Journal of Research in Pharmacy

Research Article

www.jrespharm.com

Novel sulfonamide-based compounds as dual inhibitors of hCYP1B1 and hCYP19A1 with anticancer activity against breast cancer cells

Elif İNCE-ERGÜÇ [1] *, Pınar ERASLAN-ELMA[2], Atilla AKDEMIR[3], Nilgun KARALI[2], Hande GURER-ORHAN[4]

1 Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Izmir Katip Celebi University, 35620, Izmir, Türkiye
2 Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Istanbul University, 34116, Istanbul, Türkiye
3 Department of Pharmacology, Faculty of Pharmacy, Istinye University, 34396 Istanbul, Türkiye
4 Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Ege University, 35100, Izmir, Türkiye
* Corresponding Author. E-mail: elif.ince@ikcu.edu.tr (E.İ.E); Tel. +90-232-329 35 35.

Received: 28 August 2024 / Revised: 21 November 2024 / Accepted: 23 November 2024

ABSTRACT: Cancer remains a critical global health challenge, driven by multifactorial etiologies, including oxidative stress, hormonal imbalances, and procarcinogen bioactivation. The cytochrome P450 enzymes CYP1B1 and CYP19A1 play significant roles in these processes, with CYP1B1 involved in the bioactivation of carcinogens such as DMBA and estrogens, and CYP19A1 being crucial in estrogen biosynthesis, particularly in hormone-dependent cancers. In this study, we synthesized a novel sulfonamide-based 2-indolinone compound (**7h**) and evaluated its inhibitory activity against human CYP1B1 and CYP19A1 enzymes, along with previously reported 1*H*-indole-2,3-dione 3-[4-(3-sulfamoylphenyl)thiosemicarbazones] (compounds **7-9**). Additionally, the cytotoxic effects of these compounds were tested on the MCF-7 human breast cancer cell line. R_2 trifluoromethoxy-substituted compound **7c** emerged as the most potent inhibitor of both hCYP1B1 (IC_{50} = 0.97 µM) and hCYP19A1 (IC_{50} = 6.46 µM) and demonstrated significant cytotoxicity, reducing MCF-7 cell viability to below 70% at 10 µM. R_1 methyl- substituted compound **7b**, R_1 and R_2 dimethyl- substituted compound **8b** and R_2 ethyl- substituted compound **9a** reduced MCF-7 cell viability below 60% after 24 hours of incubation at 10 µM. Molecular docking studies revealed key interactions between the compounds and enzyme active sites, correlating with their inhibitory potency. These findings suggest that the sulfonamide-based 2-indolinone derivatives, particularly compound **7c**, hold promise as dual inhibitors of CYP1B1 and CYP19A1, offering potential therapeutic benefits in the treatment of hormone-dependent and other cancers. Further studies are warranted to explore their full clinical potential.

KEYWORDS: Aromatase; CYP1B1; sulfonamides; docking

1. INTRODUCTION

Cancer, characterized by rapidly escalating incidence and mortality rates, continues to pose a formidable global health challenge. The etiology of cancer is multifactorial, encompassing oxidative stress, hormonal imbalances, radiation exposure, tobacco use, and infections [1]. Many carcinogens are procarcinogens that require metabolic activation to become cancer-causing. It is well-established that cytochrome P450 (CYP1) enzymes, particularly CYP1B1, are integral to these bioactivation processes. For instance, DMBA and endogenous estrogens are metabolized by CYP1B1 into reactive intermediates that contribute to carcinogenesis[2]. Notably, CYP1B1 expression is selectively elevated in various malignancies, including breast, lung, colon, and brain cancers, while being undetectable in normal tissues [3,4]. As a result, targeting CYP1B1 with specific inhibitors is considered a promising strategy for cancer prevention [5].

Breast cancer is one of the most prevalent cancers worldwide and a leading cause of cancer-related deaths among women. It represents about 25% of all cancer cases and 15% of cancer deaths in women [6]. The complexity of breast cancer is driven by its heterogeneity, with subtypes classified by hormone receptor status, such as estrogen receptor (ER)-positive, HER2-positive, and triple-negative breast cancer (TNBC) [7]. This diversity necessitates personalized treatment approaches, particularly for hormone-dependent subtypes like ER-positive breast cancer. One significant challenge in treating breast cancer is the role of estrogen in

How to cite this article: Ince-Erguc E, Erarslan-Elma P, Akdemir A, Karali N, Gürer-Orhon H. Novel sulfonamide-based compounds as dual inhibitors of hCYP1B1 and hCYP19A1 with anticancer activity against breast cancer cells. J Res Pharm. 2025; 29(3): 1098-1110.

ISSN: 2630-6344

https://doi.org/10.12991/jrespharm.1694239

driving tumor growth, particularly in ER-positive subtypes. Elevated estrogen levels, often regulated by the enzyme aromatase (CYP19A1), contribute to breast cancer progression [8]. Therefore, aromatase inhibitors (AIs) are essential in the treatment of postmenopausal women with ER-positive breast cancer [9–11]. However, the development of resistance to AIs and the associated side effects highlight the need for more selective and effective inhibitors [12].

In addition to the role of aromatase in estrogen biosynthesis, CYP1B1 plays a critical role in activating carcinogens, which contributes to breast cancer development. The overexpression of CYP1B1 in breast cancer makes it an attractive target for anticancer therapies, as inhibiting CYP1B1 could prevent carcinogen activation and tumor progression.

Given the critical roles of CYP19A1 and CYP1B1 in breast cancer, targeting these enzymes with novel inhibitors represents a promising therapeutic strategy. The 2-indolinone scaffold is a widely recognized pharmacophore used in the design of anticancer agents. Many drugs that contain the 2-indolinone ring such as sunutinib, pazopanib are already in clinical use for cancer treatment [13–15]. Over the past decade, considerable research has focused on 2-indolinone derivatives, which exhibit anticancer activity through various mechanisms [16,17]. Structure-activity relationship (SAR) studies have demonstrated that the introduction of alkyl or aralkyl groups at the 1-position of the indole ring, as well as methylene, imino, hydrazono, or thiosemicarbazone moieties at the 3-position, and alkyl, alkoxy, or halogen substituents at the 5-position, can significantly enhance anticancer activity [18]. Furthermore, phenyl-substituted derivatives have been found to possess superior anticancer properties compared to those substituted with alkyl or cycloalkyl groups at the thiosemicarbazone moiety. Recent investigations have revealed that 3-imino/hydrazono/thiosemicarbazone-2-indolinone derivatives bearing a phenyl sulfonamide group exhibit highly selective inhibitory effects against hCA IX and XII isozymes, which are critical targets for antitumor therapy, due to their role in tumor growth and metastasis under hypoxic conditions. While these carbonic anhydrases (CAs) are not directly related to the CYP19A1 and CYP1B1 enzymes, their involvement in cancer progression highlights the broader relevance of sulfonamide-based inhibitors in anticancer research (**Figure 1**) [19–26].

