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NOVEL IMIDAZOLE CONTAINING CHALCONE DERIVATIVES AS AN AROMATASE INHIBITOR: SYNTHESIS, DOCKING STUDIES, BIOLOGICAL SCREENING AND ADME STUDIES

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ABSTRACT

Chalcone derivatives have been proven and documented as non-steroidal anticancer agents. A study of chalcone can be performed as an aromatase inhibitor. One crucial strategy for managing estrogen-positive breast cancer is aromatase inhibition. A total of 13 derivatives of chalcone were synthesized by the aldol condensation reaction and characterized by FTIR, MS, and NMR. Molecular docking was performed by the maestro Schrodinger Suite. *In silico* ADME study was executed by the QikProp software. All 13 compounds were assessed for the cytotoxicity study in human cancer cell line MCF-7 and are subject to further aromatase inhibition assay on selected chalcone derivatives. Four compounds, 3gA, 3jA, 3kA, and 3lA were found to be more potent chalcone derivatives with their IC_{50} values of 18.13±1.19 µM, 21.71±1.61 µM, 16.36±1.47 µM and 21.06±1.87 µM, respectively. Using a fluorogenic test kit, the *in vitro* aromatase inhibitory activity of four drugs (3gA, 3jA, 3kA, and 3lA) was investigated. The values of IC_{50} for compounds 3gA, 3jA, 3kA, and 3lA were found to be 2.76±0.83 µM, 6.45±3.38 µM, 4.82±1.52 µM and 3.00±1.63 µM, respectively. Lastly, QikProp 4.8 software was used to calculate the ADME parameters (absorption, distribution, metabolism, and excretion) of synthesized compounds. It was concluded that 3gA is the most potent compound having potent aromatase inhibitory activity in comparison to Letrozole with an IC_{50} value of 2.76±0.83 µM. At the same time, 3jA and 3lA have good aromatase inhibitory activity. Therefore, For the identification of aromatase inhibitory activity, these compounds make good lead compounds for further study as anticancer agents.

Keywords: Chalcone, Imidazole, Aromatase, Cytotoxicity, ADME, Docking, MCF7.

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INTRODUCTION

The strongest endogenous estrogen is estradiol. The cytochrome P450 enzyme complex known as "aromatase" is responsible for the biosynthesis of estrogens from androgens. Premenopausal women's ovaries, pregnant women's placentas, and postmenopausal women's and men's peripheral adipose tissues all contain the highest concentrations of this enzyme.[1] It has been shown that in estrogen-dependent breast malignancies, elevated estrogen levels promote cancer cell proliferation, recurrence, and metastasis. Thus, one of the most effective ways to treat breast cancer is to reduce the quantity of estrogen generated by preventing its manufacture.[2–5] The cytochrome P450 family member aromatase (CYP19) catalyzes the biosynthesis of estrogens from androgens and is a rate-limiting enzyme. As a result, it has been acknowledged that aromatase inhibitors (AIs) are one of the commonly used drug classes for the treatment of estrogen-dependent cancer.[6–8] Aromatase inhibitors (AIs) can be categorized into two main groups based on how they work: (i) steroidal aromatase inhibitors (Androstinedion and Exemestane) which block the

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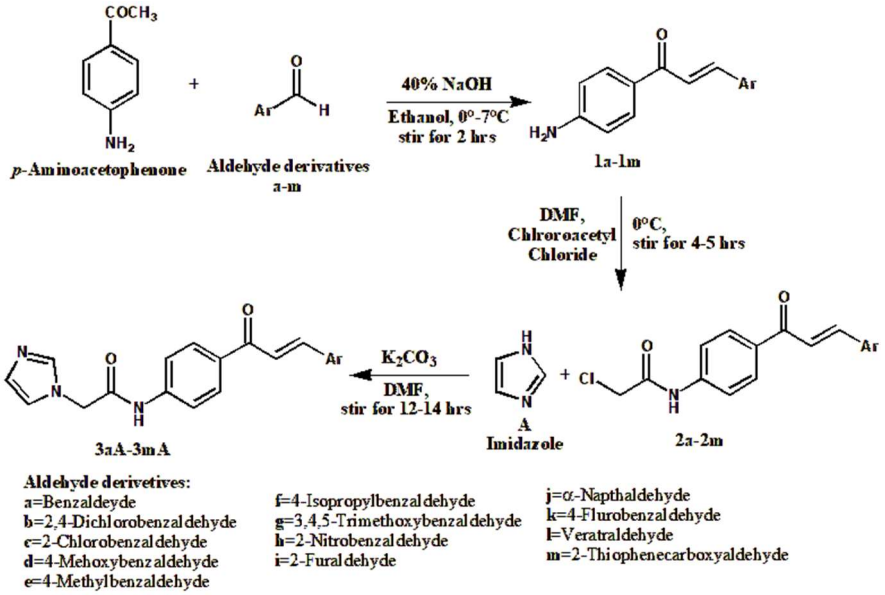
activity of the aromatase enzyme permanently; and (ii) nonsteroidal aromatase inhibitors (Letrozole, Anastrozole, and Vorozole) which give reversible inhibition effect.[7,8] While steroidal and non-steroidal aromatase inhibitors have demonstrated positive clinical results, prolonged use of these drugs can result in acquired drug resistance and serious side effects, such as cardiovascular disease, osteoporosis, and musculoskeletal pain.[9] Thus, the creation of new aromatase inhibitors is still necessary to offer preferred alternatives with better qualities. Chalcones are a core structure that is frequently studied as nonsteroidal anticancer agents.[10–12] Through its interactions with the heme iron of the aromatase enzyme, imidazole's heterocyclic nitrogen atom plays a significant role.[13,14] As a result, several imidazole derivatives containing chalcone were altered and created. Each newly synthesized chalcone derivative's aromatase inhibitory activity was evaluated. An investigation into putative binding mechanisms controlling the aromatase inhibitory effects was also done using molecular docking, which should clarify the important interactions.



