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Synthesis and In Silico Study of 5'-Chloro-2',4-Dihydroxy-3-Methoxychalcone as Potential Inhibitors of Estrogen Receptors Alpha

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Abstract
The high prevalence of breast cancer necessitates the discovery of new, more effective, and safer anticancer compounds. Chalcone, a flavonoid with promising anticancer activity, is limited by poor bioavailability and rare natural occurrence. In this study, a novel chalcone derivative, 5'-chloro-2',4-dihydroxy-3-methoxychalcone, was synthesized on a laboratory scale via Claisen-Schmidt condensation using 5-chloro-2-hydroxyacetophenone and 4-hydroxy-3-methoxybenzaldehyde as starting materials. The structure of the synthesized compound was confirmed by mass spectrometry and NMR analysis. Pharmacokinetic and toxicity profiles were predicted in silico using SwissADME, while molecular docking simulations with the ERα receptor were performed using AutoDockTools. The compound was obtained as a yellow solid with a yield of 70.15%, showed favorable ADME properties based on Lipinski's and Veber's rules, and demonstrated a binding energy of -7.82 kcal/mol to the ERα receptor, indicating potential as an anticancer agent. The novelty of this research is the successfully synthesized and characterized of this specific chalcone, which is structurally different from previously reported analogs. These findings enrich the diversity of chalcone derivatives and provide a new basis for developing synthesis-based drug candidates targeting breast cancer.

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INTRODUCTION

The high prevalence of degenerative diseases, such as cancer and drug-resistant bacterial infections encourages the discovery of new bioactive compounds that are more effective and safer. Breast cancer is one of the most prominent causes of cancer deaths in women worldwide, thus requiring more effective and selective therapeutic approaches (Rana et al., 2022). One of the main targets in breast cancer therapy is the estrogen receptor (ER), particularly the ERα subtype, which plays an important role in the growth and proliferation of breast cancer cells. The compounds are potent to inhibit ERα activity or induce apoptosis through associated molecular pathways have become a major focus in anticancer drug development (Bender et al., 2023). One of the fascinating candidates is chalcone, a flavonoid compound that exhibits a wide range of biological activities such as antioxidants, antimicrobials, and anticancer, but its

bioavailability is poor and its natural structure is relatively rare (Ekanayake et al., 2022; Singh et al., 2020). Many chemicals, including naturally derived chalcones and synthetic analogs, show significant cytotoxic levels against cultured cancer cells. The current data shows that the synthesis of chalcones and their derivatives in the laboratory is a necessary approach, as the isolation from the plant yields low quantities and requires a high amount of time and cost. The modification of the structure can increase the potency and decrease the compound toxicity (Baroroh et al., 2023; Haque et al., 2023; Rahmawati & Sofia, 2022; Safitri et al., 2021).

Several previous studies have reported the successfully synthesized chalcones and examined their biological activities, but there are remains a gap regarding the relationship between the chemical structure of chalcones and their activity and toxicity, particularly in the modified compounds by certain functional groups (Dona et al., 2019; Singh et al., 2020; Shylaja et al., 2021; Susanti & Mulyani., 2022). In addition, most of the previous studies have only focused on one type of biological activity. There are not many that have simultaneously studied the toxicity and molecular interactions of chalcones with specific protein targets using an in silico approach (Nematollahi et al., 2023; Sangpheak et al., 2019).

This study aims to synthesize the 2',4-dihydroxy-5'-chloro-3-methoxycalcone via Claisen-Schmidt condensation reaction, to evaluate the ADME parameters (absorption, distribution, metabolism, and elimination), and to predict their molecular interaction with estrogen receptor α (ERα) using a molecular docking approach. Considering the importance of chalcones and flavone biosynthesis product, many research groups worldwide have been actively involved to synthesize and evaluate various synthetic derivatives and naturally occurring chalcones (Ahsan et al., 2025). The design and synthesis of new chalcone derivatives by researcher aims to explore their structure. This approach is expected to enrich the understanding of the structure-activity relationship of chalcone and to support the development of more effective and selective synthetic drug candidates (Daina et al., 2017; Pires et al., 2015).

