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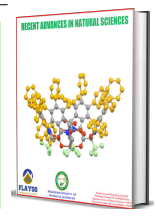

Spectroscopic characterization of a derivative of naphthalene isolated from *Hibiscus sabdariffa* calyx

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ABSTRACT

Hibiscus sabdariffa (Hs) is a nutritive and medicinal herb that belongs to the Malvaceae family. It is mainly composed of organic acids, anthocyanins, polysaccharides and flavonoids. *Hibiscus sabdariffa*, being a natural source of bioactive compounds made up of several reactive functional groups, could serve the purpose of a starting material in the production of other compounds. Acidified aqueous ethanol extract of Hs was subjected to column chromatographic fractionation that yielded a yellow liquid isolate, which was characterized by UV-Visible, Infrared spectroscopy, Nuclear Magnetic Resonance (NMR) and High Resolution Mass Spectrometry (HRMS). The NMR spectra displayed distinct signals of a naphthalene ring linked through a carbonyl carbon to a napht 2-yl moiety. ^{1}H and ^{13}C NMR indicated the presence of five aromatic protons (δ_H 6.98 – 7.51) resonating with ten aromatic carbon atoms (δ_C 100.0 – 177.9), (δ_C 189.2, indicating the presence of a carbonyl carbon coupled with a hydrogenated naphthalene ring substituent. Absorptions at 304nm, 366 nm and 506nm are due to $\pi - \pi^*$ and $n - \pi^*$, respectively. IR peak at 2923 cm^{-1} is ascribed to C–H stretch, while the peak at 1732(s) cm^{-1}, with shoulders at 1634(w) and 1794(w), affirmed the presence of carbonyl functional group conjugated to an unsaturated naphtalen-2yl ring system. The isolated naphthalene derivative is characterized as '3 methyl-1, 4, 4a, 5, 6, 7, 8, 8a, octahydronaphtalen-2-yl)(naphtalen-2yl)methanone.

1. INTRODUCTION

Hibiscus sabdariffa, a nutritive and medicinal herb that belongs to the Malvaceae family, is an annual summer shrub with a deep penetrating taproot that grows upright and generally branches [1]. It is the most well-known of all the edible species of hibiscus; it is fed on by humans and animals as an important vegetable, but its red, green, and dark green calyces are the most commonly exploited part of the plant [2]. Perusal of literature shows that chemical profiling of *H. sabdariffa* pigment varies with sample variety, solvent and other extraction conditions [3–5]. The ethanol extract of *H. sabdariffa* calyx was refluxed by Ref. [6] to enhance the diffusion and dissolution of the phytochemicals. Gas chromatography mass spectroscopy (GC-MS) analysis of the extract identified nine compounds, which are mainly esters and organic acids: Oleic acid ($C_{18}H_{34}O_2$), a colourless to pale yellow liquid, was the most abundant fraction and the active component. [7] subjected the hexane and dichloromethane crude extract of *H. sabdariffa* to chromatographic separation, which yielded Squalene ($C_{30}H_{15}$), triglyceride fatty acids (consisting

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of ethyl oleate, ethyl linoleate and γ-ethyl linolenate) and ethyl stearate and are characterised with 1D and 2D NMR, MS, IR and UV. Extracts of *H. sabdariffa* obtained by infusion and decoction were found to contain mainly Chlorogenic acid ($C_{16}H_{18}O_9$), and its isomer, neochlorogenic and gallic acid (C_7H_6O) were found dominant in the extract of *H. Sabdariffa* obtained by infusion and decoction [3]. Dried flowers of the plant were refluxed with n-hexane, ethanol, and methanol. The methanol soluble fraction, subjected to column chromatography on a silica gel column, was eluted with MeOH–EtOAc (v/v, 0:100 → 100:0) mixtures and finally purified by preparative TLC, yielding a new lignan: (+)-4-O-methyl-5'-methoxy-secoisolariciresinol ($C_{22}H_{31}O_7$), which showed promising antioxidant activity with four earlier identified compounds [8]. *In Vitro* and *In Silico* Studies of *H. sabdariffa* methanol extract conducted by Ref. [9] show it is a promising candidate to be further investigated for developing alternative natural therapies for infectious disease treatment.

