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Eco-Friendly Microwave Synthesis and Antioxidant Study of Bioactive Quinoline Scaffolds

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Abstract

A simple, efficient, and high-yielding one-pot multicomponent synthesis was developed using 5,5-dimethylcyclohexane-1,3-dione, acetanilide, and substituted benzaldehydes. The reaction mixture, with a few drops of triethylamine, was subjected to microwave irradiation at 180 W and 120°C for 5 minutes. This microwave-assisted method offers notable advantages such as reduced reaction time, excellent yields, and environmental sustainability. Microwave-Assisted Organic Synthesis (MAOS) has proven to be a powerful approach for promoting rapid and clean transformations. The synthesized compounds were structurally confirmed using IR, NMR, and Mass spectroscopy. Additionally, antioxidant activity studies were conducted, revealing promising bioactivity of the synthesized quinoline derivatives.

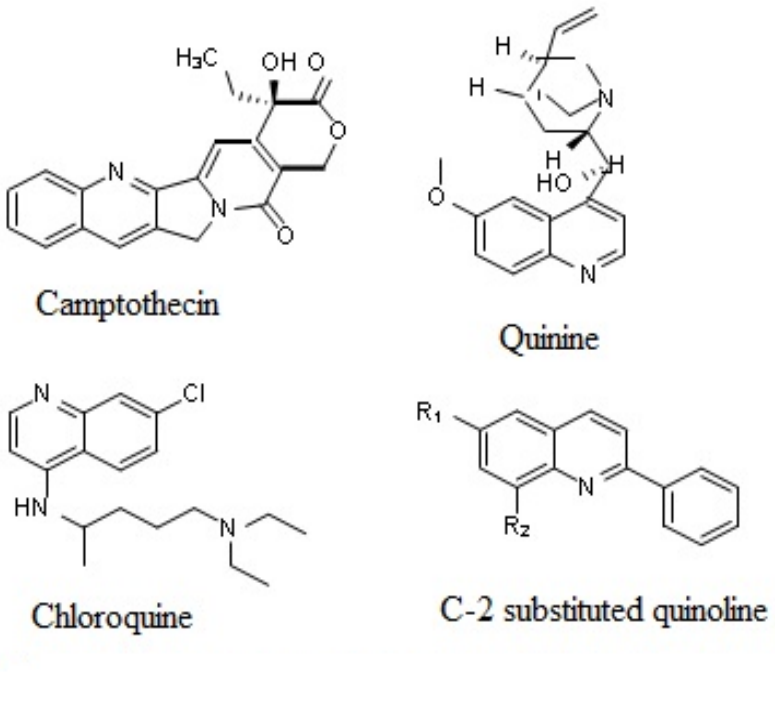
Keywords: 5,5-dimethylcyclohexane-1,3-dione, acetanilide, substituted benzaldehydes & Microwave.

1. Introduction

Quinoline nucleus occurs in various natural compounds and pharmacologically active substances exhibiting a wide range of biological activity. Quinoline and its derivatives possess diverse pharmacological agents. They play a major role in the development of new therapeutic agents. The quinoline nucleus is used to develop new therapeutic agents which makes quinoline and its derivatives constitute a significant class of heterocyclic compounds for the new drug development.

Quinoline derivatives display a wide range of significant activities such as suchasantiprion[1], antimicrobial, [2-5]antibacterial, [6]antitubercular[7-9,] and anticancer activities. [9-12]Quinolinederivatives exhibit outstanding results through various mechanisms of action such as growth inhibitors by cell cycle arrest, inhibition of angiogenesis, apoptosis, and modulation of nuclear receptor responsiveness and disruption of cell migration. [13-15] A review on anticancer candid of bioactive heterocyclic quinoline was published recently explaining the importance of quinoline.[16]

Figure - 1 illustrates promising structures of quinoline as the biologically active nucleus.



Scheme 1. Promising structures of quinolone as the biologically active nucleus

2. Results and Discussion

2.1. Synthesis 7, 7-dimethyl-1,4-diphenyl-3,4,5,6,7,8-hexahydro quinoline-2,5(1*H*, 6*H*)-dione (4a-e)

An equimolecular combination of 5,5-dimethylcyclohexane-1,3-dione, acetanilide, and a substituted benzaldehyde was thoroughly mixed with a few drops of triethylamine and irradiated in a microwave oven at 180 W and 120°C for 5 minutes **(Scheme 2).** The progress of the reaction was monitored using TLC. Upon completion, the reaction mixture was poured into ice-cold water, yielding a yellow solid. The obtained product was purified using a simple chromatographic technique.

