Malaysian Journal of Analytical Sciences (MJAS)
Published by Malaysian Analytical Sciences Society

EXPERIMENTAL AND SIMULATION OF HYDROXY CHALCONE DERIVATIVES AS ANTIOXIDANT AND DRUG-LIKE AGENTS

(Eksperimen dan Simulasi bagi Terbitan Hidroksi Kalkon sebagai Agen Antioksida dan Kesamaan Ubatan)

Wan Muhammad Haikal Wan Roshaimi[1], Siti Nabilla Aliya Mohd Nizar[1], Hifzhan Amin Azizi[1], Ahmad Saifuddin Mohamad Arshad[2], Juzaili Azizi[2], Uwaisulqarni M. Osman[3,4] and Suhana Arshad[1*]

[1]X-ray Crystallography Unit, School of Physics, Universiti Sains Malaysia, Gelugor 11800, Penang, Malaysia
[2]Centre for Drug Research, Universiti Sains Malaysia, Gelugor 11800, Penang, Malaysia
[3]Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, Kuala Nerus, Terengganu 21030, Malaysia
[4]Advanced Nano Materials Research Group (ANOMA), Ionic State Analysis (ISA) Laboratory, Universiti Malaysia Terengganu, Kuala Nerus, Terengganu 21030, Malaysia

**Corresponding author: suhanaarshad@usm.my*

Received: 30 May 2024; Accepted: 11 September 2024; Published: 29 December 2024

Abstract

Research development of antioxidant agents with drug-like properties remains a significant challenge, particularly in the limited evaluation of hydroxy chalcone derivatives. In this study, five new hydroxy chalcone derivatives were synthesized through Claisen-Schmidt condensation, achieving yields between 33% and 49%. The molecular structures of the synthesized compounds were confirmed using Infrared (IR) and Nuclear Magnetic Resonance (NMR) spectroscopy. Among the compounds, (*E*)-1-(4-bromophenyl)-3-(2-hydroxynaphthalen-1-yl)prop-2-en-1-one (**5**) exhibited superior free radical scavenging activity in the DPPH assay, while (2*E*,4*E*)-1-(anthracen-9-yl)-5-(4-hydroxy-3-methoxyphenyl)penta-2,4-dien-1-one (**2**) showed promising performance in the ABTS assay. SwissADME analysis predicted favourable drug-like properties for all compounds according to Lipinski's Rule, suggesting their potential as candidates for drug development.

Keywords: chalcone, ATR, NMR, DPPH, ABTS, SwissADME

Abstrak

Kajian mengenai perkembangan agen antioksida serta sifat keserupaan ubat merupakan cabaran besar terutamanya dalam penyelidikan yang terhad terhadap terbitan hidroksi kalkon. Dalam kajian ini, lima terbitan hidroksi kalkon baharu telah disintesis melalui tindak balas kondensasi Claisen-Schmidt dengan hasil antara 33% hingga 49%. Struktur molekul bagi sebatian yang disintesis telah disahkan menggunakan spektroskopi Inframerah (IR) dan Resonans Magnet Nuklear (NMR). Antara sebatian tersebut, (*E*)-1-(4-bromophenyl)-3-(2-hydroxynaphthalen-1-yl)prop-2-en-1-one (**5**) menunjukkan aktiviti penangkapan radikal bebas yang baik dalam ujian DPPH, manakala (2*E*,4*E*)-1-(anthracen-9-yl)-5-(4-hydroxy-3-methoxyphenyl)penta-2,4-dien-1-one (**2**) menunjukkan prestasi yang meyakinkan dalam ujian ABTS. Analisis SwissADME meramalkan sifat-sifat keserupaan ubat mengikut Peraturan Lipinski, yang menunjukkan potensi sebatian-sebatian ini sebagai calon untuk pembangunan ubat.

Kata kunci: kalkon, ATR, NMR, DPPH, ABTS, SwissADME

Introduction

Chalcones are a significant subgroup of flavonoids which are prevalent across the plant kingdom and are found in fruits, vegetables, teas, and various other plants [1]. They serve as a fundamental chemical framework for numerous naturally occurring compounds. The term "chalcone" is derived from the Greek word "chalcos," meaning "bronze," which reflects the colour characteristic of many natural chalcones [2]. Structurally, chalcones are defined as 1,3-diphenyl-2-propen-1-one derivatives featuring an open-chain unsaturated carbonyl system which comprises of two aromatic rings linked by a three-carbon α, β-unsaturated system [3-5]. They include the ketoethylenic moiety (-CO-CH=CH-) and possess a fully delocalized π-electron system along with a conjugated double bond [6,7].

Chalcones are central to a diverse range of biologically active compounds and exhibit various activities including antibacterial [8], antifungal [9], anti-inflammatory [10], anticancer [11], and antioxidant properties [12]. Despite these promising activities, there is limited research on hydroxy chalcones, particularly concerning their antioxidant potential.

This study focuses on synthesizing novel hydroxy chalcones to explore their potential antioxidant properties through in vitro DPPH assays, followed by biological simulations using molecular docking and in silico ADMET studies. The Claisen-Schmidt condensation reaction was chosen for its simplicity and efficiency, making it suitable for this short-term project. A total of five hydroxy chalcones were synthesized and designated as compounds **1**, **2**, **3**, **4** and **5**. The experimental study was conducted to corroborate the findings from the molecular docking and ADMET analyses.