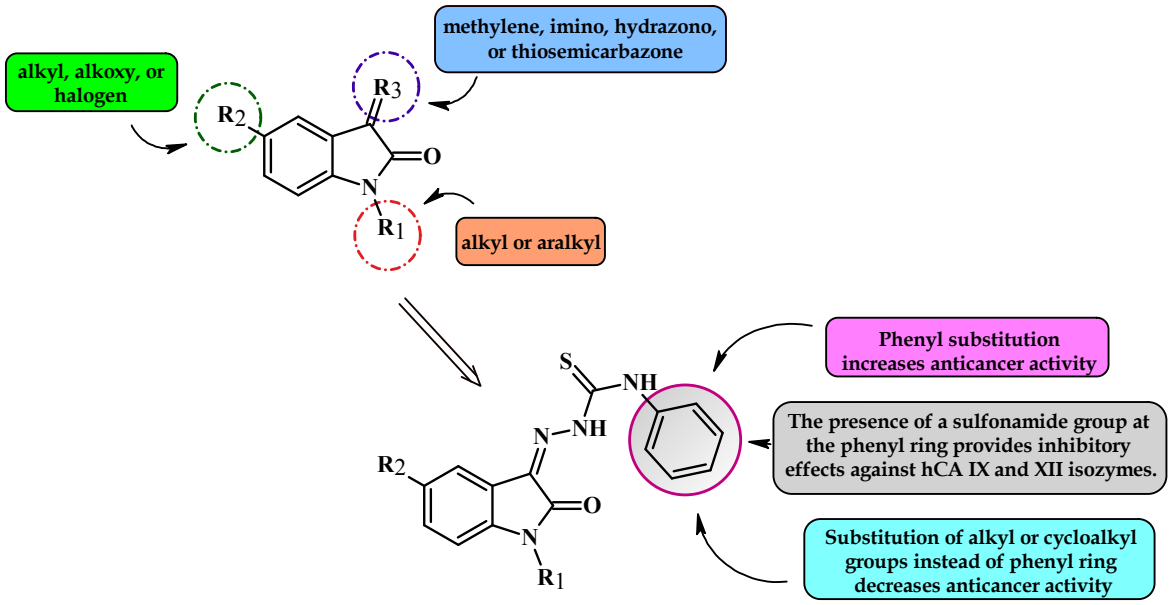

Figure 1. Structure-activity relationships of 2-indolinone derivatives

In a previous study, we reported the synthesis of several novel 1*H*-indole-2,3-dione 3-[4-(3-sulfamoylphenyl) thiosemicarbazones] (compounds **7-9**), along with molecular modeling studies for target enzymes and an evaluation of the inhibitory effects of these compounds on carbonic anhydrase (CA) isozymes. Many of the compounds tested demonstrated potent inhibition in the low nanomolar range against hCA IX and XII, with all showing selective inhibition at low nanomolar concentrations for hCA II [27].

In the current study, these previously synthesized compounds, along with a newly developed sulfonamide-based 2-indolinone compound **7h**, were evaluated for their inhibitory activity against human CYP1B1 and CYP19A1 enzymes. Additionally, the cytotoxic effects of these compounds were evaluated in

Journal of Research in Pharmacy
Research Article

the MCF-7 human breast cancer cell line. Molecular modeling studies were conducted to elucidate the interactions responsible for the observed inhibition profiles further supporting the therapeutic potential of these dual inhibitors in the treatment of hormone-dependent cancers.

2. RESULTS AND DISCUSSION

2.1. Chemistry

1*H*-Indole-2,3-diones **(1)** were reacted with methyl iodide or ethyl chloride to synthesize 1-methyl/ethyl-1*H*-indole-2,3-diones (**2** and **3**). The compound (3-sulfamoylphenyl) isothiocyanate **(5)** was prepared by treating 3-aminobenzenesulfonamide (**4)** with thiophosgene. Subsequently, 4-(3-sulfamoylphenyl) thiosemicarbazide **(6)** was synthesized through the reaction of 5 with hydrazine hydrate. The compounds 1*H*-indole-2,3-dione 3-[4-(3-sulfamoylphenyl) thiosemicarbazones] **(7-9)** were obtained by condensing **6** with **1-3**. In this study, a novel sulfonamide-based 2-indolinone compound **(7h**) carrying a sulfonic acid sodium moiety was synthesized for the first time using these methods. The other compounds (**7a-g**, **8a-f** and **9a-g**) had been previously reported by Eraslan-Elma et al.[27]. The structures of compounds were verified through elemental analysis and spectroscopic data, including IR, ^{1}H NMR, ^{13}C NMR-APT, HSQC-2D, HMBC-2D, and LCMS-ESI (**Figure 2** and **Table 1**)**.** Analysis (IR, ^{1}H NMR, and ^{13}C NMR-APT) spectra of compound **7h** are included in the supplementary material in this article. Analysis (IR, ^{1}H NMR, ^{13}C NMR-APT, HSQC-2D, HMBC-2D, and LCMS-ESI) spectra of compounds **7a-g**, **8a-f** and **9a-g** are available at supplementary material in the literature 18.

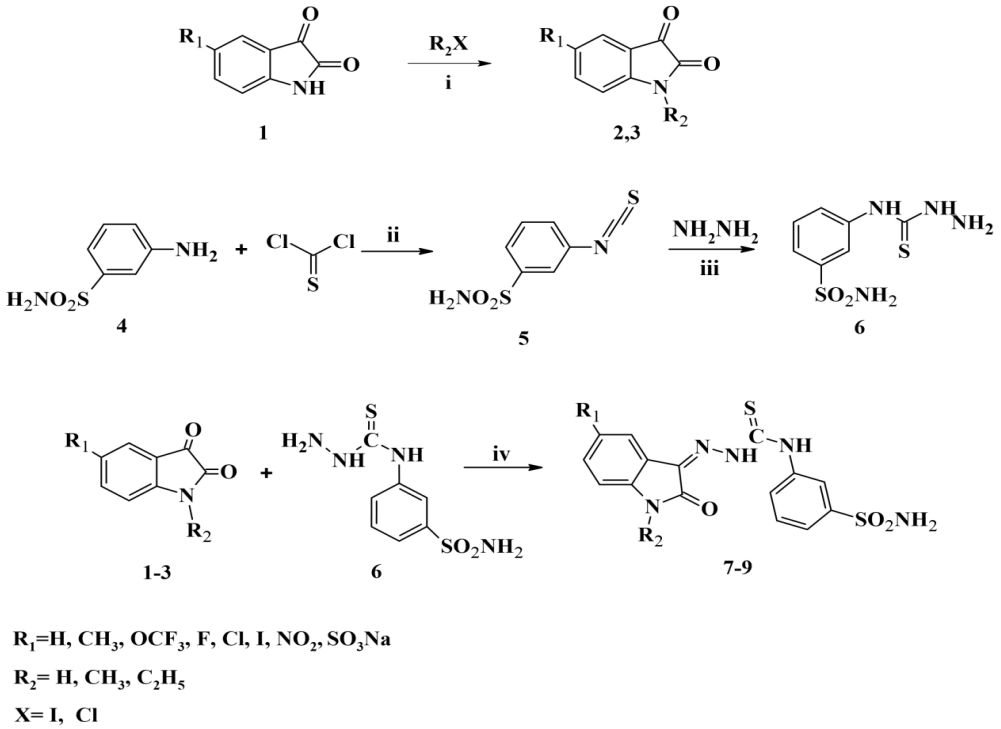

Figure 2. Synthesis of compounds **7-9**. Reagents and conditions**:** i) DMF, K_2CO_3, KI, stirred, ii) H_2O, HCl, stirred**,** iii) EtOH, stirred, cooled, iv) EtOH, H_2SO_4, reflux.

Table 1. 1*H*-indole-2,3-dione 3-[*N*-(3-sulfamoyl) phenyl]thiosemicarbazones] (**7-9**)

Comp.	R_1	R_2	Comp.	R_1	R_2	Comp.	R_1	R_2
ı	H	H	**7h**	SO_3Na	H	**9a**	H	C_2H_5
7b	CH_3	H	**8a**	H	CH_3	**9b**	CH_3	C_2H_5
7c	OCF_3	H	**8b**	CH_3	CH_3	**9c**	OCF_3	C_2H_5
7d	F	H	**8c**	OCF_3	CH_3	**9d**	F	C_2H_5
7e	Cl	H	**8d**	F	CH_3	**9e**	Cl	C_2H_5
7f	I	H	**8e**	Cl	CH_3	**9f**	I	C_2H_5
7g	NO_2	H	**8f**	I	CH_3	**9g**	NO_2	C_2H_5

