Journal of Molecular Structure 1246 (2021) 131198

Contents lists available at ScienceDirect

Journal of Molecular Structure

journal homepage: www.elsevier.com/locate/molstr

# Design, synthesis, in vitro and in silico studies of some novel triazoles as anticancer agents for breast cancer

Derya Osmaniye[a,b,*], Begüm Nurpelin Sağlık[a,b], Serkan Levent[a,b], Sinem Ilgın[c], Yusuf Özkay[a,b], Zafer Asım Kaplancıklı[a]

[a] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Anadolu University, 26470 Eskişehir, Turkey
[b] Doping and Narcotic Compounds Analysis Laboratory, Faculty of Pharmacy, Anadolu University, 26470 Eskişehir, Turkey
[c] Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Anadolu University, 26470 Eskişehir, Turkey

ARTICLE INFO

*Article history:*
Received 21 May 2021
Revised 13 July 2021
Accepted 26 July 2021
Available online 28 July 2021

*Keywords:*
Triazole
Anticancer
Aromatase
2D-NMR

ABSTRACT

Against the increasing incidence of breast cancer in postmenopausal women in recent years, a few clinically approved inhibitors and their side-effect profiles indicate the need for the development of new aromatase inhibitors. In this study, carried out to develop a new aromatase inhibitor, the triazole ring system was preferred because of its known activity in the field. The triazole ring, which is in the structure of the most commonly used aromatase inhibitors such as anastrazole and letrazole, was synthesized from the thiourea residue. Inhibitor structures were elucidated using the $^{1}$H-NMR, $^{13}$C-NMR, 2D-NMR, and HRMS spectroscopic methods. A cytotoxicity (MTT) test was performed to determine the anticancer activity of the compounds on breast (MCF7) carcinoma cell type. In addition, to determine selectivity of their action, the final compounds were screened against a healthy NIH3T3 cell line (mouse embryonic fibroblast cells). In terms of the MTT assay, it was observed that the calculated $IC_{50}$ values of compound **5e** for the NIH3T3 cell line were found to be higher than for the MCF7 cell lines. Considering the viability results, it was found that the selected compound **5e** showed a favorable safety profile and that it has anticancer activities. It was determined by *in vitro* studies that compound **5e** showed inhibition potential on the aromatase enzyme with an $IC_{50} = 0.028$ $\mu$M value. The docking study of compound **5e** revealed that there is a strong interaction between the active sites of the human aromatase enzyme and the analyzed compound.



## 1. Introduction

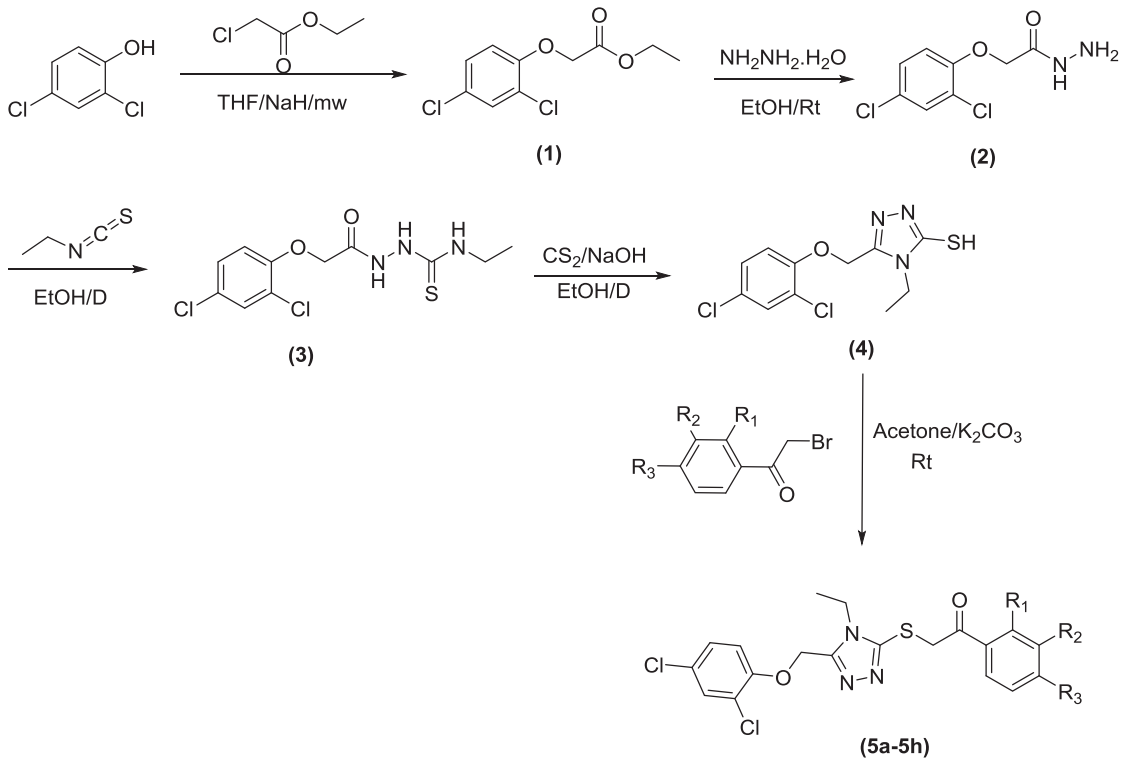
It is known that an increasing number of patients all over the world are affected by breast cancer. GLOBOCAN 2012, published by the International Agency for Research on Cancer (AIRC), stated that breast cancer is the most common type of cancer affecting women worldwide, both in terms of new cases and deaths [1]. The World Health Organization (WHO) reported that breast cancer caused more than half a million deaths in 2015 [2]. In addition, it has been reported that approximately 70-80% of the causes of breast tumors in postmenopausal women are estrogen or progesterone dependent [3]. Pharmaceutical treatment of breast cancer, including chemotherapy, Her2 antibody therapy, and endocrine therapy, are currently used clinically. Endocrine therapy, based on the ability to prevent the effect of estrogen, can be applied in 2 ways: Tamoxifen, which inhibits estrogen receptors or aromatase inhibitors (AI), which inhibit estrogen biosynthesis [1].

The aromatase enzyme, which contains heme (hemoglobine), is a microsomal enzyme complex responsible for the conversion of androgens to estrogens in peripheral tissues by aromatization of the steroid A ring [3–6]. Aromatase inhibitors, categorized as steroidal and non-steroidal blockers, have a significant role in the treatment of breast cancer in women and gynecomastia in men [7,8].

Steroidal-type agents (Type I), sometimes called “suicide inhibitors”, are androstenedione analogs that act by binding to the active site of aromatase through a competitive or irreversible process, based on a mechanism or through covalent interactions. Type II inhibitors or nonsteroidal inhibitors contain an azole structure and bind reversibly to the cytochrome P450 portion of the aromatase molecule, thus causing a reversible inhibition. The first generation of nonsteroidal aromatase inhibitors is aminoglutethimide. Problems associated with aminoglutethimide side effects and selectivity have led to the development of a second gen-

* Corresponding author at: Anadolu University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 26470 Eskişehir, Turkey.
*E-mail address:* dosmaniye@anadolu.edu.tr (D. Osmaniye).

https://doi.org/10.1016/j.molstruc.2021.131198
0022-2860/




| Compounds | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| **5a** | -H | -H | -H |
| **5b** | -H | -H | -Cl |
| **5c** | -H | -H | -F |
| **5d** | -H | -H | -Br |
| **5e** | -H | -OH | -OH |
| **5f** | -Cl | -H | -Cl |
| **5g** | -F | -H | -F |
| **5h** | -H | -Cl | -Cl |

**Scheme 1.** Synthesis pathway for obtained compounds.

eration nonsteroidal aromatase inhibitor (fadrazol-bearing imidazole structure). However, this compound still exhibits some non-selective inhibitory activity regarding progesterone, corticosterone, and aldosterone biosynthesis [9,10]. Among the third generation type II aromatase inhibitors, anastrozole, letrozole, and vorozol are the strongest, most selective, and least toxic AIs. It is known that they can reduce serum estradiol by more than 95% [11,12].