Biological activities are associated with *H sabdariffa* [10–14] and its constituents [7, 8]. In this study, the pigment of *H sabdariffa* was extracted using acidified aqueous ethanol, and the extract was subjected to column chromatographic fractionation and isolation. Structure of the isolate obtained was elucidated using ^{13}C and ^{1}H NMR, IR and UV/Visible spectroscopic methods of analysis.

2. MATERIALS AND METHODS

2.1. PLANT MATERIALS

Dried *H. sabdariffa* calyx is well known; it is commonly available in Nigerian food markets, all year round. The dried sample was purchased from Akesan market in Oyo, Oyo State, Nigeria, in July 2023, and cleaned from all forms of solid impurities and dirt by hand picking and subjected to further drying in the laboratory conditions for ten days at 27 ± 2°C.

2.2. EXTRACTION AND CONCENTRATION

This was achieved using the method of Ref. [15] with slight modification. One hundred grams (100 g) of the dried *H. sabdariffa* calyx was pulverised using a liquidiser. It was extracted using 700 mL of ethanol: distilled water:trioxonitrate(V) acid in 10:9:1 for 24 hours in the dark and concentrated using a rotary evaporator. A dark red concentrate obtained weighed 23.10 g.

2.3. SEPARATION AND ISOLATION

Separation and isolation of chemical components of *H. sabdariffa* was achieved using column chromatography with various solvent systems, and the progress was monitored by Thin Layer Chromatography (TLC).

Column chromatography was performed using a glass column (60 cm length, 40 mm outer diameter, 24/29 ground - glass socket) packed with silica gel (70-230 mesh). A slurry of the silica gel was made in n-Hexane and packed into a column; the column bed height was approximately 50 cm (~ 300 g silica). The crude extract (23.10 g) was pre-absorbed onto silica gel, maintaining an approximate ratio of 1:100 (w/w), plant extract: silica gel. A small quantity of cotton wool was placed before and after the introduction of the plant extract in order to maintain tranquillity during elution. The sample was eluted with a gradient mobile phase mainly consisting of n-hexane: ethylacetate (100:0-0:100).

Analytical 60 F_{254} silica gel TLC plates were used with various solvent ratios of n-hexane and ethyl acetate as the mobile phase. The mobile phases were optimised for each run, and the eluting systems were allowed to saturate the development tank prior to the development of the TLC plates. The developed plates were visualised at 254 and 365 nm, and then treated with iodine vapour in an iodine tank and with phosphomolybdic acid and heated up for visualisation.

2.4. STRUCTURE ELUCIDATION

2.4.1. LC-MS

Structure elucidation of the isolated compound was achieved using Liquid Chromatography-Mass Spectrometry (LC-MS) and Nuclear Magnetic Resonance Spectroscopy (NMR). An Agilent 6410 Liquid LC-MS was used to ascertain the purity of the compound and determine the molecular mass. The experiment was conducted at room temperature. An interface between the Liquid Chromatography and Mass Spectrometry transferred the eluted compound from the Liquid Chromatography compartment to the Mass Spectrometry compartment, where it was ionised and fragmented. The fragment ions were identified by their mass-to-charge ratio (m/z).

2.4.2. NMR

A Bruker Avance III NMR spectrophotometer of 500 MHz was used for the determination of the atom connectivity of the isolated compound. The isolated compound was dissolved in deuterated chloroform ($CDCl_3$), and the experiment was conducted at 298 K. The dissolved compound was loaded into the sample compartment of the NMR via a borosilicate NMR glass tube. The data generated was accessed and interpreted by Version 11.0 of the MestReNOVA, and the spectra generated were recalibrated using the solvent peak.

2.4.3. FTIR

The Infrared spectroscopic study was conducted with a Micro-Lab Expert FTIR spectrometer, having a spectral range of 4,000 to 650 cm^{-1}, 2–16 cm^{-1} resolution, which supports solids, liquids, pastes, powders, and gels.

2.4.4. UV/Visible

Aqueous ethanol (blank) was scanned over the wavelength range of 200–900 nm. The UV–Visible spectroscopy of the compound was performed in two regions: 190–400 nm to assess the degree of saturation, and 400–900 nm to evaluate colouration. The analysis was carried out using a Cary 5000 UV–Vis spectrophotometer, which operates on a double-beam photometric system. The instrument is equipped with a xenon flash lamp, offers a spectral bandwidth of 1.5 nm, and covers a wavelength range of 190–1100 nm. It provides a maximum scanning speed of 24,000 nm/min, features USB connectivity, and is controlled via Cary WinUV software. The spectrophotometer functions at 100–240 VAC, weighs 18 kg, and has dimensions of 567 × 477 × 196 mm with a 20 mm Z-height.