METHOD

Materials and Tools

The chemicals and reagents used are sodium hydroxide, hydrochloric acid, 4-hydroxy-3-methoxybenzaldehyde, 5-chloro-2-hydroxyacetophenone, distilled water, 96% ethanol, ethyl acetate, n-hexane, and silica gel. The tools used in this study are a set of laboratory glassware, synthesis tools, chromatography column, Buchner funnel, glass funnel, desiccator, hotplate, magnetic stirrer, micropipette, analytical balance, TLC plates (silica gel 60 F_{254}), vacuum pump, UV lamps (254 nm and 366 nm). The chalcone was characterized using ^{1}H-NMR and ^{13}C-NMR (Bruker, 700 MHz) using $CDCl_3$. Mass spectroscopy spectra was measured by Waters Acquity UPLC H-Class with TQD LC/MS/MS detector. The In silico study was conducted by AutoDockTools Version 1.5.7, and Asus TUF Gaming F15 laptop with Intel(R) Core(TM) i5-10300H CPU @ 2.50GHz specifications, 24.0 GB RAM, 64-bit system.

Synthesis of 5'-chloro-2',4-dihydroxy-3-methoxycalcone

The synthesis of chalcone was began by dissolving 5 mmol (0.85 g) 5-chloro-2-hydroxyacetophenone in 10 mL of 96% ethanol in a synthesis flask, then stirred using a magnetic stirrer at room temperature. Dripped 5 mL of 40% NaOH solution wisely, followed by the addition of 5 mmol (0.76 g) of 4-hydroxy-3-methoxybenzaldehyde, which was dissolved in 10 mL of 96% ethanol. The mixture was stirred for 48 hours at room temperature. After the reaction was complete, the solution mixture was acidified with 10% HCl until a precipitate formed, then cooled in a refrigerator for 24 hours. The precipitate was filtered, washed with cold distilled water, and dried in a desiccator at room temperature. The crude was purified by

column chromatography using silica gel. Elution was performed with n-hexane: ethyl acetate (8:2), and the fractions obtained were collected in vials. Each fraction was analyzed using TLC to identify the pure fraction. The pure of 5'-chloro-2',4-dihydroxy-3-methoxychalcone was characterized using mass spectroscopy, ^{1}H-NMR, and ^{13}C-NMR (Shidiq et al., 2018; Safitri et al., 2021; Mulyani et al., 2021).

In Silico Analysis of Compounds with ADME Parameters

Pharmacokinetic and toxicity evaluation of the compounds was performed in silico using SwissADME (https://www.swissadme.ch/index.php). The compound structure was entered in SMILES format to obtain predictions of ADME parameters, which were then analyzed and visualized in diagram form (Daina et al., 2017).

Molecular Docking

Molecular tethering simulations were performed using AutoDockTools 1.5.6 and AutoDock 4.2 with the ERα receptor (PDB ID: 3ERT) (Forli et al., 2016; Pratama & Siswandono, 2020). Receptor and ligand preparation included protonation, addition of Kollman charge for the receptor, and Gasteiger charge for the ligand. The grid box setup was 40×40×40, centered on the active coordinates x=30.010, y=-1.913, z=24.207 (Aldaghi et al., 2016). The molecular tethering process was carried out with the Lamarckian Genetic Algorithm method for 100 times conformational optimization. The lowest binding energy of the best conformation between the ligand and the receptor was selected for analysis using BIOVIA Discovery Studio Visualizer 2020 (Baroroh et al., 2023).

RESULTS AND DISCUSSION

Synthesis of 5'-chloro-2',4-dihydroxy-3-methoxychalcone

The synthesis of 5'-chloro-2',4-dihydroxy-3-methoxychalcone was successfully carried out via Claisen-Schmidt condensation reaction between 5-chloro-2-hydroxyacetophenone and 4-hydroxy-3-methoxybenzaldehyde using NaOH as a base catalyst in 96% ethanol (Matsjeh et al. 2017). The synthesized compound was obtained as a yellow solid with a yield of 70.15%. This result is higher compared to previous study by Shidiq et al. (2018), which reported a yield of 61.49% for similar chalcones without methoxy substituents, indicating that the presence of methoxy groups on C3 ring B can affect reaction kinetics and synthesis efficiency.

Mulyani et al. (2021) previously succeeded in synthesizing similar chalcone analogs with methoxy groups at C3 and C4 on ring B with a higher yield of 83.27%. Theoretically, these results are consistent with a study by Jumina et al. (2019), which demonstrated that polar substituents such as methoxy can increase the polarity of the reactants, affecting the rate of condensation and product formation. The implications of these results suggest that in the development of chalcones with specific biological activities, it is necessary to consider the effect of substituents on the synthesis yield and efficiency.

