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Evaluation of Antioxidant Activity of 2*H*-Chromene Derivatives through Optimized Green Synthesis

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Abstract

The increasing concern for environmental sustainability and the need for efficient and green chemical synthesis have driven the development of alternative methods for producing new compounds. In this study, we have synthesized a series of novel 2*H*-chromene derivatives using a one-pot multicomponent reaction under ultrasound sonication. The use of ultrasound sonication is a green and efficient synthetic method that utilizes sound waves to initiate chemical reactions. We optimized various reaction parameters, such as solvent, temperature, catalyst amount, and reaction time, to identify the best reaction conditions for the synthesis of the derivatives. The synthesized compounds were characterized using analytical techniques such as IR, mass, ^{1}H and ^{13}C NMR spectra, which confirmed the molecular structures of the derivatives. Furthermore, the antioxidant activities of the synthesized derivatives were evaluated and compared to those of ascorbic acid. The IC50 values of the derivatives were promising, indicating their potential as antioxidants. Overall, this study demonstrates the effectiveness of ultrasound sonication as a green and sustainable approach to the synthesis of novel compounds with potential biological activities. The use of one-pot multicomponent reactions also adds to the efficiency and simplicity of the synthesis method. These findings may have important implications for the development of new and sustainable chemical synthesis methods in the future.

Keywords: 2*H*-chromene, morpholine, ultrasound treatment, one-pot three-component reaction, green solvent, optimisation study, antioxidant assay

INTRODUCTION

The environment demands that the entire research infrastructure identifies long-term strategic goals for clean chemistry and limits the number of pollutants produced, particularly organic solvents, the recovery of which is enforced by ever-stricter rules. Pollution caused by high-efficiency procedures, solvents, catalysts, and appropriate chemicals is a conductive factor in chemical process expansion. It is preferable to carry out reactions in aqueous media to avoid reliance on environmentally hazardous chemicals. Water is the most affordable and widely available solvent. Water is, after all, a desirable medium for many organic reactions. Aqueous media reactions are often ecologically safe, free of carcinogens, easy to handle, and operate at a lower cost, which is especially important in industry [1, 2]. At the turn of the century, there is a different strategy in chemistry, with an aim of growing environmentally friendly routes into a wide range of materials using non-toxic reagents, solvents and catalysts [3]. The phrase "ideal synthesis" was recently coined to describe a process in which the desired chemical is synthesized in a single step, in quantitative yield, from easily available and inexpensive starting components in a resource-effi-

cient and ecologically friendly manner [4]. Because they have one of the aforementioned features, namely the ability to synthesise complicated compounds with maximum simplicity and brevity, one-pot multicomponent condensations constitute a feasible instrument for performing a near-ideal synthesis [5]. Organic reactions in water without the use of hazardous organic solvents have recently come to a lot of interest because water is a low-cost, non-toxic, and environmentally friendly solvent [6]. For the synthesis, our research team is attempting to avoid and minimize the use of dangerous metals, poisonous solvents, and harmful chemicals. Some of the many available environment-friendly and sustainable green synthesis approaches include bio-based techniques, microwave irradiation and ultrasonic treatment.

The chromene ring is a kind of oxygenated heterocycle found in a wide range of biologically active natural compounds. Chromene and its derivatives play an essential role in organic chemistry, both natural and synthetic. The compounds of chromene have significant biological properties, such as antimalarial [7], antimicrobial [8], anticancer [9], anticonvulsant [10], anti-virus [11], anti-influenza [12], antitubercular [13, 14], anti-inflammatory and analgesic [15], antidepressant [16], antioxidant [17, 18], anti-Alzheimer [19], antiproliferative [20], mutagenicity [21], central nervous system [22], *etc.*

We investigated the synthesis of 2*H*-chromene-3-carbonitrile in water as a green solvent in the course of our research to create new synthetic methods in water, which offers a significant benefit due to its high polarity.