Materials and Methods

Synthesis of hydroxy chalcone derivatives

The hydroxy chalcone derivatives were synthesized through Claisen-Schmidt condensation reaction between different acetophenones (0.05 mmol) and hydroxy aldehydes (0.05 mmol) with the presence of sodium hydroxide (NaOH) as the catalyst. The schematic diagrams of all compounds are shown in Scheme 1. The mixture was dissolved in 10 ml of ethanol as the solvent. NaOH solution was prepared by dissolving 4 g of NaOH in 10 mL of distilled water. The NaOH solution was added to the reaction mixture drop by drop until a precipitate was formed. The mixture was left to stir for 24 hours at room temperature in the fume hood. The progress of the chemical reaction was monitored using the thin layer chromatography (TLC) plate and was observed under UV lamp. After 24 hours of stirring, the mixture was placed in a refrigerator for another 24 hours. After that, the reaction mixture was poured into cold water and a few drops of hydrochloric acid (HCl) was added. The resultant base product was filtered and left to dry at room temperature for a few days. The precipitates formed on the filter paper were then placed in a small container. The hydroxy aldehyde derivatives used were (E)-1-(anthracen-9-yl)-3-(2,4-dihydroxyphenyl)prop-2-en-1-one (**1**), (2E,4E)-1-(anthracen-9-yl)-5-(4-hydroxy-3-methoxyphenyl)penta-2,4-dien-1-one (**2**), (E)-1-(anthracen-9-yl)-3-(2-hydroxyphenyl)prop-2-en-1-one (**3**), (E)-1-(anthracene-9-yl)-3-(4-hydroxyphenyl)prop-2-en-1-one (**4**) and (E)-1-(4-bromophenyl)-3-(2-hydroxynaphthalen-1-yl)prop-2-en-1-one (**5**).

(E)-1-(anthracen-9-yl)-3-(2,4-dihydroxyphenyl)prop-2-en-1-one (**1**), $C_{23}H_{16}O_3$. Pale orange-yellow: yield 39.26%, m.p.: 156-157 ˚C; IR (ATR-FTIR, cm^{-1}): 3053 *ν*(O-H), 2974 $\nu(\text{C-H})_{\text{aromatic}}$, 2928 *ν*(alkene=C-H), 1699 *ν*(C=O), 1670 $\nu(\text{C-H})_{\alpha,\ \beta\text{-unsaturated}}$, 1624 $\nu(\text{C=C})_{\text{aromatic}}$, 1096 *ν*(C-O). ^{1}H NMR (500 MHz; DMSOd_6; ppm): δ 8.73 (s, 1H, aromatic), 8.23 (d, J = 3.35 Hz, 1H, aromatic) 8.22 (d, *J* = 3.3 Hz, 1H, aromatic), 8.18 (d, *J* = 3.5 Hz, 1H, aromatic), 8.17 (d, *J* = 2.35 Hz, 1H, aromatic), 7.95 (d, *J* = 3.3 Hz, 1H, aromatic), 7.94 (d, J = 3.25 Hz, 1H, aromatic), 7.80 (d, *J* = 2.4 Hz, 1H, aromatic), 7.79 (s, 1H, aromatic), 7.61 (d, *J* = 1.75 Hz, 1H, aromatic), 7.59 (d, J = 2.55 Hz, H-β), 7.58 (s, 1H, aromatic), 7.57 (d, J = 2.1 Hz, H-α), 7.56 (s, 1H, aromatic), 1.37 (d, *J* = 6.1 Hz, OH), 1.37 (d, J = 6.1 Hz, OH). ^{13}C NMR (125 MHz, DMSOd_6, ppm): δ 209.64 (C=O), 135.08, 133.55, 132.35, 131.01 (Cβ), 130.55, 129.86, 129.33, 128.64, 128.55, 127.65, 127.25, 126.63, 126.21, 125.08, 124.65, 124.30 (Cα).

(2E,4E)-1-(anthracen-9-yl)-5-(4-hydroxy-3-methoxyphenyl)penta-2,4-dien-1-one (**2**), $C_{26}H_{20}O_3$.

Bright yellow solid: yield 48.93%, m.p.: 125-127 ˚C; IR (ATR-FTIR, cm-1): 2955 *v*(O-H), 2654 *v*(C-H)$_{aromatic}$, 2572 *v*(alkene=C-H), 1680 *v*(C=O), 1584 *v*(C=C)$_{aromatic}$, 1086 *v*(C-O). ^{1}H NMR (500 MHz; DMSOd_6; ppm): δ 9.60 (s, OH), 8.75 (s, 1H, aromatic), 8.20 (d, 1H), 8.18 (s, 1H), 7.79 (s, 1H, aromatic), 7.77 (d, 1H), 7.56 (s, 1H, aromatic), 7.57 (s, 1H, aromatic), 7.55 (d, J = 1.7 Hz, 1H, aromatic), 7.12 (d, J = 1.5 Hz, 1H, aromatic), 7.09 (m, 1H, aromatic), 6.89 (d, J = 1.45 Hz, 1H, aromatic), 6.88 (d, 1H, 1H), 6.87 (d, J = 1.45 Hz, 1H, aromatic), 6.81 (d, J = 1.5 Hz, 1H), 6.79 (d, J = 4.0 Hz, 1H, aromatic), 6.76 (s, 1H, aromatic), 3.75 (s, 3H). ^{13}C NMR (125 MHz, DMSOd_6, ppm): δ 199.48 (C=O), 149.76, 149.13, 148.33, 144.39 (Cβ), 135.11, 131.10, 130.90, 129.19, 128.41, 128.03, 127.86, 127.31, 126.16, 125.26, 124.29, 122.76, 116.03 (Cα), 110.86, 56.02.