Scheme-1: Synthetic Route of Imidazole Containing Chalcone Derivatives

EXPERIMENTAL

Material and Methods

For column chromatography, silica gel 60 (70–230 mesh ASTM) was used. Aluminum sheets containing silica gel 60 F_{254} were employed for analytical thin-layer chromatography (TLC). Bruker Avance III HD 500 NMR spectrometers have been utilized to record 1H-NMR and 13C-NMR spectra. Tetramethylsilane (TMS) is used to report 1H NMR and 13C NMR chemical shifts in parts per million (ppm). Using the press pellet method and potassium bromide, the produced compounds' FT-IR data were acquired on a Perkin Elmer Spectrum BSw. On a compact advion mass spectrometer, mass spectra were recorded and melting points were determined using a digital melting point apparatus and Uncorrected. The compounds 3aA–3mA were prepared in accordance with published procedures.[15–17] All chemicals were obtained from commercial sources and used without any additional purification.

General Methods of Synthesis of Chalcone Derivatives

(*E*)-1-(4-aminophenyl)-3-substitutedphenylprop-en-2-one (1a-1m)

1.35 gm (0.01 mol) of *p*-Aminoacetophenone was taken in a 250 mL conical flask and dissolved in 15 mL ethanol. To this, 10 mL of sodium hydroxide (40%) solution was added dropwise (maintaining 0˚C) with continuous stirring. then after, the equimolar of the aldehyde derivative was added in small parts (maintaining 0˚C). The solution was kept on a magnetic stirrer for 2 hours (at room temperature) stirring. After 2 hours, 100mL of ice-cold water was added to the solution with vigorous stirring. The solution was allowed to stand for 5-10 minutes and filtered using a vacuum pump. The solid mass obtained was kept for air drying. After drying, the product was collected, weighed, and %yield was calculated. TLC was performed to check the purity of the product by using Chloroform: Methanol (9.5:0.5 v/v) mobile phase.

(*E*)-2-chloro-*N*-(4-(3-(4-substitutedphenyl)acryloyl)phenyl)acetamide (2a-2m)
Compound 1(0.01 mol) was dissolved in 3 mL DMF in a conical flask and packed in an ice bath for maintaining 0˚C. Equimolar chloroacetylchloride was added using a dropping funnel with continuous stirring on a magnetic stirrer. The solution was allowed to stir for 4 -5 hours (0˚C maintained). After stirring, 100 mL of ice-cold water was added to the solution with vigorous stirring. The solution was allowed to stand for 5-10 minutes and then filtered using a vacuum pump. The solid mass obtained was kept for air drying. After drying, the product was collected and weighed and the %yield was calculated. TLC was performed to check the purity of the product by using Chloroform: Methanol (9.5:0.5 v/v) mobile phase.

(*E*)-*N*-(4-(3-(substitutedphenyl)acryloyl)phenyl)-2-(1*H*-imidazol-1-yl)acetamide (3aA-3mA)
Compound 2(0.01 mol), equimolar potassium carbonate, equimolar imidazole, and 20 mL of DMF was taken in a conical flask. The mixture was stirred for 15-16 hours on a magnetic stirrer at room temperature. After stirring, 100 mL of ice-cold water was added with vigorous stirring. The solution was allowed to stand for 5-10 minutes and then filtered using a suction pump. The solid mass obtained was kept for air drying. After drying, the product was collected, and %yield was calculated was performed to check the purity of the product by using the Chloroform: Methanol (9:1 v/v) mobile phase. All the compounds were purified by column chromatography.

Characterization Data of Synthesised Compounds:
(*E*)-*N*-(4-cinnamoylphenyl)-2-(1*H*-imidazole-1-yl)acetamide (3aA)
Yield:52% m.p.:142-150°C, IR (KBr Cm^{-1}): 3435(NH str), 2854 (C–H str), 1703 (C=O str), 1598 (C=C str), 947(trans ethylenic H), 870,,836(CH arom bend), ^{1}H-NMR (DMSO, ppm, 500MHz):4.9 (s, 2H, -CH_2-), 6.9 (d, 1H, J=5.81 Hz, imidazole), 7.1 (d, 1H, J=5.83 Hz, Imidazole), 7.6 (s, 1H, J=5.83 Hz, imidazole), 7.4-7.6 (m, 7H, 2xAr-H), 7.7 (d, 1H, =CH), 8.1 (d, 1H, J= 11.29 Hz, =CH) 10.6 (s, 1H, J=5.31 Hz, NH), EI-MS m/z:332.3 ($M+H^+$).