EXPEREMENTAL

Materials

We utilised commercially available reagents and solvents. Merk Ltd., S D Fine Chemicals Ltd., and LOBA Chemie supplied all the chemicals and solvents necessary for the synthesis. Here, all the melting points are uncorrected and determined using the open end capillary technique. TLC plates used for monitoring the completion of reaction were purchased from Merk (TLC silica gel 60 F254). The IR spectral data were measured using Bruker FT-IR alpha-t (ATR).

^{1}H NMR and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz (Bruker Avance) instrument, respectively, using TMS as an internal standard. Shimadzu mass spectrometer was utilized for the mass spectral analysis. The Perkin-Elmer 2400 CHNSO analyser was used to do the elemental analysis.

Synthesis methods

General method. In the presence of morpholine (5 mmol), a combination of dimedone (10 mmol), ethyl cyanoacetate and substituted aromatic and heteroaromatic aldehyde (10 mmol) is taken into a vessel. Water is used as a green solvent in this mixture. The mixture was stirred for 2–4 h at room temperature (30 °C). The obtained product was cooled and collected by filtering, and then the product was recrystallised from ethanol.

Ultrasonic method. A mixture of dimedone (10 mmol), ethyl cyanoacetate (10 mmol), and substituted aromatic and heteroaromatic aldehyde (10 mmol) in water (10–15 mL) with morpholine (5 mmol) as a catalyst was treated with ultrasound (33 kHz) at room temperature (30 °C). The mobile phase was *n*-hexane/ethyl acetate (60 : 40 by volume), and TLC was employed to detect reaction completion. The final product was filtered, washed with water (5 mL), dried, and recrystallised from ethanol.

Methods of analysis

4-(4-chlorophenyl)-7,7-dimethyl-2,5-dioxo-3,4,5,6,7,8-hexahydro-2*H*-chromene-3-carbonitrile (4a). Yield 96 %, Light yellow solid, mp 123 °C. IR spectrum, ν, cm^{-1}: 2900 (CH_3), 2220 (CN), 1690 (O-C=O), 1650 (C=O). ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.05 (6H, s, CH_3); 2.21-2.35 (4H, m, CH_2), 3.75 (1H, d, *J* = 8.3, CH), 4.12 (1H, d, *J* = 8.3, CH), 7.22–7.30 (4H, m, H Ar). ^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm: 25.6; 27.9; 32.6; 37.5; 51.2; 116.9; 118.7; 125.7; 125.7; 127.9; 127.9; 131.5; 147.5; 168.2; 198.4. Found, *m/z*: 330.7 $[M+H]^+$. $C_{18}H_{16}ClNO_3$. Calculated, *m/z*: 329.78. Found, %: C 65.50; H 4.90; N 4.20; O 14.50. Calculated, %: C 65.56; H 4.89; Cl 10.75; N 4.25; O 14.55.

4-(4-hydroxyphenyl)-7,7-dimethyl-2,5-dioxo-3,4,5,6,7,8-hexahydro-2*H*-chromene-3-carbonitrile (4b). Yield 90 %, yellow solid, mp 100 °C. IR spectrum, ν, cm^{-1}:3500 (OH), 2998 (CH_3), 2210 (CN), 1640 (C=O). ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.08 (6H, s, CH_3); 2.22-2.38 (4H, m, CH_2), 3.77 (1H, d, *J* = 8.4, CH), 4.52 (1H, d, *J* = 8.4, CH), 7.18–7.37 (4H, m, H Ar), 9.90 (1H, s, OH). ^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm: 25.7; 27.2; 32.1; 38.2; 51.5; 115.8; 116.8; 118.8; 129.1; 130.5; 133.5; 147.5; 155.7;

168.0; 198.9. Found, *m/z*: 312 $[M+H]^+$. $C_{18}H_{17}NO_4$. Calculated, *m/z*: 311.12. Found, %: C 70.00; H 5.30; N 4.20; O 20.50. Calculated, %: C 69.44; H 5.50; N 4.20; O 20.56.