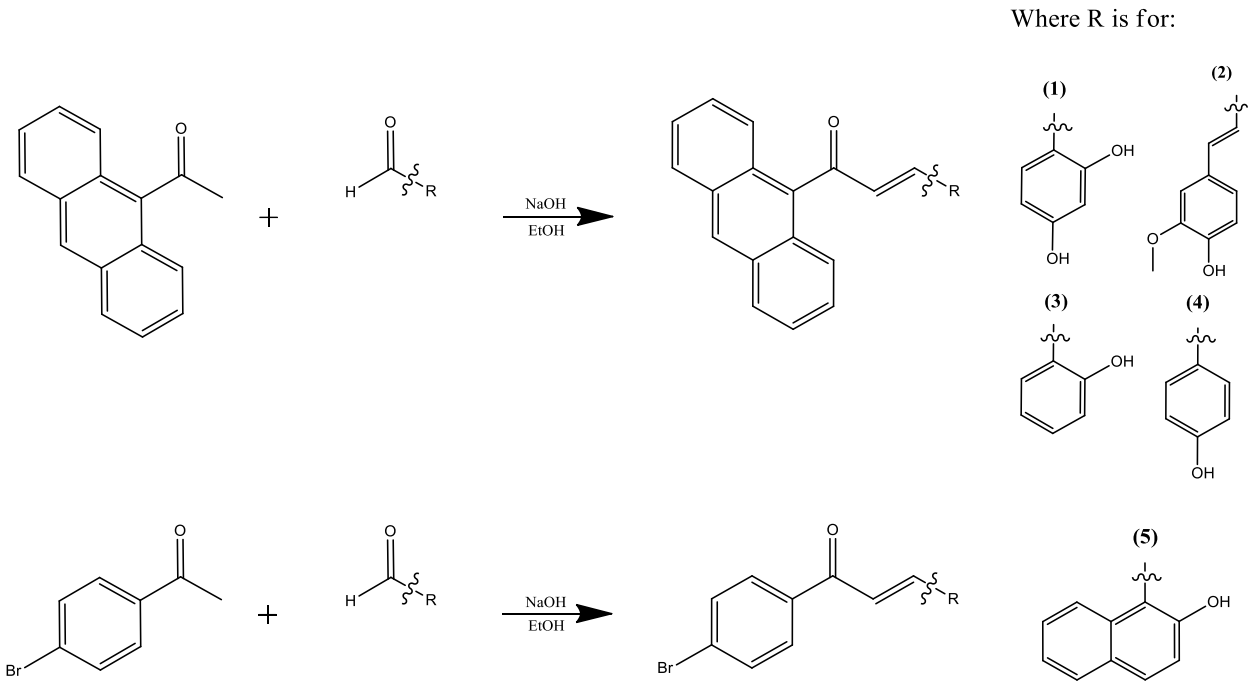
(E)-1-(anthracen-9-yl)-3-(2-hydroxyphenyl)prop-2-en-1-one (**3**), $C_{23}H_{16}O_2$. Orange-yellow solid: yield 40.78%, m.p.: 210-211 ˚C; IR (ATR-FTIR, cm^{-1}): 3351 *v*(O-H), 3050 *v*(C-H)$_{aromatic}$, 2950 *v*(alkene=C-H), 1594 *v*(C=O), 1457 *v*(C-H)$_{α, β\text{-unsaturated}}$, 1411 *v*(C=C)$_{aromatic}$, 1002 *v*(C-O). ^{1}H NMR (500 MHz; DMSOd_6; ppm): δ 8.76 (s, OH), 8.20 (d, J = 2.0 Hz, H-β), 8.18 (s, 1H, aromatic), 7.81 (s, 1H, aromatic), 7.79 (d, J = 1.6 Hz, H-α), 7.65 (d, J = 1.35 Hz, 1H, aromatic), 7.63 (d, J = 1.15 Hz, 1H, aromatic), 7.56 (m, 1H, aromatic), 7.56 (m, 1H, aromatic), 7.56 (m, 1H, aromatic), 7.56 (m, 1H, aromatic), 7.42 (s, 1H, aromatic), 7.42 (s, 1H, aromatic), 7.22 (s, 1H, aromatic), 6.80 (s, 1H, aromatic), 6.78 (s, 1H, aromatic). ^{13}C NMR (125 MHz, DMSOd_6, ppm): δ 200.10 (C=O), 157.89, 143.85 (Cβ), 135.22, 133.26, 131.07, 129.37, 129.20, 128.49, 128.46, 128.06, 127.34, 126.18, 125.30, 120.93, 119.78, 116.81 (Cα).

(E)-1-(anthracene-9-yl)-3-(4-hydroxyphenyl)prop-2-en-1-one (**4**), $C_{23}H_{16}O_2$. Bright yellow solid: 43.58%, m.p.: >213 ˚C; IR (ATR-FTIR, cm^{-1}): 3052 *v*(O-H), 2974 *v*(C-H)$_{aromatic}$, 2930 *v*(alkene=C-H), 1699 *v*(C=O), 1671 *v*(C-H)$_{α, β\text{-unsaturated}}$, 1592 *v*(C=C)$_{aromatic}$, 1096 *v*(C-O). ^{1}H NMR (500 MHz; DMSOd_6; ppm): δ 10.27 (s, OH), 8.75 (s, 1H, aromatic), 8.19 (d, J = 1.85 Hz), 8.18 (s, 1H, aromatic), 7.80 (s, 1H, aromatic), 7.78 (d, J = 1.45 Hz, 1H, aromatic), 7.56 (s, 1H, aromatic), 7.55 (d, J = 4.3 Hz, 1H, aromatic), 7.55 (s, 1H, aromatic), 7.54 (s, 1H, aromatic), 7.48 (s, 1H, aromatic), 7.47 (s, 1H, aromatic), 7.25 (d, J = 16.1 Hz, H-β), 7.05 (d, J = 16.1 Hz, H-α), 6.75 (s, 1H, aromatic), 6.73 (s, 1H, aromatic.^{13}C NMR (125 MHz, DMSOd_6, ppm): δ 199.53 (C=O), 161.11, 148.56 (Cβ), 131.68, 131.11, 129.19, 128.42, 128.03, 127.57, 127.30, 126.29, 126.15, 125.28, 124.60, 116.39 (Cα).