The IR spectrum **(Figure 1)** showed the stretching frequency at 3430 cm^{-1} and 1683, 1591 for the N-H group and carbonyl group respectively. The ^{1}H NMR spectrum **(Figure 2)**registered three singlets at 0.99 ppm, 2.29, and 1.88 for gem methyl protons, and C_6& C_8 protons respectively. The multiplet at 2.69-2.71 ppm was assigned for C_3-H & C_4-H and the aromatic protons appeared multiplet in the region 7.19-7.72 ppm. In ^{13}C NMR spectrum of compound 4**a** (**Figure 3**) showed the two methyl carbon signals observed at 26.81 ppm, the aliphatic carbons observed at 32.79, 34.99, 35.74, 43.23, and 51.12 ppm for C_8, C_4, C_3, C_9, and C_7 carbons, respectively. All the aromatic carbon signals appeared in the region of 124.92-143.01 ppm. The two carbonyl carbons, C=O (C-2) signals appeared at 169.22 and C=O (C-6) carbon signal at 198.97 ppm. The C=C double bond signals appeared at 111.54 and 152.52 ppm for C_5 and C_{10} carbons.

The mass spectrum (**Figure 4**)showed molecular ion peak at *m/z* 346 and elemental analysis Calcd. For $C_{23}H_{23}NO_2$; C 79.97, H 6.71, N4.05: Found: C 79.89, H 6.71, N 4.03. From the above spectral data, the obtained compounds as 7,7-dimethyl-1,4-diphenyl-3,4,7,8-tetrahydro quinoline-2,5(1*H*, 6*H*)-dione**(10a)**

To establish the generality and applicability of this method, various substituted benzaldehydes (**2a-e**) were subjected to the above reaction conditions to furnish the corresponding quinoline derivatives (4**b-e)** as shown in (**Scheme 2**).

Scheme 2. Synthetic route of quinoline derivatives **(4a-e)**

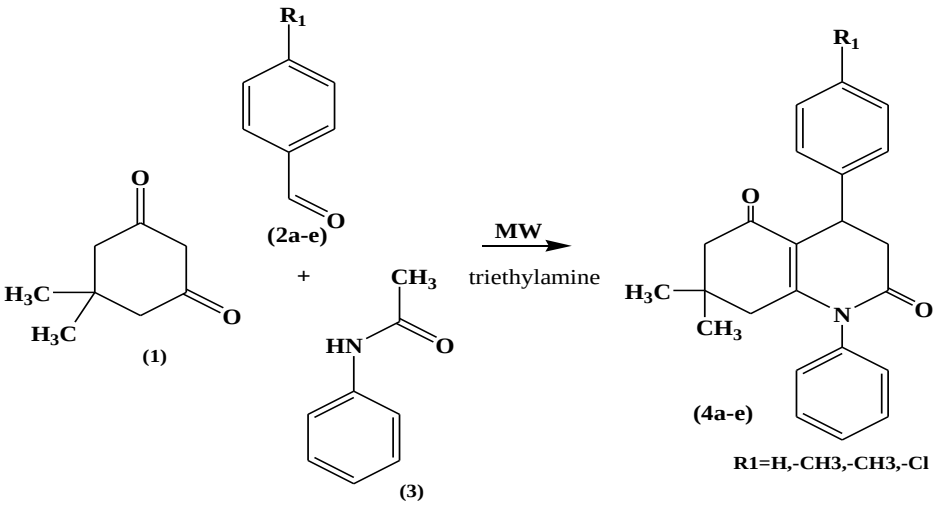


Table 2.1. Physical data of quinoline derivatives**(4a-e)**

Compounds	R_1	mp (°C)	Yield (%)	Time (min)
4a	H	235	93	6
4b	*p*-CH3	238	89	7
4c	*p*-OCH3	237	87	7
4d	*p*-Cl	240	82	8
4e	*p*-OH	242	92	6

