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Design and Synthesis of Imidazole Derivatives as Anticancer Agents and Potential Aromatase Inhibitors

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Research Article

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ABSTRACT

In this study, imidazole derivative compounds were synthesized using the Debus-Radziszewski method. The chemical structures of the compounds were characterized by spectroscopic methods. The effects of target compounds on MCF7 (CRL-3435) were examined and their IC_{50} values and percent viability were calculated. In addition, the cytotoxic effects on the L929 (CCL-1) normal cell line were evaluated in order to determine the selectivities of the compounds. Then, the inhibition values of aromatase enzyme of the compounds were calculated and compared to the reference compound. When the results were examined, it was observed that Compound Ia caused the death of breast cancer cells, although not as much as cisplatin, but did not harm healthy cells. In this respect, it was determined that compound Ia has a promising anticancer effect as an aromatase inhibitor.

Keywords: İmidazole, MTT, Anticancer, Aromatase.

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Introduction

Breast cancer is one of the most common types of cancer in our country and around the world [1]. Especially in postmenopausal women, the risk of breast cancer increases due to estrogen secretion in peripheral tissues [2]. Aromatase is a catalytic enzyme involved in the manufacture of estrogen from androgen. It catalyzes the last rate-limiting/crucial/key step in estrogen biosynthesis [3,4]. Figure 1. demonstrates the action and role of the aromatase enzyme.

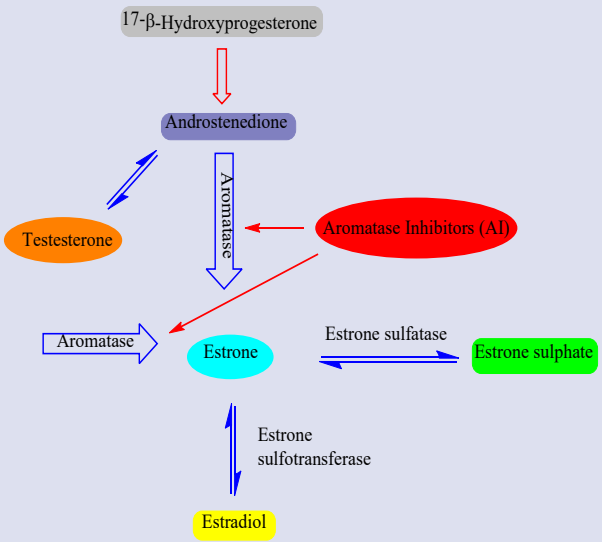

Figure 1. The action of the aromatase enzyme in synthesizing steroidal hormones.

According to their methods of action, aromatase inhibitors (AIs) can be divided into two classes. The steroidal AIs, such as exemestane and formestane (Figure 2), suppress the aromatase enzyme activity irreversibly. Nonsteroidal AIs, such as letrozole, vorozole, and anastrozole, are the second group of AIs that inhibit aromatase activity and have reversible inhibitory effects [5, 10].

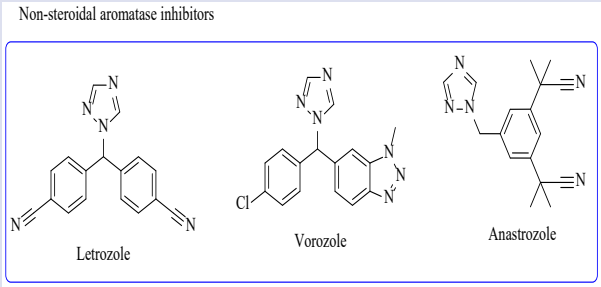

Figure 2. Non-steroidal aromatase inhibitors

Based on their clinical development order, AIs are categorized into four generations: first, second, third, and fourth generation AIs. Third generation aromatase inhibitors such as letrozole and anastrozole are the most preferred inhibitors because of their low toxicity, selectivity and effectiveness. Third-generation inhibitors,

which provide an outstanding therapeutic benefit, show almost complete specificity in practice [6].