7,7-dimethyl-2,5-dioxo-4-phenyl-3,4,5,6,7,8-hexahydro-2*H*-chromene-3-carbonitrile (4c). Yield 86 %, Pale-yellow solid, mp 105 °C. IR spectrum, ν, cm^{-1}: 2995 (CH_3), 2222 (CN), 1696 (O–C=O), 1672 (C=O). ^1H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.00 (6H, s, CH_3); 2.20–2.30 (4H, m, CH_2); 3.74 (1H, d, *J* = 8.3, CH); 4.10 (1H, d, *J* = 8.3, CH); 7.29–7.35 (5H, m, H Ar). ^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm: 25.8; 27.2; 32.1; 37.4; 38.2; 51.1; 116.8; 118.8; 125.9; 127.7; 128.6; 130.5; 140.6; 168.0; 198.9. Found, *m/z*: 296 $[M+H]^+$. $C_{18}H_{17}NO_3$. Calculated, *m/z*: 295.12. Found, %: C 73.00; H 5.85; N 4.74; O 16.40. Calculated, %: C 73.20; H 5.80; N 4.74; O 16.25.

4-(2-hydroxyphenyl)-7,7-dimethyl-2,5-dioxo-3,4,5,6,7,8-hexahydro-2*H*-chromene-3-carbonitrile (4d). Yield 80 %, Orange solid, mp 75 °C. IR spectrum, ν, cm^{-1}: 3550 (OH), 2940 (CH_3), 2220 (CN), 1665 (O–C=O), 1630 (C=O). ^1H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.00 (6H, s, CH_3); 2.21–2.33 (4H, m, CH_2); 3.70 (1H, d, *J* = 8.1, CH); 4.0 (1H, d, *J* = 8.1, CH); 6.80–7.11 (4H, m, H Ar). ^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm: 19.6; 27.2; 32.1; 37.4; 38.5; 51.1; 115.8; 116.8; 121.2; 127.2; 127.8; 129.1; 135.2; 147.1; 154.8; 168.0; 198.9. Found, *m/z*: 312 $[M+H]^+$. $C_{18}H_{17}NO_4$. Calculated, *m/z*: 311.12. Found, %: C 69.40; H 5.30; N 4.30; O 20.40. Calculated, %: C 69.44; H 5.50; N 4.50; O 20.56.

4-(3-hydroxyphenyl)-7,7-dimethyl-2,5-dioxo-3,4,5,6,7,8-hexahydro-2*H*-chromene-3-carbonitrile (4e). Yield 82 %, Golden yellow solid, mp 80 °C. IR spectrum, ν, cm^{-1}: 3520 (OH), 2945 (CH_3), 22215 (CN), 1662 (O–C=O), 1620 (C=O). ^1H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.00 (6H, s, CH_3); 2.15–2.30 (4H, m, CH_2); 3.75 (1H, d, *J* = 8.3, CH); 4.15 (1H, d, *J* = 8.3, CH); 6.95–7.05 (4H, m, H Ar); 9.29 (1H, s, OH). ^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm: 26.1; 27.2; 32.1; 37.4; 38.5; 51.1; 113.1; 116.8; 118.8; 120.3; 130.2; 135.2; 142.0; 147.1; 156.8; 168.0; 198.9. Found, *m/z*: 312 $[M+H]^+$. $C_{18}H_{17}NO_4$. Calculated, *m/z*: 311.12. Found, %: C 69.30; H 5.10; N 4.40; O 20.55. Calculated, %: C 69.44; H 5.50; N 4.50; O 20.56.

4-(3-chlorophenyl)-7,7-dimethyl-2,5-dioxo-3,4,5,6,7,8-hexahydro-2*H*-chromene-3-carbonitrile (4f). Yield 79 %, Yellow solid, mp 120 °C. IR spectrum, ν, cm^{-1}: 2935 (CH_3), 2225 (CN), 1680 (O–C=O), 1650 (C=O). ^1H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.01 (6H, s, CH_3); 1.82–2.29 (4H, m, CH2); 3.74 (1H, d, *J* = 8.3, CH); 4.20 (1H, d, *J* = 8.3, CH); 7.32–7.62 (4H, m, H Ar). ^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm: 25.3; 27.5; 32.4; 37.4; 38.7; 51.3; 118.8; 125.8; 126.2; 127.5; 130.1; 134.2; 140.0; 142.0; 147.2; 168.5; 198.9. Found, *m/z*: 330.7 $[M+H]^+$. $C_{18}H_{16}ClNO_4$. Calculated, *m/z*: 329.7 Found, %: C 65.41; H 4.85; N 4.10; O 14.40. Calculated, %: C 65.56; H 4.89; Cl 10.75; N 4.25; O 14.56.

