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Synthesis and Evaluation of Analgesic and Antioxidant Activity of 3-Phenyl Coumarin Derivatives

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A series of 3-phenyl substituted coumarin derivatives were synthesized by reacting substituted aromatic aldehyde with phenylacetic acid. The chemical composition of substituted coumarin derivatives was confirmed by FT-IR, ^{1}H NMR spectra and elemental analysis. All the 3-phenyl coumarin derivatives were docked with cyclooxygenase-2 (COX-2) enzyme (PDB code: 3Q7D). Compounds were synthesized and evaluated for *in vivo* analgesic and *in vitro* antioxidant activities depending upon the highest binding affinity. Compounds **2**, **10** and **11** exhibited significant analgesic activity, while compounds **1**, **3**, **4** and **9** showed significant antioxidant potential in the DPPH method and compounds **5** and **7** have shown excellent scavenging potential in the nitric oxide scavenging method compared to ascorbic acid.

Keywords: 3-Phenyl coumarin, Molecular docking, Analgesic activity, Antioxidant activity.

INTRODUCTION

In heterocyclic chemistry, substituted derivatives of coumarin plays a crucial role as these are parts of the structure of many medicinal compounds which can be used in the treatment of various diseases. Coumarin possess analgesic [1], antioxidant [2], anti-inflammatory [3], antimicrobial [4], anticancer [5,6], anticoagulant [7], antitubercular [8,9], antibacterial [10], anti-alzheimer [11], anticonvulsant [12], antifungal [13-15], cardio-vasucular [16] activities. Several biological activities exhibited by coumarin have generated significant interest among numerous researchers. The frequent use of analgesic drugs leads to drug resistance and toxicity issues and necessities the development of new analgesic derivatives [17].

Multiple investigations conducted by many workers [18-20] have consistently demonstrated that coumarins exhibit a high degree of selectivity towards a specific biological therapeutic target when they undergo substitution with specific functional groups at precise places. When coumarin is substituted at the C-4 position, it exhibits diminished toxicity, reduced occurrence of adverse effects, enhanced potency, decreased drug resistance and an expanded spectrum of therapeutic possibilities [19].

Moreover, researchers have also examined benzopyran derivatives as highly effective inhibitors of COX-2 [21-23]. Specifically, coumarin derivatives, which fall under the category of benzopyrans, have been demonstrated to possess significant analgesic as well as antioxidant properties [1,2].

Motivated by the inherent biological relevance of coumarin and its derivatives, in this study, 3-phenyl substituted coumarin derivatives were selected as pharmacophores with the aim of obtaining compounds with more powerful desired effects.

EXPERIMENTAL

All the reported 3-phenyl coumarin derivatives were synthesized using LR grade chemicals. Chemicals were procured from Sigma-Aldrich & Merck, USA. All the reactions were monitored by TLC and melting points. TheVeego melting point equipment (VMP MP, 32/1105) was used to check the melting point and are uncorrected. The TLC was carried out by using silica gel (Type 60 GF_{254}, Merck) and *n*-hexane:ethyl acetate (7:3) as solvent mixture. IR spectra were carried out by using Jasco FT/IR-4600) using the KBr pellet technique. ^{1}H NMR spectra were obtained using an Advance III HD 500 MHz NMR Spectrometer ($CDCl_3$-d_6, TMS) by using BRUKER compass data analysis 4.2.

Animal ethics statement: The male Swiss albino mice (150-175 g) used in this study were obtained from the animal house colony at the MAEER's, Maharashtra Institute of Pharmacy,

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Pune, India. The animals were housed in conventional plastic enclosures within a climate controlled chamber maintained at 25 ± 2 ºC and a relative humidity of 65 ± 5%. They were provided with a standard laboratory feed and unrestricted access to water. The animal experimental techniques were conducted in compliance with the Ethics Committee of the MAEER's, Maharashtra Institute of Pharmacy, Pune, India (Approval No: MIP/IAEC/Apr/10).