IR spectra of compounds **7–9** exhibited NH stretching bands of the thioamide, lactam, and sulfonamide groups in the 3105−3394 cm^{-1} region. The absorption bands due to the lactam C=O and

https://doi.org/10.12991/jrespharm.1694239
J Res Pharm 2025; 29(3): 1098-1110

thioamide C=S were observed at 1670−1707 and 1178−1229 cm^{-1} regions, respectively. Moreover, the S=O stretching bands of the sulfonamide groups appeared two separate bands at 1325−1348 and 1143−1178 cm^{-1} regions. ^{1}H NMR spectra of compounds **7-9** were observed as two separate singlets the thioamide NH protons of the thiosemicarbazone moiety at δ 10.98−11.49 and 12.56−12.89 ppm regions. While the methyl (δ 3.19-3.33 ppm region) and ethyl (δ 1.19-1.24 and 3.74-3.88 ppm regions) signals at the 1-position of the indole ring in the spectra of compounds **8a-f** and **9a-g** prove alkylation, indole NH proton signals showed as singlets in the δ 11.25−12.11 ppm region in the spectra of compound **7a-h**. The spectra of compounds **7-9** displayed a singlet sulfonamide NH protons at δ 7.43−7.71 ppm region. The phenyl C2 protons at the thiosemicarbazone moiety are least shadowed by the electron withdrawing effect of the sulfonamide and thioamide groups. Therefore, it is observed in the δ 8.05−8.33 ppm region. Whereas, phenyl C6, C4 and C5 protons were observed in the δ 7.86−8.12, 7.70−7.97 and 7.60−7.87 ppm regions, in accordance with substituent effects, cleavage patterns, and coupling constancy. Due to the anisotropic effect of the azomethine group at the 3- position of the 2-indolinone ring, the least deshielded indole C4 and C6 protons among the indole protons were observed in the δ 7.58–8.71 and 7.22–8.36 ppm regions, respectively. However, indole C7 proton signals, shielded by the mesomeric effect of the anilide group at the 2-indolinone ring, were determined in the δ 6.87-7.46 ppm region. Substituents at the 1- and 5- positions of the indole ring also caused some shifts in the indole proton signals. However, the signal sequence of indole protons is unchanged. In compounds **7a**, **8a**, and **9a**, which are unsubstituted at the 5-position of the indole ring, signals of indole C5 protons were observed at δ 7.11, 7.18 and 7.17 ppm, respectively. In the spectra of compounds **7d**, **8d** and **9d**, which have fluorine substitution at the 5-position of the indole ring, indole signals showed as double doublets or triple doublets by coupling with fluorine.

In the ^{13}C NMR-APT spectra of compounds **7-9**, thiosemicarbazone C=S, indole C2 and C3 signals were observed in the δ 176.54−177.35, 161.32−163.77 and 130.05–133.12 ppm regions, respectively. The phenyl carbon signals were determined in the order C3, C1, C5, C6, C4 and C2, starting from downfield due to the effects of sulfonamide and thioamide groups. While indole C2 and C7a carbons displayed the most downfield shift, indole C7 and C3a carbons showed the the most shielded among the indole carbons. Shifts in the indole signals were observed due to the effects of the substituents at the 5th position of the 2-indolinone ring. Indole signals of fluorine-substituted compounds (**7d**, **8d** and **9d)** displayed as separate doublets due to ^{13}C–^{19}F interaction. These data confirmed the findings of HSQC-2D (**7a**, **7d**, **7g**, and **8b**) and HMBC-2D (**7g** and **9g**). LCMS-ESI spectra of the compounds were taken using the negative ionization technique for **7d**, **7e**, **8b**, **8d**, **9a**, **9c**, and **9e**, and the positive ionization technique for **7a**, **7d**, **8b**, **8d**, **9a**, and **9e**. $[M-H]^-$ and $[M+H]^+$ peaks displayed with various relative abundances confirmed the molecular weights of all compounds.

2.2. Effects of the compounds on aromatase activity

Aromatase (CYP19A1) is a member of the cytochrome P450 (CYP) enzyme family, catalyzing the conversion of androgens to estrogens. It is a significant source of local estrogen production, especially in postmenopausal women [28,29]. Exposure to estrogens is a significant risk factor for breast cancer. Therefore, inhibiting the aromatase enzyme is a crucial strategy in treating estrogen-responsive breast cancers [30].

In the present study, the aromatase inhibitory activity of the 1*H*-indole-2,3-dione 3-[4-(3-sulfamoyl)phenyl]thiosemicarbazone derivatives (**7-9**) was evaluated using an *in vitro* assay with a fluorescence substrate and human CYP19 aromatase. Among all tested compounds, all except R_2 sodium sulfonate - substituted compound **7h** showed substantial inhibition of aromatase, indicating that methyl or ethyl substitution at 1-position decreases CYP19 inhibitory activity (Figure 3). Compounds **8e**, **9a, 9d**, **9e**, and **9f** also showed statistically significant inhibition of aromatase activity with varying potencies. However, none of the tested compounds inhibited aromatase enzyme activity as potently as the reference inhibitor compound ketoconazole (89% inhibition) at their 10 μM concentration.

Journal of Research in Pharmacy
Research Article

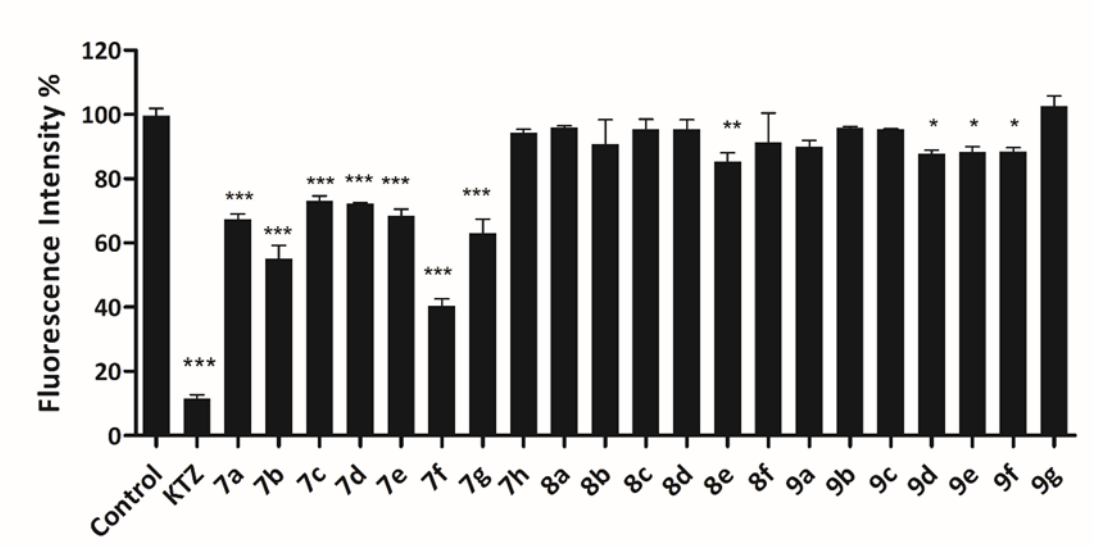

Figure 3. Effects of the 1*H*-indole-2,3-dione 3-[*N*-(3-sulfamoyl)phenyl]thiosemicarbazone] derivatives (10μM) on aromatase activity. Bars represent percentage values compared to control group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, FI: Fluorescence intensity, KTZ: Ketoconazole.

Compounds that dramatically inhibited aromatase (R_2 methyl- substituted compound **7b**; %42 inhibition and R_2 iodine- substituted compound **7f**; %58 inhibition) were further investigated at their 8 different concentrations in order to obtain IC_{50} values. We found that compounds **7b** and **7f** dose-dependently inhibited aromatase enzyme with 1.47. 10^{-5} M and 6.46. 10^{-6} M IC_{50} values, respectively (Figure 4). The most potent compound, **7f,** exhibited approximately 10-fold lower activity compared to the well-known aromatase inhibitor aminoglutethimide (IC_{50}= 0.6. 10^{-6} M) (**Figure 5**) [31].

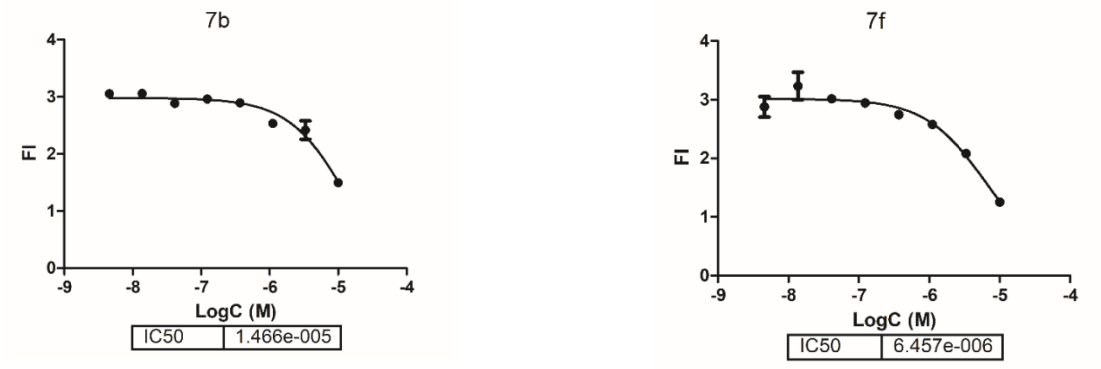

Figure 4. Aromatase inhibition curves and IC_{50} values obtained with potent compounds **7b** and **7f** (FI: Fluorescence intensity).