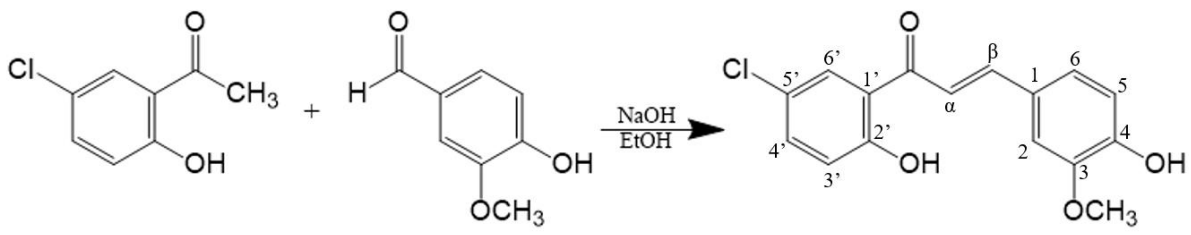


Figure 1. Reaction scheme synthesis of 5'-chloro-2',4-dihydroxy-3-methoxychalcone

The ESI-MS spectrum of 5'-chloro-2',4-dihydroxy-3-methoxychalcone was showed ion peaks at m/z of 303.05 g/mol as $[M-H]^-$ (Figure 2), which consistent with the calculated mass of 304.04 g/mol for molecular formula of $C_{16}H_{13}O_4Cl$. Based on the mass spectroscopy data analysis, it can be concluded that the compound 5'-chloro-2',4-dihydroxy-3-methoxychalcone has been formed and matches the target molecular weight.

