT AND DISCUSSION

Studies on the inhibition of carbonic anhydrase (CA) enzyme activity have demonstrated its

potential as a therapeutic approach for treating glaucoma. These studies have also aimed to clarify the enzyme's catalytic mechanisms, while revealing its distribution in tissues and its vital roles within them. These findings have led to a notable rise in the development of enzyme inhibitors and activators. Consequently, various enzyme inhibitors have been developed. Beyond its use in glaucoma treatment, CA inhibitors are also widely utilized in clinical settings for their antitumor, analgesic, anticonvulsant, antiulcer, diuretic, antibiotic, and neurological properties [49]. Our previous research has demonstrated that indole derivatives containing Schiff bases inhibit the cytosolic CA isozymes hCA I and hCA II. As a continuation of our studies, the synthesis of three new indole-derived Schiff base compounds and the investigation of their inhibitory effects on hCA I and II enzymes were evaluated [50]. This work synthesized new compounds and investigated their in vitro effects on the hCAI and hCAII isoenzymes after first doing in silico assessments of novel Schiff bases generated from indole. After purifying the CAI and CAII isoenzymes using the CNBr-activated Sepharose-4B-L-tyrosine sulfanilamide affinity chromatography method, the effects of the new indole derivative Schiff base compounds on these isoenzymes were investigated (Table 1, Figure 2-5). Compounds 2a and 2c exhibited strong inhibition effects on hCAI and hCAII isoenzymes at very low concentrations. The inhibitor concentration and % activity for the compounds 2c and 2a, which showed inhibitory effects on the hCAI and hCAII isoenzymes, respectively, were shown in graphs (Figure 2 and Figure 4). Ki values were determined for each compound at three different concentrations. The compounds were found to exhibit inhibitory effects across all three concentrations tested. Lineweaver-Burk plots were drawn to determine the Ki values and the type of inhibition. The Ki and IC_{50} values obtained for hCAI and hCAII are provided in Table 1. It has been established that the Ki values for hCAI range from 32.87 ± 10.64 to 74.86 ± 10.90 nM and for hCAII from 8.79 ± 1.92 to 60.40 ± 14.67 nM. In comparison to the control compound AZA, compound 2a displayed the strongest inhibitory activity against the hCAI isoenzyme, while compound 2c exhibited the most potent inhibition of the hCAII enzyme (Figure 2 and Figure 4) (Table 1).

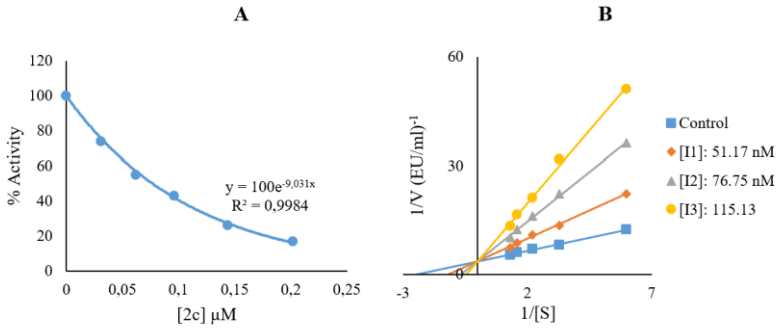

Figure 2. IC_{50} graph (A) and Lineweaver-Burk graph (B) of excellent inhibitor (**2c**) for hCAI

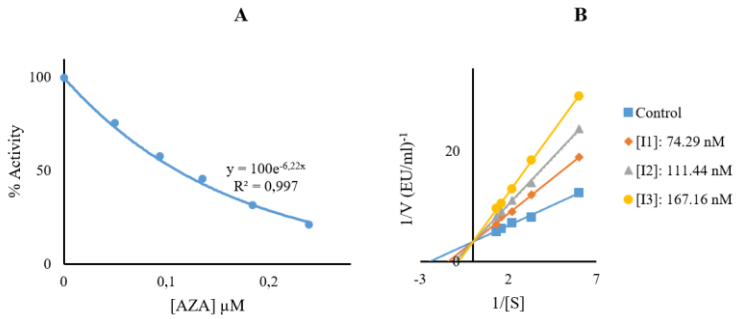

Figure 3. IC_{50} graph (A) and Lineweaver-Burk graph (B) of Asetazolamide (AZA) for hCAI

