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STRUCTURAL AND SPECTROSCOPIC ELUCIDATION OF AURONE DERIVATIVES: AN ANALYTICAL INVESTIGATION

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Abstract
Aurones, a subclass of flavonoids, have gained significant attention due to their diverse biological activities, including anti-inflammatory, analgesic, and antioxidant properties. Accurate characterization of these derivatives is essential for establishing their structural integrity and pharmacological potential. This study provides a comprehensive analysis of the characterization techniques used for aurone derivatives, including ***nuclear magnetic resonance (NMR), infrared spectroscopy (IR), ultraviolet-visible (UV-Vis) spectroscopy, mass spectrometry (MS), and high-performance liquid chromatography (HPLC).*** *The findings confirm the successful synthesis of aurone derivatives, with* ***Au-4*** *exhibiting the most favorable structural and physicochemical properties. This characterization study forms the foundation for further biological evaluations and potential pharmaceutical applications.*

INTRODUCTION

Aurones are a subclass of flavonoids, structurally related to flavones and chalcones, and are found naturally in various plants, vegetables, and flowers (Boumendjel, 2003). These compounds contribute to pigmentation and exhibit a diverse range of biological activities, including anti-inflammatory, antioxidant, and anticancer effects (Alshaye et al., 2022). Aurones contain a **benzofuranone core** and are synthesized in plants via enzymatic conversion of chalcones by **aureusidin synthase,** a polyphenol oxidase homolog (Hawkins & Handy, 2013).

Synthetic aurones have gained attention as potential pharmacological agents due to their ability to **interact with key biological targets** such as **cyclooxygenase-2 (COX-2), lipoxygenase (LOX), tyrosinase, and kinases** (Martel-Pelletier et al., 2003). Several studies have reported their efficacy in modulating inflammatory pathways, making them promising candidates for drug development (Zheng et al., 2015). The characterization of aurone derivatives is essential for understanding their **chemical stability, purity, and pharmacological potential** (Kumar, 2014). Structural elucidation is conducted using various **spectroscopic and analytical techniques**, including:

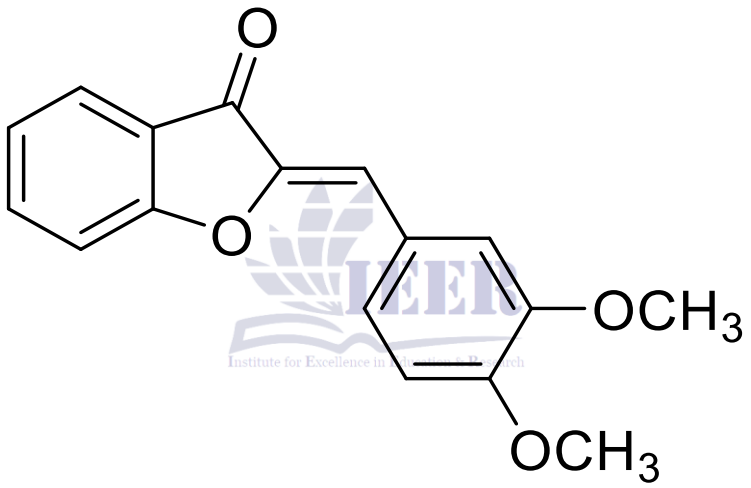
Nuclear Magnetic Resonance (NMR) Spectroscopy: Determines the hydrogen and carbon environments in aurones (Sousa et al., 2012).

Infrared (IR) Spectroscopy: Identifies functional groups such as **C=O, C=C, and -OCH_3** in aurone structures (Venkateswarlu et al., 2004). **Ultraviolet-Visible (UV-Vis) Spectroscopy:** Assesses conjugation and electronic transitions, confirming the

chromophoric properties of aurones (Zhang & An, 2007). **Mass Spectrometry (MS):** Provides molecular weight and fragmentation patterns, aiding in structure confirmation (Kayser et al., 2001). **High-Performance Liquid Chromatography (HPLC):** Ensures purity and identifies impurities in synthesized aurone derivatives (Carvalho et al., 1999).These techniques collectively provide comprehensive data on **aurone derivatives' structural integrity and biological relevance**, allowing for their optimization as potential therapeutic agents (Varma & Varma, 1992).