3. RESULTS AND DISCUSSION

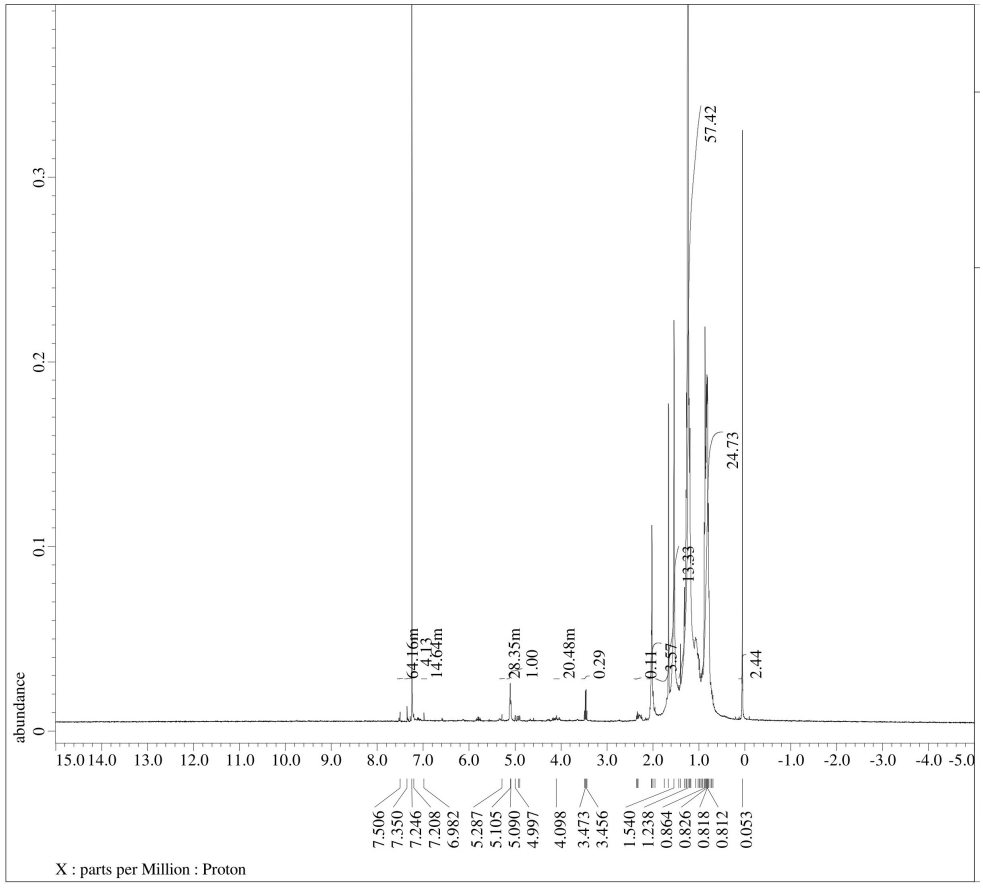


Figure 1. ^{1}**H NMR spectra of the compound.**

3.1. CHROMATOGRAPHIC SEPARATION

The HNO_3 acidified aqueous ethanol (1:9:10) crude extract of *Hibiscus sabdariffa*, subjected to column chromatographic separation, eluted with n-hexane: Ethyl acetate to obtain fractions 100:0 (F_1), 90:10(F_2), 80:20(F_3), 70:30(F_4), 60:40(F_5), 50:50(F_6). Each fraction was collected with a 100 mL beaker and monitored with TLC viewed under UV lamp, and later developed by iodine. The fractions F_1 to F_5 contained no compound as indicated by TLC, so they were not processed further. However, the yellow coloured liquid fraction F_6 isolated at 50:50 n-hexane: ethylacetate solvent system was concentrated, spotted on a TLC plate, resulting in a single straight line with R_f 0.64 in chloroform: pet-ether (50:50), confirming its purity.