(E)-1-(4-bromophenyl)-3-(2-hydroxynaphthalen-1-yl)prop-2-en-1-one (**5**). Dark yellow solid: yield 33.62%, m.p.: 83-85 ˚C; $C_{19}H_{13}BrO_2$. IR (ATR-FTIR, cm^{-1}): 3589 *v*(O-H), 3178 *v*(C-H)$_{aromatic}$, 3055 *v*(alkene=C-H), 1677 *v*(C=O), 1628 *v*(C-H)$_{α, β\text{-unsaturated}}$, 1586 *v*(C=C)$_{aromatic}$, 1069 *v*(C-O), 745 *v*(C-Br). ^{1}H NMR (500 MHz; DMSOd_6; ppm): δ 12.07 (s, OH), 10.81 (s, 1H, aromatic), 8.92 (s, 1H, aromatic), 8.93 (s, 1H, aromatic), 8.14 (d, J = 9.0 Hz, H-β), 8.13 (s, 1H, aromatic), 7.90 (d, J = 8.0 Hz, 1H, aromatic), 7.87 (d, J = 8.55 Hz, 1H, aromatic), 7.72 (m, 1H, aromatic), 7.62 (s, 1H, aromatic), 7.62 (s, 1H, aromatic), 7.25 (s, 1H, aromatic), 7.24 (d, J = 9.0 Hz, H-α).). 13C NMR (125 MHz, DMSOd6, ppm): δ 193.36 (C=O), 138.95 (Cβ), 132.19, 132.14, 131.77, 129.82, 129.32, 128.03, 127.36, 126.40, 125.32, 124.76, 124.61, 122.61, 122.03,119.18 (Cα), 112.87.

Spectroscopy analysis

Perkin Elmer Frontier Spectrometer 100 was used to acquire the Attenuated Total Reflection (ATR) spectra for all hydroxy chalcone compounds. A small amount of powder from the compounds was added to the mounting plate until it covers the exposed surface of the crystal. The pressure arm was swung over the top of the sample and the knob was rotated until it just touched the sample. The pressure arm was rotated again until the force gauge indicated a pressure between 100 and 130. All samples were scanned within the range of 600 - 4000 cm^{-1}. As for Nuclear Magnetic Resonance (NMR), 4 mg of powder from the compounds were dissolved in a small amount of an appropriate solvent in sample vials. The solvent used in this experiment was deuterated dimethyl sulfoxide (d6-DMSO). The solution was then transferred to an NMR tube to a height of approximately 4 cm. All the samples were then sent to NMR laboratory, School of Chemical Science in USM for further analysis. The ^{1}H NMR and ^{13}C NMR spectra were determined by Bruker Avance III 500 spectrometer.



Scheme 1. The synthesis route of the synthesized hydroxy chalcone derivatives **(1-5)**

DPPH assay

DPPH assay is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate antioxidant activity [13]. The free radical scavenging activity against the stable DPPH free radical was assessed using a 50 mmol/L sodium phosphate solution in a system buffered at pH 6.0 as referred to previously reported study [14]. A mixture of pure ethyl alcohol and a 50 mmol/L sodium phosphate solution buffered at pH 6 was used as the solvent with the DPPH radical and test sample prepared with the volume ratio of 1:1. The stock solution was sonicated for an hour before being stored at 4 ˚C in the refrigerator. The DPPH solution of 260 µL was added in a 96-well microplate and later the test compounds, ascorbic acid (positive control), and DMSO (negative control) were then added to the appropriate wells in a volume of 40 µL. Three duplicates of each concentration were tested. In order to give the prepared solutions time to react, the 96-well microplate was placed aside in the dark for 30 minutes at 25 ˚C. After that, the absorbance of each well was measured at 525 nm against a reagent blank (DMSO and DPPH solution) using a microplate reader spectrophotometer. The percentage of DPPH scavenging activity was calculated using the following formula:

$$\%DPPH = \left(\frac{Abs_{-ve\ control} - Abs_{test\ sample}}{Abs_{-ve\ control}}\right) \times 10 \qquad (1)$$

ABTS assay

ABTS radical cation-based assay is also among the most abundant antioxidant capacity assays that reduces the ABTS radical by reacting with the oxidized ABTS radical cation (ABTS•+). ABTS•+ cation radical was produced by the reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate (1:1) and stored in the dark at room temperature for 16 hours before used, as referred to Rajurkar & Hande [15]. ABTS•+ solution was then diluted with deionized water to obtain an absorbance of 0.7 at 734 nm. A linear curve was established, and a linear equation was used to find a concentration where absorbance was 0.7 at 734 nm. The ABTS radical cation solution of 100 µL was added in a 96-well microplate and later the test compounds, Trolox (positive control), and DMSO (negative control) were then added to the appropriate wells in a volume of 100 µL. Three duplicates of each concentration were tested. In order to give the prepared solutions time to react, the 96-well microplate was placed aside in the dark for 5

minutes. After that, the absorbance of each well was measured at 734 nm against a reagent blank (DMSO and ABTS radical cation solution) using a microplate reader spectrophotometer. The extent of decolourisation was calculated as the percentage reduction of absorbance using the following equation 2:

$$\%ABTS = \left(\frac{Abs_{-ve\ control} - Abs_{test\ sample}}{Abs_{-ve\ control}}\right) \times 100 \qquad (2)$$

***In silico* ADME study**

In silico ADME analysis was performed using SwissADME web server to evaluate the drug-likeness properties of the synthesized hydroxy chalcone derivatives [16]. Molecular structures were initially drawn and converted into Simplified Molecular Input Line Entry System (SMILES) code using JChem Web Services (version 14.9.29, ChemAxon, Budapest, Hungary). The SMILES strings were then pasted into the SwissADME interface for analysis. The software generates a comprehensive report for each compound, including various parameters related to absorption, distribution, metabolism, and excretion (ADME) properties. These reports are presented in tabular and graphical formats for easy visualization, and the data can be readily exported as Excel spreadsheets for further analysis [17].

Results and Discussion

Physical properties of hydroxy chalcone compounds

The Claisen-Schmidt condensation reaction successfully yielded five hydroxy chalcone derivatives with varying physical properties (refer to Table S1 in the Supplementary Materials section). Notably, compound **5** displayed the lowest melting point (83-85 °C), potentially due to the presence of a halogen atom in its structure. Interestingly, compounds **1**-**4**, sharing a 9-acetylanthracene functional group, exhibited comparable yields (39.26-48.93%), while compound **5**, with a different functional group, had a lower yield (33.62%). These observations are in line with previous reports where Claisen-Schmidt condensation typically affords higher yields, but hydroxy chalcones can exhibit moderate to lower yields [18, 19].