While non-steroidal aromatase inhibitors (NSAIs) do not exhibit any fatal androgenic side effects, long-term use of SAIs can result in decreased estrogen levels, which can result in infertility and osteoporosis, as well as cancer. [13]. The structure of nonsteroidal aromatase inhibitors, which consists of 2 main parts, can be explained as follows: The part in which the cytochrome P450 of the aromatase must interact with the heme-iron atom is the azole part with a nitrogen atom; the other part of the substrate that mimics the steroid ring is the bulky aryl part [11]. Moreover, imidazole or triazole-containing compounds have been reported as novel aromatase inhibitors [11,14–28].

In addition to all these, in many studies conducted in recent years, new compounds containing triazole have been synthesized and their anticancer profiles have been examined. As a result of activity studies, activity profiles ranging from 9.02 nM to 1.9 μM were obtained. It was noted that letrozole was used as the precursor compound in the design of its compounds and that there were 2 aromatic structures attached to the triazole ring [15,20,26,28].

Five-year long-term therapy that may intensify side effects (bone loss, joint pain, and cardiac events) and acquired resistance are among the reasons limiting the clinical use of third AIs [29]. Thus, the development of new non-steroidal AIs is a popular area for researchers for purposes such as limiting side effects and in-

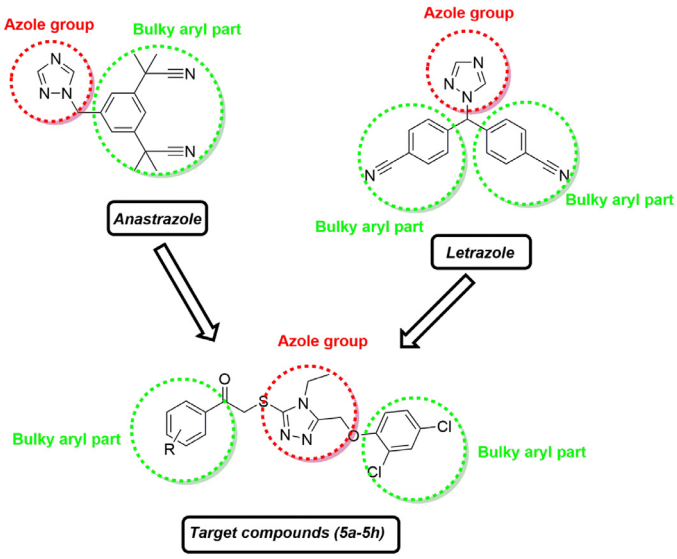



**Fig 1.** Design of target compounds.

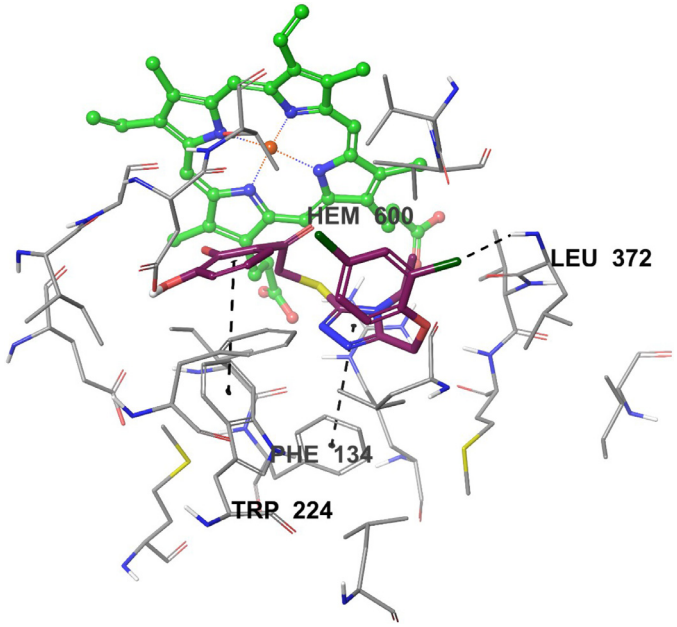



**Fig 2.** The 3D interacting mode of compound **5e** in the active region of human aromatase enzyme (PDB ID: 3EQM). The inhibitor, colored with maroon, the important residues, colored with gray, and the HEME(hemoglobine), colored with green in the active site of the enzyme are presented by tube model.

creasing clinical efficacy [30,31]. For this purpose, the aromatase activities of newly designed triazole compounds were investigated. While designing the compounds, the third generation inhibitors anastrazole and letrazole were chosen as precursors (Fig. 1). The azole group is therefore provided with a triazole ring system, and a bulky aryl part is placed on both sides of this ring system.

## 2. Result and discussion

### 2.1. Chemistry

The compounds **5a**-**5h** were obtained as presented in Scheme 1. Firstly, Ethyl 2-(2,4-dichlorophenoxy)acetate (**1**) was obtained by initiating a reaction between 2,4-dichlorophenol and ethyl 2-chloroacetate using NaH using of microwave (mw) irradiation. The phenol derivative was first reacted with NaH so that the phenolic OH could be converted into sodium salt, facilitating the progression of the reaction. Since NaH is a flammable substance with water, solvents with 99% purity and above were used in this step. In addition, the dryness of the glass materials used is of great importance. Using microwaves, compared to normal reflux procedures, shortened the experiment time by 4 times. Secondly, 2-(2,4-dichlorophenoxy)acetohydrazide (**2**) was synthesized using hydrazine hydrate. An excess of hydrazine hydrate was used in the reaction. Thirdly, the resulting hydrazide (**2**) was reacted with ethyl isothiocyanate to obtain 2-(2-(2,4-dichlorophenoxy)acetyl)-*N*-ethylhydrazine-1-carbothioamide (**3**). The ring closure reaction was then performed using 2-(2-(2,4-dichlorophenoxy)acetyl)-*N*-ethylhydrazine-1-carbothioamide (**3**) and carbondisulfide in presence of sodium hydroxide. HCl was carefully added dropwise during the finishing step. The pH was also constantly checked. It should be noted that if excess HCl is added, it is removed from obtaining the molecule in solid form. Finally, the resulting triazole compound (4) and the appropriate 2-bromoacetophenone were reacted to synthesize the target compounds. The obtained products were crystallized from ethanol using activated charcoal. In order to completely remove the activated charcoal from the environment, filtering was performed with 3 layers of filter paper. The structures of the gained compounds were demonstrated by means of spectroscopic methods, namely $^{1}$H-NMR, $^{13}$C-NMR, and HRMS (**Supplementary Data**).

In the final compounds, the triazole-linked ethyl group was recorded as 3H triplet at 1.33 ppm and as 2H quartet at 4.09 ppm in 1H-NMR and 15.51 ppm and 30.31 ppm in $^{13}$C-NMR. Hydroxy groups for compound **5e** were observed as br.s. at 5.11 ppm in $^{1}$H-NMR. Carbonyl carbons were recorded between 190.07 ppm and 194.90 ppm in $^{13}$C-NMR. All remaining protons and carbons were observed at expected values. 2D-NMR studies, namely HSQC and HMBC were performed for compound **5e** (Fig. 1), which had the most active compound of all ones obtained. Using HMBC data, it was found that the triazole carbons coded 3, 5 were recorded at 151.38 ppm and 151.03 ppm, respectively. In light of the information obtained from HMBC and HSQC, dichlorophenyl carbons (1,2,3,4,5,6) were found to be 152.38 ppm, 125.85 ppm, 129.90 ppm, 122.86 ppm, 128.58 ppm, and 116.13 ppm, respectively. Using the information obtained from HMBC and HSQC, dihydroxyphenyl carbons coded (1,2,3,4,5,6) were found to be 122.85 ppm, 158.87 ppm, 112.94 ppm, 147.43 ppm, 123.60 ppm, and 115.04 ppm, respectively. HRMS results were also well-suited with theoretical m/z values.