Molecular docking: Molecular docking study of designed 3-phenyl coumarin derivatives was carried out using Autodock Vina free software to determine the binding energy for the target enzymes active site [24]. The X-ray crystal structure of (COX) cyclooxygenase enzyme (PDB ID: 3Q7D) for analgesic activity was used. Target enzymes were retrieved from the protein data bank by the removal of cocrystallized ligand and water molecule. All compounds were docked and the compound, which has the lowest binding energy was analyzed for different intermolecular interactions. The residues with his133A, arg213A, leu238A, ser146A, arg216, phe 220A of target PDB: ID: 3Q7D were recognized for interacting with analgesic molecules substituting functional groups such as OCH_3, OC_2H_5, OH, double-bonded oxygen (=O), ring oxygen (-O-) and CH_3 results in better binding to the active site and good analgesic efficacy.

Synthesis of coumarin derivatives: An aromatic aldehyde (**I**) was converted into substituted coumarin (**IV**) using the Knoevenagel condensation reaction. An ester intermediate (**III**) was obtained by treating phenylacetic acid (II) in pyridine with an aromatic aldehyde (**I**) and $POCl_3$ (1.5 mmol) and ether. To form the substituted coumarin, ester intermediate (**III**) was further treated with KOH (4 mmol). Then, ice-cold water and dil. HCl were used to carry out the smooth progress of the reaction. The solid product was formed and then recrystallized from ethanol, producing white crystals of 3-phenyl coumarin derivative (**Scheme-I**).

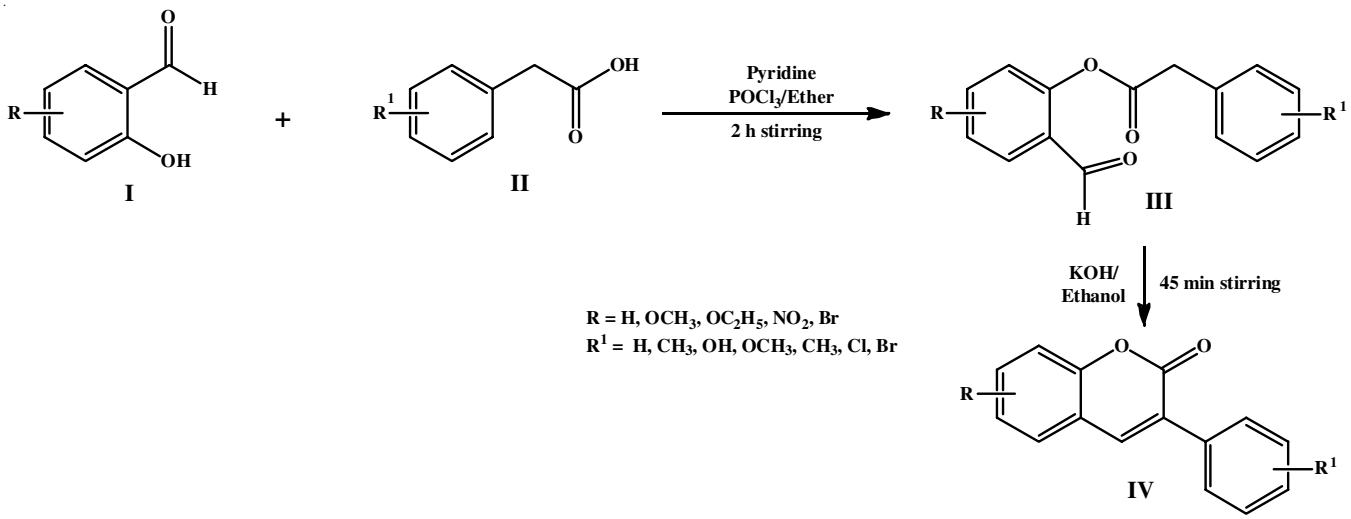
3-Mesityl-8-methoxy-2*H*-chromen-2-one (1): Yield: 89.14%, R_f = 0.45, m.p.: 294-295 ºC. IR (KBr, ν_{max}, cm^{-1}): 2923 (C-H), 1704 (C=O), 1613, 1579, 1453, 1486 (C=C), 1330 (C-O-C), 1233, 1031 (OCH_3), 852, 817, 724 (CH_3 *ortho* and *para*-substituted benzene); ^{1}H NMR (400 MHz $CDCl_3$) δ ppm: 6.96 (1H, d), 7.11 (1H, d), 7.19 (1H, d), 6.67 (1H, s), 6.67 (1H, s), 2.35 (3H, s), 2.35 (3H, s), 2.35 (3H, s), 3.73 (3H, s), 7.77 (1H, d); UV spectra: 217.88 and 269.39 nm. Elemental analysis of $C_{19}H_{18}O_3$, calcd. (found) %: C, 77.53 (77.544); H, 6.16 (6.15); O, 16.31 (16.30).

