Design, Synthesis and Molecular Docking Study of N-Heterocyclic Chalcone Derivatives as an Anti-cancer Agents

Bharti Fegade*, Shailaja Jadhav

Modern College of Pharmacy, Nigadi, Pune 411044, Maharashtra, India.

ABSTRACT

The Claisen-Schmidt condensation of 4-(aryl)-aminobenzaldehyde and 2-hydroxyacetophenone resulted in a new series of heterocyclic chalcone (4a-4g) derivatives. Nucleophilic aromatic substitution (SNAr) of 4-fluorobenzaldehyde with heterocycle amines by ultrasonication in the presence of a base and polar aprotic solvent yielded 4-(aryl)-aminobenzaldehydes. Spectral investigations were used to establish the structures of synthesized compounds. The in vitro anti-cancer activity of the synthesized derivative was evaluated against MCF-7 (breast cancer) cells by SRB assay. Compounds 4c, 4b, and 4c have a high affinity for the ER receptor binding site, whereas compounds 4c and 4g have a moderate affinity for the VEGFR-2 receptor. The GI_{50} value of 4c ((E)-1-(2-hydroxyphenyl)-3-(4-(4-methylpiperazin-1-yl)-phenyl) prop-2- en-1-one) was 44.6 uM, while the GI_{50} value of all other derivatives was greater than 80 uM. These findings lay the groundwork for additional research into the combination’s potential uses in cancer therapy.

INTRODUCTION

Worldwide cancer is the most prevalent disease which causes severe health problems and excruciating side effects produced by chemotherapy and radiation-like treatment. Development in anti-cancer therapy focuses on the drugs of natural origin with no or very few side effects and target specific molecular signaling pathways. Chalcone represents a prime group of the flavonoid family, which consist of a large number of naturally occurring biomolecules reported to possess a wide spectrum of biological activities, including antioxidant,[1] anti-cancer,[2] antiprotozoal,[3] antimalarial,[4] anti-inflammatory,[5] antitubercular,[6] antibacterial,[7] and antiviral.[8] Chalcone is the very important class of intermediate precursor for the biosynthesis of flavonoids and the synthesis of biologically active 5- and 6-membered nitrogen heterocycles.[9] Chemically chalcone consists of a 1,3-diaryl-2-propen-1-one scaffold, in which two aryl rings are joined by three carbon alpha beta-unsaturated carbonyl systems. Various substituents are added on aryl rings, which modify binding interaction with different molecular targets and thus, improve biological activities. Some well-known examples of naturally occurring anti-cancer chalcone are butein, isoliquiritigenin, and isobavachalcone, which have been isolated from the bark of Rhus verniciflua, Glycyrrhiza glabra roots, and Psoralea corylifolia, respectively.[10-13]

Several reports are available that prove the antiproliferative activity of natural and synthetic chalcone against breast cancer.[14,15] Nitrogen-containing heterocyclic compounds have great importance in drug discovery than the non-nitrogen compound. These compounds play a vital role in metabolism in the living cell and their anti-cancer effect correlates with the force of interaction of DNA and heterocyclic compounds.[16] In this study, a
A variety of heterocyclic chalcone analogs were synthesized to diversify the biological activities of typical chalcone by substituting it with various nitrogen-containing heterocycles such as morpholine, piperidine, pyrrolidine, benzimidazole, imidazole, triazole, and piperazine, at the 4th position of the B ring of chalcone. The synthesis of these heterocyclic chalcone analogs was carried out by utilizing a Claisen Schmidt condensation reaction in the presence of a standard base. These compounds were evaluated for their anti-cancer activity against breast cancer cell lines. Computational molecular docking analysis was conducted on the synthesized compounds to examine their anti-cancer efficacy.

**Materials and Methods**

**Chemicals**

The reagents and solvents for synthesis were purchased from Loba-Chem, Spectrochem, and Sigma-Aldrich, and were used as received: 4-flourobenzaldehyde, morpholine, piperidine, pyrrolidine, benzimidazole, imidazole, triazole, piperazine, 2-hydroxy-acetophenone.