(*E*)-*N*-(4-(3-(2,4-dichlorophenyl)acryloyl)phenyl)-2-(1*H*-imidazol-1-yl)acetamide (3bA)
Yield:59% m.p.:150-155°C, IR (KBr Cm^{-1}): 3430(NH str), 2926 (C–H str), 1661 (C=O str), 1598 (C=C str), 1029(trans ethylenic H), 740(CH arom bend), ^{1}H-NMR (DMSO, ppm, 500MHz):4.9 (s, 2H, -CH_2-), 6.9 (d, 1H, J=5.09 Hz, imidazole), 7.2 (d, 1H, J=6.6 Hz, Imidazole), 7.6 (s, 1H, J=10.05 Hz, imidazole), 7.7-8.0(m, 7H, 2xAr-H), 7.9 (d, 1H, J=10.49 Hz, =CH), 8.2 (d, 1H, =CH), 10.7 (s, 1H, J=5.31 Hz, NH), EI-MS m/z:400.2 ($M+H^+$).

(*E*)-*N*-(4-(3-(2-chlorophenyl)acryloyl)phenyl)-2-(1*H*-imidazol-1-yl)acetamide (3cA)
Yield:61% m.p.:130-135°C, IR (KBr Cm^{-1}): 3256 (NH str), 2829 (C–H str), 1689 (C=O str), 1595 (C=C str), 986(trans ethylenic H), 875,,845(CH arom bend), ^{1}H-NMR (DMSO, ppm, 500MHz):4.7 (s, 2H, -CH_2-), 7.1 (d, 1H, J=5.09 Hz, imidazole), 7.3 (s, 1H, J=4.44 Hz, imidazole), 7.4 (d, 1H, J=10.27 Hz, imidazole), 7 .5-7.9(m, 7H, 2xAr-H), 8.0 (d, 1H, J=10.49 Hz, =CH) 9.4 (s, 1H, J=3.64 Hz, NH), EI-MS m/z:366.3 ($M+H^+$).

(*E*)-*N*-(4-(3-(4-methoxyphenyl)acryloyl)phenyl)-2-(1*H*-imidazol-1-yl)acetamide (3dA)
Yield:71% m.p.:187-193°C, IR (KBr Cm^{-1}) : 3324(NH str), 2917 (C–H str), 1704(C=O str), 1598 (C=C str), 1030(trans ethylenic H), 748(CH arom bend), ^{1}H-NMR ($CDCl_3$, ppm, 500MHz): 3.8 (s, 3H, J=12 Hz, OCH_3), 4.9 (s, 2H, J=5.99 Hz , -CH_2-), 6.4(d, 1H, =CH), 6.9(d, 1H, J=7.88 Hz, imidazole), 7.25 (s, 1H, imidazole), 7.4-7.8(m, 8H, 2xAr-H), 8 (d, 1H, J=10.49 Hz, =CH), 8.8 (s, 1H, J=5.31 Hz, NH), EI-MS m/z:362.4 ($M+H^+$).

(*E*)-*N*-(4-(3-p-tolylacryloyl)phenyl)-2-(1*H*-imidazol-1-yl)acetamide (3eA)
Yield:71% m.p.:222-230°C, IR (KBr Cm^{-1}): 3272(NH str), 2931 (C–H str), 1689 (C=O str), 1594 (C=C str), 984(trans ethylenic H), 875,836(CH arom bend), ^{1}H-NMR ($CDCl_3$, ppm, 500MHz):2.3(s, 3H,, J=8.10 Hz, CH_3), 4.9 (s, 2H, J=4.64 Hz, -CH_2-), 6.4 (d, 1H, J=4.23 Hz, =CH), 6.5 (d, 1H, J=2.9 Hz, imidazole), 6.9 (d, 1H, J=4.15 Hz, imidazole), 7.4-7.8(m, 7H, 2xAr-H), 7.9 (d, 1H, j=4.15 Hz, =CH), 8 (d, 1H, J=5.39 Hz, Imidazole), 8.8 (s, 1H, J=3.18 Hz, NH), EI-MS m/z:346.4 ($M+H^+$).