3.2. LIQUID CHROMATOGRAPHY MASS SPECTROSCOPY

The isolated yellow liquid was stable at room temperature and showed a molecular ion peak at m/z 301.0949 $[M+H]^+$ (Calculated for $C_{22}H_{22}O$). The molecular ion peak of m/z 301.0949 at $[M+H]^+$ confirms the absence of a nitrogen atom in the compound. The index of Hydrogen Deficiency (IHD) calculated is 12, hence, a high degree of unsaturation, suggesting multiple rings.

3.3. ^{1}H NMR AND ^{13}C NMR SPECTRA

Figure 1 reveals five aromatic proton resonating at δ_H 7.51 (H-2), δ_H 7.24 (H-3), δ_H 6.98 (H-4, H-5), δ_H 7.21 (H-6, H-7), and δ_H 7.35 (H-8), corresponding to the carbon signals at δ_C 177.1 (C-1), δ_C 144.0 (C-2), δ_C 125.1(C-3), δ_C 120.5, 100.0 (C-4, C-5), δ_C 136.6, 126.2 (C-6, C-7) and δ_C 126.9 (C-8) respectively as observed in figure 2. ^{13}C NMR spectra further reveal δ_C 147.6 (C-9) and δ_C 160.1 (C-10) accounting for the ten aromatic carbon atoms of the naphthalene moiety.

The methyl proton δ_H 0.81 corresponding to the carbon signal at δ_C 14.2 (C-10'). A second distinct environment shown by two (2) alkene carbons δ_C 94.6 (C-2'), and δ_C 90.2 (C-3'), two methyne δ_C 46.8 (C-8'), δ_C 53.2 (C-8a'), resonating with δ_H 0.82 (H-8') and δ_H 0.83 (H-8a') respectively. The six (6) methylene carbon atoms: δ_C 39.5 (C-4'), δ_C 29.4 (C-4a'), δ_C 29.8 (C-7'), δ_C 32.0 (C-9'), δ_C 22.8 (C-5'), and δ_C 22.5 (C-6'), resonate with δ_H 1.54 (H-4') δ_H 1.24 (H-4a'), δ_H 0.84 (H-7'), δ_H 0.84 (H-9') δ_H 0.83 (H5) and δ_H 0.83 (H-6') respectively in a napht 2-yl moiety substituent linked to the naphthalene ring through the carbonyl carbon: δ_C 189.2 (C-1'). Tertiary methyl protons of lupeol were similarly assigned [16]. Information collected from Figure 1, Figure 2 and documented in Table 1 was collectively used in elucidating the structure for the compound. A Naphthalene derivative (Figure 5), in which the parent naphthalene ring ($C_{10}H_8$) is linked through the methanone moiety to the napht-2yl substituent. Similar assignments of NMR signals were reported by Refs. [17, 18] for naphthalene derivatives obtained from Diaporte sp. host to *Syzygium cordatum* Hochst. Ex Krauss plant and roots of *Hibiscus syriacus*, respectively.