IR spectral analysis

The IR spectrum is divided into three wavenumber regions which are far-IR spectrum (<400 cm^{-1}), mid-IR spectrum (400-4000 cm^{-1}), and near-IR spectrum (4000-13000 cm^{-1}). Although far- and near-IR spectra are also utilized to provide information about the tested materials, the mid-IR spectrum is the one that is most frequently used [20]. The analysis of IR data in the mid-IR spectrum was the focus of this work. The characteristic absorption bands that were observed in the spectra of all compounds are shown in Figure S1-S5 and tabulated in Table S2 in the Supplementary Materials section. Some of the characteristic absorption bands observed in the IR spectra of the chalcone derivatives which include the ν(O-H), ν(C=O) and $\nu(C\text{-}H)_{\alpha,\ \beta\text{-unsaturated}}$ represent the core structure of the chalcones. The ν(O-H) for all the compounds were in the range of 2900 – 3600 cm^{-1}, ν(C=O) was in the range of 1500 – 1700 cm^{-1}, and $\nu(C\text{-}H)_{\alpha,\ \beta\text{-unsaturated}}$ were in the range of 1400 – 1700 cm^{-1}. These values are in the range of the reported findings of a previous study [20]. Moreover, the aromatic ν(C=C) and ν(C-O) were observed within the ranges of 1400 – 1650 cm^{-1} and 1000 – 1100 cm^{-1}, respectively. Additionally, the ν(C-Br) was observed at 745 cm^{-1} in the spectrum of compound **5**.

NMR spectral analysis

The proton and carbon spectra are the two most crucial types of NMR spectra for determining organic structures. They provide details on functional groups, the quantity of hydrogens and carbons in a molecule, and how those hydrogens and carbons are linked to one another. ^{1}H NMR is used to determine the types and number of hydrogen atoms present in a compound whereas ^{13}C NMR is used to determine the type and number of carbon atoms in a compound. It is performed to determine the content and purity of the hydroxy chalcone structures. The NMR spectra of the reported compounds are listed in Figure S6-S15 in the Supplementary Materials section. Based on the ^{1}H NMR spectra, the hydroxy chalcone compounds **1**, **3**, **4** and **5** have 16 protons or hydrogen atoms each while compound **2** has 19 protons. The H_α protons displayed a greater chemical shift than the H_β counterparts at higher fields. This is because the carbonyl group in the system is primarily responsible for polarizing the C=C double

bond, which results in a higher electron density at position α than position β. The coupling constants of H_α and H_β that were both below 15 Hz indicated that they are in *cis* configuration while coupling constants above 15 Hz indicated that they are in *trans* configuration [21]. The structures of the synthesized compounds were further characterized using ^{13}C-NMR, of which compounds **1**, **3** and **4** have 23 carbons each, while compound **2** has 26 carbons and compound **5** has 19 carbons. These confirm the structures of all the hydroxy chalcone.

***In vitro* DPPH assay analysis**

The free radical scavenging capacity of all synthesized hydroxy chalcone derivatives was evaluated using the DPPH assay with ascorbic acid as the positive control. In DPPH assay, a lower IC_{50} value was interpreted as a higher antioxidant activity of a compound. As shown in Figure 1, all compounds exhibited a lower activity compared to that of ascorbic acid (IC_{50} = 6.21 µg/mL). Interestingly, compounds **1** and **3** displayed no detectable activity, while compound **4**, which is the structural isomer of **3**, showed a moderate activity (IC_{50} = 260.07 µg/mL). This suggests that the presence and position of the hydroxy groups may influence the antioxidant potential, with dihydroxylated compounds have a lower antioxidant activity compared to the monohydroxylated compounds [22].

Among the synthesized compounds, compound **2** exhibited the strongest antioxidant activity (IC_{50} = 48.37 µg/mL), likely due to the combined effect of the methoxy group on the aromatic ring and the potential for enhancing hydrogen bonding between the rings, stabilizing its structure [23]. Compound **5** displayed a moderate activity (IC_{50} = 65.36 µg/mL), potentially as a result of the bromine atom (Br) at the end of the side chain, which aligns with previous findings suggesting improved antioxidant activity for halogenated derivatives [24].

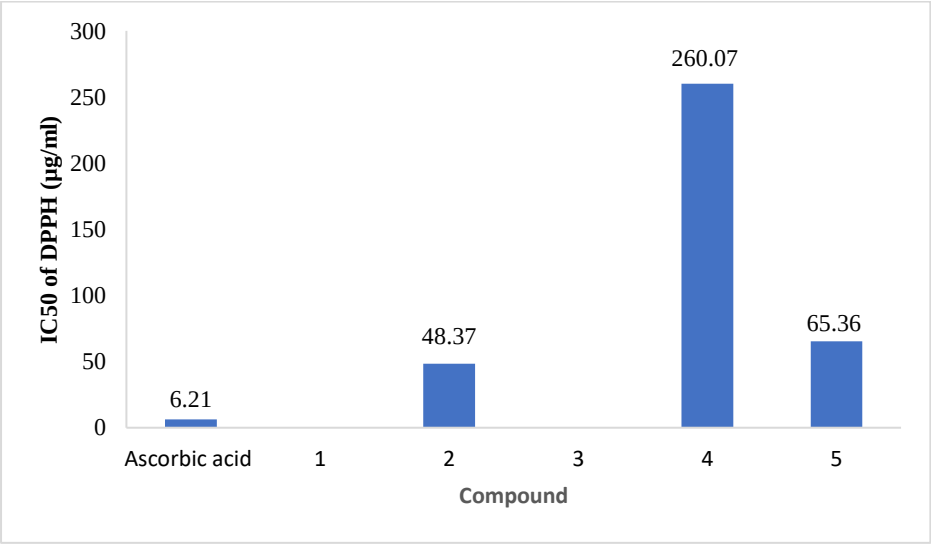


Figure 1. DPPH radical scavenging activities of chalcone compounds

***In Vitro* ABTS radical cation-based Assay Analysis**

The hydroxy chalcone compounds were also determined by using ABTS assay for their radical scavenging capabilities with the use of Trolox as a standard for reducing power assay. Similarly, the ABTS reducing capabilities of all synthesized compounds were evaluated by determining their IC_{50} values. Based on Figure 2, Trolox served as a standard for reducing power assay with IC_{50} value of 8.68 µg/ml. It was observed that **4** was the only compound that did not have an antioxidant activity in ABTS assay due to its weak antioxidant agent. Meanwhile, **5** showed the highest antioxidant activity with IC_{50} value of 77.37 µg/ml. The measurements of the ABTS assay showed lower antioxidant activities of compounds compared to DPPH assay results. The solubility of a molecule in a particular