### 2.2. Cytotoxicity test

The MTT test, one of the most preferred cytotoxicity tests, is based on the principle that metabolically active cells convert the pale yellow MTT color to a spectrophotometrically measured blue formazan salt [32]. The antitumor potential of the novel triazole derivatives against the MCF-7 cell line at various concentrations (1000 µM, 316 µM, 100 µM, 31.6 µM, 10 µM, 3.16 µM, 1 µM, 0.316 µM) was evaluated. In addition, the NIH3T3 cells were applied as a healthy cell line reference. $IC_{50}$ values and the selectivity indices (SI) of compounds against the MCF7 cell line are shown in Table 1. According to the obtained MTT test results, compounds **5b, 5c**, and **5d** showed activity with $IC_{50}$ = 11.003 ± 0.112 µM, 15.235 ± 0.470 µM and 13.587 ± 0.297 µM values against the MCF7 cell line, respectively. The efficacy of these compounds on the MCF7 cell line is very valuable, but the selectivity indices calculated according to the MTT test result performed with the healthy cell line (NIH3T3) are 3.443, 1.528, 10.007, respectively. On the other hand, compound **5e** was the most active derivative in the series with $IC_{50}$ = 7.501 ±

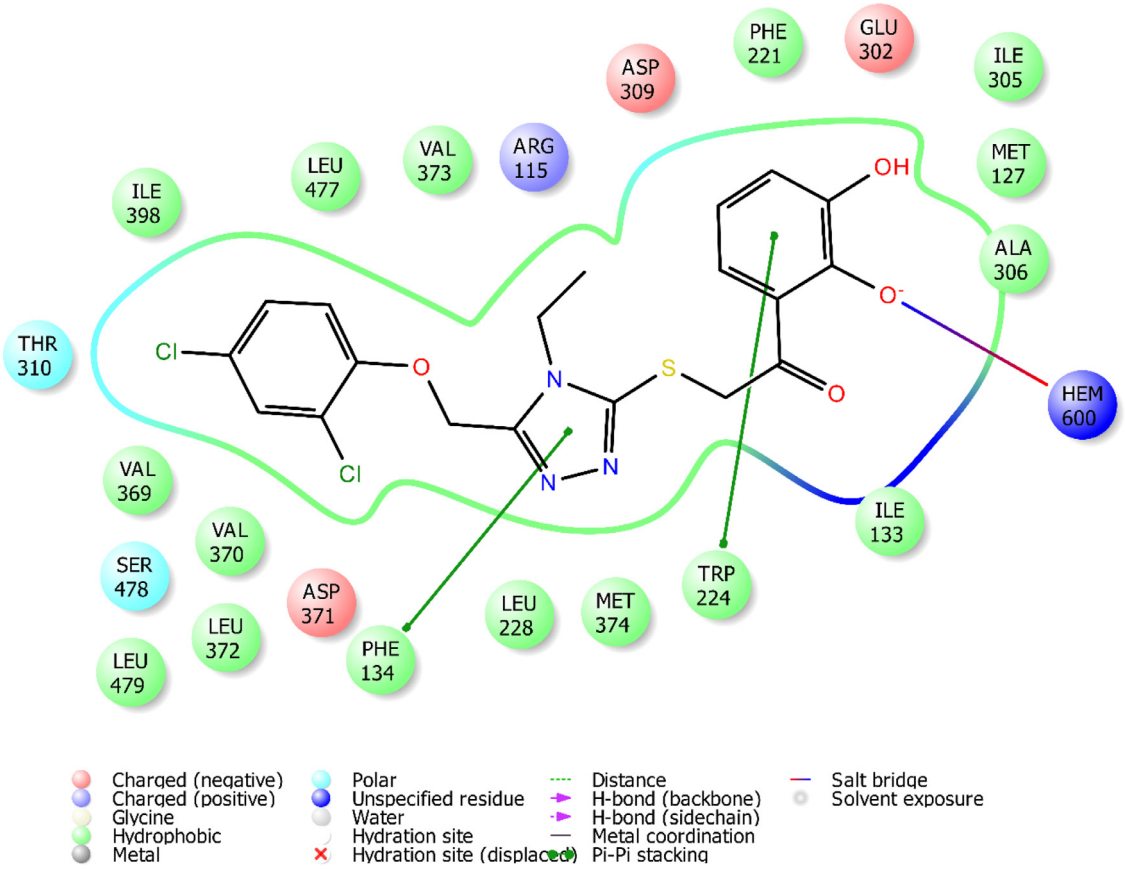



**Fig 3.** The 2D interacting mode of compound **5e** in the active region of human aromatase enzyme (PDB ID: 3EQM).

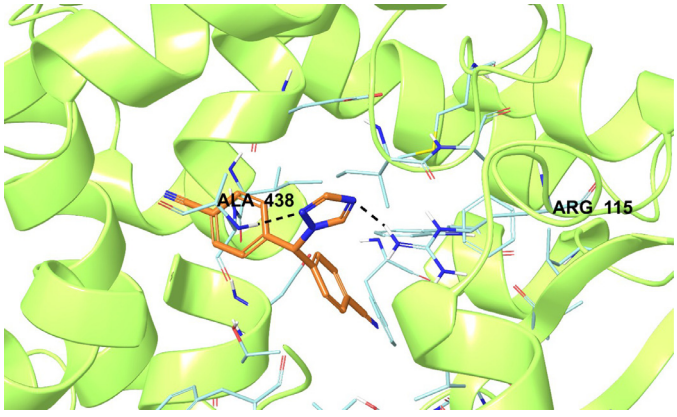



**Fig 4.** The 3D interacting mode of letrazole in the active region of human aromatase enzyme (PDB ID: 3EQM). The inhibitor, colored with orange, the important residues, colored with blue in the active site of the enzyme are presented by tube model.

**Table 1**
$IC_{50}$ values (μM) of compounds (**5a-5h**) against MCF7 and NIH3T3 cell lines.

| Compounds | MCF7 | NIH3T3 | SI* |
|---|---|---|---|
| **5a** | 20.411±0.497 | 187.685±2.322 | 9.195 |
| **5b** | 11.003±0.112 | 37.885±0.991 | 3.443 |
| **5c** | 15.235±0.470 | 23.286±1.879 | 1.528 |
| **5d** | 13.587±0.297 | 135.965±2.811 | 10.007 |
| **5e** | **7.501±0.305** | 387.685±1.325 | **51.684** |
| **5f** | 33.762±0.936 | 34.782±1.091 | 1.030 |
| **5g** | 39.395±1.797 | 40.896±0.541 | 1.038 |
| **5h** | 37.159±0.837 | 53.662±2.073 | 1.444 |
| **Dox** | 16.385±0.658 | 1141.688±5.268 | 69.678 |

* Selectivity index (SI)=[$IC_{50}$(NIH3T3)/$IC_{50}$(MCF7)], n=4

**Table 2**
$IC_{50}$ values (μM) of compound **5e** and letrazole.

| Compounds | Aromatase inhibition |
|---|---|
| **5e** | 0.028±0002 |
| **Letrazole** | 0.024±0.001 |

0.305 μM. In addition, compound **5e** resulted in a safe profile with a selectivity index value of 51.684.

### 2.3. Aromatase inhibition assay

The *in vitro* aromatase inhibition activity of the most active compound, compound **5e**, the results of which are presented in Table 2, was assessed by the commercial fluorometric assay kit (Aromatase-CYP19A Inhibitor Screening kit, Bio Vision). Letrozole was used as the reference drug. According to the activity result, compound **5e** shows activity with an $IC_{50}$ value of 0.028 μM, while letrazole showed activity with a value of 0.024 μM. When compared with the reference drug letrazole, it was observed that compound **5e** has a similar level of aromatase inhibitor activity with letrazole.