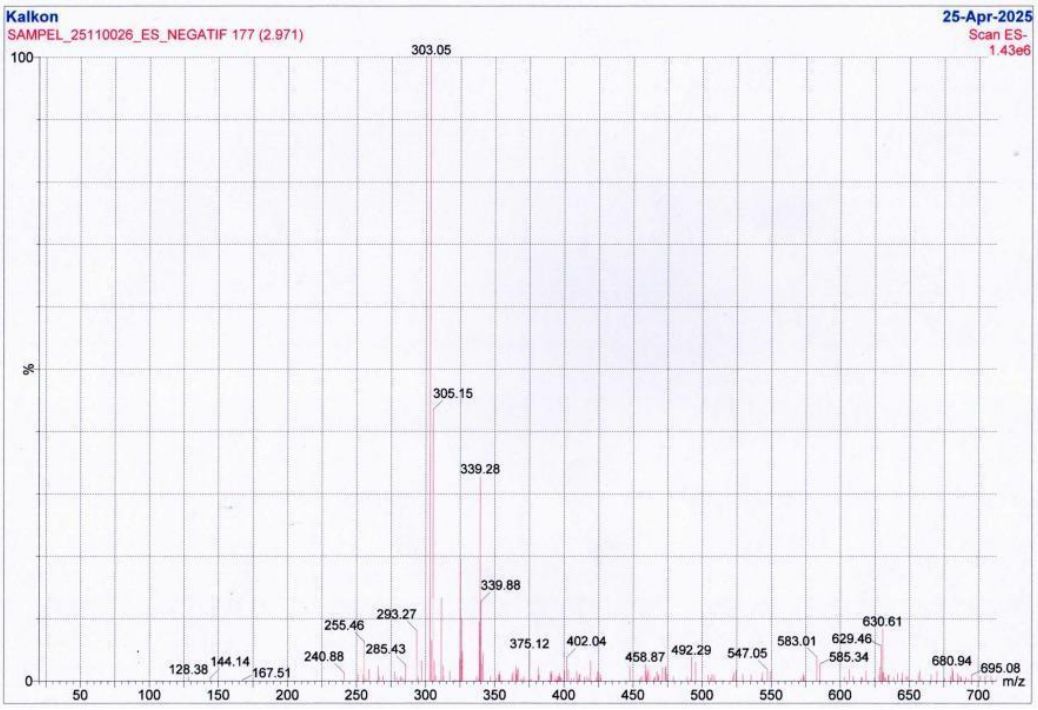


Figure 2. Mass spectrum ESI(-) of 5'-chloro-2',4-dihydroxy-3-methoxychalcone

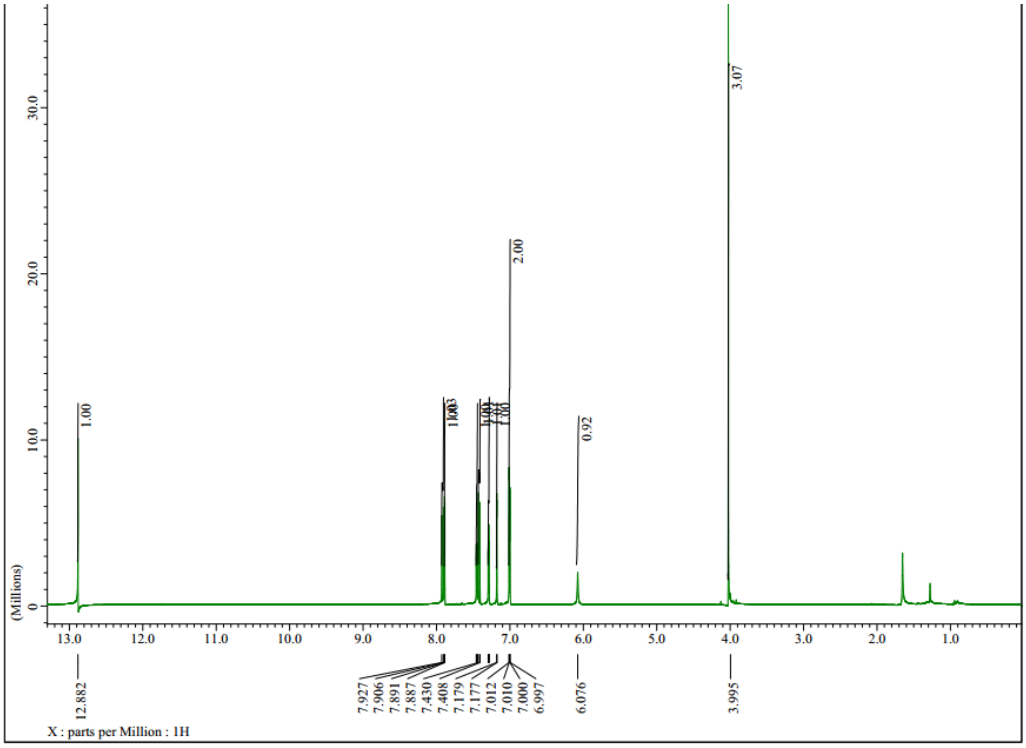


Figure 3. ^{1}H-NMR spectra of 5'-chloro-2',4-dihydroxy-3-methoxychalcone (700 MHz, $CDCl_3$)