Heterocyclic rings containing nitrogen atoms can form an ionic bond with the iron part of hemoglobin and thus perform a very important function by suppressing the aromatase enzyme (HES). In addition to the contribution of the heterocyclic nitrogen atom to the activity, the presence of hydrogen bond acceptors in these structures is an important feature in terms of providing interactions in bonding algebra [7,8].

Nitrogen-containing heterocyclic rings are actively researched in the field of drug discovery, particularly in cancer research [9]. As possibly nonsteroidal AIs, triazole and imidazole rings are employed. The heterocyclic nitrogen atom of triazole and imidazole is significant because it coordinates the aromatase enzyme's heme iron. Some imidazole and triazole compounds have been produced and tested as antiaromatase drugs in the past [11-15].

New imidazole derivative compounds with a structure comparable to letrozole were created in this work. The imidazole ring is employed instead of the triazole structure, and the imidazole ring is given an additional heteroaromatic group (Figure 3.). All final chemicals created on MCF7 (CRL-3435) breast cancer cells were tested for cell viability and cytotoxicity using the MTT assay. The aromatase inhibition potentials of the compounds were evaluated in a fluorimetric *in vitro* assay.

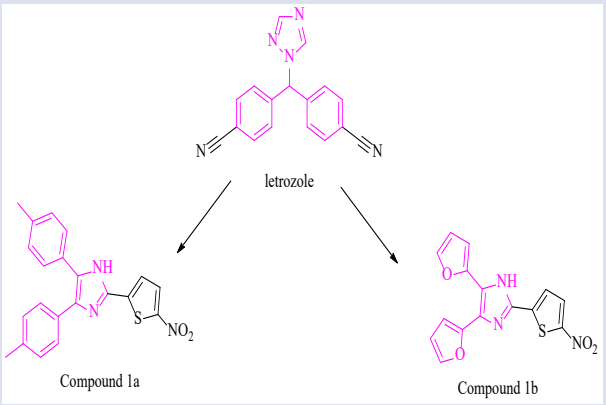

Figure 3. Chemical structures of letrozole and design strategy of compound 1a and 1b

Materials and Methods

Chemistry

4,5-Disubstituted-2-(5-nitrothiophen-2-yl)-1H-imidazole (1a-1b): The mixture of benzyl derivative compound (4,4'-dimethylbenzyl (97 %) and α-furyl (98 %)) (0.02 mol) and thiophene aldehyde (98 %) derivative compound (0.02 mol), ammonium acetate (> 98 %) (0.12 mol) and 10 mL acetic acid (> 99 %) is boiled under reflux for 3 hours with stirring. The product is precipitated by pouring the mixture into ice water. The raw product is washed with plenty of water and dried. The product is crystallized from aqueous ethanol. The synthesis mechanism of the compounds is given in Figure 4.