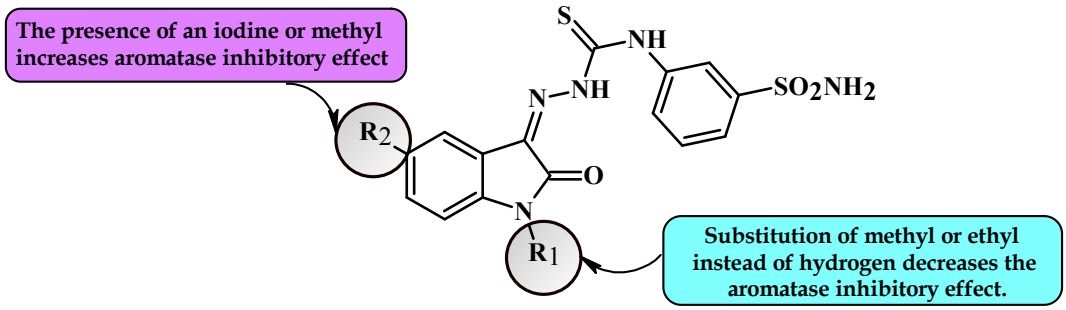

Figure 5. Structure-activity relationships on aromatase inhibitory effects of compounds **7-9**

Journal of Research in Pharmacy
Research Article

2.3. Effects of the compounds on catalytic CYP1 activity

Many known carcinogens are procarcinogens, needing bioactivation to induce carcinogenic effects. It's widely recognized that CYP1 enzymes play a pivotal role in these bioactivation processes. For example, DMBA and endogenous estrogens are converted into reactive metabolites through CYP1B1, thereby causing carcinogenic effects [2]. Consequently, the development of CYP1B1 inhibitors has been proposed as a significant strategy for chemoprevention [5].

For this reason CYP1 inhibitory activities of the compounds were tested. Among 21 tested compounds, only nine compounds showed statistically significant inhibition on microsomal CYP1 enzymes. However, none of them was effective as much as ketoconazole which is a known CYP1 inhibitor (Figure 6).

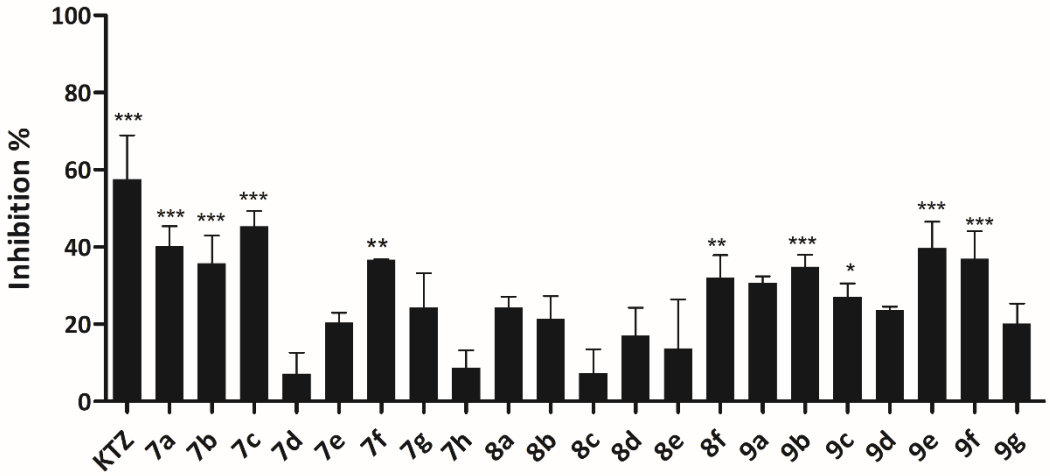

Figure 6. Effects of the 1*H*-indole-2,3-dione 3-[4-(3-sulfamoyl)phenyl] thiosemicarbazone] derivatives (10 μM) on microsomal CYP1 activity. Bars represent percentage inhibition values compared to control group from three different experiments * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (KTZ: Ketoconazole).

In addition, we found that fluorine substitution at the 5-position decreased the inhibitory activity comparing to chlorine- and iodine- substituted derivatives at the same position. With 45% inhibition, compound **7c** without substituents at the 1-position and trifluoromethoxy- substituted at the 5-position has the highest microsomal CYP1 inhibitory effect compared to other compounds. We further investigated inhibitory activity of compound **7c** on the human recombinant CYP1B1 enzyme at 8 different concentrations and the enzyme inhibition curve was obtained. We found that compound **7c** dose-dependently inhibits CYP1B1 enzyme with $9.7.10^{-7}$ M IC_{50} value (Figure 7). Compared to previously reported sulfonamide-based inhibitors of CYP1B1, compound **7c** demonstrates a significantly lower IC_{50} value (9.7×10^{-7} M vs. 2.5×10^{-6} M reported by Chun et al. [5].The introduction of a trifluoromethoxy group at the 5-position of compound 7c resulted in the highest inhibitory effect on CYP1B1 ($IC_{50} = 9.7 \times 10^{-7}$ M). This enhancement can be attributed to the electron-withdrawing nature of the trifluoromethoxy group, which stabilizes the interaction between the compound and the enzyme's active site. By contrast, the 5-position methyl group in compound 7b did not provide the same level of activity, suggesting that larger, more polar substituents at this position play a critical role in optimizing the enzyme-binding affinity.

Journal of Research in Pharmacy
Research Article

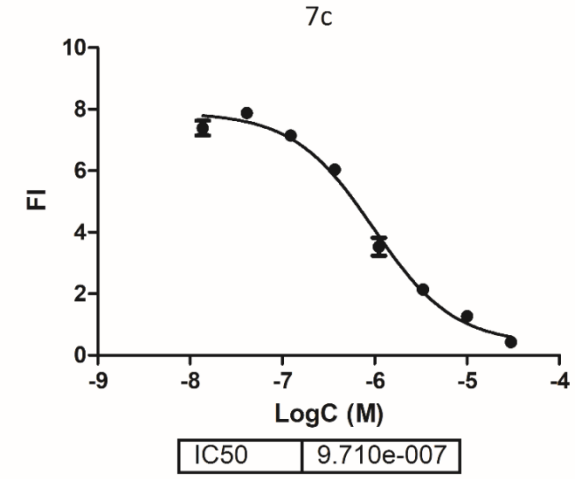

Figure 7. Inhibitory effect of the compound **7c** on human recombinant CYP1B1 activity (FI: Fluorescence intensity)

Furthermore, as microsomes contain many CYP isozymes like CYP1A1 and CYP1A2 beside CYP1B1, possible inhibitory activity of the compound **7c** against other isozymes should also be investigated to enlighten its selectivity and specificity (Figure 8).