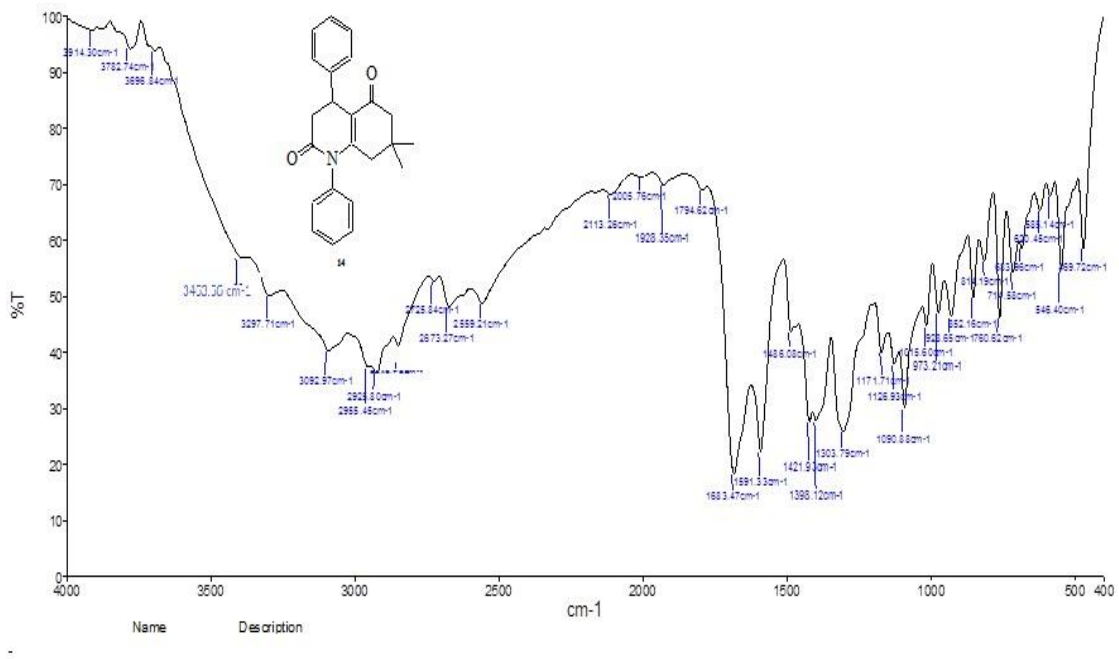


Figure 1 -IR spectrum of7,7-dimethyl-1,4-diphenyl-3,4,7,8-tetrahydroquinoline-2,5(1*H*, 6*H*)-dione (4a)

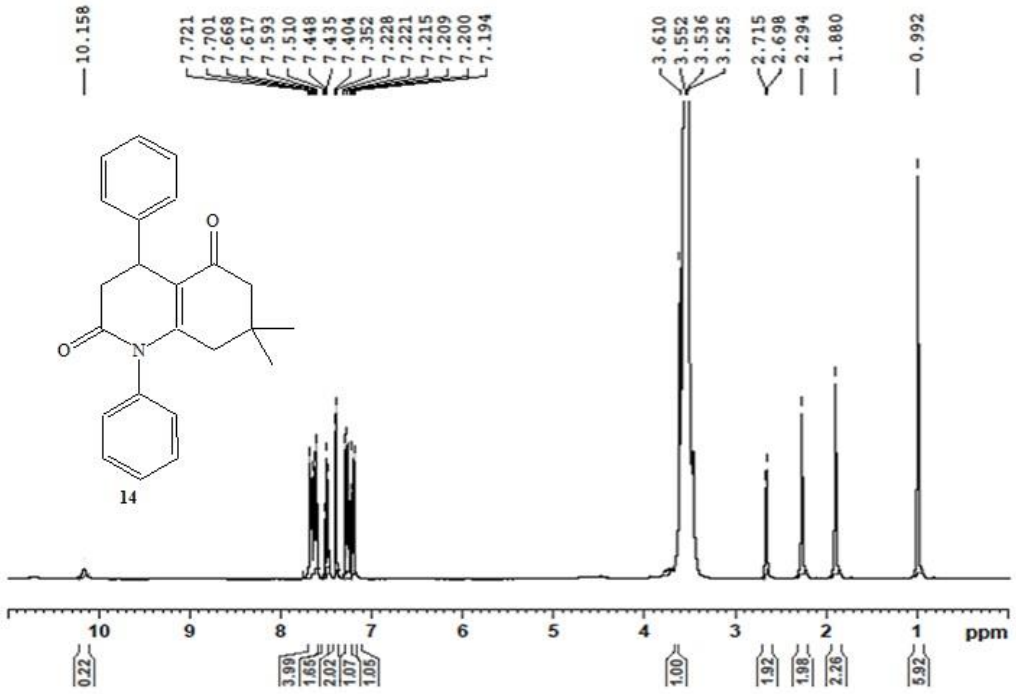


Figure 2 - ^{1}H NMR spectrum of7,7-dimethyl-1,4-diphenyl-3,4,7,8-tetrahydroquinoline-2,5(1*H*, 6*H*)-dione (4a)