4-(furan-2-yl)-7,7-dimethyl-2,5-dioxo-3,4,5,6,7,8-hexahydro-2*H*-chromene-3-carbonitrile (4g). Yield 84 %, Brown solid, mp 85 °C. IR spectrum, ν, cm^{-1}: 2945 (CH_3), 2224 (CN), 1660 (O–C=O), 1635 (C=O). ^1H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.04 (6H, s, CH_3); 1.82–2.29 (4H, m, CH_2); 3.74 (1H, d, *J* = 8.2, CH); 4.30 (1H, d, *J* = 8.2, CH); 6.11 (1H, d, *J* = 8, H Ar); 6.33 (1H, dd, *J* = 12, H Ar); 7.49 (1H, d, *J* = 8, H Ar). ^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm: 27.0; 27.2; 32.1; 36.4; 36.8; 51.1; 105.8; 110.0; 116.8; 118.8; 141.2; 147.1; 151.8; 168.0; 198.8. Found, *m/z*: 286 $[M+H]^+$. $C_{16}H_{15}NO_4$. Calculated, *m/z*: 285. Found, %: C 67.30; H 5.20; N 4.80; O 22.40. Calculated, %: C 67.36; H 5.30; N 4.91; O 22.43.

4-(4-(dimethylamino)phenyl)-7,7-dimethyl-2,5-dioxo-3,4,5,6,7,8-hexahydro-2*H*-chromene-3-carbonitrile (4h). Yield 80 %, Orange solid, mp 118 °C. IR spectrum, ν, cm^{-1}: 2944 (CH_3), 2225 (CN), 1664 (O–C=O), 1647 (C=O). ^1H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.04 (6H, s, CH_3); 1.85–2.32 (4H, m, CH_2); 3.02 (6H, s, CH_3); 3.74 (1H, d, *J* = 8.3, CH); 4.14 (1H, d, *J* = 8.3, CH); 6.68–7.26 (4H, m, H Ar). ^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm: 25.8; 27.2; 32.1; 37.4; 38.2; 41.3; 51.1; 112.8; 116.8; 118.9; 128.6; 130.1; 147.1; 148.3; 168.0; 198.9. Found, *m/z*: 339.4 $[M+H]^+$. $C_{20}H_{22}N_2O_3$. Calculated, *m/z*: 338.4. Found, %: C 70.85; H 6.50; N 8.0; O 14.20. Calculated, %: C 70.99; H 6.55; N 8.28; O 14.18.

4-(1H-indol-3-yl)-7,7-dimethyl-2,5-dioxo-3,4,5,6,7,8-hexahydro-2*H*-chromene-3-carbonitrile (4i). Yield 91 %, Dark yellow solid, mp 152 °C. IR spectrum, ν, cm^{-1}: 2940 (CH_3), 2218 (CN), 1661 (O–C=O), 1642 (C=O). ^1H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.00 (6H, s, CH_3); 1.82–2.29 (4H, m, CH_2); 3.74 (1H, d, *J* = 8.3, CH); 4.13 (1H, d, *J* = 8.3, CH); 6.97–7.58 (4H, m, H Ar); 7.18 (1H, s, CH); 10.80 (1H, s, NH). ^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm: 25.5; 27.4; 32.6; 37.7; 39.3; 51.3; 111.1; 111.7; 118.8; 119.9; 121.7; 123.0; 126.3; 147.1; 168.0; 198.7. Found, *m/z*: 335.0 $[M+H]^+$. $C_{20}H_{18}N_2O_3$. Calculated, *m/z*: 334.13. Found, %:

C 71.80; H 5.40; N 8.40; O 14.33. Calculated, %: C 71.84; H 5.43; N 8.38; O 14.35.