3-(3,4-Dimethoxyphenyl)-6-nitro-2*H*-chromen-2-one (2): Yield: 79.43%, R_f = 0.79, m.p.: 327-328 ºC; IR (KBr, ν_{max}, cm^{-1}): 2883 (C-H), 1705 (C=O), 1579 (NO_2), 1333 (C-O-C), 1200, 1014 (OCH_3), 1663, 1500, 1480, 1448 (C=C); ^{1}H NMR (400 MHz $CDCl_3$) δ ppm: 7.30 (1H, d), 8.25 (1H, d), 8.32 (1H, s), 7.50 (1H, s), 7.14 (1H, d), 6.6 (1H, d), 3.706 (6H, s), 7.14 (1H, s); UV spectra: 206.67, 232.42, 312.21 nm, Elemental analysis of $C_{17}H_{13}NO_6$, calcd (found) %: C, 62.39 (62.38); H, 4.00 (4.01); N, 4.28 (4.29); O, 29.33 (29.31).

6-Bromo-3-(4-hydroxyphenyl)-8-methoxy-2*H*-chromen-2-one (3): Yield: 75.18%, R_f = 0.84, m.p.: 347-348 °C; IR (KBr, ν_{max}, cm^{-1}): 2980 (C-H), 1654 (C=O), 1654, 1579, 1475, 1448, (C=C), 1275 (C-O-C), 1203, 1079 (OCH_3), 574 (C-Br); ^{1}H NMR (400 MHz $CDCl_3$) δ ppm: 3.87 (3H, s), 6.67 (1H, s), 7.31 (1H, s), 8.00 (1H, s), 7.36 (2H, d), 6.80 (2H, d), 4.5 (1H, s); UV spectra: 226.10, 265.15, 345.76 nm, Elemental analysis of $C_{16}H_{11}BrO_4$, calcd. (found) %: C, 55.36 (55.362); H, 3.19 (3.194), Br, 23.02 (23.017), O, 18.43 (18.425).

6-Bromo-3-(3,4-dichlorophenyl)-2*H*-chromen-2-one (4): Yield: 89.26%, R_f = 0.89, m.p.: 369 °C; IR (KBr, ν_{max}, cm^{-1}): 3042, 2878 (C-H), 1672 (C=O), 1610, 1561, 1467 (C=C), 1278 (C-O-C), 762, 703 (C-Cl), 1348, 539 (C-Br); ^{1}H NMR (400 MHz $CDCl_3$) δ ppm: 7.24 (1H, d), 7.62 (1H, d), 7.80 (1H, s), 7.21 (1H, s), 7.09 (1H, d), 7.37 (1H, d), 8.16 (1H, s); UV spectra: 22.12, 251.21 and 337.58 nm, Elemental analysis of $C_{15}H_7BrClO_2$, calcd. (found) %: C, 48.69 (48.82); H, 1.91 (1.911); Br, 21.59 (21.65); Cl, 19.16 (18.951); O, 8.65 (8.665).