**Instrumentation**

The digital melting point apparatus determined melting points. FTIR spectra were obtained by Agilent Cary 630 FTIR Spectrometer for the range 4000–450 cm⁻¹. Waters Alliance E 2695/HPLC-TQD and UPLC/XEVO G2-XS QTOF Mass spectrometer were used to detect mass spectra. ¹H and ¹³C-NMR spectra were recorded by Brucker Avance II 400 MHz and Brucker Avance Neo 500 MHz spectrometers. The synthesis of intermediate aldehydes was carried out using PCI Analytics ultrasonicator. The synthesized compounds were tested in vitro for anti-cancer efficacy at ACTREC’s Anti-Cancer Drug Screening Facility (ACDSF) (Tata Memorial Centre, Navi Mumbai, India).

**Synthesis of Target Compounds**

The target chalcone was synthesized by Claisen-Schmidt condensation of 4-(aryl)-aminobenzaldehyde 2a-g and 2-hydroxyacetophenone 3 in the presence of a base. Nucleophilic aromatic substitution (SNAr) reaction of haloarenes with amines is a challenging process that is usually limited to fluorides or chlorides with a strong electron-withdrawing group, such as the nitro group at para position.[17] Using a polar aprotic solvent like DMSO and potassium carbonate as the base, 4-(aryl)-aminobenzaldehyde was produced via nucleophilic aromatic substitution (SNAr) reaction of 4-flurobenzaldehyde with various five and six membered heterocyclic amines under the influence of ultrasonic irradiation. The SNAr reaction on 4-fluorobenzaldehyde 1 was sped up from 5 hours to 15 minutes using ultrasound, and yields of 4-alkyl-(aryl)aminobenzaldehydes were 15–30 percent greater than in tests using a 5 hours thermal heating of the reaction mixture at 100 degrees Celsius.[18]

In the current study, various 4-(aryl)-aminobenzaldehydes were synthesized by sonicating a mixture of 4-flurobenzaldehyde, amine R, potassium carbonate, and DMSO for 30 minutes and then heating it at 100°C for 1 to 3 hours. The Claisen-Schmidt condensation of these substituted benzaldehydes with 2-hydroxyacetophenone was catalysed by a base, yielding chalcone 4a-g.[19] The complete synthesis route is depicted in Scheme 1.

### Step 1: Synthesis of 4-(aryl)-aminobenzaldehyde (2a-g)

In a DMSO solution of N-heterocyclic compound (20 mmol) and 4-flurobenzaldehyde (20 mmol), potassium carbonate (3.04 g; 1 mmol) was added (20 cm³). For 30 minutes, the reaction mixture was sonicated. The reaction mixture was heated at 100°C for 1–3 hours, stirring after sonication. The reaction mixture was cooled to 60°C before being poured into 400 cm³ of distilled water, where solid crystals were...
rapidly separated. These crystals were filtered and utilized to make chalcone.

**Step 2: General procedure for the synthesis of chalcone (1,3-diphenylpropenone) derivatives (4a-g)**

2-hydroxycetophenone was added to a solution of ethanol (ml) and LiOH H2O (0.02 mole) and magnetically swirled for 10 minutes at room temperature. The appropriate (E)-arylaminobenzaldehyde was progressively added to the reaction mixture with stirring. The reaction was continued until all of the starting material had been consumed. TLC was used to examine the completion of the reaction. Crushed ice was added once the reaction was completed, and the reaction mixture was acidified with glacial acetic acid. The separated solid product was filtered and washed with a small amount of cold ethanol. The product was purified by column chromatography on silica gel (120 mesh, Merck), with a mixture of ethyl acetate and n-hexane as the mobile phase.