4-(4-hydroxy-3-methoxyphenyl)-7,7-dimethyl-2,5-dioxo-3,4,5,6,7,8-hexahydro-2*H*-chromene-3-carbonitrile (4j). Yield 87 %, Orange solid, mp 115 °C. IR spectrum, ν, cm^{-1}: 3550 (OH), 2945 (CH_3), 2221 (CN), 1668 (O–C=O), 1636 (C=O). ^1H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.00 (6H, s, CH_3); 1.85–2.31 (4H, m, CH_2); 3.74 (1H, d, *J* = 8.3, CH); 3.78 (3H, s, CH_3); 4.10 (1H, d, *J* = 8.3, CH); 6.73–6.78 (3H, m, H Ar), 9.93 (s 1H, OH). ^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm: 26.1; 27.2; 32.1; 37.4; 38.2; 51.4; 56.1; 109.8; 115.5; 116.7; 118.7; 121.4; 134.2; 145.9; 147.1; 147.5; 168.0; 198.9. Found, *m/z*: 342 $[M+H]^+$. $C_{19}H_{19}NO_5$. Calculated, *m/z*: 341.3. Found, %: C 66.80; H 5.55; N 4.00; O 23.40. Calculated, %: C 66.85; H 5.61; N 4.10; O 23.43.

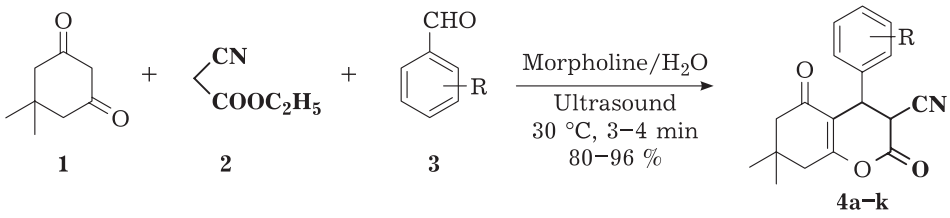
7,7-dimethyl-4-(4-nitrophenyl)-2,5-dioxo-3,4,5,6,7,8-hexahydro-2*H*-chromene-3-carbonitrile (4k). Yield 89 %, Yellow solid, mp 150 °C. IR spectrum, ν, cm^{-1}: 2947 (CH_3), 2225 (CN), 1667 (O–C=O), 1638 (C=O). ^1H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.00 (6H, s, CH_3); 1.82–2.29 (4H, m, CH_2); 3.74 (1H, d, *J* = 8.3, CH); 4.1 (1H, d, *J* = 8.3, CH); 7.70–8.17 (4H, m, H Ar). ^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm: 25.8; 27.2; 32.1; 37.4; 38.2; 51.1; 118.7; 123.8; 128.6; 145.1; 146.7; 147.4; 168.0; 198.9. Found, *m/z*: 341 $[M+H]^+$. $C_{18}H_{16}N_2O_5$. Calculated, *m/z*: 340.0. Found, %: C 63.50; H 4.70; N 8.20; O 23.60. Calculated, %: C 63.53; H 4.74; N 8.23; O 23.50.

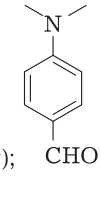
RESULTS AND DISCUSSIONS

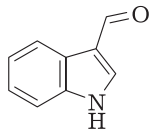
Scheme 1 illustrates the common synthetic route for different 2*H*-chromene derivatives using the optimized reaction conditions. Here, we have taken 10 mmol of dimedone, 10 mmol of ethyl cyanoacetate and 10 mmol of substituted aromatic and heteroaromatic aldehyde, and the mixture was treated with ultrasonic waves for 5 min using morpholine as a catalyst and water as a solvent.