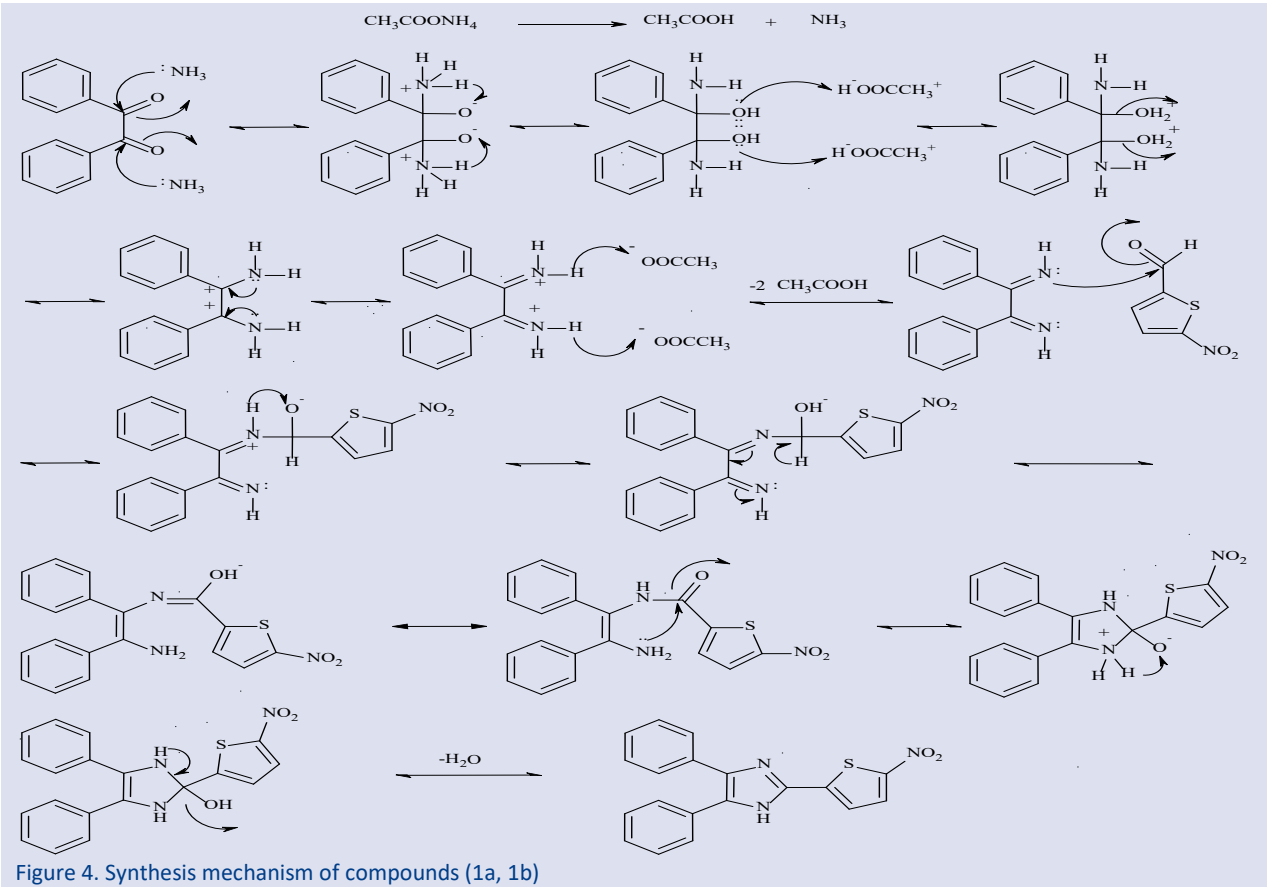

Figure 4. Synthesis mechanism of compounds (1a, 1b)

4,5-Bis(4-methylphenyl)-2-(5-nitrothiophen-2-yl)-1*H*-imidazole (1a); Yield: 77 %. ^{1}H NMR (500 MHz, DMSO-d_6): *δ*: 2.30 (3H, s, CH_3), 2.36 (3H, s, CH_3), 7.13 (2H, d, 5.76 Hz, Aromatic CH), 7.27 (2H, d, 5.79 Hz, Aromatic CH), 7.38-7.45 (4H, m, Aromatic CH), 7.68-7.69 (1H, m, Thiophen CH), 8.17-8.18 (1H, m, Thiophen CH), 12.60 (1H, s, NH). ^{13}C NMR (125 MHz, DMSO-d_6): *δ*: 21.34, 21.51, 123.49, 124.94, 127.57, 128.66, 129.39, 129.84, 130.09, 130.51, 131.93, 139.41, 142.11. HRMS (m/z): $[M+H]^+$ calcd for $C_{21}H_{17}N_3O_2S$ 376.1114; found: 376.1110.