6-Bromo-3-mesityl-2*H*-chromen-2-one (5): Yield: 91.50%, R_f = 0.82, m.p.: 343 °C; IR (KBr, ν_{max}, cm^{-1}): 3042, 2878 (C-H), 1672 (C=O), 1610, 1561, 1467 (C=C), 1278 (C-O-C), 703, 762 (C-Cl), 1348, 539 (C-Br); ^{1}H NMR (400 MHz $CDCl_3$) δ ppm: 7.62 (1H, d), 7.80 (1H, d), 7.09 (1H, s), 6.67 (1H, s), 6.67 (1H, s), 2.35 (3H, s), 2.35 (3H, s), 2.35 (3H, s),



Scheme-I: General scheme for the synthesis of coumarin derivatives

7.77 (1H, d); UV spectra: 220.61, 253.64 and 335.76 nm, Elemental analysis of $C_{18}H_{15}BrO_2$, calcd. (found) %: C, 62.99 (62.995); H, 4.41 (4.405); Br, 23.28 (23.281); O, 9.32 (9.318).

3-(3-Hydroxyphenyl)-6-nitro-2*H*-chromen-2-one (6): Yield: 75.26%, R_f = 0.63, m.p.: 283 °C; IR (KBr, ν_{max}, cm^{-1}): 3070, 2591, 2850 (C-H), 1665 (C=O), 1625, 1523, 1470 (C=C), 1530, 1342 (NO_2), 1291 (C-O-C), 1215, 1020 (OH); ^{1}H NMR (400 MHz $CDCl_3$) δ ppm: 6.58 (1H, s), 6.98 (1H, s), 7.46 (1H, s), 8.56 (1H, s), 8.38 (1H, s), 8.16 (1H, s), 7.09 (1H, d), 6.89 (1H, d), 5.1 (1H, s). UV spectra: 231.10 and 301.21 nm, Elemental analysis of $C_{15}H_9NO_5$, calcd. (found) %: C, 63.61 (63.618); H, 3.20 (3.203); N, 4.95 (4.946); O, 28.24 (28.23).

6-Bromo-3-(4-fluorophenyl)-2*H*-chromen-2-one (7): Yield: 89.67%, R_f = 0.57, m.p.: 319 °C; IR (KBr, ν_{max}, cm^{-1}): 3041, 2878 (C-H), 1673 (C=O), 1610, 1560, 1463 (C=C), 1115 (C-O-C), 1276 (C-F), 538 (C-Br); ^{1}H NMR (400 MHz $CDCl_3$) δ ppm: 6.62 (1H, s), 6.98 (1H, d), 7.62 (1H, s), 7.80 (1H, s), 7.09 (1H, s), 7.40 (1H, s), 8.16 (1H, d), 6.97 (1H, d). UV spectra: 230, 250 and 336.97 nm, Elemental analysis of $C_{15}H_8BrFO_2$, calcd. (found) %: C, 56.45 (56.45); H, 2.53 (2.527); Br, 25.04 (25.038); F, 5.95 (5.953); O, 10.03 (10.021).

3-(4-Bromophenyl)-8-ethoxy-2*H*-chromen-2-one (8): Yield: 90.23%, R_f = 0.94, m.p.: 345 °C; IR (KBr, ν_{max}, cm^{-1}): 3038, 2850 (C-H), 1706 (C=O), 1620, 1588, 1500, 1490 (C=C), 1219, 1066 (O-C_2H_5), 1141 (C-O-C), 557 (C-Br); ^{1}H NMR (400 MHz $CDCl_3$) δ ppm: 1.33 (3H, t), 4.02 (2H, q), 6.59 (1H, d), 6.87 (1H, t), 7.18 (1H, d), 7.60 (1H, s), 7.21 (2H, d), 7.45 (2H, d); UV spectra: 221.21, 264.55 and 339.70 nm, Elemental analysis of $C_{17}H_{13}BrO_3$, calcd. (found) %: C, 59.15 (59.157); H, 3.80 (3.796); Br, 23.15 (23.148); O, 13.90 (13.897).