Reaction optimisation

Solvents are known to significantly affect the yield of a chemical reaction. Therefore, we investigated the synthesis of derivatives utilising the solvents listed in Table 1. This led us to hypothesize that using water as a solvent produced the highest yield. The yield of the product as a function of temperature was also investigated, as shown in Table 2. It has been discovered that an increase in temperature causes an increase in the reaction time required to produce yield, and that the yield gained decreases as the temperature rises. Thus, the optimal temperature for studying the impact of catalyst was determined to be 30 °C, where the highest yield of 96 % was attained in three minutes. Product yield was assessed in relation to the quantity of catalyst used. It was determined that 5 mmol of catalyst provided the highest yield in relation to time (Table 3). It has



R = 4-Cl (**a**); 4-OH (**b**); -H (**c**); 2-OH (**d**); 3-OH (**e**); 3-Cl (**f**); (**g**);  (**h**);

 (**i**); 4-OH 3-OCH_3 (**j**); 4-NO_2 (**k**)

Scheme 1. Synthetic route to different 2H-chromene derivatives using dimedone (**1**), ethyl cyanoacetate (**2**), substituted aromatic and heteroaromatic aldehyde (**3**).

TABLE 1

Effect of different solvents on reaction yield and time in the synthesis of **4a***

No.	Solvent	Time, min	Yield, %
1	Solvent free	10	None
2	Water	03	96
3	Ethanol	07	81
4	Methanol	07	70
5	Acetone	11	45
6	*n*-Hexane	18	–
7	Toluene	21	–

* Reaction conditions: compound 1(10 mmol), compound 2 (10 mmol), compound 3 (10 mmol), morpholine (5 mmol), solvent 10 mL, temperature 30 °C, ultrasound irradiation.

TABLE 2

Effect of temperature on reaction yield and time in the synthesis of **4a***

No.	Solvent	Temperature, °C	Time, min	Yield, %
1	Water	30	03	96
2	Water	40	05	90
3	Water	50	05	87
4	Water	60	06	83
5	Water	70	06	80

* Reaction conditions: compound 1 (10 mmol), compound 2 (10 mmol), compound 3 (10 mmol), morpholine (5 mmol), solvent 10 mL, ultrasonic treatment.

TABLE 3

Effect of catalyst amount on reaction yield and time in the synthesis of **4a***

No.	Amount of morpholine, mmol	Time, min	Yield, %
1	3	10	Trace
2	5	03	96
3	10	05	89
4	15	06	83
5	20	06	80
6	25	06	77
7	30	06	73

* Reaction conditions: compound 1 (10 mmol), compound 2 (10 mmol), compound 3 (10 mmol), solvent (water) 10 mL, temperature 30 °C, ultrasonic treatment.

TABLE 4

Effect of time on reaction yield in the synthesis of **4a** under optimal conditions*

No.	Solvent	Time, min	Yield, %
1	Water	03	96
2	Water	05	91
3	Water	10	88
4	Water	15	85
5	Water	20	82

* Reaction conditions: compound 1 (10 mmol), compound 2 (10 mmol), compound 3 (10 mmol), morpholine (5 mmol), solvent 10 mL, temperature 30 °C, ultrasonic treatment.

been shown that exceeding the optimal quantity resulted in a discernible loss in yield, and a smaller quantity of catalyst resulted in a negligible yield even after 10 minutes of reaction time (Table 4). All subsequent reactions employed the optimised reaction conditions to produce 2*H*-chromene derivatives (Table 5).

Comparison of conventional method and ultrasonic treatment

Ultrasonic treatment is a type of irradiation that uses ultrasound to see how it affects a certain reaction. When the reaction was executed in the traditional manner, it produced low product yields and took longer to complete, but when it was executed under ultrasonic treatment, this method was shown to be superior to the conventional procedure in terms of the yield of derivatives (see Table 5).

Free radical scavenging by the compounds determined using DPPH analysis

Antioxidants convert the stable free radical DPPH to DPPH-H, which decreases the absorbance of the DPPH radical relative to the DPPH-H form. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen-donating ability. The changes in the color (from deep violet to light yellow) of the standard and compounds **4a–k** were measured at 515 nm on a spectrophotometer. Stock solutions were prepared by dissolving 1 mg of DPPH in 1 mL of methanol. Then, different concentrations of **4a–k** 25, 50, 75, 100, and 125 μg were prepared by dissolving in 3 mL methanol. The DPPH solution was prepared just before the measurements. Then, 3 mL of the test sample and 1 mL of the DPPH solution were mixed and

TABLE 5

Synthesis of 2*H*-chromene-3-carbonitrile derivatives under both conventional and ultrasonic conditions*