reaction medium can be the cause of the variation in DPPH and ABTS radical scavenging capabilities. The ABTS assays are typically conducted in aqueous solution, while the DPPH assays are typically carried out in a medium containing 50% ethanol. The substances that are less active in the ABTS assay than the DPPH assay, are those that are less soluble in water [22]. Overall, the antioxidant activities of the compounds were comparable in the DPPH and ABTS assay reaction mixtures despite the solubility issues. This indicates that the synthesized compounds have dependable and significant antioxidant characteristics.

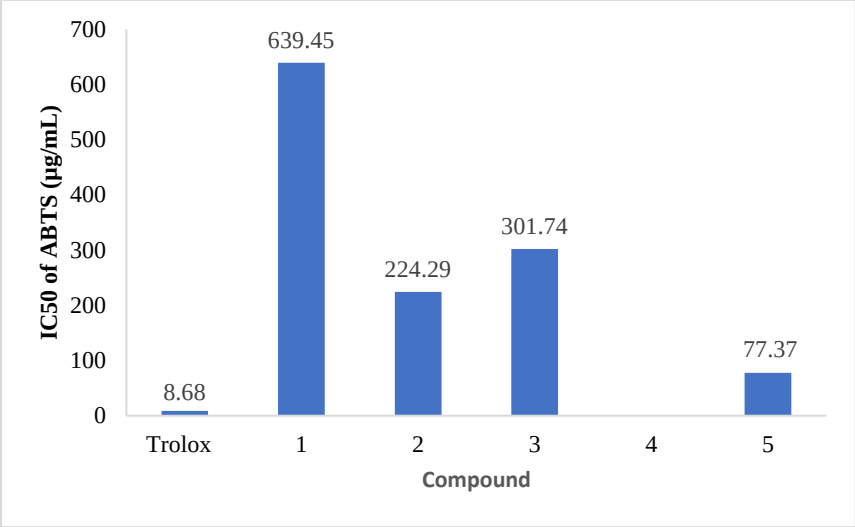


Figure 2. Graph of IC50 values of active compounds in ABTS assay

ADME prediction

The estimation of drug absorption, distribution, metabolism, and excretion (ADME) is currently supported by computer-aided drug development. The percentage of pharmacokinetics-related failures in the clinical phase has been found to be significantly reduced by the first assessment of ADME properties in the discovery phase [17]. Five hydroxy chalcones derivatives were analysed using SwissADME web tool to study their physiochemical properties and drug likeliness. The physiochemical properties of hydroxy chalcone compounds are listed in Table 1 which include molecular weight, number of heavy atoms, number of aromatic heavy atoms, fraction Csp^3, number of rotatable bonds, number of H-bond acceptors, number of H-bond donors, and topological polar surface area (TPSA). Open babel version 2.3.0 was used to calculate the results [16]. The general characteristics of the hydroxy chalcone derivatives revealed that all the compounds have a molecular weight of less than 500 g/mol, which is a prime property to be called as drug likeliness of small molecules [25].

Table 1. The physiochemical properties of hydroxy chalcone compounds.

Compound	MW (g/mol)	Num. Heavy Atoms	Num. Arom. Heavy Atoms	Fraction Csp^3	Num. Rotatable Bonds	Num. H-Bond Acceptors	Num. H-Bond Donor	TPSA (Å²)
1	340.37	26	20	0.00	3	3	2	57.53
2	380.44	29	20	0.04	5	3	1	46.53
3	324.37	25	20	0.00	3	2	1	37.30
4	324.37	25	20	0.00	3	2	1	37.30
5	353.21	22	16	0.00	3	2	1	37.30

This SwissADME section provides access to five distinct rule-based filters, each of which has a unique set of qualities that characterize a compound as being drug-like. The Lipinski filter is the pioneer rule-of-five implemented with the Ghose, Veber, Egan and Muegge methods. Based on the results, all compounds met the drug likeliness according to Lipinski's rules. The Lipinski filter is the first of five rules that characterize compounds based on physicochemical property profiles such as Molecular Weight (MW) less than 500, MLOGP ≤ 4.15, N or O ≤ 10, NH or OH ≤ 5 [25]. The target compound will not be taken into consideration as a potential candidate for drug development if more than two rules of the filters are violated. It is also observed that compounds **1**, **3**, **4** and **5** are in accordance with the Ghose, Veber, and Egan rules. Furthermore, this drug-likeliness assessment reveals that all the compounds have moderate probability of being developed into an oral drug with respect to bioavailability, where the compounds have a moderate bioavailability score of 0.55 [26,27].

The boiled egg allows for the evaluation of brain penetration (BBB) and passive gastrointestinal absorption (HIA). The white portion of the egg indicates a high likelihood of passive absorption via the gastrointestinal tract and the yolk indicates a high likelihood of brain penetration. Additionally, the points are coloured red if anticipated to be a non-substrate of P-gp (PGP-) and blue if predicted to be actively effluxes by P-gp (PGP+) [17]. As illustrated in Figure 3, all compounds have passive gastrointestinal absorption. While the rest of the compounds are BBB permeable, only compound **2** is P-gp substrate, thus unable to cross the BBB.