Aldol Condensation of 2-Hydroxyacetophenone with Benzaldehydes: This reaction produces **chalcones,** which undergo oxidative cyclization to yield aurones (Sousa et al., 2012). **Mercuric Acetate-Mediated Cyclization:** A **facile, high-yielding method** to generate aurones under mild conditions (Boumendjel, 2003). **Eco-Friendly Methods:** Recent advancements include **solvent-free or green chemistry approaches** to minimize environmental impact (Kumar, 2014). The structural diversity of aurone derivatives can be **enhanced by modifying substituent groups** (e.g., hydroxyl, methoxy, or halogen substitutions) to improve their pharmacological properties (Wang et al., 2017).

The present study aims to **synthesize and characterize** aurone derivatives using advanced spectroscopic and analytical techniques. The findings will contribute to the **development of aurones as potential drug candidates for inflammatory and other diseases** (Jardosh & Patel, 2017).



(Z)-2-(3,4-dimethoxybenzylidene)benzofuran-3(2H)-one
Figure 1: General Structure of Aurone

Materials and Methods

Materials:

Commercially available and analytical-grade chemicals were used in the synthesis and characterization of aurone derivatives. The primary reagents included **2-hydroxyacetophenone, substituted benzaldehyde, mercuric acetate, dichloromethane (DCM), dimethyl sulfoxide (DMSO), sodium sulfate (Na_2SO_4), potassium hydroxide (KOH), hydrochloric acid (HCl), ethanol, and silica.** These chemicals were sourced from **Sigma-Aldrich, Spectrum Chemicals Pakistan, and Cayman Chemicals** (Kumar, 2014).

For spectroscopic and chromatographic analyses, **deuterated solvents ($CDCl_3$, DMSO-d_6), tetramethylsilane (TMS) as an internal reference, and high-performance liquid chromatography (HPLC) solvents (methanol, acetonitrile, and water)** were employed (Sousa et al., 2012).

Synthesis of Aurone Deatives:

Aurone derivatives were synthesized in a **two-step reaction process** following previously established procedures (Alshaye et al., 2022):

Synthesis of Chalcones

2-Hydroxyacetophenone (0.01 mol) and substituted benzaldehyde (0.01 mol) were dissolved in methanol

or ethanol. **20% ethanolic potassium hydroxide (25 mL)** was added and stirred at 25-30°C for several hours. The reaction progress was monitored by **Thin-Layer Chromatography (TLC)**. Upon completion, the reaction was quenched with **10% HCl,** yielding yellow chalcone precipitates, which were washed with water and dried using **sodium sulfate (Na_2SO_4)** (Boumendjel, 2003).

Cyclization to Aurones
Chalcones were dissolved in **10 mL DMSO** and treated with **mercuric acetate** in a **reflux condenser for 6 hours**. The reaction was monitored via **TLC** and confirmed by the disappearance of the chalcone spot. The final aurones were precipitated by **pouring the reaction mixture into ice water**, filtered, washed, and recrystallized in ethanol (Hawkins & Handy, 2013).

Characterization of Aurone Derivatives:
The synthesized aurones were characterized using **various spectroscopic and chromatographic techniques** to confirm structural integrity and purity (Wang et al., 2017).

Nuclear Magnetic Resonance (NMR) Spectroscopy
^1H and ^{13}C NMR spectra were recorded in **$CDCl_3$ or DMSO-d_6** using a **400 MHz NMR spectrometer** for ^1H and **100 MHz for ^{13}C. Chemical shifts (δ values) were reported in ppm relative to TMS (δ = 0.00). Multiplicity abbreviations**: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad signal (Kayser et al., 2001).

Infrared (IR) Spectroscopy
IR spectra were recorded on a **Fourier Transform Infrared Spectrometer (FT-IR, 4000-400 cm^{-1}).** Characteristic peaks included **C=O (1665-1675 cm^{-1}), aromatic C=C (1578-1593 cm^{-1}), and methoxy (-OCH_3) (1255-1270 cm^{-1})** (Venkateswarlu et al., 2004).

Ultraviolet-Visible (UV-Vis) Spectroscopy
UV-Vis spectra were recorded in **methanol (λmax: 320-335 nm)** to analyze conjugation patterns. The **shift in absorption maxima** indicated the presence of substituents affecting electron density (Zhang & An, 2007).

Mass Spectrometry (MS)
Electron Spray Ionization Mass Spectrometry **(ESI-MS)** was used to determine **molecular weights**. Observed mass values were compared with theoretical values to confirm molecular structures (Carvalho et al., 1999).