No.	Compound	-R	Ultrasonic treatment		Conventional method	
			Time, min	Yield, %	Time, min	Yield, %
1	**4a**	4-Cl	03	96	132	56
2	**4b**	4-OH	03	90	138	49
3	**4c**	-H	04	86	128	54
4	**4d**	2-OH	04	80	130	45
5	**4e**	3-OH	04	82	131	55
6	**4f**	3-Cl	03	79	138	52
7	**4g**	Furfural	03	84	140	38
8	**4h**	4-$N(CH_3)_2$	04	80	158	46
9	**4i**	Indole-3-carbaldehyde	04	91	154	58
10	**4j**	4-OH, 3-OCH_3	04	87	148	49
11	**4k**	4-NO_2	03	89	134	50

* Reaction conditions: compound 1 (10 mmol), compound 2 (10 mmol), compound 3 (10 mmol), morpholine (5 mmol), solvent (water) 10 mL, temperature 30 °C, ultrasonic treatment.

placed for incubation for 30 min at 30 °C. The absorbances of incubated test solutions and control (without sample) were measured at 515 nm. Ascorbic acid was used as the standard. The experiment was carried out in triplicate. The IC50 values (μg/mL) defined as the concentration corresponding to 50 % growth inhibition were calculated by Graph pad prism 7.0. for each sample, as well as for the standard, and are represented in Table 6.

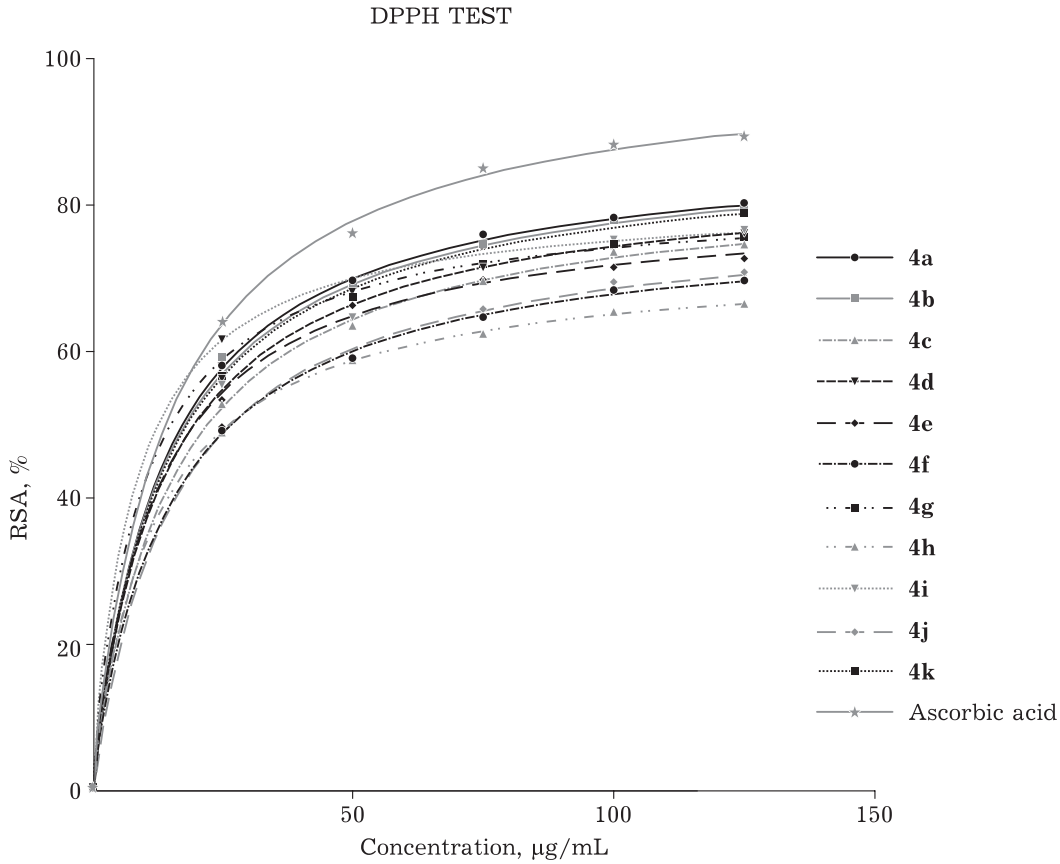


Fig. 1. DPPH radical scavenging activity (RSA) of compounds **4a–k.**

TABLE 6

Antioxidant scavenging activity of compounds **4a–k** on DPPH* free radical at different concentrations