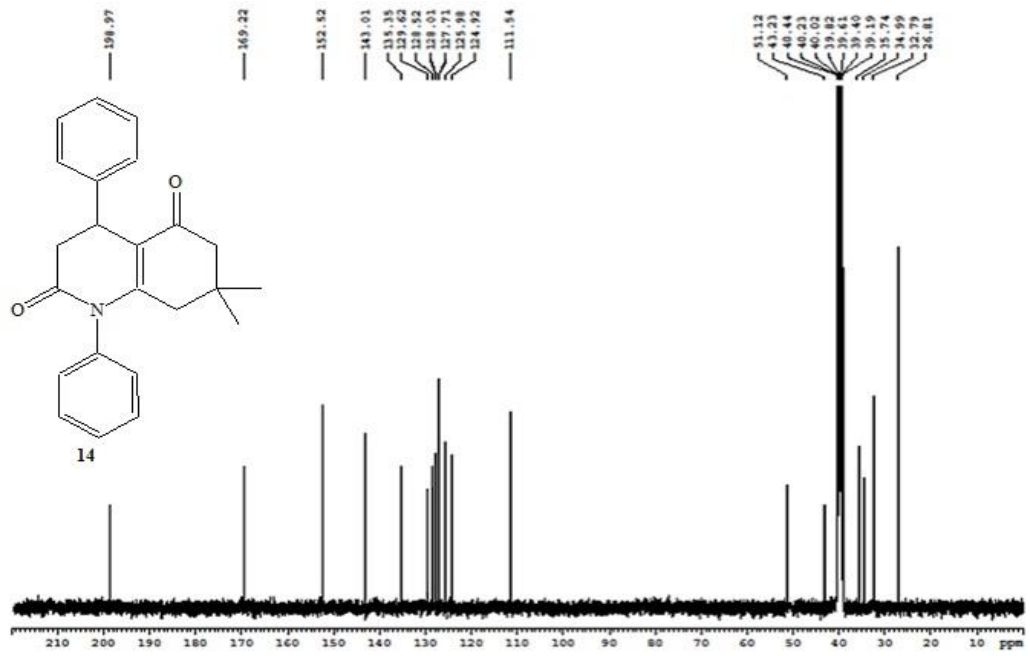


Figure 3- ^{13}C NMR spectrum of7, 7-dimethyl-1,4-diphenyl-3,4,7,8-tetrahydroquinoline-2,5(1*H*, 6*H*)-dione (4a)

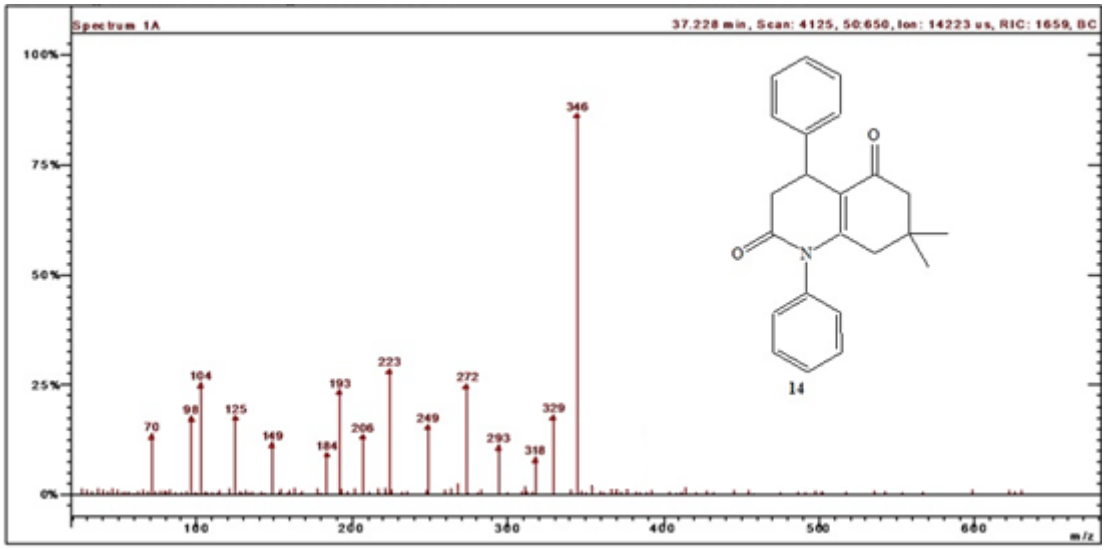


Figure 4 -Mass spectrum of 7,7-dimethyl-1,4-diphenyl-3,4,7,8-tetrahydro quinoline-2,5(1*H*, 6*H*)-dione (4a)

2.2. Antioxidant activity

Experiments were carried out to explore the free radical scavenging ability of the newly synthesized compounds along with standard anti-oxidant Vitamin C with the hope of developing potential antioxidants and therapeutic reagents for respiratory diseases such as asthma, emphysema, and asbestosis.[17] The 50% inhibitory concentration (IC_{50}) values of compounds **4a-4e** and **4e, 4c** are 32.04, 38.09, 31.02, 34.44, 37.01 µg/ml in hydroxyl radical and 34.05, 39.30, 31.60, 38.14, 38.60 µg/ml in

superoxide radical. Whereas standard antioxidant Vitamin C showed their IC_{50} value is 25.00 and 20.00 µg/ml respectively (**Table 2 & 3**). From the above results, it can be concluded that appreciable scavenging activity was exhibited by the target compounds when compared with the standard vitamin C. The compounds **4e** and **4c** showed better activities compared to the other compounds. Further, the results obtained from the two different radical assays confirmed that the synthesized compounds are more effective in arresting the formation of superoxide radicals than the hydroxyl radical. The lower IC_{50} values observed in the antioxidant assays did demonstrate that these compounds have a strong potential to be applied as scavengers to eliminate the radicals **Figure 5 & 6**.