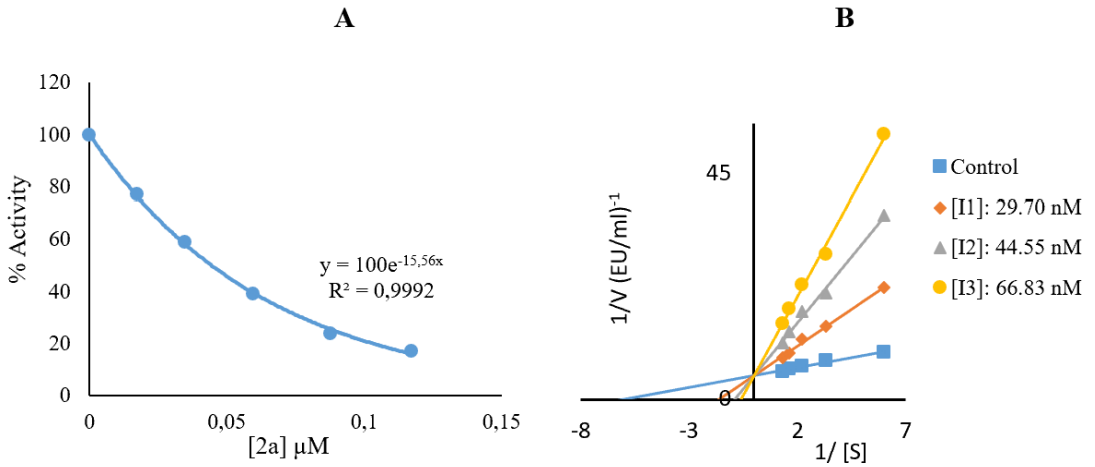

Figure 4. IC_{50} graph (A) and Lineweaver-Burk graph (B) of excellent inhibitor (**2a**) for hCAII

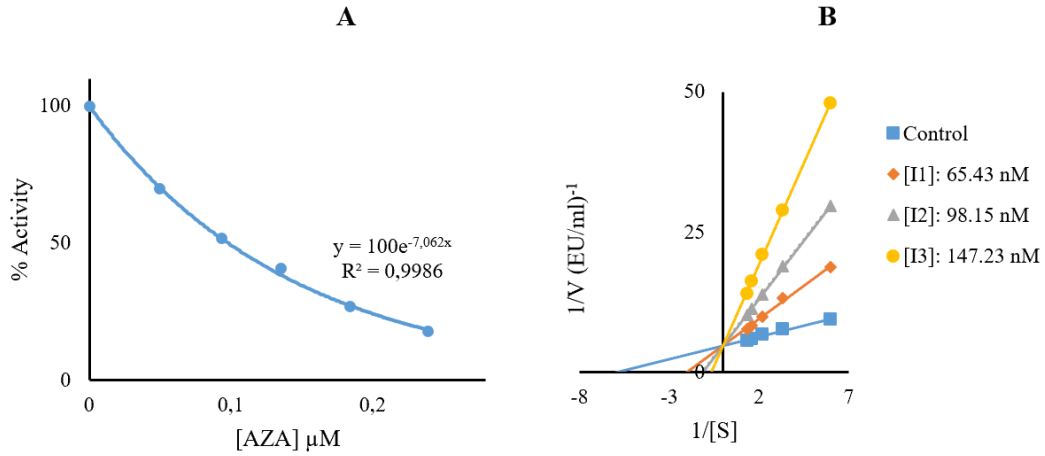

Figure 5. IC_{50} graph (A) and Lineweaver-Burk graph (B) of Asetazolamide (AZA) for hCAII

Table 1. The Ki constants and IC_{50} values determined for compounds (2a-c) and acetazolamide (AZA) drugs having inhibitory effects on hCAI and hCAII.