### 2.4. Molecular docking

Molecular docking studies were achieved to find the binding modes of compound **5e** on the human aromatase enzyme active site. Therefore, the crystal structure of the human aromatase en-

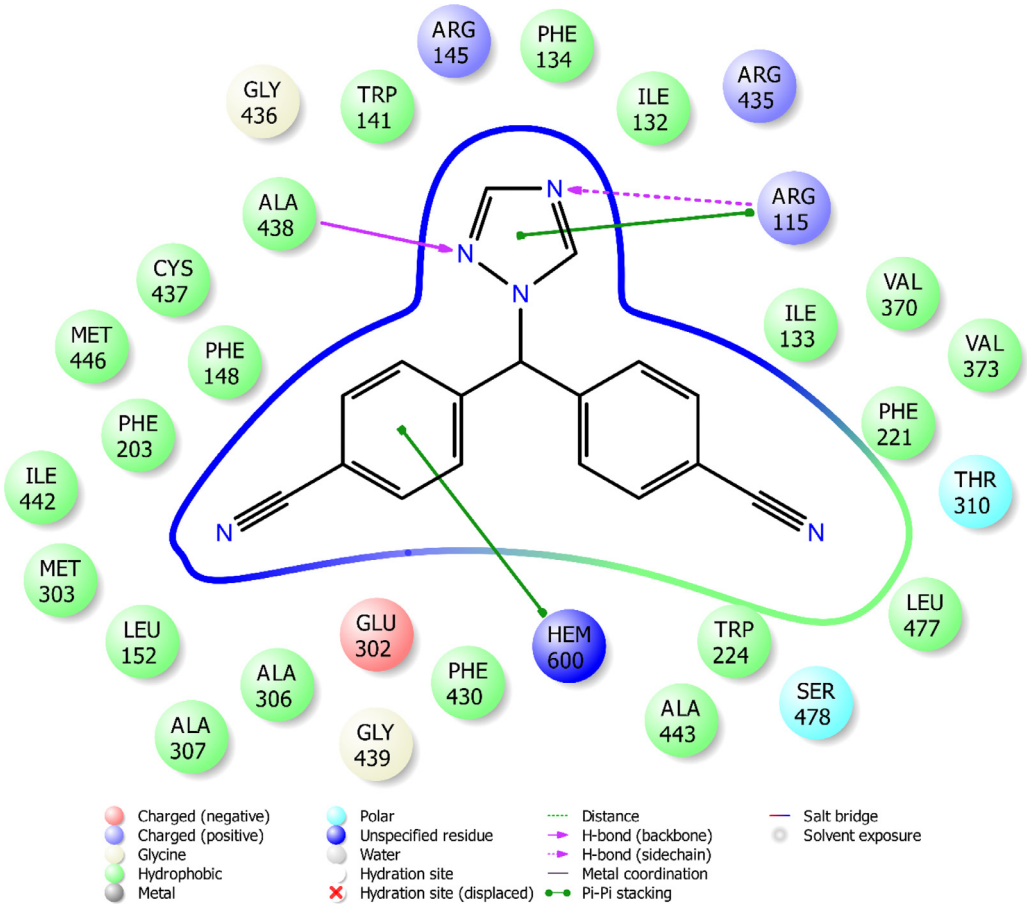



**Fig 5.** The 2D interacting mode of letrazole in the active region of human aromatase enzyme (PDB ID: 3EQM).

zyme (PDB ID: 3EQM) [33] was recovered from the Protein Data Bank server (www.pdb.org). The 2D and 3D docking poses of compound **5e** can be seen in Figs 2–3. The 2D and 3D docking poses of letrazole are shown in Figs. 4–5.

As seen in the docking poses, compound **5e** properly settled down in the enzyme active site. According to the molecular docking results, this compound displayed many interactions. The first of these belonged to triazole. This ring formed a $\pi$-$\pi$ interaction with the phenyl of Phe134. The other interaction belonged to the dihydroxyphenyl ring. There was a $\pi$-$\pi$ interaction between the phenyl and the indole of Trp224. When the 3D pose of compound **5e** (Fig. 2) is examined, it can be observed that the Cl atom in the 2nd position of the phenyl forms a halogen bond with the hydroxy group of the Leu372. Another interaction took place between the hydroxy group in the 2nd position of the phenyl ring and the hemoglobine in the enzyme active site. A salt bridge formed between these 2 structures.

As seen in the docking poses, letrozole is settled down in the enzyme active site properly. It interacted with the active site in a similar position to compound **5e**. For letrozole, it is seen that benzonitrile ring makes $\pi$–$\pi$ interaction with hemoglobin. In compound **5e**, this interaction was observed as a salt bridge. Another $\pi$–$\pi$ interaction is detected between 1,2,4-triazole and Arg115. Likewise, this 1,2,4-triazole ring forms two hydrogen bonds through its second and fourth nitrogen atoms with amino groups of Ala438 and Arg115, respectively.

When looking at the results obtained, the triazole ring interacted with the enzyme active site. In addition, the dichlorophenyl ring contributed significantly to the activity by making a halogen bond in the 2-Cl substituent in the structure. Unlike other compounds, the dihydroxy phenyl ring in compound **5e** creates the $\pi$-$\pi$ interaction itself, while one of the OH groups interacted with the hemoglobine molecule, which is very important for the aromatase enzyme active site.

## 3. Conclusion

Aromatase inhibitors are frequently used in hormone-dependent breast cancer cases, especially in postmenopausal women. The most distinctive feature of this group is that they contain azole groups in their structures and that the azole group is bound to 2 aromatic structures. For this purpose, the aromatase activities of newly designed triazole compounds were investigated. Compound **5e** showed activity against the MCF7 cell line with an $IC_{50}$ = **7**.501 ± 0.305 μM. In addition, this compound showed similar efficacy to letrazole by inhibiting the aromatase enzyme with a value of $IC_{50}$ = 0.028 μM. When compound **5e** was analyzed in terms of its chemical properties, it was observed that it had a 2,4-dihydroxyphenyl ring that was different from the other derivatives in the series. This is thought to have been caused by the fact that the substituent of the hydroxy group of the phenyl ring activated the compounds biologically. The superiority of this compound could be explained by the molecular modeling studies. According to the results of docking studies, compound **5e** bounded to the active site by forming additional interaction (salt bridge) via its substituent of the hydroxy atom at the 2nd position with hemoglobine. In addition, the arrangement that occurred in the conformation of the compound originating from this substituent enabled the chlorine atom of the compound to form a halogen bond.

When looking at the data obtained, the fact that the compounds show anticancer activity in relation to breast cancer is observable. Compound **5e** is very important for both its anticancer activity and aromatase inhibitory property. In summary, structural modifications can be further made on the basis of the new triazoles to look for compounds with higher inhibitory activity against the human aromatase enzyme.

## 4. Experimental

### 4.1. Chemistry

All reagents purchased from commercial suppliers were used without additional purification. (M.p.), and the melting points determined on the Mettler Toledo-MP90 Melting Point System are uncorrected. For 1H-NMR (nuclear magnetic resonance), a Bruker DPX 300 FT-NMR spectrometer was used and for 13C-NMR, a Bruker DPX 75 MHz spectrometer (Bruker Bioscience, Billerica, MA, USA). The coupling constants (J) were expressed in Hertz (Hz). Mass spectra were recorded on an LCMS-IT-TOF (Shimadzu, Kyoto, Japan) using the ESI method.

#### 4.1.1. Synthesis of ethyl 2-(2,4-dichlorophenoxy)acetate (**1**)

2,4-Dichlorophenol (4.859 gr, 0.030 mol) was dissolved in acetone (ACS reagent) (≥99.5%). Sodium hydride (0.864 gr, 0.036 mol) was added in reaction mixture as 4 portions. Ethyl 2-chloroacetate (3.69 mL, 0.036 mol) was added to the reaction medium; then, the reaction was continued in the microwave reactor for 20 m. At the end of the reaction, the solvent was removed. The filtrate was washed with water, dried, and recrystallized from EtOH. The product was obtained in 80% yield.