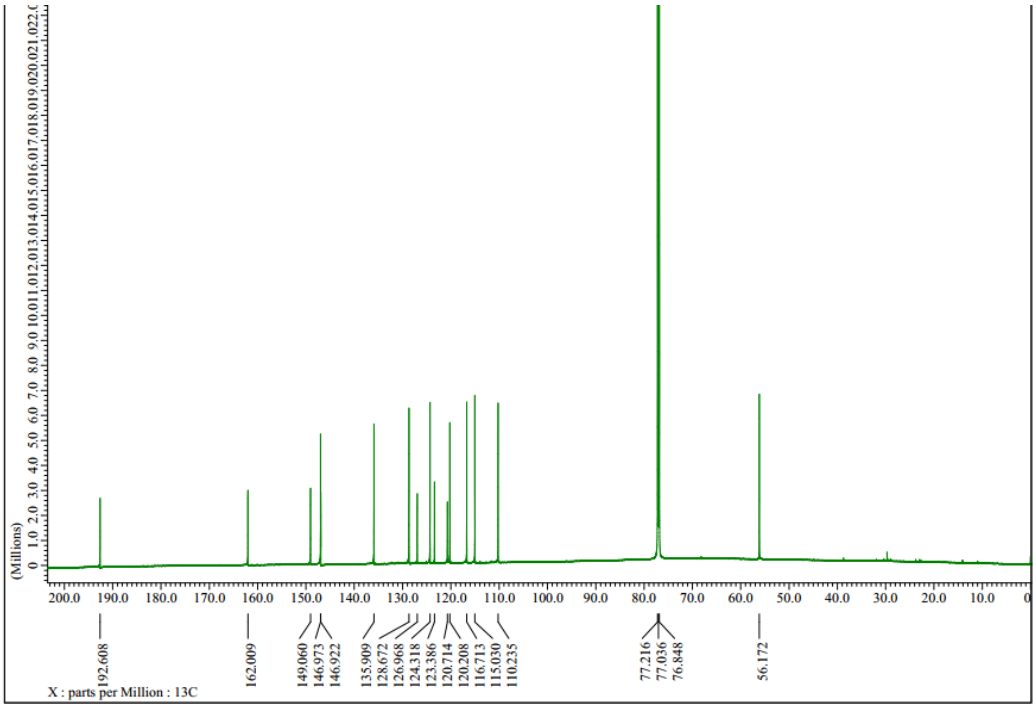


Figure 4. ^{13}C-NMR spectra of 5'-chloro-2',4-dihydroxy-3-methoxychalcone (175 MHz, $CDCl_3$)

Table 1. ^{1}H-NMR and ^{13}C-NMR spectra of 5'-chloro-2',4-dihydroxy-3-methoxychalcone

Atom Number	δ ^{1}H-NMR (ppm)	Integration and Multiplicity type	δ ^{13}C-NMR (ppm)
1	-	-	124.3
2	7.18 (*J* = 1.4 Hz)	1H, d	110.2
3	-	-	149.0
4	-	-	146.9
5	7.01 (*J* = 8.4 Hz)	1H, d	115.0
6	7.29 (*J* = 1.4; 7.7 Hz)	1H, dd	120.7
1'	-	-	123.3
2'	-	-	162.0
3'	7.00 (*J* = 9.1 Hz)	1H, d	116.7
4'	7.45 (*J* = 2.8; 9.1 Hz)	1H, dd	135.9
5'	-	-	126.9
6'	7.89 (*J* = 2.8 Hz)	1H, d	128.6
A	7.42 (*J* = 15.4 Hz)	1H, d	120.2
B	7.91 (*J* = 14.7 Hz)	1H, d	146.9
C=O	-	-	192.6
OCH_3	3.99	3H, s	56.1
2'-OH	12.8	1H, s	-
4-OH	6.07	1H, s	-

The synthesized compound 5'-chloro-2',4-dihydroxy-3-methoxychalcone, is a novel molecule whose structure was elucidated using spectroscopic techniques. As this compound represents a novel structure that has not been widely reported, the spectral data were interpreted primarily based on the proposed theoretical structure, with literature data serving as a supportive reference to strengthen the analysis. The ^{13}C-NMR data (Table 1) showed the synthesized chalcone contain 16 carbon atoms. This spectrum confirmed the 16 different carbon atoms of the 5'-chloro-2',4-dihydroxy-3-methoxychalcone.

The ^{1}H-NMR data showed the chalcone contain 13 non-equivalent protons, and corresponding to the protons environment of the 5'-chloro-2',4-dihydroxy-3-methoxychalcone. The chemical shifts at 7.42 ppm (15.4 Hz) and 7.91 ppm (14.7 Hz) indicate the presence of a typical C=C bond of chalcone with trans configuration. The protons with trans configuration will generate the coupling constants of 12-19 Hz. The nearly identical coupling constant value indicates that the two protons affect each other and are in a adjacent.

Chemical shift data at 7.18 ppm (J = 1.4 Hz), 7.01 ppm (J = 8.4 Hz), 7.29 ppm (J = 1.4; 7.7 Hz) has a proton integration of one for each shifts and the resulting coupling constants is appropriate. This also explains that the three protons are in the same environment in ring A of 5'-chloro-2',4-dihydroxy-3-methoxychalcone. The chemical shift at 7.00 ppm (J = 9.1 Hz), 7.45 ppm (J = 2.8; 9.1 Hz), 7.89 ppm (J = 2.8 Hz) were protons in ring B and also have the appropriate coupling constant.