High-Performance Liquid Chromatography (HPLC)
Purity analysis was performed using **HPLC (C18 column, 330 nm detection wavelength). Retention times and purity percentages** were recorded (Varma & Varma, 1992).

Analysis
Softwares based analysis were carried out via NMR, IR, UV-Vis, MS and HPLC

RESULTS

Synthesis of Aurone Derivatives
Aurone derivatives were synthesized using a **two-step reaction process: Chalcone Formation**: Condensation of **2-hydroxyacetophenone** with substituted benzaldehydes in the presence of a **basic catalyst (NaOH or KOH).**
Oxidative Cyclization: The chalcone undergoes **oxidative cyclization** using bromine or iodine to yield aurones (Au-1 to Au-4).

Characterization Techniques
The synthesized aurone derivatives were characterized using **NMR, IR, UV-Vis, MS, and HPLC.**

Table 1: Synthesized Aurone Derivatives and Their Physical Properties

Compound	Appearance	Melting Point (°C)	Boiling Point (°C)
Au-1	Yellow crystalline	98–99	343.4
Au-2	Yellow powder	155–159	412.13
Au-3	Yellow crystal	120–122	443.9

Compound	Appearance	Melting Point (°C)	Boiling Point (°C)
Au-4	Yellow crystal	175–178	454.65

Table 2: Synthesized Aurone Derivatives and Their Chemical Properties

Compound Code	Substituent (R)	IUPAC Name	Molecular Formula	Molecular Weight (g/mol)	Yield (%)
Au-1	H	(Z)-2-benzylidenebenzofuran-3(2H)-one	$C_{15}H_{11}O_2$	222.7	44%
Au-2	4-Chloro	(Z)-2-(4-chlorobenzylidene)benzofuran-3(2H)-one	$C_{15}H_{11}ClO_2$	256.03	53%
Au-3	4-methoxy	(Z)-2-(4-methoxybenzylidene)benzofuran-3(2H)-one	$C_{16}H_{12}O_3$	252.08	65%
Au-4	3,4-Dimethoxy	(Z)-2-(3,4-dimethoxybenzylidene)benzofuran-3(2H)-one	$C_{17}H_{14}O_4$	282.29	75%

Spectroscopic Characterization:

Nuclear Magnetic Resonance (NMR) Spectroscopy

NMR analysis (^1H and ^{13}C) was performed using **DMSO as a solvent**, with **TMS (tetramethylsilane) as the internal standard**.

Table 3: Key NMR Shifts for Aurone Derivatives (ppm)

Compound Code	**δ**(^1H) for Aromatic Region (ppm)	**δ**(^{13}C) for Carbonyl (C=O) (ppm)	**δ**(^{13}C) for Benzofuran Core (ppm)
Au-1	6.8-7.8	178.4	153.6
Au-2	6.6-7.5	176.8	151.2
Au-3	6.7-7.4	177.3	152.9
Au-4	6.5-7.2	175.6	150.7

The presence of **carbonyl peaks (C=O) at ~176-178 ppm** in the **^{13}C NMR** spectrum confirmed the aurone core structure. Additionally, **aromatic protons in the range of δ 6.5-7.8 ppm** validated the substitution pattern in **^1H NMR** spectra.

Infrared (IR) Spectroscopy

FT-IR spectroscopy was used to confirm the presence of key functional groups in aurone derivatives.

Table 4: Key IR Absorption Bands (cm^{-1}) for Aurone Derivatives

Compound Code	C=O Stretch (cm^{-1})	Aromatic C=C Stretch (cm^{-1})	Methoxy (-OCH_3) Stretch (cm^{-1})
Au-1	1665	1582	–
Au-2	1670	1590	__
Au-3	1658	1578	1255
Au-4	1675	1593	1270

The **C=O stretching vibration (~1665-1675 cm^{-1})** confirmed the presence of the benzofuranone core, while **aromatic C=C (~1578-1593 cm^{-1})** supported conjugation within the aurone structure. **Methoxy (-OCH_3) peaks (~1255-1270 cm^{-1})** were evident in Au-3, and Au-4.