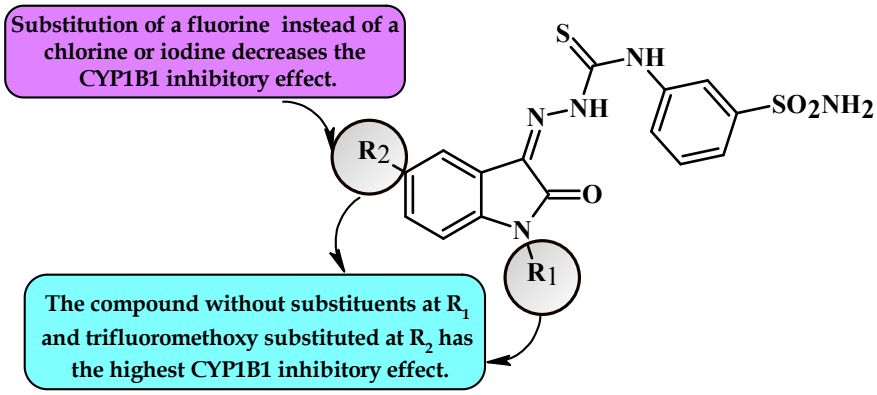

Figure 8. Structure-activity relationships on CYP1B1 inhibitory effects of compounds **7-9**

2.4. Cytotoxicity of the compounds

The cytotoxicity of the novel derivatives was assessed in MCF-7 cells using MTT assay. Almost all compounds (10 µM) were found to decrease cell viability with varying potencies. Compounds which have methyl substitution at 5-position (**7b**, **8b** and **9b**) showed statistically significant decrease in cell proliferation and showed potent cytotoxic activity. In addition, compounds without substituents at 5-position of indole ring, namely compounds **7a**, **8a** and **9a**, caused dramatic and significant reduction in cell proliferation. Compounds **7b**, **8b** and **9a** reduced MCF-7 cell viability below 60% after 24 h incubation. Furthermore, among the tested compounds, **7c** stood out with both strong CYP1B1 inhibition and significant cytotoxicity against MCF-7 cells, reducing cell viability to below 70% at 10 µM. This dual action positions **7c** as a promising candidate for further development, particularly as a treatment for hormone-dependent breast cancers (Figures 9 and 10).

https://doi.org/10.12991/jrespharm.1694239
J Res Pharm 2025; 29(3): 1098-1110

Journal of Research in Pharmacy
Research Article

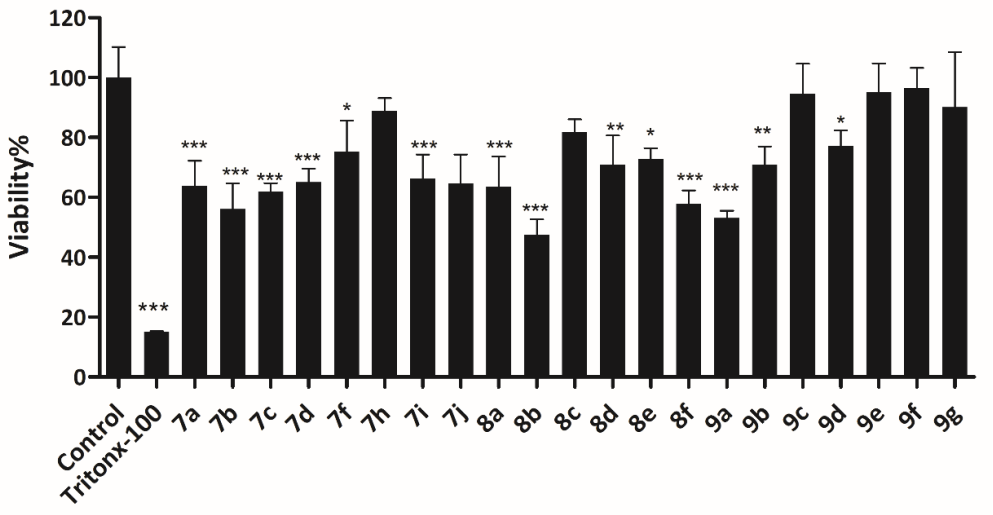

Figure 9. Effects of the 1*H*-indole-2,3-dione 3-[4-(3-sulfamoyl)phenyl]thiosemicarbazone] derivatives (10 µM) on MCF-7 cell viability evaluated via MTT assay. Bars show percentage viability values compared to control group from four different experiments. Statistical analysis of samples was performed by comparing with control group, TritonX-100 used as positive cytotoxic control group.*p < 0.05, ** p < 0.01, *** p < 0.001.

The observed cytotoxicity of compound 7b in MCF-7 cells (62% cell viability at 10 µM) may be linked to its dual inhibition of CYP19A1 and CYP1. By reducing estrogen production via CYP19A1 inhibition, compound 7b decreases the availability of estrogen needed for tumor growth. Simultaneously, the inhibition of CYP1 prevents the bioactivation of carcinogenic estrogens, thereby reducing DNA damage and subsequent tumor progression. This dual action may explain the significant reduction in cell viability observed in our assays.

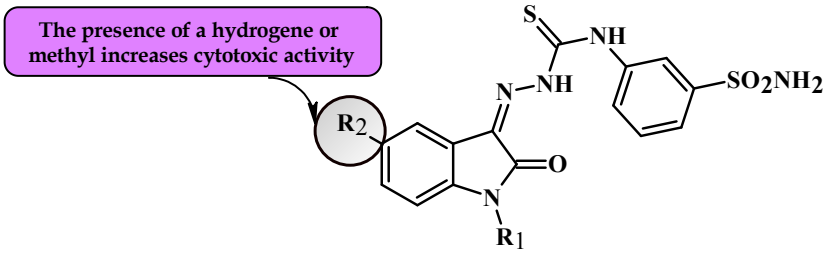

Figure 10. Structure-activity relationships on cytotoxic activities of compounds **7-9** against MCF-7 cells.

2.5. Molecular modelling

In molecular modeling studies, we have investigated the possible binding relationships of compounds **7c**, **7a** and **9e** which have the highest activity in hCYP1B1 enzyme inhibition assays. The indole ring of compound **7c** located close to the HEM group and the sulfonamide moiety formed hydrogen bonds with the side chains of Asn228 and Gln332 (**Figure 11**). In addition the sulfonamide moiety had hydrophobic interactions with Phe 231, Phe 268 and the HEM group. As with compound **7c**, the indole rings of compound **7a** and **9e** were close to the HEM group (**Figure 11**). The sulfur atom of thiosemicarbazides interacted the side chain of Asp333 as well as the phenil moiety formed hydrophobic interactions with Phe231 and Phe268. In addition the sulfonamide groups of the ligands made hydrogen bonds with either Ser127 (**7a**) or leu224 and Asn228 (**9e**).Compounds **7b** and **7f** showed the highest activity in hCYP19A1 enzyme inhibition assays. The sulfonamide moiety of compound **7f** made hydrogen bonds with side chains of Arg192 and Asp309 (**Figure 12**). This compound adopted a linear conformation and the indole ring was close to the HEM group. Other molecules similarly bind to the active site.

https://doi.org/10.12991/jrespharm.1694239
J Res Pharm 2025; 29(3): 1098-1110

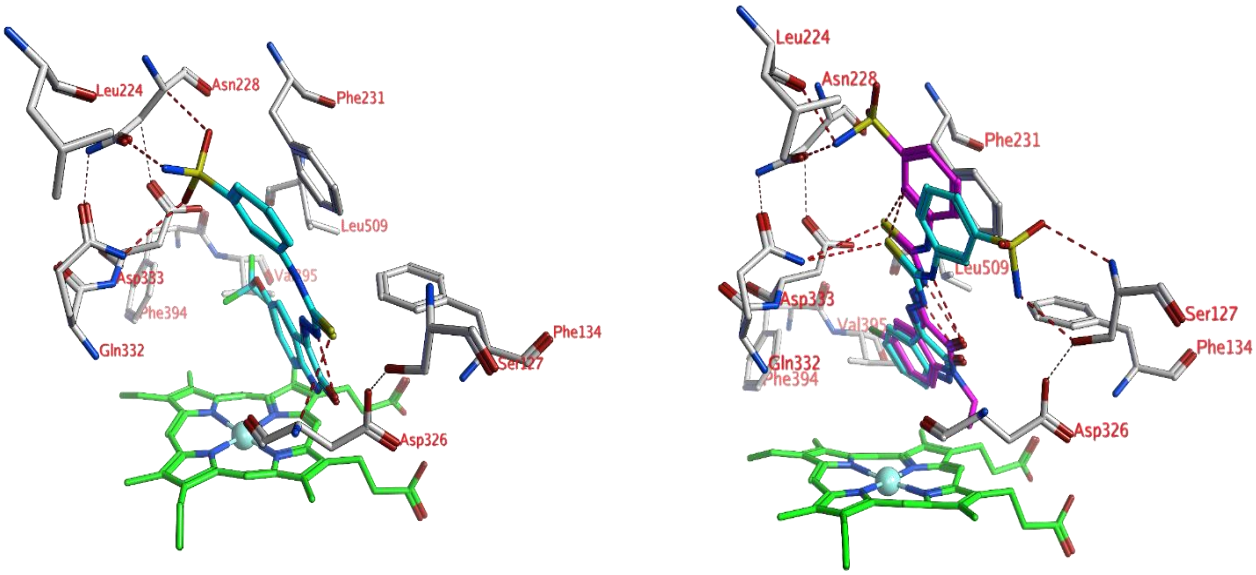

Figure 11. The docked poses of compounds **7c** (left) **and 7a** and **9e** (right) forming interaction in the active site of hCYP1B1. The heme group is represented in green, iron is displayed in turquoise, amino acids are shown in grey, ligands are displayed in turquoise or purple. Hydrogen bonds are indicated with red dashed lines.