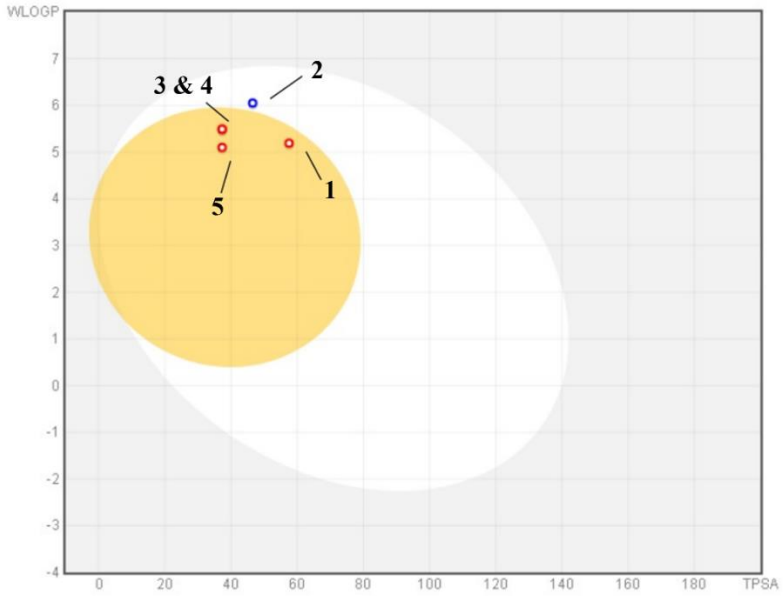


Figure 3. Schematic representation of perceptive evaluation of passive gastrointestinal absorption (HIA) and brain penetration (BBB) with molecules in the WLOGP-versus-TPSA using BOILED-Egg

Conclusion

In this study, five hydroxy chalcone derivatives were successfully synthesized via Claisen-Schmidt condensation. Characterization by IR and NMR spectroscopy confirmed the presence of the characteristic chalcone core structure. Notably, compounds **2** and **5** displayed promising antioxidant activity in both DPPH and ABTS assays, suggesting their potential for further development as antioxidants. Molecular docking and in silico ADMET analyses were performed to corroborate the experimental results with the ADME analysis predicting favourable drug-likeness properties for all compounds as according to Lipinski's Rule. By integrating both experimental and simulation

methods, we were able to not only validate the antioxidant activity of the chalcones but also predict their behaviour in a biological system, thus enhancing the robustness of our conclusions and identifying promising candidates for further development. While the moderate bioavailability score warrants further investigation, these findings highlight the potential of these hydroxy chalcone derivatives for a continued exploration as therapeutic agents.

Supplementary Materials

Table S1. The physical properties of hydroxy chalcone compounds; Figure S1. ATR spectrum of (E)-1-(anthracen-9-yl)-3-(2,4-dihydroxyphenyl)prop-2 en-1-one (**1**); Figure S2. ATR spectrum of (2E,4E)-1-(anthracen-9-yl)-5-(4-hydroxy-3-methoxyphenyl)penta-2,4-dien-1-one (**2**); Figure S3. ATR spectrum of (E)-1-(anthracen-9-yl)-3-(2-hydroxyphenyl)prop-2-en-1-one (**3**); Figure S4. ATR spectrum of (E)-1-(anthracene-9-yl)-3-(4-hydroxyphenyl)prop-2-en-1-one (**4**); Figure S5. ATR spectrum of (E)-1-(4-bromophenyl)-3-(2-hydroxynaphthalen-1-yl)prop-2-en-1-one (**5**); Table S2. The ATR spectral data of all compounds; Figure S6. ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of (E)-1-(anthracen-9-yl)-3-(2,4-dihydroxyphenyl)prop-2-en-1-one (**1**); Figure S7. ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of (E)-1-(anthracen-9-yl)-3-(2,4-dihydroxyphenyl)prop-2-en-1-one (**1**); Figure S8. ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of (2E,4E)-1-(anthracen-9-yl)-5-(4-hydroxy-3-methoxyphenyl)penta-2,4-dien-1-one (**2**); Figure S9. ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of (2E,4E)-1-(anthracen-9-yl)-5-(4-hydroxy-3-methoxyphenyl)penta-2,4-dien-1-one (**2**); Figure S10. ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of (E)-1-(anthracen-9-yl)-3-(2-hydroxyphenyl)prop-2-en-1-one (**3**); Figure S11. ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of (E)-1-(anthracen-9-yl)-3-(2-hydroxyphenyl)prop-2-en-1-one (**3**); Figure S12. ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of (E)-1-(anthracene-9-yl)-3-(4-hydroxyphenyl)prop-2-en-1-one (**4**); Figure S13. ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of (E)-1-(anthracene-9-yl)-3-(4-hydroxyphenyl)prop-2-en-1-one (**4**); Figure S14. ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of (E)-1-(4-bromophenyl)-3-(2-hydroxynaphthalen-1-yl)prop-2-en-1-one (**5**); Figure S15. ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of (E)-1-(4-bromophenyl)-3-(2-hydroxynaphthalen-1-yl)prop-2-en-1-one (**5**).

Acknowledgements

The authors are grateful to the Malaysian Government and Universiti Sains Malaysia (USM) for the laboratory facilities and chemicals provided to conduct this work. Thanks to Nippon Sheet Glass Foundation for Materials Science and Engineering No. 304.PFIZIK.6501366.N120 for funding this research.

References

1. Salehi, B., Quispe, C., Chamkhi, I., El Omari, N., Balahbib, A., Sharifi-Rad, J., ... and Les, F. (2021). Pharmacological properties of chalcones: a review of preclinical including molecular mechanisms and clinical evidence. *Frontiers in Pharmacology*, 11: 592654.
2. Zhuang, C., Zhang, W., Sheng, C., Zhang, W., Xing, C., and Miao, Z. (2017). Chalcone: a privileged structure in medicinal chemistry. *Chemical reviews*, 117(12): 7762-7810.
3. Nizar, S. N. A. M., Rosli, M. M., Samsuri, S. A. M., Razak, I. A., and Arshad, S. (2023). Involvement of halogen and polyaromatic substituents in chalcone derivatives as dye sensitizers in solar cell applications. *New Journal of Chemistry*, 47(12): 5804-5815.
4. Mohd Nizar, S. N. A. M., Rosli, M. M., Mohamad Samsuri, S. A., Abdul Razak, I. and Arshad, S. (2023). Research perspectives on the structure-property relationship of polyaromatic pyrene-based chalcone derivatives as dye-sensitizers in DSSC applications. *Physica Scripta*, 99:015917.
5. Gaitry Chopra, P. K. P. (2016). Chalcones: A brief review. *International Journal for Research in Applied Science and Engineering Technology*, 6573(6):2249–3905.
6. Mohd Nizar, S. N. A., Ab Rahman, S. N. F., Zaini, M. F., Anizaim, A. H., Abdul Razak, I. and Arshad, S. (2021). The photovoltaic performance of sensitizers for organic solar cells containing fluorinated chalcones with different halogen substituents. *Crystals*, 11(11), 1357.
7. Goyal, K., Kaur, R., Goyal, A., and Awasthi, R. (2021). Chalcones: A review on synthesis and