4,5-Di(furan-2-yl)-2-(5-nitrothiophen-2-yl)-1*H*-imidazole (1b): Yield: 78 %. ^{1}H NMR (500 MHz, DMSO-d_6): *δ*: 6.85-6.86 (2H, m, Furan CH), 7.67-7.68 (2H, m, Furan CH), 7.76-7.80 (1H, m, Furan CH), 8.12-8.16 (1H, m, Furan CH), 8.25 (2H, s, Thiophen CH), 12.50 (1H, s, NH). ^{13}C NMR (125 MHz, DMSO-d_6): *δ*: 107.60, 108.75, 114.05, 118.27, 120.04, 125.32, 126.71, 131.05, 131.55, 149.10, 151.39. HRMS (m/z): $[M+H]^+$ calcd for $C_{15}H_9N_3O_4S$ 328.0334; found: 328.0640.

Anticancer Activity

The anticancer activities of compounds 1a and 1b were determined by the absorbance values obtained from MTT assays. The MTT method was performed as previously described [16]. The cells were seeded at a density of 1×10^4 cells/well and treated with different concentrations between 2-100µM for each and incubated for 48 h. Untreated cells were used as control. Following incubation, the cells were treated with 20 µL of MTT solution (5 mg/mL in PBS, Sigma) and incubated at 37 °C for 3 h to let the metabolically active cells reduce MTT dye into formazan crystals. The formazan crystals were dissolved in DMSO (Sigma). The reduction of MTT was quantified by measuring the absorbance at 540 nm with a microplate reader (Thermo, Germany). Datas were represented as mean ± standard deviation (± SD). The results obtained were evaluated with the MCF-7 breast cancer cell line versus the L929 (CCL-1) normal fibroblast cell line. In this section, cisplatin was used as a reference drug in cell lines.

Aromatase Inhibition Assay

This method was carried out according to the kit procedure (BioVision, Aromatase (CYP19A) Inhibitor Screening Kit (Fluorometric)). The compounds were dissolved in DMSO and added to the assay in at least 8 concentrations ranging from 1000 µM to 7.81 µM. Recombinant Human Aromatase stock was prepared by reconstituting with 1 ml of Aromatase Assay Buffer. The contents were mixed thoroughly by vortexing to obtain a homogeneous solution and transfered the solution to a 15 ml conical tube. The volume was brought to 2450 µl with Aromatase Assay Buffer and 50 µl of NADPH Production System (100X) was added for a final total volume of 2.5 ml. Letrozole was used as a positive inhibition control. For solvent control, a small aliquot of Aromatase Assay Buffer containing the organic solvent used to dissolve the test compounds were prepared. Reaction wells containing test compounds and corresponding no inhibitor controls (which may also serve as a solvent control), as well as a background control (containing no fluorogenic Aromatase Substrate) were prepared. The plate was incubated for at least 10 min at 37°C to allow test ligands to interact with aromatase. After incubation, 30 µl of the Aromatase Substrate/NADP+ mixture was added to each well. Immediately (within 1 min), the fluorescence at Ex/Em = 488/527 nm was measured. The experiment was carried out in three repetitions.

Results and Discussion

In this study, 2,4,5-trisubstituted imidazole derivatives were obtained by boiling the diketone derivatives in acetic acid in the presence of ammonium acetate under reflux using the Debus-Radziszewski method. The general synthesis scheme of the compounds is shown in Figure 5. The structures of the compounds were elucidated by spectroscopic methods. Compound 1a has the p-tolyl structure. The -CH_3 protons in this structure were seen as singlet 2.30-2.36 ppm. Protons of the thiophene ring were observed in the range of 7.68-8.25 ppm. The NH protons of the imidazole ring were observed as singlet at 12.50 and 12.60 ppm.