Compounds	IC_{50} (nM)				Ki (nM) ± STD	
	hCAI	R^2	hCAII	R^2	hCAI	hCAII
2a	59.09	0.9991	44.55	0.9971	34.20 ± 7.09	8.79 ± 1.92
2b	196.70	0.9947	165.38	0.9889	74.86 ± 10.90	60.40 ± 14.67
2c	76.75	0.9975	64.48	0.9971	32.87 ± 10.64	14.45 ± 4.28
AZA	111.44	0.9939	98.15	0.9976	89.70 ± 9.43	24.98 ± 7.71

Molecular docking studies revealed that the binding energies of compounds 2a-c to the hCA-I and hCA-II enzymes ranged from from -6.8 to -8.8 kcal/mol. The binding energy of the reference drug AZA for the hCAI enzyme is -5.7 kcal/mol, and for the hCAII enzyme, it is -6.1 kcal/mol (Table 2). All compounds were observed to bind with lower binding energies than the reference drug AZA.

When the interactions of the compounds with the hCAI enzyme were evaluated, hydrogen bonds and pi interactions were observed in the interactions of all compounds and acetazolamide with the active site of the hCAI enzyme. In compound 2b, hydrogen bonds are predominant and there are fewer hydrophobic interactions. Considering the in vitro hCAI activity of 2b, it is thought that hydrophobic

interactions may play an important role in enzyme inhibition activity. In compounds 2a and 2c, pi interactions are also seen in addition to hydrogen bonds. There are hydrogen bonds and pi interactions in the interaction of the reference drug acetazolamide with the hCAI enzyme's active site (Figure 6).

Table 2. The binding energy and protein-ligand interaction for compounds (2a-c) and acetazolamide (AZA) drugs having inhibitory effects on hCAI and hCAII

Compunds	Binding energy for hCA-I (kcal/mol)	Protein-ligand interaction (for hCA-I)	Binding energy for hCA-II (kcal/mol)	Protein-ligand interaction (for hCA-II)
2a	-6.9	VAL A:239, LYS A:170, PRO A:240, TYR A:7	-7.2	PHE A:231, PRO A:237, GLU A:236, GLY A:63, GLU A:239
2b	-6.8	GLY A:63, GLN A:242, HIS A:243, LYS A:170, SER A:231, PRO A:241	-7.8	PHE A:231
2c	-7.8	LEU A:231, HIS A:94, ALA A:121, PHE A:91, ASN A:69, HIS A:200	-8.8	TYR A:7, PHE A:231, LYS A:170, ASN A:32, VAL A:242
AZA	-5.7	LEU A:131, ALA A:121, HIS A:100, GLU A:92, HIS A:96, THR A:199, HIS A:94, HIS A:119	-6.1	PHE A:231, HIS A:4, LYS A:140, GLY A:63, TYR A:7, GLU A:239, ASN A:11