**[(E)-1-(2-hydroxyphenyl)-3-(4-(piperidin-1-yl)phenyl)prop-2-en-1-one (4b)]**

Orange colour, yield: 84%, mp: 176°C, λ_max: 446, IR (KBr, cm⁻¹): 2918 (C-H), 1610 (C=O), 1540 (C=C), 1370 (C-N), 1HNMR (500 MHz, CDCl₃): 6.95 (t, 1H, J = 7.5 Hz, H₂), 7.01 (d, 2H, J = 8.35 Hz, Ha, Hb), 7.24 (s, 1H, Hf), 7.43 (500 MHz, CDCl₃): 2.30 (3H, -CH₃), 2.50 (t, 4H, J = 5 Hz, H₂), 3.29 (t, 4H, J = 5 Hz, H₁), 6.83 (d, 2H, J = 8.8 Hz, Hf), 6.9 (t, 1H, J = 7.55 Hz, Hc), 6.99 (d, 1H, J = 8.05 Hz, Ha), 7.43 (m, 2H, J = 12.45, Hc, Hb), 7.51 (d, 2H, J = 8.75 Hz, Hf), 7.86 (m, 2H, J = 15.25 Hz, Hb, Hδ), 13.1 (s, 1H, -OH); 13CNMR (500 MHz, CDCl₃): 18.47 (-CH₃), 46.05 (C- 2°), 54.65 (C- 5°'), 57.88 (C- 3°'), 114.57 (C- 3° & 5'), 115.71 (C-3), 118.44 (C- β), 118.70 (C- 5), 120.27 (C- 1), 124.69 (C- 1°), 129.50 (C- 6), 130.59 (C- 2° & 6'), 135.85 (C- 4), 145.91 (C- γ), 152.88 (C- 4°) 163.47 (C- 2), 193.53 (C- α), ESI MS ES⁺ (m/z): 323 [M+H⁺] +; 322 calculated [M⁺] for C₂₀H₂₂N₂O₂.

**[(E)-1-(2-hydroxyphenyl)-3-(4-(pyrrolidin-1-yl)phenyl)prop-2-en-1-one (4c)]**

Red crystalline solid, yield: 87%, mp: 210°C, λ_max: 446, IR (KBr, cm⁻¹): 2918 (C-H), 1612 (C=O), 1514 (C=C), 1377 (C-N), 1HNMR (500 MHz, CDCl₃): 2.02 (s, 4H, J = 6.5 Hz, H₂), 3.35 (s, 4H, J = 6.5 Hz, H₂). 6.54 (d, 2H, J = 9 Hz, Hf), 6.9 (t, 1H, J = 7.5 Hz, Hc), 7.00 (d, 1H, J = 8 Hz, Ha), 7.44 (m, 2H, J = 15 & 7.5 Hz, Ha, Hb), 7.55 (d, 2H, J = 8.5 Hz, He), 7.92 (m, 2H, J = 12.5 Hz, Hb, Hδ), 13.27 (s, 1H, -OH); 13CNMR (500 MHz, CDCl₃): 25.45 (C- 3°'4°'), 47.61 (C- 2°'5°'), 111.84 (C- 3° & 5°'), 113.54 (C- 3), 118.45 (C- β), 118.54 (C- 5), 120.49 (C- 3'), 121.81 (C- 1'), 129.34 (C- 6), 131.11 (C- 2' & 6'), 135.53 (C-4), 146.92 (C- γ), 163.49 (C- 2), 193.43 (C- α); TOF MS ES⁺ (m/z): 294.15 [M+H⁺]; 293.14 calculated [M⁺] for C₁₉H₁₉NO₃.

**[(E)-1-(2-hydroxyphenyl)-3-(4-(1H-imidazol-1-yl)phenyl)prop-2-en-1-one (4d)]**

Red crystalline solid, yield: 87%, mp: 210°C, λ_max: 446, IR (KBr, cm⁻¹): 2918 (C-H), 1612 (C=O), 1514 (C=C), 1377 (C-N), 1HNMR (500 MHz, CDCl₃): 6.94 (t, 1H, J = 7.5 Hz, Hc), 7.04 (d, 1H, J = 8.38 Hz, Ha), 7.52 (t, 2H, J = 7.24 & 8.38 Hz, Hb), 7.69 (d, 1H, J = 15.52 Hz, Ha), 7.8 (m, 4H, J = 8.8 & 9 Hz, He & Hf), 7.92 (2, 2H, J = 7.76 & 15.44 Hz, Hd & Hδ), 8.15 (s, 1H, H₃), 8.65 (s, 1H, H₂), 12.13 (s, 1H, -OH); 13CNMR (500 MHz, CDCl₃): 117.73 (C- 3), 117.94 (C-β), 118.89 (C- 1), 119.14 (C- 3° & 5°'), 120.14 (C- 5), 128.62 (C- 6), 129.05 (C- 2' & 6'), 133.41 (C- 1'), 135.64 (C- 4), 137.34 (C- 4'), 139.99 (C- 5°'), 142.46 (C- γ), 151.97 (C- 3°' 4°'), 162.65 (C- 2), 192.33 (C- α); TOF MS ES⁺ (m/z): 292.11 [M+H⁺]; 291.10 calculated [M⁺] for C₁₉H₁₉NO₃.
Synthesis of N-Heterocyclic Chalcone derivatives