3-(2,4-Dichlorophenyl)-8-ethoxy-2*H*-chromen-2-one (9): Yield: 88.37%, R_f = 0.49, m.p.: 334 °C; IR (KBr, ν_{max}, cm^{-1}): 3038, 2850 (C-H), 1705 (C=O), 1586, 1560, 1477, 1405 (C=C), 1218, 1066 (O-C_2H_5), 1110 (C-O-C), 766, 657 (C-Cl), ^{1}H NMR (400 MHz $CDCl_3$) δ ppm: 1.33 (3H, t), 3.89 (2H, q), 6.93 (1H, d), 7.11 (1H, t), 7.19 (1H, d), 7.30 (1H, s), 7.15 (1H, d), 7.28 (1H, d), 7.99 (1H, d). UV spectra: 222.62, 263.80, 341.72 nm, Elemental analysis of $C_{17}H_{12}Cl_2O_3$, calcd. (found) %: C, 61.098 (60.92); H, 3.619 (3.61); Cl, 20.927 (21.15); O 14.354 (14.32).

6-Bromo-3-*p*-tolyl-2*H*-chromen-2-one (10): Yield: 82.98%, R_f = 0.47, m.p.: 315 °C; IR (KBr, ν_{max}, cm^{-1}): 3020, 2880 (C-H), 1685 (C=O), 1560, 1465 (C=C), 1175 (C-O-C), 537 (C-Br), 767, 696 (CH_3 mono substituted benzene), ^{1}H NMR (400 MHz $CDCl_3$) δ ppm: 6.85 (1H, d), 7.12 (1H, d), 7.66 (1H, s), 7.70 (1H, s), 7.63 (1H, d), 7.01 (2H, d); UV spectra: 219.33, 253.12 and 334.72 nm, Elemental analysis of $C_{16}H_{11}BrO_2$, calcd. (found) %: C, 60.98 (60.098); H, 3.52 (3.518); Br, 25.35 (25.35); O, 10.15 (10.147).

8-Ethoxy-3-(3,4-dimethoxyphenyl)-2*H*-chromen-2-one (11): Yield: 81.49%, R_f = 0.61, m.p.: 326 °C; IR (KBr, ν_{max}, cm^{-1}): 2892 (C-H), 1718.57 (C=O), 1637, 1585, 1508, 1475 (C=C), 1325 (C-O-C), 1265, 1003 (OCH_3), 1219, 1069 (OC_2H_5), ^{1}H NMR (400 MHz $CDCl_3$) δ ppm: 1.33 (3H, t) 3.96 (2H, q), 6.96 (1H, d), 8.16 (1H, d), 7.11 (1H, s), 7.19 (1H, d), 6.87 (1H, d), 6.66 (1H, d), 6.82 (1H, d), 3.73 (6H, s).UV spectra: 222.49, 259.41 and 344.47 nm, Elemental analysis of $C_{19}H_{18}O_5$, calcd. (found) %: C, 69.93 (76.963); H, 5.56 (5.550); O, 24.51 (24.498).

8-Ethoxy-3-*p*-tolyl-2*H*-chromen-2-one (12): $C_{18}H_{16}O_3$, Yield: 77.34%, R_f = 0.52, m.p.: 280 °C; IR (KBr, ν_{max}, cm^{-1}): 2892 (C-H), 1718 (C=O), 1650, 1521, 1420 (C=C), 1298, 1050 (OC_2H_5), 1296 (C-O-C), 773, 716 (CH_3 mono substituted benzene), ^{1}H NMR (400 MHz $CDCl_3$) δ ppm: 1.38 (3H, t) 3.84 (2H, q), 6.86 (1H, d), 8.11 (1H, d), 7.16 (1H, s), 7.14 (1H, d), 7.34 (1H, d), 7.01 (1H, d), 7.06 (1H, d), 7.30 (1H, d), 2.26 (3H, t). UV spectra: 218.95, 265.02 and 340.93 nm, Elemental analysis of $C_{18}H_{16}O_3$, calcd. (found) %: C, 77.12 (76.963); H, 5.75 (5.741); O, 17.12 (17.076).