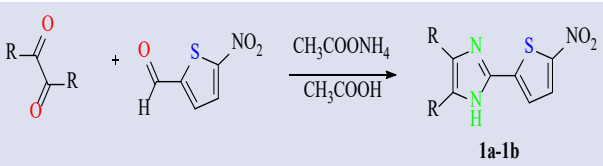

Figure 5. Chemical structure and general procedure for the synthesis of the final compounds 1a-1b

The anticancer activity results of compounds 1a-1b against MCF7 (CRL-3435) and L929 (CCL-1) are presented in Table 1. When the cytotoxic effects of the synthesized compounds on the MCF7 (CRL-3435) cell line were evaluated, it was determined that the compound 1a had promising effects in the series. Two different diketone derivatives, 4,4'-dimethylbenzyl and α-furyl were used in the synthesis of the compounds. It has been determined that the anticancer effect potential of the 4,4'-dimethylbenzyl derivative is particularly promising in terms of activity.

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

	MCF7		*L929*	
Comp.	***IC50***	***Percent Vitality***	***IC50***	***Percent Vitality***
1a	82.7	43.3 ± 1.03	>100	63.5 ± 6.03
1b	>100	62.2 ± 8.8	>100	60.6 ± 3.02
Cisplatin	69.75	28.32 ± 3.78	>100	53.7 ± 4.83

The anti-aromatase activity of compounds 1a and 1b against the reference drug letrozole was evaluated using the commercial fluorimetric assay kit, Aromatase-CYP19A

(BioVision). The results found are shown in Table 2. The compound 1a one was found to have a higher activity with an IC_{50} value of 55.780 ± 1.980 µM.

Table 2. IC_{50} (µM) values of compounds

Compounds	Aromatase Inhibition (IC_{50})
1a	55.780 ± 1.980
1b	>100
Letrozole	0.114 ± 0.003

Conclusion

In this study, two imidazole derivative, 4,5-bis(4-methylphenyl)-2-(5-nitrothiophen-2-yl)-1*H*-imidazole (1a) and 4,5-di(furan-2-yl)-2-(5-nitrothiophen-2-yl)-1*H*-imidazole (1b), were synthesized. The anticancer effects of the compounds were evaluated on the MCF7 (CRL-3435) breast cancer cell line. Two different diketone derivatives, 4,4'-dimethylbenzyl and α-furyl were used in the synthesis of the compounds. When the effects of the compounds on the aromatase enzyme were evaluated, it was found that compound 1a had moderate activity, while the activity of compound 1b on the aromatase enzyme was not found.

Conflicts of interest

The authors declare that there are no conflicts of interest.

References

[1] Lekgau K., Raphoko L.A., Lebepe C.M., Mongokoana D.F., Leboho T.C., Matsebatlela T. M., ... & Nxumalo W., Design and Synthesis of 6-Amino-quinoxaline-alkynyl as Potential Aromatase (CYP19A1) Inhibitors, *J. Mol. Struct*., 1255 (2022) 132473.

[2] Osmaniye D., Karaca Ş., Kurban B., Baysal M., Ahmad I., Patel H., ... Kaplancıklı Z. A., Design, synthesis, molecular docking and molecular dynamics studies of novel triazolothiadiazine derivatives containing furan or thiophene rings as anticancer agents, *Bioorg. Chem.,* 122 (2022) 105709.

[3] Sayyad N. B., Sabale P. M., Umare M. D., Bajaj K. K., (2022). Aromatase Inhibitors: Development and Current Perspectives, *Indian J. Pharm. Educ. Res.*56 (2022) 311-320.

[4] Shah V., Bhaliya J., Patel G. M., In silico docking and ADME study of deketene curcumin derivatives (DKC) as an aromatase inhibitor or antagonist to the estrogen-alpha positive receptor (Erα+): potent application of breast cancer, *Struct. Chem.,* 33 (2022) 571-600.

[5] Rashdan H. R., Shehadi I. A., Triazoles Synthesis & Applications as Nonsteroidal Aromatase Inhibitors for Hormone-Dependent Breast Cancer Treatment, *Heteroatom Chem.,* (2022).