(*E*)-2-(1*H*-imidazol-1-yl)-*N*-(4-(3-(4-isopropylphenyl)acryloyl)phenyl)acetamide (3fA)
Yield:87% m.p.:175-180°C, IR (KBr Cm^{-1}): 3321 (NH str), 2959 (C–H str), 1683 (C=O str), 1601(C=C str), 986 (trans ethylenic H), 819 (CH arom bend),^{1}H-NMR ($CDCl_3$, ppm, 500MHz):1.1 (d, 6H, J=25, $(-CH3)_2$, 2.8-2.9(sept, 1H,, J=4.18 Hz, -CH-), 4.8 (s, 2H, J=8.14 Hz, $-CH_2-$), 7.1 (d, 1H, J=8.52 Hz, =CH), 7.2 (d, 1H, J=3.54 Hz, Imidazole), 7.4 (d, 1H, J=4.8 Hz, imidazole), 7.5-7.8(m, 9H, 2xAr-H and =CH), 7.9 (d, 1H, J=7.43 Hz, imidazole), 8 (d, 1H, J=5.39 Hz, Imidazole), 9.6 (s, 1H, J=3.53 Hz, NH), EI-MS m/z:374.4 ($M+H^+$).

(*E*)-2-(1*H*-imidazol-1-yl)-*N*-(4-(3-(3,4,5-trimethoxyphenyl)acryloyl)phenyl)acetamide (3gA)
Yield:88% m.p.:180-185°C, IR (KBr Cm^{-1}): 3321 3400 (NH str), 2941 (C–H str), 1711 (C=O str), 1598 (C=C str), 997(trans ethylenic H), 826 (CH arom bend),^{1}H-NMR ($CDCl_3$, ppm, 500MHz): 3.8 (s, 6H, $(OCH3)_2$, 3.9 (s, 3H, OCH_3), 4.8 (s, 2H, J=5.26 Hz, $-CH_2-$), 6.8 (s, 2H, J=6.15 Hz, Ar-H), 7.1 (d, 1H, J=2.93, Hz Imidazole), 7.2 (s, 1H, J=2.17 Hz, imidazole), 7.3(s, 1H, J=3.99 Hz, Imidazole), 7.6-7.8 (m, 4H, J=12 Hz, Ar-H), 8.1 (s, 1H, J=3.20 Hz, NH), EI-MS m/z:422.4 ($M+H^+$).

(*E*)-2-(1*H*-imidazol-1-yl)-*N*-(4-(3-(4-nitrophenyl)acryloyl)phenyl)acetamide (3hA)
Yield:69% m.p.:145-52°C, IR (KBr Cm^{-1}): 3435 (NH str), 2935 (C–H str), 1699 (C=O str), 1599(C=C str), 975(trans ethylenic H), 854,,830(CH arom bend),^{1}H-NMR (DMSO, ppm, 500MHz): 4.9 (s, 2H, J=11.15 Hz, $-CH_2-$), 6.9 (d, 1H, J=5.37 Hz, =CH), 7.1 (d, 1H, J=5.54 Hz, Imidazole), 7.7-8.2 (m, 7H, 2xAr-H and =CH), 8.3(d, 1H, j=12.69 Hz, imidazole), 10.7 (s, 1H, J=5.74 Hz, NH), EI-MS m/z:377.4 ($M+H^+$).

(*E*)-*N*-(4-(3-(furan-2-yl)acryloyl)phenyl)-2-(1*H*-imidazol-1-yl)acetamide (3iA)
Yield:89% m.p.:210-215°C, IR (KBr Cm-^{1}) : 3300(NH str), 3113 (C–H str), 1704 (C=O str), 1599 (C=C str), 968, 926(trans ethylenic H), 883, 818(CH arom bend), ^{1}H-NMR (DMSO, ppm, 500MHz): 4.79(s, 2H, J=13.72 Hz, -CH2-), 6.6-6.7 (t, 1H, J=5.62 Hz, furan), 6.9 (d, 1H, J=5.9 Hz, =CH), 7.1 (d, 1H, J=5.87 Hz, imidazole), 7.5(d, 1H, J=11.97 Hz, imidazole), 7.6 (s, 1H, J=7.78 Hz, imidazole), 7.8(d, J=6.54 Hz, furan), 7.4(m, 5H, Ar-H and furan), 8.1 (d, 1H, j=12.71 Hz, =CH), 10.7 (s, 1H, J=6.17 Hz, NH), EI-MS m/z:322.3 ($M+H^+$).

(*E*)-2-(1*H*-imidazol-1-yl)-N-(4-(3-(naphthalen-1-yl)acryloyl)phenyl)acetamide (3jA)
Yield:90% m.p.:222-230°C, IR (KBr Cm^{-1}): 3435 (NH str), 2923 (C–H str), 1702 (C=O str), 1651 (C=C str), 913(trans ethylenic H), 834(CH arom bend), ^{1}H-NMR (DMSO, ppm, 500MHz): 4.9 (s, 2H, J=8.75 Hz, $-CH_2-$), 6.9 (d, 1H, J=4.44 Hz, =CH), 7.7-7.8 (d, 1H, J=9.87 Hz, Imidazole), 7.1-7.6 (m, 4H, J=20.67, Ar-H), 8.1-8.4 (m, 6H, J=14.8 Hz & 19.5 Hz, Napth-H), 8.5 (d, 1H, j=4.69 Hz, =CH), 10.7 (s, 1H, J=6.17 Hz, NH), EI-MS m/z:382.4 ($M+H^+$).