#### 4.1.2. Synthesis of 2-(2,4-dichlorophenoxy)acetohydrazide (**2**)

Ethyl 2-(2,4-dichlorophenoxy)acetate (1) (5.952 gr, 0.024 mol) was dissolved in ethanol (absolute) (50 mL). A solution of hydrazine hydrate in ethanol was added dropwise to the reaction medium. After the dropping was completed, the reaction was stirred for 5 h at room temperature. The precipitated product was filtered. The hydrazide (**2**) was washed 3 times with cold ethanol (95%) to remove the excess of hydrazine hydrate. The product was obtained in 75% yield.

#### 4.1.3. Synthesis of 2-(2-(2,4-dichlorophenoxy)acetyl)-N-ethylhydrazine-1-carbothioamide (**3**)

2-(2,4-Dichlorophenoxy)acetohydrazide (**2**) (4.914 gr, 0.021 mol) and isothiocyanatoethane (1.827 gr, 0.021 mol) were dissolved in ethanol (absolute) (40 mL). The reaction mixture was refluxed for 10 h. After completion of the reaction, the mixture was cooled and the precipitated compound was filtered and recrystallized from ethanol. The product was obtained in 83% yield.

#### 4.1.4. Synthesis of 5-((2,4-dichlorophenoxy)methyl)-4-ethyl-4H-1,2,4-triazole-3-thiol (**4**)

2-(2-(2,4-dichlorophenoxy)acetyl)-N-ethylhydrazine-1-carbothioamide (**3**) (5.778 gr, 0.018 mol) was dissolved in ethanol (absolute) (40 mL). Sodium hydroxide solution (0.840 gr, 0.021 mol) in ethanol and carbon disulfide (1.270 mL, 0.021 mol) were added to the reaction vial. The reaction mixture was refluxed for 5 h. After completion of the reaction, the mixture was poured in ice water. The reaction medium is acidified with 20% HCl to pH 2. The precipitated product was filtered, then dried and recrystallized from EtOH. The product was obtained in 80% yield.

#### 4.1.5. Synthesis of target compounds (**5a-5h**)

5-((2,4-Dichlorophenoxy)methyl)-4-ethyl-4*H*-1,2,4-triazole-3-thiol (**4**) (0.485 gr, 1.6 mmol) and the appropriate 2-bromoacetophenone (1.6 mmol) were reacted in the presence of potassium carbonate in acetone (≥99.5%) at room temperature. After completion of the reaction, the solvent was removed under reduced pressure. The residue was washed with water to remove potassium carbonate, then dried and recrystallized from EtOH.

*4.1.5.1. 2-((5-((2,4-Dichlorophenoxy)methyl)-4-ethyl-4H-1,2,4-triazol-3-yl)thio)-1-phenylethan-1-one (**5a**).* Yield: 85 %, M.P.: 167.8-169.2 °C. $^1$H-NMR (300 MHz, DMSO-$d_6$): $\delta$ = 1.33 (3H, t, $J$=7.2 Hz, -$CH_2\underline{CH_3}$), 4.09 (2H, q, $J$=7.2 Hz, -$\underline{CH_2}CH_3$), 5.00 (2H, s, -$CH_2$-), 5.41 (2H, s, -$CH_2$-), 7.42-7.43 (2H, m, 1,2,4-Trisubstituebenzene), 7.56 (2H, t, $J$=7.5 Hz, Monosubstituebenzene), 7.61 (1H, dd, $J_1$=0.8 Hz, $J_2$=1.9 Hz, 1,2,4-Trisubstituebenzene), 7.69 (1H, t, $J$=7.4 Hz, Monosubstituebenzene), 8.03 (2H, d, $J$=7.2 Hz, Monosubstituebenzene). $^{13}$C-NMR (75 MHz, DMSO-$d_6$): $\delta$ = 15.51, 33.03, 40.99, 61.19, 116.14, 122.83, 125.87, 128.59, 129.30, 129.31, 129.93, 134.25, 135.72, 151.17, 152.36, 193.14. HRMS (m/z): [M+H]+ calcd for $C_{19}H_{17}N_3O_2SCl_2$: 422.0491; found: 422.0489.

*4.1.5.2. 1-(4-Chlorophenyl)-2-((5-((2,4-dichlorophenoxy)methyl)-4-ethyl-4H-1,2,4-triazol-3-yl)thio)ethan-1-one (**5b**).* Yield: 79 %, M.P.: 199.4-201.3 °C. $^1$H-NMR (300 MHz, DMSO-$d_6$): $\delta$ = 1.33 (3H, t, $J$=7.2 Hz, -$CH_2\underline{CH_3}$), 4.09 (2H, q, $J$=7.2 Hz, -$\underline{CH_2}CH_3$), 4.98 (2H, s, -$CH_2$-), 5.41 (2H, s, -$CH_2$-), 7.42-7.43 (2H, m, 1,2,4-Trisubstituebenzene), 7.61 (1H, br.s., 1,2,4-Trisubstituebenzene), 7.63 (2H, d, $J$=8.7 Hz, 1,4-Disubstituebenzene), 8.04 (2H, d, $J$=8.6 Hz, 1,4-Disubstituebenzene). $^{13}$C-NMR (75 MHz, DMSO-$d_6$): $\delta$ = 15.50, 30.45, 40.85, 61.18, 116.14, 122.83, 125.88, 128.59, 129.42, 129.93, 130.81, 134.45, 139.15, 150.73, 151.21, 152.36, 192.71. HRMS (m/z): [M+H]+ calcd for $C_{19}H_{16}N_3O_2SCl_3$: 456.0102; found: 456.0096.

*4.1.5.3. 2-((5-((2,4-Dichlorophenoxy)methyl)-4-ethyl-4H-1,2,4-triazol-3-yl)thio)-1-(4-fluorophenyl)ethan-1-one (**5c**).* Yield: 82 %, M.P.: 191.7-192.9 °C. $^1$H-NMR (300 MHz, DMSO-$d_6$): $\delta$ = 1.33 (3H, t, $J$=7.2 Hz, -$CH_2\underline{CH_3}$), 4.09 (2H, q, $J$=7.2 Hz, -$\underline{CH_2}CH_3$), 4.99 (2H, s, -$CH_2$-), 5.41 (2H, s, -$CH_2$-), 7.38 (2H, d, $J$=8.8 Hz, 1,4-Disubstituebenzene), 7.42-7.43 (2H, m, 1,2,4-Trisubstituebenzene), 7.61 (1H, dd, $J_1$=0.8 Hz, $J_2$=1.9 Hz, 1,2,4-Trisubstituebenzene), 8.11 (2H, dd, $J_1$=4.8 Hz, $J_2$=8.8 Hz, 1,4-Disubstituebenzene). $^{13}$C-NMR (75 MHz, DMSO-$d_6$): $\delta$ = 15.51, 29.80, 40.86, 61.18, 116.14, 116.36 (d, $J$=21.9 Hz), 122.83, 125.88, 128.59, 129.92, 131.98 (d, $J$=9.6 Hz), 132.52 (d, $J$=2.5 Hz), 150.79, 151.19, 152.36, 165.77 (d, $J$=252.2 Hz), 192.24. HRMS (m/z): [M+H]+ calcd for $C_{19}H_{16}N_3O_2FSCl_2$: 440.0397; found: 440.0389.