[6] Çevik U. A., Celik I., Mella J., Mellado M., Özkay Y., Kaplancıklı Z. A., Design, Synthesis, and Molecular Modeling Studies of a Novel Benzimidazole as an Aromatase Inhibitor, *ACS omega*. 7 (2022) 16152-16163.

[7] Osmaniye D., Levent S., Sağlık B. N., Karaduman A. B., Özkay Y., Kaplancıklı Z. A., Novel imidazole derivatives as potential aromatase and monoamine oxidase-B inhibitors against breast cancer, *New J. Chem*., 46 (2022) 7442-7451.

[8] Ammazzalorso A., Gallorini M., Fantacuzzi M., Gambacorta N., De Filippis B., Giampietro L., ... Amoroso R., Design, synthesis and biological evaluation of imidazole and triazole-based carbamates as novel aromatase inhibitors, *Eur. J. Med. Chem.,* 211 (2021) 113115.

[9] Acar Çevik U., Celik I., Işık A., Ahmad I., Patel H., Özkay Y., & Kaplancıklı Z. A., Design, synthesis, molecular modeling, DFT, ADME and biological evaluation studies of some new 1,3,4-oxadiazole linked benzimidazoles as anticancer agents and aromatase inhibitors, *J. Biomol. Struct. Dyn.,* (2022) 1.

[10] Ana G., Kelly P. M., Malebari A. M., Noorani S., Nathwani S. M., Twamley B., ... Meegan, M. J., Synthesis and Biological Evaluation of 1-(Diarylmethyl)-1H-1, 2, 4-Triazoles and 1-(Diarylmethyl)-1H-Imidazoles as a Novel Class of Anti-Mitotic Agent for Activity in Breast Cancer, *Pharma.,* 14 (2021) 169.

[11] Asadi P., Khodarahmi G., Farrokhpour H., Hassanzadeh F., Saghaei L., Quantum mechanical/molecular mechanical and docking study of the novel analogues based on hybridization of common pharmacophores as potential anti-breast cancer agents, *Res. Pharm. Sci.,* 12 (2017) 233.

[12] Mojaddami A., Sakhteman A., Fereidoonnezhad M., Faghih Z., Najdian A., Khabnadideh S., ... Rezaei Z. Binding mode of triazole derivatives as aromatase inhibitors based on docking, protein ligand interaction fingerprinting, and molecular dynamics simulation studies, *Res. Pharm. Sci.,* 12 (2017) 21.

[13] Song Z., Liu Y., Dai Z., Liu W., Zhao K., Zhang T., ... & Dai Y., Synthesis and aromatase inhibitory evaluation of 4-N-nitrophenyl substituted amino-4H-1,2,4-triazole derivatives, *Bioorg. Med. Chem.,* 24 (2016) 4723-4730.

[14] Adhikari N., Amin S. A., Jha T., & Gayen S., Integrating regression and classification-based QSARs with molecular docking analyses to explore the structure-antiaromatase activity relationships of letrozole-based analogs, *Can. J. Chem.,* 95 (2017) 1285-1295.

[15] Acar Çevik U., Sağlık B. N., Osmaniye D., Levent S., Kaya Çavuşoğlu B., Karaduman A. B., ... & Kaplancıklı Z. A., Synthesis and docking study of benzimidazole–triazolothiadiazine hybrids as aromatase inhibitors, *Arch. Pharm.,* 353 (2020) e2000008.

[16] Acar Çevik U., Sağlık B.N., Osmaniye D., Levent S., Kaya Çavuşoğlu B., Karaduman A.B., ... & Kaplancıklı Z.A., Synthesis, anticancer evaluation and molecular docking studies of new benzimidazole-1,3,4-oxadiazole derivatives as human topoisomerase types I poison, *J. Enzyme Inhib. Med. Chem.,* 35 (2020) 1657-1673.