(*E*)-*N*-(4-(3-(4-fluorophenyl)acryloyl)phenyl)-2-(1*H*-imidazol-1-yl)acetamide (3kA)
Yield:69% m.p.:146.150°C, IR (KBr Cm^{-1}): 3274 (NH str), 2832 (C–H str), 1688 (C=O str), 1598 (C=C str), 982(trans ethylenic H), 875,,822(CH arom bend), ^{1}H-NMR (DMSO, ppm, 500MHz): 4.6 (s, 2H, J=5.74 Hz, $-CH_2-$), 7.1 (d, 1H, J=11.17 Hz, imidazole), 7.2(s, 1H, J=11.17 Hz, imidazole), 7.5 (d, 1H, J=6.03 Hz, imidazole), 7.6-7.7 (m, 10H, J=20.67 Hz, Ar-H and =CH), 8.0 (d, 1H, j=10.42 Hz, =CH), 9.3 (s, 1H, J=4.28, Hz NH), EI-MS m/z:350.3 ($M+H^+$).

(*E*)-*N*-(4-(3-(3,4-dimethoxyphenyl)acryloyl)phenyl)-2-(1*H*-imidazol-1-yl)acetamide (3lA)
Yield:71% m.p.:198-201°C, IR (KBr Cm^{-1}): 3436 (NH str), 2926 (C–H str), 1705 (C=O str), 1598 (C=C), 1023(trans ethylenic H), 806(CH arom bend), ^{1}H-NMR ($CDCl_3$, ppm, 500MHz): 3.9 (S, 6H, J=18.63 Hz, 2xOCH_3), 4.8 (s, 2H, J=4.22 Hz, $-CH_2-$), 6.8 (d, 1H, J=2.96 Hz, =CH), 7.1 (d, 1H, J=3.38 Hz, =CH), 7.6-7.8 (m, 6H, Ar-H and imidazole), 7.6-7.8 (m, 3H, Ar-H), 7.9(d, 1H, J=6.03 Hz, imidazole), 8.0 (s, 1H, NH), EI-MS m/z:392.3 ($M+H^+$).

(*E*)-2-(1*H*-imidazol-1-yl)-*N*-(4-(3-(thiophen-2-yl)acryloyl)phenyl)acetamide (3mA)
Yield:71% m.p.:165-131°C, IR (KBr Cm^{-1}): 3435(NH str), 2925 (C–H str), 1653 (C=O str), 1598 (C=C str), 966(trans ethylenic H), 820(CH from bend), ^{1}H-NMR (DMSO, ppm, 500MHz): 4.9(s, 2H, J=4.22 Hz,

-CH_2-), 6.9 (d, 1H, J=2.96 Hz, =CH), 7.2 (d, 1H, J=9.96 Hz, =CH), 7.5-7.9 (m, 10H, Ar-H, thiophene and imidazole), 8.1 (s, 1H, J=9.07 Hz, imidazole), 10.6 (s, 1H, NH), EI-MS m/z:338.3 ($M+H^+$).

Molecular Docking Simulations

The Schrodinger suite was utilized to perform molecular docking simulation on the synthesized compounds (3aA-3mA) in order to comprehend the binding affinities of different atom/aromatic ring/functional groups present in the Aromatase enzyme receptor's active site. The protein data bank[18] provided the X-ray crystal structure of human placental aromatase complexed with the third-generation aromatase inhibitor exemestane, a medication used to treat breast cancer. Using a series of steps, including the addition of hydrogen atoms, the removal of water molecules and tautomer optimization, cap termini, co-crystallized ligand, and H-bonding network optimization, the protein preparation wizard (Schrodinger LLC) in Maestro-13.8 has been utilized to refine and prepare protein structure. Using Epik v7, the protonation states of the entire system were brought to neutral pH, and the OPLS2005 force field was used to optimize the geometry up to a maximum of 2 Å RMSD. The chemical structures of the designed compounds were constructed using Maestro 13.8, and force field OPLS2005 was utilized to optimize their energy. Schrodinger, LLC, New York, NY's LigPrep (version 4.7) was utilized to investigate the ligands with every potential tautomer while preserving their stereochemistry. It generated multiple conformations using the maestro's config module. All the compounds were generated into their ionization states using the Epik program. With the help of the Glide docking program, to determine the binding locations of protein 3S7S, docking studies were performed with the energy-minimized ligands. For the cantered corresponding ligand, the protein preparation wizard was utilized, utilizing an outer grid box measuring 25×25×25 $Å^3$ and an internal grid box measuring 10×10×10 $Å^3$. A potential molecular docking with a van der Waals radii scale of 0.7 was carried out during the first gliding docking stage. 5.0 Å of ligand poses' residues were free to move during the first refining stage. In order to dock the ligands to the binding site, the extra precision (XP) glide algorithm was employed. The optimal position for ligand-receptor interactions was determined by calculating the affinity and binding with the least G-score, which was then tested. [19–21]