The docking results indicated in **Figures 11** and **12** show the possible localization and binding relationships of the hCYP1B1 and hCYP19A1 active sites of the compounds which were tested. It has predicted that many of these compounds can bind to the active site in this way.

Our docking studies identified critical interactions in the CYP1B1 and CYP19A1 active sites, including hydrogen bonds with Asn228, Gln332 (CYP1B1), and Arg192, Asp309 (CYP19A1). These residues play key roles in inhibitor binding, as highlighted in previous studies. For example, Fabris et al. noted similar hydrogen bond and π-π interactions with CYP1B1 residues, particularly Phe231, essential for binding affinity [32]. Yi et al. demonstrated that hydrophobic interactions with Phe268 and Val395 further enhance CYP1B1 specificity[33]. Our findings align with these results and suggest avenues for optimizing selectivity and potency in CYP-targeted inhibitors.

3. CONCLUSION

In summary, a series of 1*H*-indole-2,3-dion 3-thiosemicarbazones bearing 3-sulfamoylphenyl moiety were evaluated towards CYP1 and aromatase enzymes. Compounds that lack methyl or ethyl substitutions at the 1-position of the indole ring, except **7h**, showed potent inhibition on aromatase enzyme. Among these compounds, compound **7b** bearing a methyl group at the 5-position of indole ring and **7f** bearing an iodine group at the position 5- of indole ring exhibited more effective inhibition than the other molecules. Compound **7c** bearing a trifluoromethoxy group at the 5-position of indole ring had the highest microsomal CYP1 inhibitory effect compared to other compounds. We also screened the inhibitory activity of **7c** on the human recombinant CYP1B1 enzyme and determined that **7c** is an effective CYP1B1 inhibitor. The cytotoxic activity of these derivatives was tested on the MCF-7 human breast cancer cell line, and compound **7c** significantly inhibited cell proliferation. By the support of molecular modelling studies, we explained that the synthesized compounds possessed similar binding modes in aromatase and CYP1B1 enzyme active sites.

Journal of Research in Pharmacy
Research Article

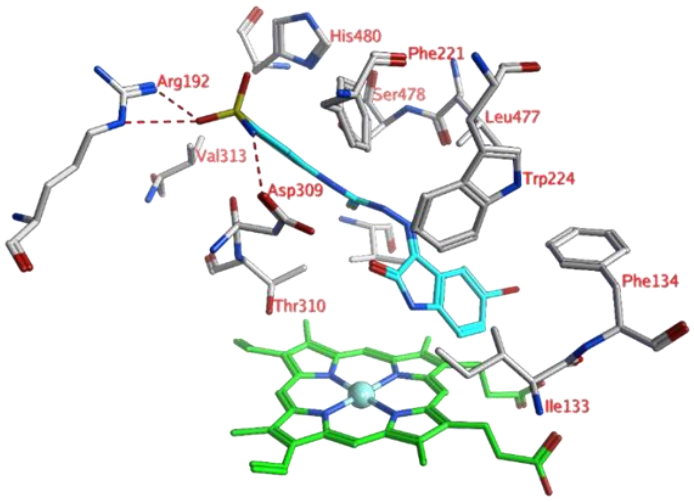

Figure 12. The docked poses of compound **7f** forming interaction with the active site of hCYP19A1. The heme group is represented in green, iron is displayed in turquoise, amino acids are shown in grey, ligands are displayed in turquoise. Hydrogen bonds are indicated with red dashed lines.

4. MATERIALS AND METHODS

4.1. Chemistry

The synthesis and chemical identification of the compounds **7-9** were described by Eraslan Elma et.al [27]. The spectral data of the novel compound **7h** was identified below.

Sodium 2-oxo-3-{2-[(3-sulfamoylphenyl)carbamothioyl]hydrazinylidene}-2,3-dihydro-1H-indole-5-sulfonate (7h).

Yellow powder (95%): mp >300 °C; IR (KBr): 3298, 3188, 3165 (NH), 1707 (C=O), 1618, 1589, 1543, 1479 (C=N, C=C), 1336, 1149 (S=O), 1229 (C=S). ^{1}H NMR (DMSO-d_6/300 MHz) ppm: 6.87 (1H, d, *J:* 7.80 Hz, ind. C7-H), 7.43 (2H, s, SO_2NH_2), 7.59 (1H, d, *J:* 7.8 Hz, phen. C5-H), 7.70 (1H, d, *J:* 8.3 Hz, phen. C4-H), 7.88 (1H, d, *J:* 8.3 Hz, phen. C6-H), 8.10 (1H, t, *J:* 1.9 Hz, phen. C2-H), 7.63 (1H, dd, *J:* 8.3, 1.9 Hz, ind. C6-H), 8.15 (1H, d, *J:* 1.9 Hz, ind. C4-H), 11.24 (1H, s, N4-H), 11.30 (1H, s, ind. N-H), 12.82 (1H, s, N2-H). ^{13}C APT (DMSO-d_6/125 MHz): 110.39 (ind. C7), 120.01 (ind. C4), 119.58 (ind. C3a), 123.30 (phen. C2); 123.47 (phen. C4), 129.39 (ind. C6), (phen. C6), 129.41 (phen. C5, C6), 133.12 (ind. C3), 139.51 (phen. C1), 142.87 (ind. C5), 143.92 (phen. C3), 144.75 (ind. C7a), 163.48 (ind. C2), 177.09 (CS). Anal. Calcd for $C_{15}H_{12}N_5NaO_6S_3.H_2O$: C, 36.36; H, 2.84; N, 14.13 Found: C, 36.68; H, 2.98; N, 13.74.

4.2. Cell culture and reagents

MCF-7 cells were generously provided by Prof. Ana Soto from Tufts Institute and cultured at 37°C in a 5% CO2 atmosphere in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS). All chemical reagents used for the biological activity assays were obtained from Sigma-Aldrich (St. Louis, MO, USA) and Invitrogen (Oregon, USA). The final concentration of DMSO in the cell culture medium was kept below 0.5% v/v.

4.3. Aromatase activity assay

The aromatase inhibitor activity of the synthesized compounds was evaluated following the procedure described by Ozcan-Sezer et al. [32] using a commercially available CYP19A/MFC screening kit from Corning. In this assay, the fluorescent substrate 7-methoxy-4-trifluoromethyl coumarin (MFC) is metabolized by aromatase to HFC in the presence of an NADPH-generating system. A decrease in fluorescence intensity reflects the inhibitory effect on aromatase. Enzyme reactions were conducted in black 96-well plates, with the compounds dissolved in acetonitrile. Fluorescence was measured at an excitation wavelength of 409 nm and an emission wavelength of 530 nm. The percent inhibition was calculated by comparing the fluorescence of treated wells to that of control wells without the inhibitor. The IC_{50} values for each compound were determined using GraphPad Prism 5 software.