pharmacological activities. *Journal of Applied Pharmaceutical Science*, 11(1): 001-014.

8. Božić, D. D., Milenković, M., Ivković, B., and Cirković, I. (2014). Antibacterial activity of three newly-synthesized chalcones & synergism with antibiotics against clinical isolates of methicillin-resistant Staphylococcus aureus. *Indian Journal of Medical Research*, 140(1): 130-137.
9. Gupta, D., and Jain, D. K. (2015). Chalcone derivatives as potential antifungal agents: Synthesis, and antifungal activity. *Journal of Advanced Pharmaceutical Technology & Research*, 6(3): 114-117.
10. Herencia, F., Ferrándiz, M. L., Ubeda, A., Domínguez, J. N., Charris, J. E., Lobo, G. M. and Alcaraz, M. J., (1998). Synthesis and anti-inflammatory activity of chalcone derivatives. *Bioorganic & Medicinal Chemistry Letters*, 8:10.
11. Constantinescu, T., and Lungu, C. N. (2021). Anticancer activity of natural and synthetic chalcones. *International Journal of Molecular Sciences*, 22(21): 11306.
12. Lahsasni, S. A., Al Korbi, F. H., and Aljaber, N. A. A. (2014). Synthesis, characterization and evaluation of antioxidant activities of some novel chalcones analogues. *Chemistry Central Journal*, 8: 1-10.
13. Santos, J. S., Brizola, V. R. A., and Granato, D. (2017). High-throughput assay comparison and standardization for metal chelating capacity screening: A proposal and application. *Food Chemistry*, 214: 515-522.
14. Rajurkar, N. S., and Hande, S. M. (2011). Estimation of phytochemical content and antioxidant activity of some selected traditional Indian medicinal plants. *Indian Journal of Pharmaceutical Sciences*, *73*(2): 146.
15. Daina, A., Michielin, O., and Zoete, V. (2017). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific Reports*, *7*(1): 42717.
16. Ranjith, D. and Ravikumar, C. J. (2019). SwissADME predictions of pharmacokinetics and drug-likeness properties of small molecules present in *Ipomoea mauritiana* Jacq. *Journal of Pharmacognosy and Phytochemistry*, 8(5):2063–2073.
17. Kozłowska, J., Potaniec, B., and Anioł, M. (2020). Biotransformation of hydroxychalcones as a method of obtaining novel and unpredictable products using whole cells of bacteria. *Catalysts*, 10(10): 1167.
18. Pambudi, W., Haryadi, W., and Matsjeh, S. (2019). The effectiveness of hydroxychalcone synthesis by using NaOH and NaOH + ZrO_2 montmorillonite catalyst through conventional and microwave assisted organic synthesis (MaOS) method. *Journal of Physics: Conference Series*, 1155: 012074.
19. Nandiyanto, A. B. D., Oktiani, R., and Ragadhita, R. (2019). How to read and interpret FTIR spectroscope of organic material. *Indonesian Journal of Science and Technology*, 4(1): 97-118.
20. Aksöz, B. E. and Ertan, R. Fabad (2012). Spectral properties of chalcones II. *Journal of Pharmaceutical Sciences*, 37(4): 205-216.
21. AlNeyadi, S. S., Amer, N., Thomas, T. G., Ajeil, R. A., Breitener, P., and Munawar, N. (2020). Synthesis, characterization, and antioxidant activity of some 2-methoxyphenols derivatives. *Heterocyclic Communications*, 26(1): 112-122.
22. de Souza Farias, S. A., da Costa, K. S., & Martins, J. B. (2021). Analysis of conformational, structural, magnetic, and electronic properties related to antioxidant activity: revisiting flavan, anthocyanidin, flavanone, flavonol, isoflavone, flavone, and flavan-3-ol. *ACS omega*, *6*(13): 8908-8918.
23. Karmaker, N., Lira, D. N., Das, B. K., Kumar, U., and Rouf, A. S. S. (2017). Synthesis and antioxidant activity of some novel benzimidazole derivatives. *Dhaka University Journal of Pharmaceutical Sciences*, 16(2): 245-249.
24. Riyadi, P. H., Sari, I. D., Kurniasih, R. A., Agustini, T. W., Swastawati, F., Herawati, V. E., and Tanod, W. A. (2021). SwissADME predictions of pharmacokinetics and drug-likeness properties of small molecules present in spirulina platensis. *IOP Conference Series: Earth and Environmental Science*, 890: 012021.

25. Rajasagaran, R., Mohamad Arshad, A. S., Azizi, J., Mohd Nizar, S. N. A., and Arshad, S. (2023). (E)-3-([2, 2'-bithiophen]-5-yl)-1-(4-fluorophenyl) prop-2-en-1-one: Synthesis and structural elucidation of chalcone derivatives for potential antioxidant activity. *Molecular Crystals and Liquid Crystals*, 758(1): 97-110.

26. Rajasagaran, R., Mohamad Arshad, A. S., Azizi, J., Mohd Nizar, S. N. A., and Arshad, S. (2023). Structural elucidation, in vitro and in silico biological evaluations of new fluorinated chalcone derivatives for potential antioxidant activity. *Letters in Organic Chemistry*, 20(12): 1136-1150.