Table – 2. The *in vitro* antioxidant activities of compounds **4a-e** by Hydroxyl radical method

Compounds	**% Inhibition at different concentrations**					**IC_{50}µg/ml**
	Hydroxyl Radical Scavenging Activity					
	10µg/ml	20µg/ml	30µg/ml	40µg/ml	50µgml	
4a	15.2	24.2	35.6	43.8	62.3	32.04
4b	21.0	30.1	38.9	50.6	70.6	38.09
4c	24.8	38.2	60.2	78.6	93.5	31.02
4d	22.5	32.4	48.3	52.7	75.1	34.44
4e	26.6	39.8	65.2	86.7	97.9	37.01
Vitamin-C	32.6	42.5	62.3	81.4	94.6	25.00

***CT- Vitamin-C**

Table – 5.3. The *in vitro* antioxidant activities of compounds **a-e** by Superoxide radical method

Compounds	**% Inhibition at different concentrations**					**IC_{50}µg/ml**
	Superoxide Radical Scavenging Activity					
	10µg/ml	20µg/ml	30µg/ml	40µg/ml	50µgml	
4a	17.3	24.5	33.5	52.5	61.3	34.05
4b	24.3	33.6	48.5	68.2	85.6	39.30
4c	31.2	42.5	59.3	75.1	95.7	31.60
4d	28.7	40.1	53.8	70.6	79.5	38.14
4e	33.5	46.7	63.9	82.4	98.6	38.60
Vitamin-C	29.9	51.8	71.3	93.8	98.9	20.00