***In silico* ADME Pharmacokinetic Studies**

For the *in silico* ADME prediction investigations, the QikProp (v4.8) module of the program was utilized. This module helps evaluate the theoretical ADME features of the ligands to forecast the drug-likeness (Lipinski RO5) values of the ligands. Aqueous solubility (QPlogS), octanol/water partition coefficient (QPlogPo/w), water/gas partition coefficient (QPlogPw), and H-bond acceptor and donor were among the pertinent properties that the software computed.[13]

***In vitro* Cytotoxicity Assay**

The primary rationale for choosing the MCF-7 cell line of breast cancer is that it functions as a model for estrogen receptor-positive (ER+) breast cancers due to its expression of the estrogen receptor (ER), which makes these cells estrogen-sensitive. The aim of this study was to evaluate the chalcones' inhibitory action against the enzyme aromatase, which is crucial for the development of estrogen receptor-positive breast cancer (ER+) by aromatizing and changing to estrone from androstenedione. MCF-7 was well matched to the study's aims and objectives because the triple-negative breast cancer cell line (MDA-MB-231) does not express any hormone receptors.[22,23]

The cell line of MCF-7 human breast adenocarcinoma was acquired from NCCS, Pune, India, under passage number 39. The flask was then propagated and allowed to thaw in a T-25 flask that had five passages of DMEM media, 5% FBS, MEM non-essential amino acid HEPES solution, and an antibiotic-antimycotic solution. Using the MTT assay, all chalcone derivatives at concentrations of 10, 25, 50, 100, 200, and 300μM were assessed for the growth inhibition activity against the MCF-7 breast cancer cell line, following the ATCC protocol. Since it is a common medication used to treat breast cancer, a non-steroidal aromatase inhibitor of third-generation letrozole was used as a reference drug.[24] Graph pad prism software (ver.7) was used to measure the IC_{50} values based on the nonlinear graph of percent cell inhibition vs. log concentration.

***In vitro* Aromatase Inhibitory Assay**

The aromatase inhibitory effect of four compounds (3gA, 3jA, 3kA, and 3lA) was evaluated using the Aromatase (CYP19A) Inhibitor Screening Kit (ab284522) according to the manufacturer's instructions. Briefly, the reaction was carried out in a 96-well plate format with a total reaction volume of 100 µL. Each reaction mixture contained 50 µL of recombinant aromatase enzyme, 30 µL of aromatase substrate, and 20 µL of letrozole or the test sample (positive control). The test compounds were tested at various concentrations (1.6 µM, 3.2 µM, 6.25 µM, 12.5 µM, and 25 µM) in separate reactions to determine their inhibitory effect at these concentrations. Baseline correction was applied to a reaction mixture that contained only the aromatase substrate (30 µL) and aromatase assay buffer (70 µL). The Varioskan Flash Spectral Scanning Multimode Reader (Thermo Scientific) was used to analyze the reaction mixture. The reaction mixture was incubated for 10 minutes at 37°C, allowing the inhibitor (test compound or letrozole) to react with the aromatase enzyme. Following incubation, the fluorescence was immediately (within 1 minute) measured at excitation/emission wavelengths of 488/527 nm in kinetic mode for 60 minutes using a plate reader. IC_{50} values were calculated using GraphPad Prism software (version 7) by plotting a nonlinear regression curve of percentage inhibition versus the logarithm of compound concentration.

RESULTS AND DISCUSSION

Chemistry

The first step was performed with aldol condensation and the chalcone derivatives were synthesized (1a-1m). In the second step, Chalcone derivatives were reacted with the chloroacetyl chloride and different chloroacetamide chalcone derivatives were synthesized (2a-2m). In the last step, a Total of 13 compounds of imidazole containing chalcone derivatives were synthesized (3aA-3mA). The route of reaction is depicted in scheme 1. The physicochemical parameter and spectroscopical characterization results are in good consonance with their structure and are shown in the section of the Experiment.

Molecular Docking

For docking studies, the human placental aromatase crystal structure complexed with the breast cancer drug exemestane (PDB Id. 3S7S) was chosen. Table -1 provides the docking score with binding energy for each chalcone derivative containing the aromatase enzyme (PDB ID: 3S7S), along with the corresponding intermolecular energy, hydrogen bonding, electrostatic energy, and hydrophobic interaction. Fig. (1) shows the interaction between Exemestane and the aromatase enzyme. When compared to the chosen ligand, the 3jA derivative of the 3aA–3mA series of compounds exhibited the lowest binding energy; the docking score for this series of chalcone derivatives ranged from -8.7 to -6.1 kcal/mol. The "N" atom of the compound 3iA and 3mA imidazole ring is shown in Fig.-2 and 3 to exhibit both aromatic hydrophobic and H-bonding interactions with the amino acids MET374 and ALA438. Other derivatives, including 3dA, 3eA, 3gA, 3hA, 3iA, 3kA, 3lA, and 3mA, also showed significantly stronger binding energies than the ligand.