4.4. 7-Ethoxyresorufin O-dealkylation assay

The 7-ethoxyresorufin O-dealkylation (EROD) assay was conducted using a spectrofluorometric method [33] with minor modifications. In this assay, 7-ethoxyresorufin, a CYP1 selective substrate, is converted to resorufin by CYP1 enzymes, and the fluorescence intensity of resorufin is measured. Fenobarbital-induced hepatic microsomes and/or human recombinant CYP1s were used as CYP donors. Briefly, each 250 µL incubation contained 90 mM KH2PO4/K2HPO4 (pH 7.4), 3.70 mM 7-ethoxyresorufin, human recombinant CYP enzyme (1.25 pmol CYP1B1) or hepatic rat microsomes, 0.22 mM NADPH, and the test compounds. The reaction was initiated by the addition of NADPH, and incubation was performed at 30°C for microsomes or 37°C for recombinant CYP enzymes. After 10 minutes of incubation, the reaction was stopped by the addition of 500 µL ice-cold acetone. Samples were centrifuged, and fluorescence intensity was determined at excitation and emission wavelengths of 537 nm and 583 nm, respectively. The percentage inhibition values of the compounds were calculated compared to the control group.

4.5. MTT assay

The cytotoxic activity of the synthesized compounds was assessed using the MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)[34]. This assay relies on the reduction of MTT by mitochondrial succinate dehydrogenases in viable cells, forming purple formazan crystals, which are then quantified by measuring optical density. MCF-7 cells were plated in 96-well plates at a density of 6,000 cells per well and incubated for 24 hours at 37°C with 5% CO2. Cells were treated with the synthesized compounds at a concentration of 10 µM for 24 hours. The experiment included a control group (cells with medium only), a vehicle control, and a positive control (15 µM Triton X-100). After treatment, the medium was removed, and the cells were washed with phosphate-buffered saline (PBS) before incubation with MTT (1 mg/ml) for 4 hours at 37°C. The MTT solution was discarded, and the resulting formazan crystals were solubilized in DMSO. Absorbance was measured at 550 nm using a microplate reader. The percentage of cell viability was calculated as the ratio of the absorbance of treated cells to that of the control, which was set to 100%.

4.6. Molecular modelling studies

4.6.1. Preparation of human CYP19A1 and CYP1B1 crystal structures for docking studies

The crystal structures of hCYP19A1 in complex with the aromatase inhibitor 4-androstene-3-17-dione (pdb entry: 3s79; 2.75 Å) and hCYP1B1 in complex with α-naphthoflavone (pdb entry: 3pm0; 2.70 Å) were obtained from the Protein Data Bank. All water molecules were deleted from their crystal structures Hydrogens atoms were added using the protonate 3D tool of the MOE software package (v. 2016.0802, Chemical Computing Group Inc., Montreal) and subsequently the obtained structure was energy-minimized (AMBER12:EHT force field) [35]. The RMSD value of the protein Cα-atoms after the energy minimization was low (RMSD<0.1 Å) indicating that the protein structure did not change significantly. The protein was saved as a mol2-file.

4.6.2. Preparation of ligands for docking studies

The three-dimensional structures of synthesized compounds were built using MOE software package (v2016.08, Chemical Computing Group, Inc, Montreal, Canada). The protanation of the ligands were generated according to pH 7 and energy minimization protocol was applied using the MMFF94x forcefield. The ligands were saved as mol2-files.

4.6.3. Docking studies

All ligands were docked into the hCYP19A1 (pdb entry: 3s79) and hCYP1B1 (pdb entry: 3pm0; 2.70 Å) structures using the P450 enzyme settings(chemscore.p450_csd.params) and the ChemScore scoring function in the GOLD suite software package (v5.6, CCDC, Cambridge, UK). The binding pocket was defined as all amino acids within 5.5Å of the cocrystallized ligand. For each ligand 25 dockings were performed with default settings and the best scoring five poses were identified.

4.7. Statistical analyses

Statistical analysis was carried out using GraphPad Prism 5.0 software. Results are presented as the mean ± SD. Differences among group means were evaluated using one-way ANOVA followed by Tukey's post-hoc test. Statistical significance was defined as $p < 0.05$, with a 95% confidence interval. Levels of significance are indicated as follows: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

Acknowledgements: This work was supported by The Scientific Research Projects Coordination Unit of Istanbul University (TSA-2017-25638) and the Scientific and Technological Research Council of Turkey (TÜBİTAK) Grant 117S065. Ege University FABAL facilities are used for biological activity assays. Authors are grateful to Prof. Hilmi Orhan for providing microsomes.

Author contributions: Concept – E.İ.E., H.G.O.; Design – E.İ.E., N.K., H.G.O.; Supervision – N.K., H.G.O.; Resources – H.G.O.,N.K.; Materials – H.G.O.,N.K.; Data Collection and/or Processing – E.İ.E, P.E.E, A.A; Analysis and/or Interpretation – E.İ.E, P.E.E, A.A; Literature Search – E.İ.E, P.E.E; Writing – E.İ.E, P.E.E; Critical Reviews – A.A., N.K., H.G.O.

Conflict of interest statement: The authors declare no conflicts of interest.

REFERENCES

[1] Anand P, Kunnumakkara AB, Sundaram C, Harikumar KB, Tharakan ST, Lai OS, Sung B, Aggarwal BB. Cancer is a preventable disease that requires major lifestyle changes. Pharm Res. 2008; 25(9): 2097-2116. https://doi.org/10.1007/S11095-008-9661-9.

[2] Cavalieri E, Rogan E. The molecular etiology and prevention of estrogen-initiated cancers: Ockham's Razor: Pluralitas non est ponenda sine necessitate. Plurality should not be posited without necessity. Mol Aspects Med. 2014; 36: 1–55. https://doi.org/10.1016/j.mam.2013.08.002

[3] McFadyen, MC, McLeod HL, Jackson FC, Melvin WT, Doehmer J, Murray GI. Cytochrome P450 CYP1B1 protein expression: A novel mechanism of anticancer drug resistance. Biochem Pharmacol. 2001; 62(2): 207–212. https://doi.org/10.1016/s0006-2952(01)00643-8

[4] Kim T, Park H, Yue, W, Wang, JP, Atkins KA, Zhang Z, Rogan EG, Cavalieri EL, Mohammad KS, Kim S, Santen RJ, Aiyar SE. Tetra-methoxystilbene modulates ductal growth of the developing murine mammary gland. Breast Cancer Res Treat. 2011; 126(3): 779–789. https://doi.org/10.1007/s10549-010-1301-5

[5] Chun YJ, Kim S. Discovery of cytochrome P450 1B1 inhibitors as new promising anti-cancer agents. Med Res Rev. 2003; 23(6): 657-668. https://doi.org/10.1002/med.10050.

[6] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018; 68: 394–424. https://doi.org/10.3322/CAAC.21492.

[7] Perou CM, Borresen-Dale AL. Systems Biology and Genomics of Breast Cancer. Cold Spring Harb Perspect Biol. 2011; 3: a003293–a003293. https://doi.org/10.1101/cshperspect.a003293.

[8] Leung YK, Lau KM, Mobley J, Jiang Z, Ho SM. Overexpression of cytochrome P450 1A1 and its novel spliced variant in ovarian cancer cells: Alternative subcellular enzyme compartmentation may contribute to carcinogenesis. Cancer Res. 2005; 65(9): 3726-3734. https://doi.org/10.1158/0008-5472.CAN-04-3771.

[9] Stocco C. Aromatase expression in the ovary: hormonal and molecular regulation. Steroids. 2008; 73(5): 473-487. https://doi.org/10.1016/J.steroids.2008.01.017.

[10] Lazaros L, Xita N, Kaponis A, et al. The association of aromatase (CYP19) gene variants with sperm concentration and motility. Asian J Androl. 2011; 13(2): 292-297. https://doi.org/10.1038/AJA.2010.144.

[11] Kharb R, Haider K, Neha, Yar MS. Aromatase inhibitors: Role in postmenopausal breast cancer. Arch Pharm. (Weinheim). 2020; 353(8): e2000081. https://doi.org/10.1002/ARDP.202000081.

[12] Miller WR, Larionov AA. Understanding the mechanisms of aromatase inhibitor resistance. Breast Cancer Res. 2012; 14: 201. https://doi.org/10.1186/BCR2931.

[13] Leoni A, Locatelli A, Morigi R, Rambaldi M. 2-Indolinone a versatile scaffold for treatment of cancer: a patent review (2008-2014). Expert Opin Ther Pat. 2016; 26: 149–173. https://doi.org/10.1517/13543776.2016.1118059.