***CT- Vitamin-C**

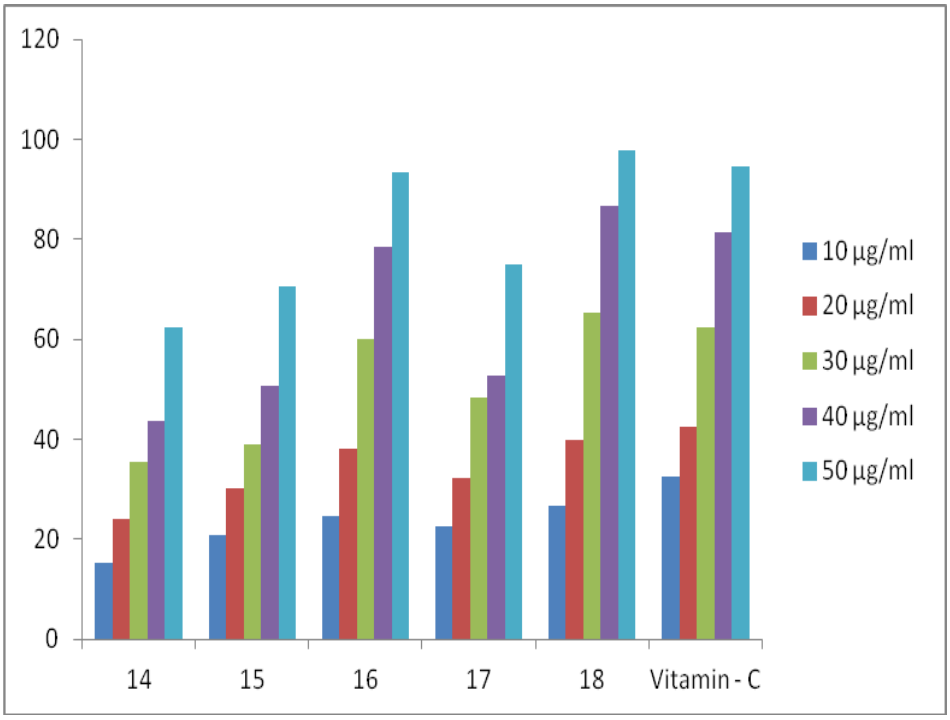


Figure 5.5. Hydroxyl radical scavenging activity of compounds **10a-e**

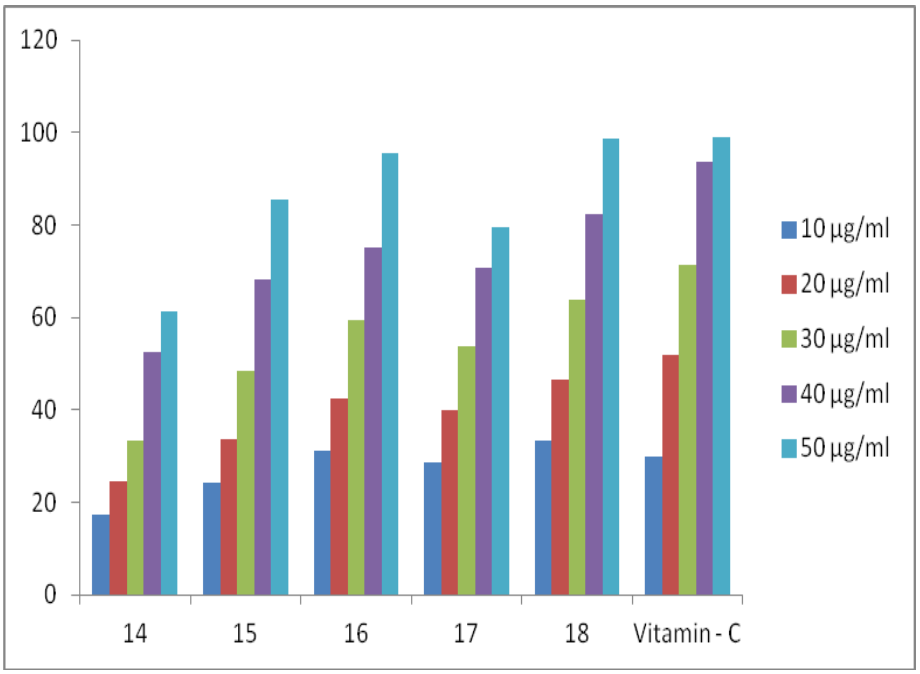


Figure 6. Superoxide radical scavenging activity of compounds **4a-e**

3. Experimental section

3.1Materials required

Chemicals

o-Methylbenzaldehyde, *p*-methylbenzaldehyde, *p*-methoxybenzaldehyde, *p*-chlorobenzaldehyde, *o*-hydroxybenzaldehyde, 5,5-dimethylcyclohexane-1,3-dione, and N-phenyl acetamidehydrate, were purchased from Sigma Aldrich.

Spectral Measurements

All melting points were taken in open capillaries and are uncorrected. The ^{1}H NMR spectra of compounds were recorded at room temperature with a Bruker 400 NMR spectrometer at 400 MHz Proton decoupled ^{13}C NMR spectra were obtained at room temperature using a Bruker 400 NMR spectrometer. The mass spectra were recorded using a Thermo Fischer LC-Mass spectrometer in fast atom bombardment (FAB) mode. IR spectra are acknowledged in the SHIMADZU FT-IR 8400S spectrometer using KBr pellets. Microwave reactions were performed in Ragas Microwave Synth System [RG3IL], complete with glass door, 700 Watt delivered power, exhaust system, triple safety interlocks, magnetic stirrer, and automatic temperature control.

Thin layer chromatography technique, glass plates coated with silica gel containing calcium sulfate were employed. The slurry of silica gel was prepared by mixing silica gel with water and the slurry was mixed homogeneously analytical TLC plates were coated uniformly by using this slurry, dried, and used.

3.2. Synthesis of quinoline derivatives (4a-e)

General procedure

A mixture of 5,5-dimethyl cyclohexane-1,3-dione (**1**,0.14 mg, 0.001 mmol), N-phenyl acetamide hydrate (**9**, 0.08 mg, 0.001 mmol) and substituted benzaldehyde (**2**, 0.1 mL) under microwave irradiation at 180W,120°C for suitable time. The reaction was monitored every 30s time interval on TLC. After that, the reaction mixture was quenched in distilled water and filtered. The obtained yellow product was purified by column chromatography.

(i) 7,7-Dimethyl-1,4-diphenyl-3,4,7,8-tetrahydroquinoline2,5(1*H*,6*H*)-dione (4a)

Time-6 min, yield: 93%, mp: 235° C, IR (KBr, cm^{-1}): 3666 (NH, stretching, broad), 1670 (C=O, ring carbonyl, strong), 2360 (CN stretching & 1548 C=C stretching). ^{1}H NMR (DMSO-d_6) δ: δ0.99,(2s, gem methyl protons), 2.29 (s, 1H, C_6–H), 2.70,(s, 1H, C_8–H), 1.88 (m, C_3–H&C_4–H) & 7.19-7.72 (d, J = 8.0 Hz) (m, 4H, Ar–H).^{13}C NMR (400 MHz, $CDCl_3$) δ : δ 20.99, 26.83, 29.56, 32.56, 32.87, 40.32, 50.65, 112.11, 127.99, 128.62, 134.85, 144.68, 149.85, 195.21.HR LCMS (m/z): 363 [M+2]; Anal Calcd. For $C_{24}H_{29}NO_2$; C 79.30, H 8.04, N 3.85: Found: C 79.23, H 8.02, N 3.84.