Entry	Compounds	Concentration, μg/mL					IC50**
		25	50	75	100	125	
1	**4a**	58.18±0.12	69.29±0.12	76.10±0.12	76.56±0.11	78.37±0.12	13.08±3
2	**4b**	56.86±0.11	69.17±0.12	74.79±0.12	77.93±0.18	78.91±0.12	13.73±3
3	**4c**	52.84±0.68	63.51±0.17	69.61±0.24	73.67±0.18	74.63±0.12	15.01±3
4	**4d**	55.59±0.24	64.75±0.12	71.56±0.12	75.38±0.12	76.23±0.13	13.62±3
5	**4e**	53.44±0.18	66.34±0.18	69.89±0.12	71.52±0.18	72.75±0.11	12.09±3
6	**4f**	49.22±0.12	59.17±0.24	64.79±0.18	68.41±0.24	69.73±0.24	15.04±3
7	**4g**	59.25±0.12	67.48±0.27	71.91±0.12	74.79±0.12	75.61±0.12	9.54±3
8	**4h**	49.26±0.18	58.81±0.29	62.4±0.18	65.47±0.12	66.54±0.12	12.69±3
9	**4i**	61.64±0.12	69.53±0.12	73.75±0.18	75.38±0.12	75.98±0.12	7.95±3
10	**4j**	49.7±0.12	59.21±0.17	65.82±0.11	69.53±0.12	70.25±0.22	15.34±3
11	**4k**	56.39±0.12	68.45±0.12	74.43±0.12	77.17±0.11	78.41±0.24	13.78±3
12	Ascorbic acid	64.15±0.12	76.1±0.12	85.18±0.12	88.17±0.12	89.36±0.12	14.42±3

* Results are expressed as mean of triplicates ± standard deviation.

** The IC50 values defined as the concentration corresponding to 50 % growth inhibition, μg/mL.

The radical scavenging activity (RSA) was calculated using formula:

$$\% \text{ Inhibition} = [(AB - AA)/AB] \cdot 100$$

where AB is the absorption of blank solution and AA is the absorption of the test sample. Figure 1 shows DPPH radical scavenging activity of compounds **4a–k**. In this Figure, graphs represent the comparative study of the standard and the synthesised compounds.

The results shown in Table 6 revealed that all the compounds exhibited good radical scavenging activities compared to the standard antioxidants like ascorbic acid. The antioxidant assay based on the IC50 values obtained as a result of DPPH free radical scavenging activity is a well-documented method. Using this method, we carried out the analysis of the antioxidant assay for the synthesized 2*H*-chromene derivatives (**4a–k**). From the results, we observed that IC50 of **4j**, **4f** and **4c** had higher values than ascorbic acid. Out of this, **4j** has the highest value, followed by **4f** and **4c**. We believe that this is due to the variations in proton-electron transfer in the compounds because of differences in their structure and stability.

CONCLUSION

Herein, the use of water and the ultrasonicated synthesis method was employed to synthesize 2*H*-chromene-3-carbonitrile derivatives. This green synthesis resulted in higher product yields, shorter reaction time and ease of setup in comparison to the traditional synthesis method. The optimisation of synthesis reveals that water is found to be the choice of solvent, as it is an environment-friendly solvent and it allows highly satisfactory product yield. These synthesised derivatives were then tested for their antioxidant activity. Results of antioxidant activity tests show that **4j** derivative has the highest IC50 value with respect to ascorbic acid, which we believe is due to variations in proton-electron transfer in the compounds because of differences in their structure and stability. The results indicate that derivatives **4j**, **4f** and **4c** show promising applications as antioxidant agents.

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