[14] Motzer RJ, Hutson TE, Tomczak P, Michaelson MD, Bukowski RM, Rixe O, Oudard S, Negrier S, Szczylik C, Kim ST, Chen I, Bycott PW, Baum CM, Figlin RA. Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. N Engl J Med. 2007; 356: 115–124. https://doi.org/10.1056/NEJMOA065044.

[15] Sternberg CN, Davis ID, Mardiak J, Szczylik C, Lee E, Wagstaff J, Barrios CH, Salman P, Gladkov OA, Kavina A, Zarbá JJ, Chen M, McCann L, Pandite L, Roychowdhury DF, Hawkins RE. Pazopanib in locally advanced or metastatic renal cell carcinoma: Results of a randomized phase III trial. J Clin Oncol. 2023; 41: 1957–1964. https://doi.org/10.1200/JCO.22.02622.

[16] Varun, Sonam, Kakkar R. Isatin and its derivatives: A survey of recent syntheses, reactions, and applications. Medchemcomm. 2019; 10: 351–368. https://doi.org/10.1039/C8MD00585K.

[17] Eldeeb M, Sanad EF, Ragab A, Ammar YA, Mahmoud K, Ali MM, Hamdy NM. Anticancer effects with molecular docking confirmation of newly synthesized isatin sulfonamide molecular hybrid derivatives against hepatic cancer cell lines. Biomedicines. 2022; 10(3):722. https://doi.org/10.3390/BIOMEDICINES10030722.

[18] Chaudhari P, Bari S, Surana S, Shirkhedkar A, Wakode S, Shelar S, Racharla S, Ugale V, Ghodke M. Logical synthetic strategies and structure-activity relationship of indolin-2-one hybrids as small molecule anticancer agents: An overview. J Mol Struct. 2022; 1247 : 131280. https://doi.org/10.1016/J.MOLSTRUC.2021.131280.

[19] Güzel-Akdemir Ö, Akdemir A, Karalı N, Supuran CT. Discovery of novel isatin-based sulfonamides with potent and selective inhibition of the tumor-associated carbonic anhydrase isoforms IX and XII. Org Biomol Chem. 2015; 13(23): 6493-6499. https://doi.org/10.1039/C5OB00688K.

[20] Eldehna WM, Fares M, Ceruso M, Ghabbour HA, Abou-Seri SM, Abdel-Aziz HA, Abou El Ella DA, Supuran CT. Amido/ureidosubstituted benzenesulfonamides-isatin conjugates as low nanomolar/subnanomolar inhibitors of the tumor-associated carbonic anhydrase isoform XII. Eur J Med Chem. 2016; 110: 259-266. https://doi.org/10.1016/J.EJMECH.2016.01.030.

[21] Karalı N, Akdemir A, Göktaş F, Eraslan Elma P, Angeli A, Kızılırmak M, Supuran CT. Novel sulfonamide-containing 2-indolinones that selectively inhibit tumor-associated alpha carbonic anhydrases. Bioorg Med Chem. 2017; 25(14): 3714-3718. https://doi.org/10.1016/j.bmc.2017.05.029

[22] Eldehna WM, Al-Ansary GH, Bua S, Nocentini A, Gratteri P, Altoukhy A, Ghabbour H, Ahmed HY, Supuran C T. Novel indolin-2-one-based sulfonamides as carbonic anhydrase inhibitors: Synthesis, in vitro biological evaluation against carbonic anhydrases isoforms I, II, IV and VII and molecular docking studies. Eur J Med Chem. 2017; 127: 521-530. https://doi.org/10.1016/j.ejmech.2017.01.017.

[23] Eldehna WM, Nocentini A, Al-Rashood ST, Hassan GS, Alkahtani HM, Almehizia AA, Reda AM, Abdel-Aziz HA, Supuran CT. Tumor-associated carbonic anhydrase isoform IX and XII inhibitory properties of certain isatin-bearing sulfonamides endowed with in vitro antitumor activity towards colon cancer. Bioorg Chem. 2018; 81: 425-432. https://doi.org/10.1016/j.bioorg.2018.09.007

[24] Abo-Ashour MF, Eldehna WM, Nocentini A, Ibrahim HS, Bua S, Abou-Seri SM, Supuran CT. Novel hydrazido benzenesulfonamides-isatin conjugates: Synthesis, carbonic anhydrase inhibitory activity and molecular modeling studies. Eur J Med Chem. 2018; 157: 28-36. https://doi.org/10.1016/j.ejmech.2018.07.054

[25] Eldehna WM, Abo-Ashour MF, Nocentini A, El-Haggar RS, Bua S, Bonardi A, Al-Rashood ST, Hassan GS, Gratteri P, Abdel-Aziz HA, Supuran CT.Enhancement of the tail hydrophobic interactions within the carbonic anhydrase IX active site via structural extension: Design and synthesis of novel N-substituted isatins-SLC-0111 hybrids as carbonic anhydrase inhibitors and antitumor agents. Eur J Med Chem. 2019; 162: 147-160. https://doi.org/10.1016/j.ejmech.2018.10.068

[26] George RF, Bua S, Supuran CT, Awadallah FM. Synthesis of some N-aroyl-2-oxindole benzenesulfonamide conjugates with carbonic anhydrase inhibitory activity. Bioorg Chem. 2020; 96: 103635. https://doi.org/10.1016/j.bioorg.2020.103635

[27] Eraslan-Elma P, Akdemir A, Berrino E, Bozdağ M, Supuran CT, Karalı N. New 1H-indole-2,3-dione 3-thiosemicarbazones with 3-sulfamoylphenyl moiety as selective carbonic anhydrase inhibitors. Arch Pharm (Weinheim). 2022; 355(8): e2200023. https://doi.org/10.1002/ardp.202200023

[28] Jordan C, Furr BJA, An Introduction to the Regulation of Sex Steroids for the Treatment of Cancer, in: B.J.A. Jordan, V.C and Furr (Ed.), Horm. Breast Prostate Cancer, Humana Press, 2006: pp. 1–16.

[29] Santen RJ, Brodie H, Simpson ER, Siiteri PK, Brodie A. History of aromatase: saga of an important biological mediator and therapeutic target. Endocr Rev. 2009;30(4):343-375. https://doi.org/10.1210/er.2008-0016.

[30] Bhatia N, Thareja S. Aromatase inhibitors for the treatment of breast cancer: An overview (2019-2023). Bioorg Chem, 2024; 151: 107607. https://doi.org/10.1016/j.bioorg.2024.107607

[31] Wouters W, De Coster R, Goeminne N, Beerens D, van Dun J. Aromatase inhibition by the antifungal ketoconazole. J Steroid Biochem. 1988; 30(1-6): 387-389. https://doi.org/10.1016/0022-4731(88)90128-8

[32] Fabris M, Silva ML, de Santiago-Silva KM, de Lima Ferreira Bispo M, Goes Camargo P. CYP1B1: A promising target in cancer drug discovery. Anticancer Agents Med Chem. 2023; 23: 981–988. https://doi.org/10.2174/1871520623666230119103914.

[33] Yi L, Huang X, Yang M, Cai J, Jia J, Peng Z, Zhao Z, Yang F, Qiu D. A new class of CYP1B1 inhibitors derived from bentranil, Bioorganic Med Chem Lett. 2023; 80: 129112. https://doi.org/10.1016/j.bmcl.2022.129112.

[34] Ozcan-Sezer S, Ince E, Akdemir A, Ceylan ÖÖ, Suzen S, Gurer-Orhan H. Aromatase inhibition by 2-methyl indole hydrazone derivatives evaluated via molecular docking and in vitro activity studies. Xenobiotica. 2019; 49(5): 549-556. https://doi.org/10.1080/00498254.2018.1482029

[35] Burke MD, Mayer RT. Ethoxyresorufin: direct fluorimetric assay of a microsomal O-dealkylation which is preferentially inducible by 3-methylcholanthrene. Drug Metab Dispos. 1974; 2(6): 583-588.

[36] Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983; 65(1-2): 55-63. https://doi.org/10.1016/0022-1759(83)90303-4

[37] Labute P. Protonate3D: assignment of ionization states and hydrogen coordinates to macromolecular structures. Proteins. 2009; 75(1): 187-205. https://doi.org/10.1002/prot.22234