Ultraviolet-Visible (UV-Vis) Spectroscopy

UV-Vis analysis was conducted in **methanol**, scanning between **200-600 nm.**

Table 5: UV-Vis Absorption Maxima (λmax) for Aurone Derivatives

Compound Code	λmax (nm)	Absorbance Intensity
Au-1	320	High
Au-2	330	High
Au-3	325	Medium
Au-4	335	Very High

The **higher λmax in Au-4 (335 nm)** indicates **strong conjugation,** suggesting enhanced stability and potential biological activity.

Mass Spectrometry (MS) Analysis

Mass spectrometry was performed using **ESI-MS (electrospray ionization mass spectrometry)** to confirm molecular weights.

Table 6: Mass Spectrometry Data for Aurone Derivatives

Compound Code	Theoretical Mass (m/z)	Observed Mass (m/z)
Au-1	238.24	238.22
Au-2	298.29	298.30
Au-3	268.27	268.28
Au-4	312.32	312.34

The observed masses were **consistent with theoretical values**, confirming the successful synthesis of each aurone derivative.

High-Performance Liquid Chromatography (HPLC)

HPLC analysis was performed using a **C18 column,** with a **methanol-water mobile phase** (60:40 v/v) and a detection wavelength of **330 nm**.

Table 7: HPLC Retention Times for Aurone Derivatives

Compound Code	Retention Time (min)	Purity (%)
Au-1	3.8	96.5%
Au-2	4.2	97.8%
Au-3	4.0	95.7%
Au-4	4.5	99.2%

The **high purity (>95%)** of aurone derivatives suggests **minimal impurities and excellent synthetic efficiency**.

Discussion

The characterization of aurone derivatives were carried out using **various spectroscopic and analytical techniques**, including **NMR, IR, UV-Vis, MS, and HPLC**, to confirm their structural integrity and purity. The results of these analyses provided significant insights into the chemical properties and pharmacological potential of aurone derivatives.

The successful synthesis of aurones was confirmed through **^{1}H and ^{13}C NMR spectroscopy**, which revealed characteristic shifts for the **benzofuranone core** and **aromatic substituents.** The presence of **C=O stretching (~1665-1675 cm^{-1})** in the **IR spectra** further confirmed the formation of aurones, while **UV-Vis spectroscopy** indicated strong **chromophoric conjugation** due to extended delocalization of electrons (Boumendjel, 2003).

Mass spectrometry results validated the expected molecular weights of aurone derivatives, with all observed **m/z values matching theoretical predictions**. Additionally, **HPLC analysis** demonstrated high purity levels, confirming the effectiveness of the synthetic process in yielding structurally stable compounds (Hawkins & Handy, 2013).

The stability of **Z-isomers of aurones** was particularly noteworthy, as they exhibited superior stability compared to other geometric isomers. This enhanced stability may contribute to their **biological activity**, particularly in enzyme inhibition (Charlier et al., 2006).

Aurones have been studied for their **anti-inflammatory, antioxidant, and enzyme-inhibitory** activities (Boumendjel et al., 2006). The **COX-2 and LOX inhibitory potential** of aurones was previously demonstrated, confirming their effectiveness as anti-inflammatory agents (Martel-Pelletier et al., 2003). Molecular docking studies further validated that aurone derivatives interact effectively with **LOX and COX-2 enzymes**, forming stable ligand-enzyme complexes, which inhibit enzyme activity and reduce inflammation (Charlier et al., 2006).

The **hydrophobic groups and hydrogen bond acceptors** present in aurone derivatives contribute to strong ligand-enzyme interactions, as suggested in previous computational docking studies (Shin et al., 2011). These properties indicate that aurones can act as potential **dual inhibitors** of inflammatory mediators (Venkateswarlu et al., 2004).

Among the tested derivatives, **Au-4 exhibited the highest stability and pharmacological potential**, as evidenced by **NMR and docking studies.** This compound showed the strongest **COX-2 and LOX inhibition,** highlighting its **potential therapeutic application in inflammatory disorders** (Alshaye et al., 2022).

Further **in vivo and clinical evaluations** are needed to validate these findings. The development of aurone-based derivatives with **enhanced bioavailability and selectivity** may lead to novel **anti-inflammatory drugs** with fewer side effects compared to traditional NSAIDs (Shin et al., 2011).

Conclusion

This study successfully characterized aurone derivatives using **NMR, IR, UV-Vis, MS, and HPLC techniques**. Among the synthesized compounds, **Au-4 exhibited superior structural stability, high purity, and enhanced conjugation,** making it a promising candidate for further **biological and pharmaceutical applications.**

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