Chemical shift at 3.99 ppm was corresponding as protons of methoxy group (OCH_3) with total proton integration of three and singlet. Chemical shift at 12.8 and 6.07 ppm were the protons of hydroxy group at positions 2' and 4. These signal showed protons integration one for each signal and singlet (Meilinda et al., 2018). The ^{1}H and ^{13}C-NMR data showed the number of protons and carbons that corresponded to the structure of 5'-chloro-2',4-dihydroxy-3-methoxychalcone. The structural analysis using ^{1}H-NMR, ^{13}C-NMR, and mass spectrometry was confirmed the successfully synthesis, with spectral data in good agreement with the theoretical structure of the target compound (Mulyani et al., 2021).

In Silico Analysis of Compounds with ADME Parameters

In silico analysis using SwissADME showed that the synthesized chalcone has pharmacokinetic and toxicological profiles that support its potential as an oral drug candidate. Based on Lipinski's rule, the chalcone complies with all oral biovailability parameters: molecular weight 304.04 g/mol (<500), logP 3.31 (<5), two hydrogen bond donors (≤) and four hydrogen bond acceptors (≤10), with no violation of Lipinski's criteria (0 violation). Compliance with Lipinski's rule indicates the potential of the compound for use in oral dosage forms and systemic distribution should be further assessed through buffer solubility or fat solubility. This compliance indicates good biological membrane penetration ability and systemic distribution. In addition, the chalcone also complied with Veber's rule, with a Topological Polar Surface Area (TPSA) value of 66.76 Å² (≤140 Å²) and four rotational bonds (≤10), indicating a high possibility for efficient oral absorption (Anusionwu et al., 2024; D. Ranjith & C. Ravikumar, 2019).

Table 2. Physicochemical and ADME parameters

Compound	Lipinski's rule				Veber's rule		Inhibitor
	MW (g/mol)	H-bond acceptors	H-bond donors	LogP	Rotatable bonds	TPSA	
5'-chloro-2',4-dihydroxy-3-methoxychalcone	304.04	4	2	3.31	4	66.76 Å²	CYP1A2, CYP2C9, CYP2C19, CYP3A4

The 5'-chloro-2',4-dihydroxy-3-methoxychalcone compound in Figure 5 is predicted to exhibit the ability to penetrate the Blood Brain Barrier (BBB), indicating potential for central nervous system therapy, although caution is needed in non-central applications (Chlebek et al., 2019; Ndombera et al., 2019; Sagitasa et al., 2021). The absence of substrativity towards P-glycoproteins indicates the potential for good systemic bioavailability. However, its ability as an inhibitor of several CYP1A2, CYP2C9, CYP2C19, and CYP3A4 enzymes signals a risk of metabolic interactions that need to be further reviewed in vitro. Structural toxicity review via

SwissADME showed no PAINS alerts as well as only one mild violation of the leadlikeness parameter (XLOGP3 > 3.5), which was tolerable. A bioavailability score of 0.55 also reinforced the favourable systemic distribution profile. Overall, the synthesized chalcone has preliminary characteristics that support its development as a drug candidate with a low risk of toxicity.