*4.1.5.4. 1-(4-Bromophenyl)-2-((5-((2,4-dichlorophenoxy)methyl)-4-ethyl-4H-1,2,4-triazol-3-yl)thio)ethan-1-one (**5d**).* Yield: 88 %, M.P.: 207.9-209.0 °C. $^1$H-NMR (300 MHz, DMSO-$d_6$): $\delta$ = 1.33 (3H, t, $J$=7.2 Hz, -$CH_2\underline{CH_3}$), 4.09 (2H, q, $J$=7.2 Hz, -$\underline{CH_2}CH_3$), 4.98 (2H, s, -$CH_2$-), 5.41 (2H, s, -$CH_2$-), 7.42-7.43 (2H, m, 1,2,4-Trisubstituebenzene), 7.61 (1H, d, $J$=1.7 Hz, 1,2,4-Trisubstituebenzene), 7.77 (2H, d, $J$=8.5 Hz, 1,4-Disubstituebenzene), 7.96 (2H, d, $J$=8.6 Hz, 1,4-Disubstituebenzene). $^{13}$C-NMR (75 MHz, DMSO-$d_6$): $\delta$ = 15.51, 30.31, 40.83, 61.18, 116.13, 122.83, 125.88, 128.38, 128.58, 129.93, 130.88, 132.37, 134.77, 150.73, 151.21, 152.36, 192.93. HRMS (m/z): [M+H]+ calcd for $C_{19}H_{16}N_3O_2SCl_2Br$: 499.9596; found: 499.9583.

*4.1.5.5. 2-((5-((2,4-Dichlorophenoxy)methyl)-4-ethyl-4H-1,2,4-triazol-3-yl)thio)-1-(2,4-dihydroxyphenyl)ethan-1-one (**5e**).* Yield: 77 %, M.P.: 203.7-205.8 °C. $^1$H-NMR (300 MHz, DMSO-$d_6$): $\delta$ = 1.33 (3H, t, $J$=7.2 Hz, -$CH_2\underline{CH_3}$), 4.09 (2H, q, $J$=7.2 Hz, -$\underline{CH_2}CH_3$),

4.79 (2H, s, -$CH_2$-), 5.11 (2H, br.s., -OH), 5.41 (2H, s, -$CH_2$-), 6.56 (1H, d, *J*=8.3 Hz, 1,2,4-Trisubstituebenzene), 7.22 (1H, d, *J*=1.6 Hz, 1,2,4-Trisubstituebenzene), 7.34 (1H, d, *J*=10.0 Hz, 1,2,4-Trisubstituebenzene), 7.42 (2H, br.s., 1,2,4-Trisubstituebenzene), 7.61 (1H, br.s., 1,2,4-Trisubstituebenzene). $^{13}$C-NMR (75 MHz, DMSO-$d_6$): $\delta$ = 15.55, 39.40, 40.95, 61.24, 112.90, 115.04, 116.13, 122.84, 122.85, 123.60, 125.85, 128.58, 129.90, 147.43, 151.03, 151.38, 152.38, 158.87, 190.06. HRMS (m/z): [M+H]+ calcd for $C_{19}H_{17}N_3O_4SCl_2$: 454.0390; found: 454.0378.

*4.1.5.6. 2-((5-((2,4-Dichlorophenoxy)methyl)-4-ethyl-4H-1,2,4-triazol-3-yl)thio)-1-(2,4-dichlorophenyl)ethan-1-one* (***5f***). Yield: 80 %, M.P.: 175.7-177.7 °C. $^1$H-NMR (300 MHz, DMSO-$d_6$): $\delta$ = 1.33 (3H, t, *J*=7.2 Hz, -$CH_2\underline{CH_3}$), 4.09 (2H, q, *J*=7.2 Hz, -$\underline{CH_2}CH_3$), 4.82 (2H, s, -$CH_2$-), 5.41 (2H, s, -$CH_2$-), 7.42-7.43 (2H, m, 1,2,4-Trisubstituebenzene), 7.56 (1H, dd, $J_1$=2.0 Hz, $J_2$=8.4 Hz, 1,2,4-Trisubstituebenzene), 7.61 (1H, d, *J*=1.9 Hz, 1,2,4-Trisubstituebenzene), 7.76 (1H, d, *J*=2.0 Hz, 1,2,4-Trisubstituebenzene), 7.83 (1H, d, *J*=8.4 Hz, 1,2,4-Trisubstituebenzene). $^{13}$C-NMR (75 MHz, DMSO-$d_6$): $\delta$ = 15.37, 32.31, 42.72, 61.12, 116.12, 122.82, 125.88, 128.01, 128.59, 129.93, 130.54, 131.90, 132.03, 135.77, 137.24, 150.50, 151.28, 152.33, 194.90. HRMS (m/z): [M+H]+ calcd for $C_{19}H_{15}N_3O_2SCl_4$: 489.9712; found: 489.9697.

*4.1.5.7. 2-((5-((2,4-Dichlorophenoxy)methyl)-4-ethyl-4H-1,2,4-triazol-3-yl)thio)-1-(2,4-difluorophenyl)ethan-1-one* (***5g***). Yield: 82 %, M.P.: 192.7-194.6 °C. $^1$H-NMR (300 MHz, DMSO-$d_6$): $\delta$ = 1.33 (3H, t, *J*=7.2 Hz, -$CH_2\underline{CH_3}$), 4.09 (2H, q, *J*=7.2 Hz, -$\underline{CH_2}CH_3$), 4.87 (2H, d, *J*=2.5 Hz, -$CH_2$-), 5.41 (2H, s, -$CH_2$-), 7.27 (1H, td, $J_1$=2.2 Hz, $J_2$=8.4 Hz, 1,2,4-Trisubstituebenzene), 7.42-7.43 (2H, m, 1,2,4-Trisubstituebenzene), 7.48 (1H, td, $J_1$=2.5 Hz, $J_2$=9.4 Hz, 1,2,4-Trisubstituebenzene), 7.61 (1H, d, *J*=1.3 Hz, 1,2,4-Trisubstituebenzene), 7.97 (1H, td, $J_1$=6.7 Hz, $J_2$=8.7 Hz, 1,2,4-Trisubstituebenzene). $^{13}$C-NMR (75 MHz, DMSO-$d_6$): $\delta$ = 15.46, 30.88, 44.01 (d, *J*=7.9 Hz), 61.17, 105.79 (t, *J*=26.8 Hz), 112.99, 113.15, 116.14, 122.82, 125.88, 128.59, 129.92, 133.31 (q, *J*=5.1 Hz), 150.71, 151.22, 152.35, 165.76 (d, *J*=252.8 Hz), 165.94 (d, *J*=251.8 Hz), 190.13. HRMS (m/z): [M+H]+ calcd for $C_{19}H_{15}N_3O_2F_2SCl_2$: 458.0303; found: 458.0290.

*4.1.5.8. 2-((5-((2,4-Dichlorophenoxy)methyl)-4-ethyl-4H-1,2,4-triazol-3-yl)thio)-1-(3,4-dichlorophenyl)ethan-1-one* (***5h***). Yield: 76 %, M.P.: 165.8-166.9 °C. $^1$H-NMR (300 MHz, DMSO-$d_6$): $\delta$ = 1.33 (3H, t, *J*=7.2 Hz, -$CH_2\underline{CH_3}$), 4.09 (2H, q, *J*=7.2 Hz, -$\underline{CH_2}CH_3$), 4.99 (2H, s, -$CH_2$-), 5.41 (2H, s, -$CH_2$-), 7.42-7.43 (2H, m, 1,2,4-Trisubstituebenzene), 7.61 (1H, d, *J*=1.7 Hz, 1,2,4-Trisubstituebenzene), 7.84 (1H, d, *J*=8.4 Hz, 1,2,4-Trisubstituebenzene), 7.97 (1H, dd, $J_1$=2.0 Hz, $J_2$=8.4 Hz, 1,2,4-Trisubstituebenzene), 8.25 (1H, d, *J*=2.0 Hz, 1,2,4-Trisubstituebenzene). $^{13}$C-NMR (75 MHz, DMSO-$d_6$): $\delta$ = 15.51, 30.49, 40.79, 61.17, 116.13, 122.82, 125.88, 128.59, 128.89, 129.92, 130.85, 131.65, 132.34, 135.94, 137.01, 150.58, 151.25, 152.35, 192.08. HRMS (m/z): [M+H]+ calcd for $C_{19}H_{15}N_3O_2SCl_4$: 489.9712; found: 489.9692.