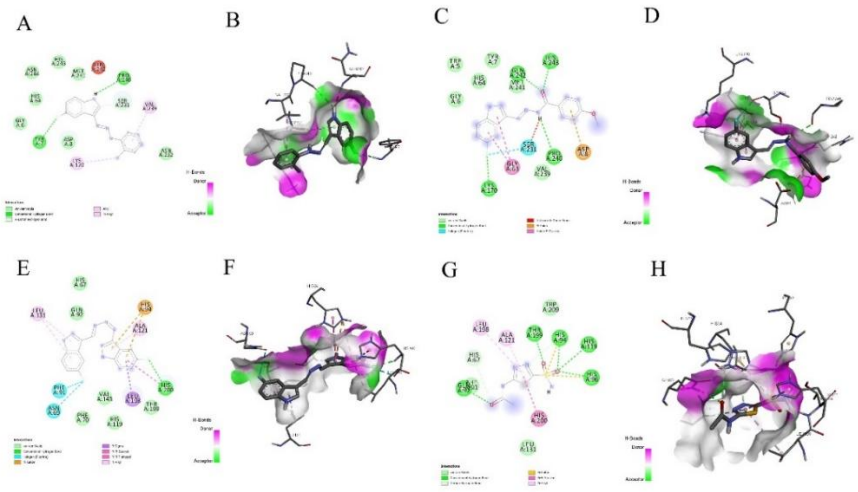

Figure 6. The active site of the hCA I enzyme contains 2D molecular docking images of compounds 2a (A), 2b (C), 2c (E), and the reference drug acetazolamide (G), as well as 3D molecular docking images of compounds 2a (B), 2b (D), 2c (F), and the reference drug acetazolamide (H). Interactions of compound 2a:VAL A:239(π interactions), LYS A:170(alkyl interactions), PRO A:240(H-bonds), TYR A:7(H-bonds), interactions of compound 2b: GLY A:63 (π-alkyl interactions), GLN A:242(H-bonds), HIS A:243(H-bonds), LYS A:170(H-bonds), SER A:231(Halogen interactions), PRO A:241 (H-bonds), Interactions of compound 2c: LEU A:231 (π interactions), HIS A:94 (π interactions), ALA A:121 (π interactions), PHE A:91 (Halogen interactions), ASN A:69(Halogen interactions), HIS A:200 (π interactions and H-bonds), interactions of reference drug acetazolamide; LEU A:131(π interactions), ALA A:121(π interactions), HIS A:100(π interactions), GLU A:92(H-bonds), HIS A:96, (H-bonds), THR A:199(H-bonds), HIS A:94(H-bonds), HIS A:119 (H-bonds)

When the interactions of compounds with hCAII enzyme are evaluated, hydrogen bonds and pi interactions are observed in the interactions of compounds 2a and 2b with the active site of hCAII enzyme. Hydrophobic interaction with Phe231 is seen as common in all compounds and acetazolamide. Hydrophobic interaction with Gly63, Glu239 is similar in compounds 2a and acetazolamide. Moreover, the low binding energy of compound 2a is low compared to acetazolamide. The fact that this compound has a more potent activity compared to acetazolamide is correlated with molecular docking study, and it can be thought that the hydrophobic interactions observed in the interaction of the compound with the enzyme contribute to the activity. It was observed that compound 2b has both hydrophobic and hydrogen bond interactions with Phe231 in the active site of the enzyme. Although its binding energy to the enzyme is high, it is thought that its lower activity compared to acetazolamide and compound 2a contributes to the activity due to hydrophobic interactions and hydrogen bonds. In the interactions of compound 2c with protein, more hydrophobic interactions are observed compared to acetazolamide. Compound 2c binds to the target protein with lower binding energy and correlates with in vitro activity. (Figure 7).