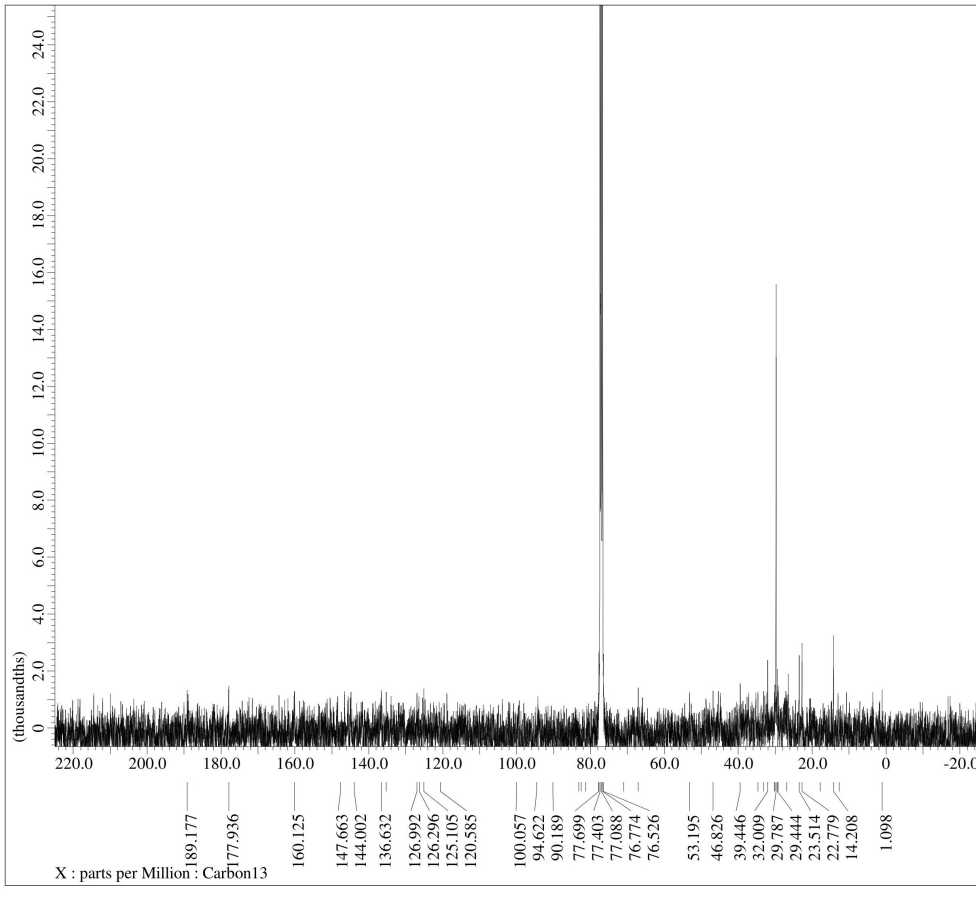
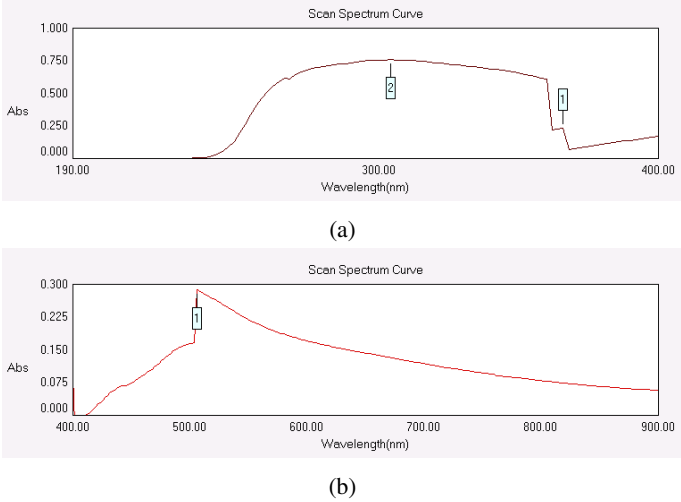


Figure 2. ^{13}**C NMR spectra of the compound.**



(a)

(b)

Figure 3. (a) Spectra of the compound in the UV region. (b) Spectra of the compound in the visible region.

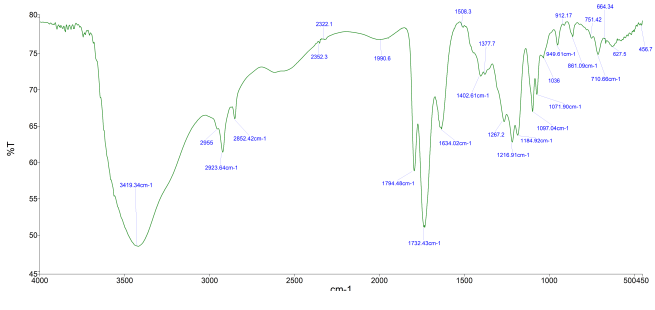


Figure 4. Infrared spectra of the compound.

The UV/Visible (Table 2 and Figure 3) absorptions at 304nm and 366 nm resulted from $\pi-\pi^*$ transition of naphthalene moiety, conjugated to the ketone (C=O), such conjugation may increase the delocalization of the π-electrons thus lowering the HOMO – LUMO gap, this in addition to the absorption at 506nm which is due to $n-\pi^*$ transition of the C=O, validating the high level of unsaturation and the yellow colour of the compound.