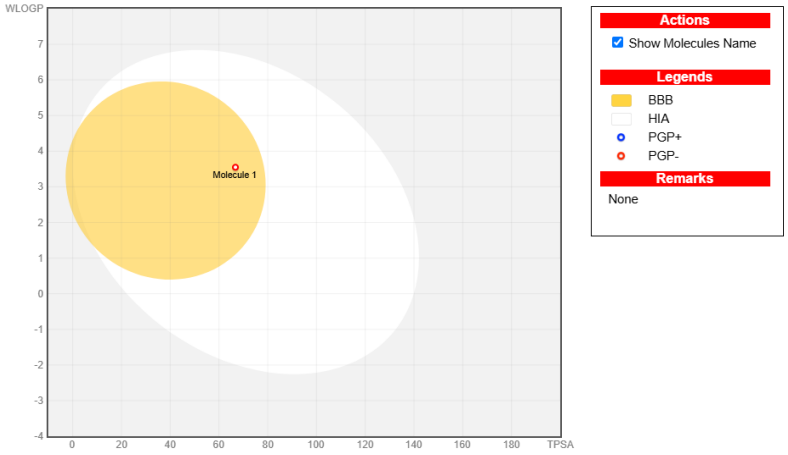


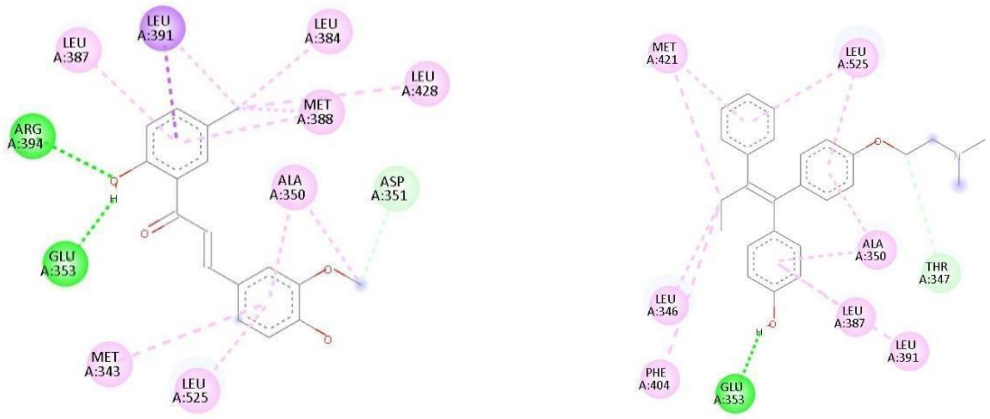
Figure 5. The BOILED-Egg pharmacokinetic parameters of the test compound include Human Gastrointestinal Absorption (HIA), Blood Brain Barrier (BBB), and P-glycoprotein (P-gp).

Simulation of Molecular Docking to Estrogen Receptor Alpha

The molecular docking data analysis showed that the 5'-chloro-2',4-dihydroxy-3-methoxychalcone has a lower affinity to ERα, with an interaction energy of -7.82 kcal/mol, compared to the 4-hydroxytamoxifen (4OHT), which showed an energy of -11.90 kcal/mol (Table 3). For further study on the role of ligands against ERα, the interaction with important residues of Erα could be performed (Fig. 6). The difference affinity of 4OHT with 5'-chloro-2',4-dihydroxy-3-methoxychalcone was influenced by the complexity and range of hydrophobic interactions. The 4OHT was formed the broader interactions with crucial residues such as Phe404, Met421, and Leu525, which strengthens the stability of the complex (Shtaiwi et al., 2019). Ligands designed to be ERα antagonists should at least have hydrogen interactions with Glu353 or Arg394 and the absence of interaction with His524 might be a marker of potential antagonism to ERα (Shylaja et al., 2021). The compound of 5'-chloro-2',4-dihydroxy-3-methoxychalcone did not show hydrogen interactions with the His524 residue, so it is feasible that the chalcone act as ERα antagonists. To confirm the occupancy of each residue by the chalcone it can be further analyzed using molecular dynamics simulations.

Table 3. Free energy and intramolecular interactions of molecular docking

Compounds	ΔG (kcal/mol)	Molecular Interactions	
		Hydrogen Bond	Alkyl
5’-chloro-2’,4-dihydroxy-3-methoxychalcone	-7.82	Arg394, Glu353, Asp351	Leu391, Ala350, Leu384, Met388,, Leu428, Leu387, Met343, Leu525
4-hydroxytamoxifen	-11.90	Glu353, Thr347	Leu346, Met421, Phe404, Leu346, Leu387, Ala350, Leu525



(a) (b)

Figure 6. Ligand-ligand interaction towards ERα; (a) 5'-chloro-2',4-dihydroxy-3-methoxychalcone, (b) 4OHT

CONCLUSION

The study successfully synthesized 5'-chloro-2',4-dihydroxy-3-methoxychalcone with a yield of 70.15% via Claisen-Schmidt condensation. The compound demonstrated a favorable pharmacokinetic and toxicity profile in silico, and molecular docking showed a promising interaction with the ERα receptor. These results highlight the compound's potential as a new drug candidate for degenerative and infectious disease therapy. The impact of this study lies in expanding the structural diversity of chalcones and providing a valuable scaffold for further drug development targeting diseases with limited treatment options.

RECOMMENDATIONS

In order to maximise the potential of the synthesised chalcones, practitioners and researchers are advised to continue in vitro and in vivo biological tests and explore other structural modifications to enrich the activity and safety of the compounds. Academics can utilise these findings as a basis for developing interdisciplinary research with a data triangulation approach. Future research should also examine pharmacodynamic aspects and clinical applications, so that kalkon is increasingly ready to be developed as an innovative drug candidate for degenerative and infectious diseases.

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