(ii) 7,7- Dimethyl-1-phenyl-4-(*p*-tolyl)-3,4,7,8-tetrahydroquinoline-2,5 (1*H*,6*H*)-dione (4b)

Time-7 min, yield: 89%, mp: 238° C, IR (KBr, cm^{-1}): 3397 (NH, stretching), 1695 (C=O, ring carbonyl, strong), 2461 (CN stretching &1521 (C=C stretching,).^{1}H NMR (DMSO-d_6) δ: δ0.99,(2s, gem methyl protons), 2.29 (s, 1H, C_6–H), 2.35,(s, 1H, C_8–H), 1.90 (m,C_3–H&C_4–H),3.42 (s, 3H, OCH_3),&6.69-7.51 (d, J = 8.0 Hz) (m, 4H, Ar–H). ^{13}C NMR (400 MHz, $CDCl_3$)δ : δ 26.20, 29.57, 32.30, 32.59, 40.52, 50.72, 63.17, 112.17, 113.84, 128.96, 139.96, 149.57, 156.84, 194.96.HR LCMS (m/z): 379 [M+]. Anal Calcd. For $C_{24}H_{25}NO_2$; C 80.19, H 7.01, N 3.89: Found: C 80.11, H 7.10, N 3.89.

(iii)7,7-Dimethyl-1-phenyl-4-(*p*-methoxyphenyl)-3,4,7,8-tetrahydro quinoline-2,5(1*H*,6*H*)-dione (4c)

Time -7 min, yield: 87%, mp: 237° C, IR (KBr, cm^{-1}): 3338-3383 (NH, stretching, broad), 1683(C=O, ring carbonyl, strong), 3476, (OH, stretching), 2385, (CN stretching &1482 (C=C stretching,).^{1}H NMR ($CDCl_3$) δ0.99,(2s, gem methyl protons), 2.29 (s, 1H, C_6–H), 2.35,(s, 1H, C_8–H), 1.90 (m, C_3–H&C_4–H),5.35 (s, 3H, OCH_3), &6.69-7.51 (d, J = 8.0 Hz) (m, 4H, Ar–H).^{13}C NMR (400 MHz, $CDCl_3$)δ : δ 26.92, 29.51, 32.61, 33.27, 40.51, 50.60, 111.47, 118.94, 130.37, 130.91, 146.96, 150.04, 194.96. HRLCMS (m/z): 395 [M+]. Anal Calcd. For $C_{24}H_{25}NO_3$; C 76.77, H 6.71, N 3.73: Found: C 76.70, H 6.71, N 3.71.

(iv). 7,7-Dimethyl-1-phenyl-4-(*p*-chlorophenyl)-3,4,7,8-tetrahydro quinoline-2,5(1*H*,6*H*)-dione (4d)

Time - 8 min, yield: 82%, mp: 240° C, IR (KBr, cm^{-1}): 3359-3443 (NH, stretching, broad), 1689 (C=O, ring carbonyl, strong), 2109 (CN stretching &1486 (C=C stretching,).^{1}H NMR ($CDCl_3$) δ: ^{1}H NMR ($CDCl_3$) δ: 0.99,(2s, gem methyl protons), 2.29 (s, 1H, C_6–H), 2.69,(s, 1H, C_8–H), 1.90 (m, C_3–H&C_4–H),3.58 (s, 3H, OCH_3), &6.69-7.51 (d, J = 8.0 Hz) (m, 4H, Ar–H).^{13}C NMR (400 MHz, $CDCl_3$) : δ 27.66, ,31.76, 41.65, 51.14, 61.47, 104.65, 115.17-156.59, 187.40.HR LCMS (m/z): 427 [M+]; Anal Calcd. For $C_{23}H_{22}ClNO_2$; C 72.79, H 5.83, N 3.68: Found: C 72.65, H 5.83, N 3.68.

(v). 7,7-Dimethyl-1-phenyl-4-(*p*-hydroxyphenyl)-3,4,7,8-tetrahydro quinoline-2,5- (1*H*,6*H*)-dione (4e)

Time - 6 min, yield: 92%, mp: 242° C, IR (KBr, cm^{-1}): 3359-3443 (NH, stretching, broad), 1689 (C=O, ring carbonyl, strong), 2109 (CN stretching &1486 (C=C stretching,).^{1}H NMR ($CDCl_3$) δ: δ0.99,(2s, gem methyl protons), 2.29 (s, 1H, C_6–H), 2.69,(s, 1H, C_8–H), 1.90 (m, C_3–H&C_4–H),3.58 (s, 3H, OCH_3), &6.69-7.51 (d, J = 8.0 Hz) (m, 4H, Ar–H).^{13}C NMR (400 MHz, $CDCl_3$) :δ 27.66, ,31.76, 41.65, 51.14, 61.47, 104.65, 115.17-156.59, 187.40.LCMS (m/z): 379 [M+]. Anal Calcd. For $C_{23}H_{23}NO_3$; C 76.43, H 6.41, N 3.87: Found: C 76.36, H 6.41, N 3.87.