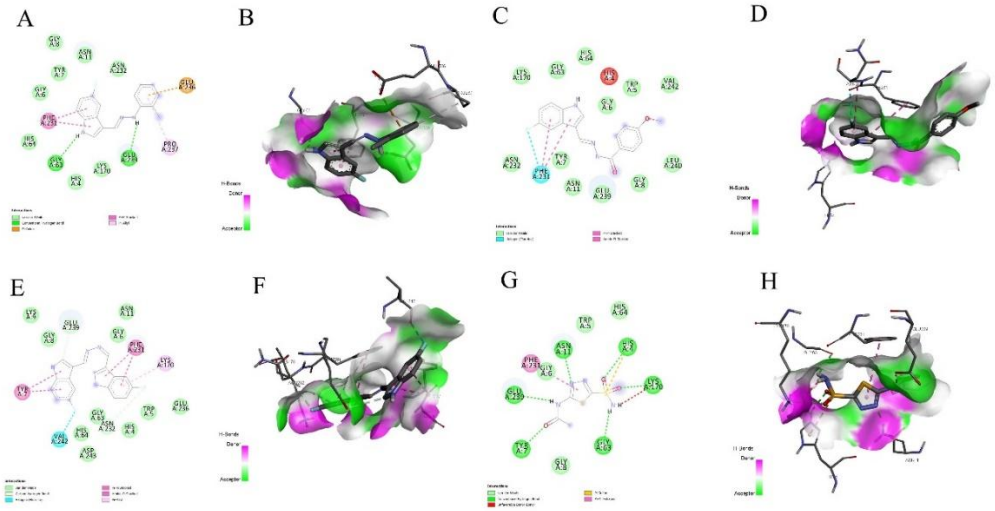

Figure 7. The hCA II enzyme's active site contains 2D molecular docking images of compounds 2a (A), 2b (C), 2c (E), and the reference drug acetazolamide (G). The hCA II enzyme's active site contains 3D molecular docking images of compounds 2a (B), 2b (D), 2c (F), and the reference drug acetazolamide (H). Interactions of compound 2a: PHE A:231(π interactions), PRO A:237, GLU A:236 (π interactions), GLY A:63(H-bonds), GLU A:239(H-bonds), interactions of compound 2b: PHE A:231(Halogen interactions and H-bonds), interactions of compound 2c: TYR A:7 (π interactions), PHE A:231 (π interactions), LYS A:170 (π interactions), ASN A:32 (H-bonds), VAL A:242 (Halogen interactions), interactions of reference drug acetazolamide; PHE A:231(π interactions), HIS A:4(H-bonds), LYS A:140(H-bonds),, GLY A:63(H-bonds), TYR A:7(H-bonds), GLU A:239(H-bonds), ASN A:11(H-bonds)

Conclusion

In this study, three different indole-derived Schiff base compounds were synthesized, and the inhibitory profiles of the synthesized compounds against the hCA I and hCA II enzymes were investigated. The investigated compounds exhibited more effective inhibitory profiles compared to AZA which is utilized as a reference drug. Consequently, the nnovel compounds inhibited hCAI and hCAII activities at lower concentrations. The synthesized compounds demonstrated effective inhibition of cytosolic carbonic anhydrase isoforms hCA I and II, with Ki values ranging from 32.28±7.09 to

74.86±10.90 nM for hCA I and 8.79±1.92 to 60.40±14.64 nM for hCA II. Compound 2a exhibited the most potent inhibitory effect on both isoenzymes. Docking studies were used to confirm the in vitro results, and interactions with enzymes were shown. The lower activity of compound 2b compared to 2a and 2c is believed to be due to the intramolecular hydrogen bond formed between the carbonyl oxygen and the hydrogen on the hydrazine nitrogen in 2b. This bond reduces the molecule's ability to form hydrogen bonds with the enzyme, leading to decreased activity. Even if our findings are encouraging, we intend to carry out more research to examine the cytotoxicity and mode of action of these substances in order to create an ideal and trustworthy hCA I and II enzyme inhibitor. Therefore, we believe that these novel 5-fluoroindolylmethylene hydrazone derivatives hold great potential as lead compounds for the development of improved CA inhibitors.

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AUTHOR CONTRIBUTIONS

Concept: C.K., N.F., E.D., H.Ş.; Design: C.K., N.F., E.D., H.Ş.; Control: C.K., N.F., E.D., H.Ş.; Sources: C.K., N.F., E.D., H.Ş.; Materials: C.K., N.F., E.D., H.Ş.; Data Collection and/or Processing: C.K., N.F., E.D., H.Ş.; Analysis and/or Interpretation: C.K., N.F., E.D., H.Ş.; Literature Review: C.K., N.F., E.D., H.Ş.; Manuscript Writing: C.K., N.F., E.D., H.Ş.; Critical Review: C.K., N.F., E.D., H.Ş.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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