Table-1: Docking Score of Synthesised Chalcone Derivatives

Compound Code	Docking score	Glide energy	Glide model	XP H Bond
3aA	-5.395	-51.604	-67.692	-0.757
3bA	-5.942	-44.44	-70.935	-0.423
3cA	-5.395	-51.604	-67.692	-0.757
3dA	-7.104	-50.921	-75.641	-0.35
3eA	-7.121	-47.652	-71.586	-0.287
3fA	-4.555	-48.038	-72.579	0
3gA	-6.012	-51.306	-74.643	0
3hA	-6.275	-54.557	-86.579	-2.55
3iA	-7.679	-40.543	-60.661	-0.993
3jA	-8.778	-52.542	-77.996	-0.159
3kA	-7.632	-46.899	-68.493	-0.388
3lA	-6.124	-52.371	-73.592	-0.193
3mA	-7.839	-50.937	-70.253	-0.869
Exemestane	-8.252	-42.387	-58.509	-0.7

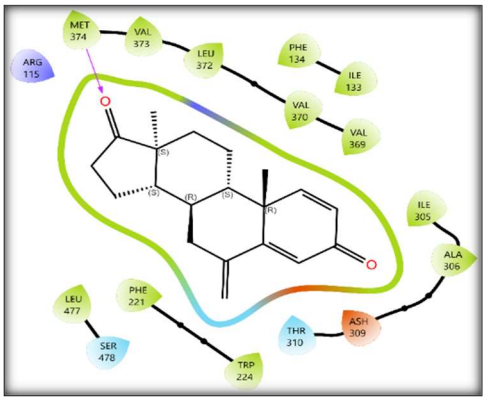


Fig.-1: Ligand-Target interaction of Exemestane

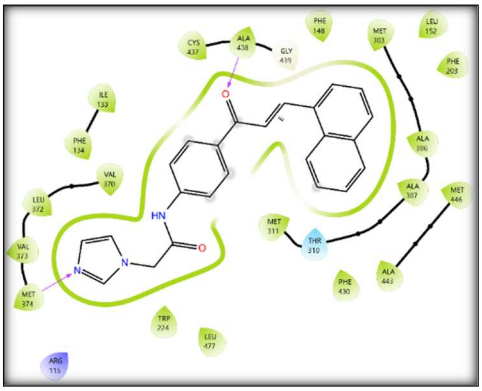


Fig.-2: Ligand-Target Interaction of 3iA

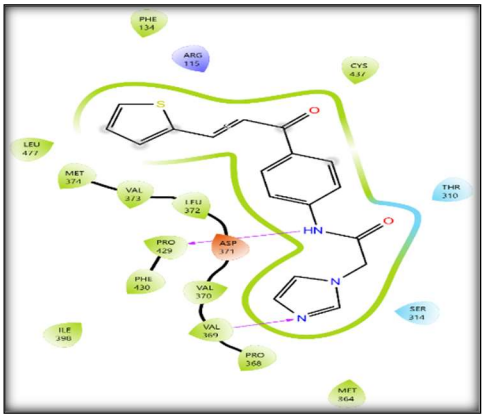


Fig.-3: Ligand-Target Interaction of 3Ma

In Silico ADME Prediction

The QikProp module (V5.1, 2023) of Maestro software was used to test the ADME properties of all analogs. The results showed that all chalcone derivative parameters, such as Lipinski's rule of 5(LRoF), water/gas partition coefficient (QPlogpw), Hydrogen bond donor/acceptor, predicted octanol/gas partition coefficient, aqueous solubility (QPlogS) and log (QPlogPoct), coefficient of octanol/water partition (QPlogPo/w)[25] were within their standard values. Table-2 displays the obtained values for all of the analogues, which are within the typical range. As a result, each analog had attributes that were appropriate for the ADME.

In vitro Assay for Cytotoxic Activity (MTT Assay)

Thirteen chalcone derivatives were synthesized and evaluated for their *in vitro* cytotoxicity assay on the MCF7 cell line by the MTT assay. The IC_{50} values were calculated using the Graph Pad Prism software

(ver. 7) by nonlinear graph (% cell inhibition vs. log concentration). On the other hand, compounds 3gA, 3jA, 3kA, and 3lA were discovered to be more effective derivatives of chalcone, with IC_{50} values of 18.13±1.19 µM, 21.71±1.61 µM, 16.36±1.47 µM, and 21.06±1.87 µM, respectively. Throughout the MTT assay, letrozole was used as the standard compound. Furthermore, letrozole's IC_{50} value varied according to the estimation technique. Letrozole IC_{50} values were found to be 30.39±1.41 µM. Four compounds (3gA, 3jA, 3kA, and 3lA) were selected for additional *in vitro* aromatase inhibitory activity based on the MTT assay results (Table-3).