### 4.2. Cytotoxicity test

The anticancer activity of compounds **5a-5h** was screened according to MTT experiments using Doxorubicin as reference. MTT assays were performed as previously described. [34–38]. The NIH3T3 cell line was used to determine the cytotoxic properties of the compounds on healthy cells.

### 4.3. Aromatase inhibition assay

*In vitro* aromatase inhibition tests were performed using a kit procedure (Bio Vision, Aromatase (CYP19A) Inhibitor Screening Kit (Fluorometric)), in accordance with the method previously reported by our study group [39,40]. The compounds were dissolved in dimethyl sulfoxide (DMSO) and added to the assay in at least 7 concentrations ranging from $10^{-3}$-$10^{-9}$ M. Letrozole was used as a positive inhibition control.

### 4.4. Molecular docking

In this study, the structure based in the silico docking method, including protein-ligand interaction analysis, was applied to determine the binding and interaction points of compound **5e** to the human aromatase enzyme active site. The crystal structure of human aromatase (PDB ID: 3EQM) [33] was regained from the Protein Data Bank server (www.pdb.org).

The structures of the ligands were constructed by means of the *Schrödinger Maestro* [41] interface and were then acquiesced to the *Protein Preparation Wizard* protocol of the *Schrödinger Suite 2016 Update 2* [42]. The ligands were set by *LigPrep 3.8* [43] to assign the protonation states at pH 7.4 ± 1.0 and the atom types appropriately, as well. Bond instructions were assigned and hydrogen atoms were added to the structures. The grid generation was designed using *Glide 7.1* [44]. Flexible docking runs were achieved with single precision docking mode (SP).

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.molstruc.2021.131198.

## CRediT authorship contribution statement

**Derya Osmaniye:** Conceptualization, Methodology, Software, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Begüm Nurpelin Sağlık:** Methodology. **Serkan Levent:** Formal analysis. **Sinem Ilgın:** Methodology. **Yusuf Özkay:** Supervision, Conceptualization, Investigation, Visualization. **Zafer Asım Kaplancıklı:** Supervision, Conceptualization, Investigation, Writing – original draft, Writing – review & editing.

## References

[1] Y. Yamaguchi, N. Nishizono, D. Kobayashi, T. Yoshimura, K. Wada, K. Oda, Evaluation of synthesized coumarin derivatives on aromatase inhibitory activity, Bioorg. Med. Chem. Lett. 27 (2017) 2645–2649.
[2] A.J. Nielsen, S. Raez-Villanueva, D.J. Crankshaw, A.C. Holloway, J. McNulty, Synthesis of $\alpha$-methylstilbenes using an aqueous Wittig methodology and application toward the development of potent human aromatase inhibitors, Bioorg. Med. Chem. Lett. 29 (2019) 1395–1398.
[3] A.M. Prior, X. Yu, E.-J. Park, T.P. Kondratyuk, Y. Lin, J.M. Pezzuto, D. Sun, Structure-activity relationships and docking studies of synthetic 2-arylindole derivatives determined with aromatase and quinone reductase 1, Bioorg. Med. Chem. Lett. 27 (2017) 5393–5399.
[4] P.C. Sharma, K.K. Bansal, A. Sharma, D. Sharma, A. Deep, Thiazole-containing compounds as therapeutic targets for cancer therapy, Eur. J. Med. Chem. 188 (2020) 112016.
[5] S.-O. Zaraei, A.R. Abduelkarem, H.S. Anbar, S. Kobeissi, M. Mohammad, A. Ossama, M.I. El-Gamal, Sulfamates in drug design and discovery: pre-clinical and clinical investigations, Eur. J. Med. Chem. 179 (2019) 257–271.