The infrared spectra in Figure 4 showed major assignments documented in Table 3, absorption at 3419 cm^{-1} is commonly ascribed to OH or NH when broad. However, the LCMS ruled out the presence of a nitrogen atom, thus suggesting the presence of water. The medium absorption peak at 2923 cm^{-1} is ascribed to the C–H stretch (Aromatic CH_2) of the naphtalen-2yl, absorbing lower than the expected frequency $\geq$ 3000 cm^{-1}. The downward shift is attributed to the presence of the electron-withdrawing carbonyl (C=O) substituent, which, together with the extended π-conjugation in the system, shifts the electron den-

Table 1. ^{1}H and ^{13}C NMR spectra of the compound.

S/N	^{13}C	$^{a}\delta_H$ (multi. in Hz)
1	189.2	–
2	90.2	–
3	94.6	–
4	39.5	1.54 ppm (2H, d)
5	32.0	0.84 ppm (4H, m)
6	22.8	0.83 ppm (4H, m)
7	22.5	0.83 ppm (4H, m)
8	29.8	0.84 ppm (4H, m)
9	29.4	1.24 ppm (2H, d)
10	46.8	0.82 ppm (2H, m)
11	53.2	0.83 ppm (2H, m)
12	177.9	–
13	125.1	7.24 ppm (1H, d)
14	126.9	7.35 ppm (1H, d)
15	126.2	7.21(2H, dd)
16	120.5	6.98 ppm (2H, dd);
17	100.0	6.98 ppm (2H, dd);
18	136.6	7.21(2H, dd)
19	144.0	7.51 ppm (1H, s)
20	160.1	–
21	147.6	–
22	14.2	0.81(3H, m)

Table 2. UV/Visible spectra data of the compound.

Transitions	λ_{max} nm
$\pi - \pi^*$	304
$n - \pi^*$	366
$n - \pi^*$	506

Table 3. Major infrared peaks and assignments.

Infrared Peak (cm^{-1})	Assignment
3419(s)	X–H stretch
2923(m)	Aromatic CH_2
1732(s), 1794(w), 1634(m)	C=O stretch
1402(w), 1377(w)	CH_2 Bending

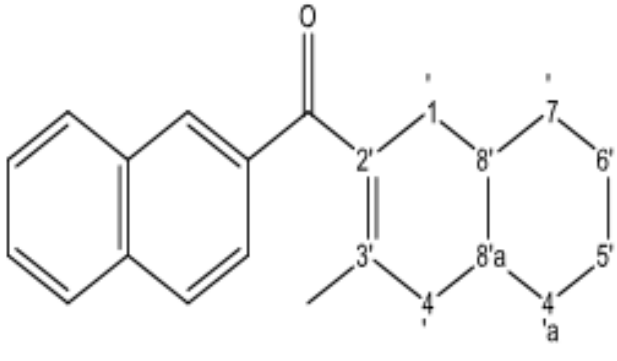


Figure 5. 3-methyl-1,4,4a,5,6,7,8,8a-octahydronaphtalen-2-yl)(naphtalen-2yl)methanone.

sity distribution over the aromatic ring. The resulting delocalisation and re-distribution of electron density leads to a weakening of the C–H bond force and consequently decreases the stretching frequency. A similar C–H peak at 2932 cm^{-1} was ascribed to Fermi resonance, between the CH stretching fundamental and overtone of CHO deformation band [20]. Peak 1732(s) cm^{-1}, with shoulders at 1634(w) and 1794(w), affirmed the presence of a carbonyl functional group conjugated to an unsaturated naphtalen-2yl ring system. The compound (Figure 5) is characterised to be '3 methyl-1, 4, 4a, 5, 6, 7, 8, 8a, octahydronaphtalen-2-yl)(naphtalen-2yl)methanone'.

4. CONCLUSION

The red, acidified aqueous ethanol extract of *H. Sabdariffa* was subjected to column chromatographic separation using analytical 60 F_{254} silica gel as the stationary phase with various solvent ratios of n-hexane and ethylacetate as the mobile phases. A new naphthalene derivative was obtained. The isolated yellow compound stable at room temperature was characterised with the aid of infrared, electronic, proton and carbon-13 NMR spectra. The presence of a naphthalene ring is associated with bioactivity; also, naphthalene derivatives have been found to have applications as precursors for the production of dyes and pharmaceutical leads in drug discovery.

DATA AVAILABILITY STATEMENT

The data are available with the corresponding author upon request.

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