5.3.3 Antioxidant studies

DPPH radical scavenging assay

The synthesized moieties were evaluated by radical scavenging activity using 1,1-diphenyl-2-picrylhydrazil (DPPH) for the free charge followed by the previously reported method.[17] Briefly, a 0.0001M solution of DPPH in DMSO was made and 10mL of prepared solution was supplemented to 100ml of the solutions of all compounds in DMSO at various concentrations (100µL, 200µL, 300µL, 400µL). The solutions were shaken well & made to stand the solutions at room temperature for 30 min. The absorbance was examined at 517 nm in a UV-VIS spectrophotometer as ascorbic acid was employed as a reference. If the absorbance value is low means then the free radical scavenging activity will be very high. The scavenging DPPH radical activity was calculated by utilizing the given principle

DPPH scavenging effect (% inhibition) = $[A_0\text{-}A_t/ A_0]$ x100

Where A_0 = absorbance of the control and A_t absorbance of tested samples at a particular time. IC_{50} = concentration of the compound required to inhibit 50% of DPPH• production.

Hydroxyl radical scavenging assay

The hydroxyl (OH•) radical scavenging performances of the complexes have been inspected by the Nash method. [18] *In vitro* hydroxyl radicals have formed by a Fe^{3+}-ascorbic acid system. The hydroxyl radical recognition was measured by the sum of formaldehyde produced during the corrosion reaction with DMSO and it was absorbed at 412 nm. In the test tubes the combination of 1.0 mL Fe–EDTA result (0.13% FAS and 0.26% EDTA), 0.5 mL EDTA result (0.018%), and 1.0 mL DMSO (0.85% v/v DMSO in 0.1 M phosphate buffer, pH 7.4) were supplemented. The reaction was started by the addition of 0.5 mL ascorbic acid (0.22%) and it was nurtured at 80–90º C in a water bath for 15 min. After incubation, the reaction was concluded by adding 1.0 mL icy trichloroacetic acid (17.5% w/v). Then 3.0 mL of Nash reagent was supplemented to all tubes and the tubes were kept at room temperature for 15 minutes. The concentration of the color produced during the reaction was recorded at 412 nm by spectrophotometrically next to a blank reagent. Additionally, the proportion of scavenging activity was designed utilizing the given principle:

Percentage of scavenging activity = $[(A_0 - A_C)/A_0] \times 100$

(A_0 and A_C are the absorbances in the nonexistence and existence of the complexes, correspondingly). The 50% of scavenging activity (IC_{50}) can be designed by using the percentage of scavenging activity results.

Superoxide radical scavenging assay

A superoxide radical was inspected obliquely utilizing the system of MET-VitB2-NBT.[19,20] The aqueous mixture containing 0.5 mL of 3.3×10^{-5} M VitB2, 1 mL of 2.3×10^{-4} M NBT (Nitro blue-tetrazolium), 1 mL of 0.05M MET (Methionine), and the compounds of a variety of concentrations were set with 0.067 M phosphate barrier (Na_2HPO_4–NaH_2PO_4, pH = 7.8). They were clarified by a luminous lamp with a steady intensity light at 25ºC. The optical absorbance (A) of the result was measured at 560 nm with different lighting eras. All the examinations were repeated in triplicate and are stated as the mean and standard deviation.[19]

All the examinations were repeated in triplicate and a variety of concentrations of the complexes were prepared which was used to mend a concentration of the compounds to show around 50% of scavenging activity. Additionally, the proportion of scavenging activity was designed by the given rule:

Percentage of scavenging activity = $[(A_0 - A_C)/A_0] \times 100$ (A_0 and A_C are the absorbances in the nonexistence and existence of the complexes, correspondingly). The 50% of scavenging activity (IC_{50}) can be designed by the proportion of scavenging activity results.

4. Conclusion

we reported synthesis, spectral characterization, and antioxidant studies of quinoline derivatives. The milder reaction conditions, availability of starting materials, short reaction times, high yields, and clean product formation are the most important advantages of this procedure. Synthesized quinoline derivatives **(4a-e)**were characterized by IR, NMR, and Mass spectral techniques and it confirmed their structure. From the results, it can be concluded that appreciable scavenging activity was exhibited by the target compounds when compared with the standard vitamin C.

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