[6] M.M. Ghorab, M.S. Alsaid, N. Samir, G.A. Abdel-Latif, A.M. Soliman, F.A. Ragab, D.A. Abou El Ella, Aromatase inhibitors and apoptotic inducers: design, synthesis, anticancer activity and molecular modeling studies of novel phenothiazine derivatives carrying sulfonamide moiety as hybrid molecules, Eur. J. Med. Chem. 134 (2017) 304–315.
[7] H. Kang, X. Xiao, C. Huang, Y. Yuan, D. Tang, X. Dai, X. Zeng, Potent aromatase inhibitors and molecular mechanism of inhibitory action, Eur. J. Med. Chem. 143 (2018) 426–437.
[8] N.M. Raghavendra, D. Pingili, S. Kadasi, A. Mettu, S. Prasad, Dual or multi-targeting inhibitors: the next generation anticancer agents, Eur. J. Med. Chem. 143 (2018) 1277–1300.
[9] K. Bonfield, E. Amato, T. Bankemper, H. Agard, J. Steller, J.M. Keeler, D. Roy, A. McCallum, S. Paula, L. Ma, Development of a new class of aromatase inhibitors: design, synthesis and inhibitory activity of 3-phenylchroman-4-one (isoflavanone) derivatives, Bioorg. Med. Chem. 20 (2012) 2603–2613.
[10] S. Rampogu, A. Baek, R. Bavi, M. Son, G.P. Cao, R. Kumar, C. Park, A. Zeb, R.M. Rana, S.J. Park, K.W. Lee, Identification of novel scaffolds with dual role as antiepileptic and anti-breast cancer, IEEE/ACM Trans. Comput. Biol. Bioinform. 16 (2019) 1663–1674.
[11] F. Kalalinia, M. Jouya, A.K. Komachali, S.M. Aboutourabzadeh, G. Karimi, J. Behravan, K. Abnous, L. Etemad, H. Kamali, F. Hadizadeh, Design, synthesis, and biological evaluation of new azole derivatives as potent aromatase inhibitors with potential effects against breast cancer, Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents) 18 (2018) 1016–1024.
[12] R. Pingaew, P. Mandi, V. Prachayasittikul, S. Prachayasittikul, S. Ruchirawat, V. Prachayasittikul, Synthesis, molecular docking, and QSAR study of sulfonamide-based indoles as aromatase inhibitors, Eur. J. Med. Chem. 143 (2018) 1604–1615.
[13] K. Kaur, V. Jaitak, Recent development in indole derivatives as anticancer agents for breast cancer, Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents) 19 (2019) 962–983.
[14] M.J. Brier, D.L. Chambless, J. Chen, J.J. Mao, Ageing perceptions and non-adherence to aromatase inhibitors among breast cancer survivors, Eur. J. Cancer 91 (2018) 145–152.
[15] J. Doiron, A.H. Soultan, R. Richard, M.M. Touré, N. Picot, R. Richard, M. Cuperlović-Culf, G.A. Robichaud, M. Touaibia, Synthesis and structure-activity relationship of 1- and 2-substituted-1,2,3-triazole letrozole-based analogues as aromatase inhibitors, Eur. J. Med. Chem. 46 (2011) 4010–4024.
[16] M. Di Matteo, A. Ammazzalorso, F. Andreoli, I. Caffa, B. De Filippis, M. Fantacuzzi, L. Giampietro, C. Maccallini, A. Nencioni, M.D. Parenti, D. Soncini, A. Del Rio, R. Amoroso, Synthesis and biological characterization of 3-(imidazol-1-ylmethyl)piperidine sulfonamides as aromatase inhibitors, Bioorg. Med. Chem. Lett. 26 (2016) 3192–3194.
[17] R. Wang, H.-F. Shi, J.-F. Zhao, Y.-P. He, H.-B. Zhang, J.-P. Liu, Design, synthesis and aromatase inhibitory activities of novel indole-imidazole derivatives, Bioorg. Med. Chem. Lett. 23 (2013) 1760–1762.
[18] S. Yahiaoui, C. Pouget, C. Fagnere, Y. Champavier, G. Habrioux, A.J. Chulia, Synthesis and evaluation of 4-triazolylflavans as new aromatase inhibitors, Bioorg. Med. Chem. Lett. 14 (2004) 5215–5218.
[19] P. Marchand, M.L.e Borgne, M. Palzer, G.L.e Baut, R.W. Hartmann, Preparation and pharmacological profile of 7-(alpha-azolylbenzyl)-1H-indoles and indolines as new aromatase inhibitors, Bioorg. Med. Chem. Lett. 13 (2003) 1553–1555.
[20] Z. Song, Y. Liu, Z. Dai, W. Liu, K. Zhao, T. Zhang, Y. Hu, X. Zhang, Y. Dai, Synthesis and aromatase inhibitory evaluation of 4-N-nitrophenyl substituted amino-4H-1, 2, 4-triazole derivatives, Bioorg. Med. Chem. 24 (2016) 4723–4730.
[21] M. Di Matteo, A. Ammazzalorso, F. Andreoli, I. Caffa, B. De Filippis, M. Fantacuzzi, L. Giampietro, C. Maccallini, A. Nencioni, M.D. Parenti, Synthesis and biological characterization of 3-(imidazol-1-ylmethyl) piperidine sulfonamides as aromatase inhibitors, Bioorg. Med. Chem. Lett. 26 (2016) 3192–3194.
[22] J. Song, Z. Wu, B. Wangtrakuldee, S.R. Choi, Z. Zha, K. Ploessl, R.H. Mach, H. Kung, 4-(((4-Iodophenyl) methyl)-4 H-1, 2, 4-triazol-4-ylamino)-benzonitrile: a potential imaging agent for aromatase, J. Med. Chem. 59 (2016) 9370–9380.
[23] A.M. Andrianov, G.I. Nikolaev, I.A. Kashin, Y.V.eE. Kornoushenko, S.A. Usanov, Molecular modeling of novel non-steroidal aromatase inhibitors containing 1, 2, 4-triazole, Matematicheskaya Biologiya i Bioinformatika 13 (2018) 290–307.
[24] M. Ouassaf, S. Belaidi, K. Lotfy, I. Daoud, H. Belaidi, Molecular docking studies and ADMET properties of new 1.2. 3 triazole derivatives for anti-breast cancer activity, J. Bionanosci. 12 (2018) 26–36.
[25] P.M. Sable, L.C. Potey, Synthesis and antiproliferative activity of imidazole and triazole derivatives of flavonoids, Pharm. Chem. J. 52 (2018) 438–443.
[26] C. Chamduang, R. Pingaew, V. Prachayasittikul, S. Prachayasittikul, S. Ruchirawat, V. Prachayasittikul, Novel triazole-tetrahydroisoquinoline hybrids as human aromatase inhibitors, Bioorg. Chem. 93 (2019) 103327.
[27] S.P. Avvaru, M.N. Noolvi, U.A. More, S. Chakraborty, A. Dash, T.M. Aminabhavi, K.P. Narayan, V. Sutariya, Synthesis and anticancer activity of thiadiazole containing thiourea, benzothiazole and imidazo[2,1-b][1,3,4]thiadiazole scaffolds, Med. Chem. (2020).
[28] A. Ammazzalorso, M. Gallorini, M. Fantacuzzi, N. Gambacorta, B. De Filippis, L. Giampietro, C. Maccallini, O. Nicolotti, A. Cataldi, R. Amoroso, Design, synthesis and biological evaluation of imidazole and triazole-based carbamates as novel aromatase inhibitors, Eur. J. Med. Chem. 211 (2021) 113115.
[29] M. Fantacuzzi, B. De Filippis, M. Gallorini, A. Ammazzalorso, L. Giampietro, C. Maccallini, Z. Aturki, E. Donati, R.S. Ibrahim, E. Shawky, Synthesis, biological evaluation, and docking study of indole aryl sulfonamides as aromatase inhibitors, Eur. J. Med. Chem. 185 (2020) 111815.
[30] V. Prachayasittikul, R. Pingaew, A. Worachartcheewan, S. Sitthimonchai, C. Nantasenamat, S. Prachayasittikul, S. Ruchirawat, V. Prachayasittikul, Aromatase inhibitory activity of 1, 4-naphthoquinone derivatives and QSAR study, EXCLI J. 16 (2017) 714.
[31] U. Acar Çevik, B. Kaya Çavuşoğlu, B.N. Sağlık, D. Osmaniye, S. Levent, S. Ilgın, Y. Özkay, Z.A. Kaplancıklı, Synthesis, docking studies and biological activity of new benzimidazole-triazolothiadiazine derivatives as aromatase inhibitor, Molecules 25 (2020) 1642.
[32] M. Varache-Lembège, S. Moreau, S. Larrouture, D. Montaudon, J. Robert, A. Nuhrich, Synthesis and antiproliferative activity of aryl-and heteroaryl-hydrazones derived from xanthone carbaldehydes, Eur. J. Med. Chem. 43 (2008) 1336–1343.
[33] D. Ghosh, J. Griswold, M. Erman, W. Pangborn, Structural basis for androgen specificity and oestrogen synthesis in human aromatase, Nature 457 (2009) 219–223.
[34] D. Osmaniye, S. Levent, C.M. Ardıç, Ö. Atlı, Y. Özkay, Z.A. Kaplancıklı, Synthesis and anticancer activity of some novel benzothiazole-thiazolidine derivatives, phosphorus, sulfur, and silicon and the related elements, 193 (2018) 249-256.
[35] D. Osmaniye, B.K. Çelikateş, B.N. Sağlık, S. Levent, U.A. Çevik, B.K. Çavuşoğlu, S. Ilgın, Y. Özkay, Z.A. Kaplancıklı, Synthesis of some new benzoxazole derivatives and investigation of their anticancer activities, Eur. J. Med. Chem. 210 (2021) 112979.
[36] D. Osmaniye, S. Levent, A.B. Karaduman, S. Ilgın, Y. Özkay, Z.A. Kaplancıklı, Synthesis of new benzothiazole acylhydrazones as anticancer agents, Molecules 23 (2018) 1054.
[37] U.A. Çevik, B.N. Sağlık, C.M. Ardıç, Y. Özkay, Ö. Atlı, Synthesis and evaluation of new benzimidazole derivatives with hydrazone moiety as anticancer agents, Turkish J. Biochem. 43 (2018) 151–158.
[38] A.E. Evren, L. Yurttaş, B. Eksellı, G. Akalın-Cıftcı, Novel tri-substituted thiazoles bearing piperazine ring: synthesis and evaluation of their anticancer activity, Lett. Drug Des. Discovery 16 (2019) 547–555.
[39] U. Acar Çevik, B. Kaya Çavuşoğlu, B.N. Sağlık, D. Osmaniye, S. Levent, S. Ilgın, Y. Özkay, Z.A. Kaplancıklı, Synthesis, docking studies and biological activity of new benzimidazole- triazolothiadiazine derivatives as aromatase inhibitor, Molecules 25 (2020).
[40] U. Acar Çevik, B.N. Sağlık, D. Osmaniye, S. Levent, B. Kaya Çavuşoğlu, A.B. Karaduman, Y. Özkay, Z.A. Kaplancıklı, Synthesis and docking study of benzimidazole-triazolothiadiazine hybrids as aromatase inhibitors, Arch. Pharm. (Weinheim) 353 (2020) e2000008.
[41] MaestroM. Maestro, version 10.6, Schrödinger, LLC, New York, NY, 2016.
[42] Schrödinger, LLC, New York, NY, (2016).
[43] LigPrep, Schrödinger, LLC, New York, NY, 2016.
[44] Glide, Schrödinger, LLC, New York, NY, 2016.