130.98 (C-4''), 133.6 (C-2''), 136.61 (C-4), 138.86 (C-4''), 143.6 (C-γ), 163.62 (C-2), 193.35 (C-α). ESI MS ES⁺ [m/z]: 291 [M+H]⁺; 290.11 calculated [M⁺] for C₁₈H₁₄N₂O₂.

(E)-3-(4-(1H-benzo[d]imidazol-1-yl)phenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one (4g)

Yellow crystalline solid, yield: 94%, mp: 192°C, λmax: 336, IR (KBr, cm⁻¹): 3122 (Ar-C), 1638 (C=O), 1562 (C≡C), 1343 (C-N). ¹HNMR (500 MHz, CDCl₃): 6.97 (t, 1H, J = 8 Hz, Hε), 7.05 (d, 1H, J = 8.5 Hz, Hα), 7.38 (m, 2H, J = 3 Hz, H₃, H₄), 7.52 (t, 1H, J = 8 Hz, Hb), 7.61 (t, 3H, J = 8.5 & 5.5 Hz, Hf & Hg), 7.72 (d, 1H, J = 15 Hz, Hδ), 7.87 (d, 2H, J = 8.5 Hz, He), 7.90 (m, 1H, J = 3 Hz), 7.94 (t, 2H, J = 15.5 & 9.1 Hz, Hβ & Hδ), 8.17 (s, 1H, Hδ), 12.75 (s, 1H, -OH); ¹³CNMR (500 MHz, CDCl₃): 110.49 (C-7''), 118.76 (C-1''), 119.95 (C-5), 120.90 (C-4''), 121.20 (C-5'' & 6''), 123.20 (C-3), 124.08 (C-2' & 6), 129.65 (C-6), 130.30 (C-3' & 5'), 133.6 (C-2''), 136.61 (C-4), 138.86 (C-4'), 139.35 (C-α); TOF MS ES⁺ [m/z]: 341.13 [M+H]⁺; 340.12 calculated [M⁺] for C₁₈H₁₄N₂O₂.

In-vitro Anti-cancer Activity

All the newly synthesized compounds were screened for their in vitro anti-cancer activity against MCF-7 cancer cell lines by SRB assay, using Adriamycin as a standard drug. The cell lines were grown in RPMI 1640 medium containing 10% fetal bovine serum and 2 mM L-glutamine. For the present screening experiments, cells were inoculated into 96 well microtiter plates at 5000 cells per well. After cell inoculation, the microtiter plates were incubated at 37°C, 5% CO₂, 95% air, and 100% relative humidity for 48 hours. After 24 hours before adding experimental drugs. Experimental drugs were solubilized in an appropriate solvent to prepare a stock of 10⁻² concentrations. At the time of the experiment, four 10-fold serial dilutions were made using the complete medium. Aliquots of 10 µL of these different drug dilutions were added to the appropriate microtiter wells already containing 90 µL of the medium, resulting in the required final drug concentrations. After compound addition, plates were incubated at standard conditions for 48 h., and the assay was terminated by the addition of cold trichloroacetic acid (TCA). Cells were fixed in situ by the gentle addition of 50 µL of cold 30% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 minutes at 4°C. The supernatant was discarded; the plates were washed five times with tap water and air-dried. Sulforhodamine B (SRB) solution (50 µL) at 0.4% (w/v) in 1% acetic acid was added to each of the wells, and plates were incubated for 20 minutes at room temperature. After staining, the unbound dye was recovered and the residual dye was removed by washing five times with 1% acetic acid. The plates were air-dried.