Table-2: *In Silico* ADME Properties of Chalcone Derivatives (3aA-3mA)

Compound ID	donorHB	accept	QPlogPoct	QPlogPw	QPlogPo/w	QPlogS	Violation of LRO5
Std Value	NMT 5.0	NMT 10.0	8 - 43	5 - 48	-2.0 - 6.0	6.0 - 0.5	Nil
3aA	1	6.5	18.737	11.546	3.813	-5.41	0
3bA	1	6.5	19.441	11.313	4.31	-6.163	0
3cA	1	6.5	18.737	11.546	3.813	-5.41	0
3dA	1	7.25	18.903	11.899	3.454	-4.885	0
3eA	1	6.5	18.573	11.433	3.686	-5.368	0
3fA	1	6.5	19.68	11.22	4.363	-6.238	0
3gA	1	8.75	20.733	12.554	3.697	-5.489	0
3hA	3	7.5	22.495	15.408	2.418	-4.707	0
3iA	1	7	17.176	11.857	2.706	-4.037	0
3jA	1	6.5	20.214	12.366	4.3	-5.978	0
3kA	1	6.5	18.327	11.556	3.635	-5.238	0
3lA	1	8	19.675	12.242	3.548	-5.28	0
3mA	1	6.5	17.589	11.316	3.324	-4.836	0

Table-3: IC_{50} Value of Synthesized Compounds (*In Vitro* Cytotoxicity Assay)

Compound Code	IC_{50} (µM)
3aA	26.97±2.12
3bA	60.33±3.69
3cA	35.16±2.56
3dA	39.49±3.09
3eA	26.73±2.11
3fA	28.71±1.79
3gA	18.13±1.19
3hA	42.98±3.54
3iA	32.02±2.41
3jA	21.71±1.61
3kA	16.36±1.47
3lA	21.06±1.87
3mA	86.29±4.29

In vitro Aromatase Inhibitory Assay

Four chalcone derivatives, namely 3gA, 3jA, 3kA, and 3lA, were evaluated for their ability to inhibit *in vitro* aromatase activity using the Fluorogenic Kit for aromatase inhibition testing. It was determined that the untreated group's fluorescence intensity represented 100% aromatase activity. the concentration ranges for the standard compounds and test strength were as follows: 1.6 µM, 3.2 µM, 6.25 µM, 12.5 µM, 25 µM, and 50 µM. The fluorogenic substrate used in the experiment was converted into a highly fluorescent metabolite observable in the visible spectrum (Ex / Em = 488/527 nm) and detected using a Thermo scientific Varioscan Flash Spectral Scanning Multimode reader in percent inhibition mode. Graph Pad Prism software (ver.9) was used to measure the IC_{50} values based on the nonlinear graph that was plotted between the log concentration and percent inhibitions. IC_{50} value data is shown as mean ± SEM. Using a student t-test, the mean of IC_{50} of the chosen compounds was compared to letrozole, the reference medication.[13,24] The IC_{50} value of letrozole was obtained to be 0.98 ±0.03 µM. Compounds 3gA 2-(1H-imidazol-1-yl)-N-(4-(3-(3,4,5-trimethoxyphenyl)acryloyl)phenyl)acetamide and 3lA N-(4-(3-(3,4-dimethoxyphenyl)acryloyl)phenyl)-2-(1H-imidazol-1-yl)acetamide were found to be 2.76±0.83 µM and

3.00±1.63 µM, respectively compared with Letrozole quite higher. For the remaining Chalcone derivatives with similar activity, the IC_{50} values were 3jA (6.457±3.381µM) and 3kA (4.823±1.523 µM), in that order.

CONCLUSION

Molecular docking was carried out on a set of synthesized 13 imidazole derivatives. According to the QikProp software results, all synthesized compounds exhibited drug-like properties. An aldol condensation reaction was used to synthesize chalcones from bases. Finally, 13 compounds were assessed for cytotoxicity *in vitro* on the MCF-7 breast cancer cell line. Among the four compounds tested, 3gA, 3jA, 3kA, and 3lA were found to be potent in an acceptable range with reference drug letrozole having a 30.39±1.41 µM IC_{50} value. Using a fluorogenic assay kit, the *in vitro* aromatase inhibitory activity of the compounds 3gA, 3jA, 3kA, and 3lA was ascertained. The IC_{50} values of compounds 3gA 2-(1H-imidazol-1-yl)-N-(4-(3-(3,4,5-trimethoxyphenyl)acryloyl)phenyl)acetamide and 3lA N-(4-(3-(3,4-dimethoxyphenyl)acryloyl)phenyl)-2-(1H-imidazol-1-yl)acetamide were found to be 2.76 ± 0.83 µM and 3.00 ± 1.63 µM. Compounds 3gA and 3lA showed comparable aromatase inhibitory activity to the reference compound letrozole (IC_{50} = 0.98 ± 0.03 µM). The -OCH_3 group on the chalcone ring is the common structural element that inhibits aromatase. However, it was believed that the other two compounds, 3jA, and 3kA, had comparable inhibitory effects against letrozole. The imidazole's "N" and the amino acid's C=O interacted in the docking results, confirming that the result matched the *in vitro* aromatase inhibition activity. Their noteworthy aromatase inhibition properties thus provide them attractive options for the creation of aromatase inhibitors.

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CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest.

AUTHOR CONTRIBUTIONS

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