Docking Study

A molecular docking study was conducted against estrogen receptor (ERα) and vascular endothelial growth factor receptor (VEGFR-2) to discover and analyze the new chalcones' binding affinity, binding mechanism, and molecular interactions in the active site of estrogen receptors and vascular endothelial growth factor receptor. The X-ray crystallographic structures of ER and VEGFR-2 (PDB ids 2IOG and 1YWN, respectively) were retrieved with 1.60 A and 1.71 A resolutions from the Protein Data Bank. AutoDock 4.2.6 was used to carry out docking experiment and calculated binding energy of chalcones within the ER and VEGFR receptor binding site. The protein was isolated from the ligand. Water molecules were removed from the protein, and the polar hydrogen atoms and Kollman charges were added to the protein. The final prepared file was minimized by UCSF Chimera software and saved in PDBQT format for further analysis.

All chalcone derivatives were drawn in ChemDraw 20. These derivatives were imported to ChemBioDraw Ultra, subjected to MM2 and MMFF94 energy minimization, and saved in a PBD file. Final energy minimization was done by the UCSF chimera.

The area of the protein structure to be mapped was then selected using the three-dimensional grid box. The coordinates 16.732, 33.121, and 12.166 were used to center the grid box (x, y, and z, respectively). The Lamarckian Genetic Algorithm (LGA) was utilized during docking simulation for energy optimization and minimization. A collection of grid maps was constructed using AutoGrid 4.2 based on the available atom kinds. The run time of the genetics algorithm was set to 100, and the rest of the settings remained unchanged. The three-dimensional grid box was constructed around the active site of the target receptor. The box's dimensions were set to 60, 60, 60 A. The ER receptor grid box was oriented on the coordinates 22.630, -2.08, and 27.131 (x, y, and z, respectively), while the VEGFR2 receptor grid box was centered on the coordinates 3.50, 34.51, and 15.32 (x, y, and z, respectively). The Lamarckian Genetic Algorithm (LGA) was utilized during docking simulation for energy optimization and minimization. A collection of grid maps was constructed using AutoGrid 4.2.6 based on the available atom types. The genetic algorithm run was set to 50, and the rest of the settings parameters were kept as default. The visualization analysis of docked complexes of chalcone and ligand into ER and VEGFR2 receptor pocket was done by LigPlot and Biovia Discovery.
RESULT AND DISCUSSION

Chemistry

TLC was used to determine the purity of chalcone using a mobile phase of n-hexane: ethyl acetate (7:3). TLC showed the presence of a single compound. Experimental determination of the melting point of the new derivative was done by open capillary tubes. The structures of all the compounds were confirmed by IR, $^1$HNMR, $^{13}$CNMR, and MS spectroscopic techniques.

The IR spectra confirmed numerous essential characteristics of the synthesized molecules. The presence of the carbonyl group was illustrated by the peaks in the range of 1600–1642 cm$^{-1}$. Stretching was observed as a broad peak between 3300 and 3410 cm$^{-1}$ for the hydroxyl group. A C-N stretch was mostly seen in the range of 1343–1377 cm$^{-1}$. The well-known structural characteristics were proposed by $^1$HNMR spectroscopy. The proton of hydroxyl group appeared in the most downfield region of the spectrum, with a chemical shift of 12.2–13.27 ppm. The H-$\alpha$ and H-$\beta$ protons about the carbonyl group of chalcone appear as doublets (J=15Hz) and have values of 7.43-7.72 ppm and 7.86-7.95 ppm, respectively. The large J value (15 Hz) confirms the trans-type geometrical isomerism at the double bond. The carbonyl group of chalcone appears at 192-193 in its $^{13}$CNMR spectrum. The hydroxyl group of chalcone gives a characteristic peak at 163 in the $^{13}$CNMR spectrum. The mass spectra of all chalcone represent the base peaks that correspond to [M+H]$^+$.

In Vitro Anticancer Screening

The in vitro anti-cancer activity of new series of chalcone was evaluated by SRB assay against a human breast cancer cell line, MCF-7. Adriamycin was used as a positive control. The results of anti-cancer activity of investigated compounds have been expressed as the GI$_{50}$ values in uM, where GI$_{50}$ refers to the concentration of the synthetic compound necessary for 50% inhibition of cell growth. In vitro anti-cancer screening showed that the N-methyl piperazine chalcone derivative (4c) exhibits good growth inhibition of MCF-7 cell lines with a GI$_{50}$ value of 44.6 uM. All other synthesized chalcones of these series showed poor growth inhibition with a GI$_{50}$ value greater than 80 uM. The effects of adriamycin and chalcone derivatives on MCF-7 cell lines are shown in Fig 1.

Docking Study

Molecular docking is used to anticipate the ligand’s most prevalent binding interaction with the protein target with a known three-dimensional structure. In this study, a series of chalcone were docked in the active binding site of ER-$\alpha$ and VEGFR-2. A high estrogen level is associated with an increased risk of hormone-related breast cancer, which mediates distinct transcriptional responses by binding with estrogen receptors that are opposite effects of normal cellular processes.$^{[22]}$ The aberrant expression of Estrogen Receptor $\alpha$-positive particularly affects around 70% of primary breast cancer patients is one of the key causes of breast cancer.$^{[23,24]}$ Research findings reveal the overexpression of the VEGFR-2 receptor in breast cancer, which is responsible for angiogenesis.$^{[25,26]}$ As an outcome, treating breast cancer by targeting ER-$\alpha$ and VEGFR-2 in malignant cells might be beneficial.

Molecular docking was kicked off by control docking of co-crystallized IOG and LIF into the crystal structure of ER-$\alpha$ (PBD ID: 2IOG) and VEGFR-2 (PBD ID: 1YWN), respectively. The docking score was observed by the interaction between the active site residues of the target.
ER-α and VEGFR-2 with the chalcone derivatives and control compound displayed in Table 1. The chalcone derivatives 4b, 4c, and 4g had stronger binding interactions with the ER than the control compound IOG (Fig. 2), while the other chalcone had moderate binding with the receptor active site. The carbonyl and hydroxyl groups of the chalcone derivative form a strong hydrogen bond with the ER-active site. The carbonyl and hydroxyl groups of 4b and 4c form hydrogen bonds with LYSS31 and ASP351, respectively. The compound 4g showed different binding interactions than 4c and 4b, that is, with PRO325 and LEU327, and gave an excellent docking score compared to the control ligand, LIF. Docking studies of 4a-4g within the active site of VEGFR-2 showed moderate binding energies compared

Table 1: Docking score and GI_{50} values of synthesized compound against MCF-7

<table>
<thead>
<tr>
<th>Compound code</th>
<th>Human ER-α receptor</th>
<th>VEGFR-2 receptor</th>
<th>GI_{50} (µM) against MCF-7</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>-8.80</td>
<td>-7.10</td>
<td>&gt;80</td>
</tr>
<tr>
<td>4b</td>
<td>-9.44</td>
<td>-7.82</td>
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<tr>
<td>4c</td>
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<td>-11.24</td>
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</table>
with the control compound LIF. The chalcone 4b, 4c and 4g bind more strongly to VEGFR-2 than the other derivatives by showing more negative binding energy (Fig. 3). The major hydrogen binding interaction of chalcone with the VEGFR-2 binding site is due to the presence of the carbonyl group. The carbonyl group of chalcone 4c and 4g form 2 hydrogen bonds with GLU883 and LYS866. The nitrogen of the N-methyl piperazine ring of compound 4c forms an additional hydrogen bond with ILE023.

The study reveals the evolution of N-methyl piperazine chalcone derivative (4c) as an anti-breast cancer candidate. The docking study for 4c was in perfect accord with the cytotoxic study in terms of molecule access to the enzyme’s active regions. The compound 4b and 4g showed good docking score with estrogen receptor but failed to show the in vitro anti-cancer activity. Chalcone is a key step in synthesizing a variety of heterocyclic compounds. Many significant derivatives with a range of pharmacological properties will be produced using this synthesis technique of N-heterocyclic substituted chalcone.

REFERENCES