Analgesic activity: MvtexAnalgesiometer (MVTEX/MAEER/MIP LAB/AM-02) was used for the evaluation of the analgesic activity of synthesized derivatives using Eddy's hot plate method. Male Swiss albino mice were divided into 10 different groups. Food was withdrawn 12 h prior to drug administration till the completion of the experiment. The animals were weighed and numbered appropriately. Basal reaction time was recorded by placing the animals on a hot plate and recording the time until either licking or jumping. To the standard group pentazocine (0.1 mL) 10 mg/kg was administered. The test groups were given orally (0.2 mL, 100 mg/kg). Analgesic activity of synthesized compounds was studied at equimolar doses. The reaction time was recorded at 15, 30, 60 and 120 min following oral administration of the standard or the test compound. A cut off period of 15 s was observed to prevent tissue damage of the tail of animals.

Statistical analysis: The mean value ± SEM was calculated for each parameter and results were analyzed statistically by ANOVA followed by Dunnett's test.

Antioxidant activity (DPPH method): A 50 µg/mL test solution was added to the test tube volume was adjusted to 3 mL followed by the addition of 150 µg/mL DPPH solution and then kept in dark for 15 min. Control was made by adding only 150 µg/mL DPPH solution in to methanol. The absorbance was recorded at 517 nm.

$$\text{Inhibition (\%)} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

Nitric oxide scavenging method: A reaction mixture (5 mL) was prepared containing sodium nitroprusside 1mL of (5 mM) and 2 mL of drug solution at different concentrationsand then incubated at 25 ºC for 180 min in front of polychromatic light source (25 W tungsten lamp). The produce nitrite ion (NO^-) was assayed at 30 min intervals by mixing 1 mL of Griss reagent. The absorbance was recorded at 546 nm.

RESULTS AND DISCUSSION

A series of coumarin derivatives was successfully synthesized, purified and their structures were confirmed by spectral analysis. The compounds have been tested for their analgesic activity and antioxidant activity.

Molecular docking: The newly synthesized 3-phenyl coumarin derivatives were acquired to undergo docking in the

active site of the cyclooxygenase-2 (COX-2) enzyme. By using molecular modelling, we theoretically find the binding mode of 12 new ligands at the active site of naproxen, which is an analgesic drug used for the suppression of COX-2 (PDB ID: 3Q7D) enzyme and structurally made by joining two aromatic rings with carboxylic acid and methoxy functional groups. . These studies were done to find out intermolecular ligand-receptor interactions using 2.40 Å resolution as the main template for docking studies (Table-1). Compound **10** shows three hydrogen bonds with His133A, Asn144A, Tyr147A amino acids at a distance 2.433 Å, 2.022 Å and 2.078 Å, respectively (Fig. 1a), also it has formed 1 hydrophobic and 49 van der Waals interaction and one pi-stacking interaction. Compound **12** has formed one hydrogen bond interaction with His242A and hydrophobic as well as van der Waal interaction with amino acid Asp239A, Leu238A, Thr237A ranging from distance 3.1-4.8 Å in the protein cavity as shown in Fig. 1b. Compound **1** has formed maximum van der Waals interactions (88) as compared to other molecules (Fig. 1c).

Analgesic activity: The dose-dependent study was carried out for three compounds on 10 groups of animals including the standard compound pentazocine. With reference to the docking studies, out of 12 compounds, three compounds (compounds **2**, **10** and **11**) showed good interaction with the COX-2 protein molecule when compared to the standard. Therefore, these substances were chosen to test for analgesic activity. From Table-2, it is clear that significant analgesic activity is not seen for a longer duration in test drug groups 10 & 11 (*i.e.* at 120 min), whereas analgesia lasts for a longer duration in test drug group 02 till 120 min. Compound **2** have shown very good result due to substitution with NO_2 and OCH_3 groups. Whereas compounds **10** and **11** showed moderate activity due to substitution with CH_3, Br and OC_2H_5 groups. Hence, it is concluded that compound **2** containing a substitution of electron-withdrawing NO_2 group on the coumarin ring and electron-donating methoxy group on phenyl ring are able to show excellent analgesic activity. Compounds **10** and **11** contain electron withdrawing Br group on the coumarin ring and electron donating OCH_3, CH_3 group on the phenyl ring shows moderate analgesic activity.

TABLE-1
DOCKING STUDIES FOR ANALGESIC ACTIVITY

Ligand molecules	Energy of molecule	ΔG binding energy	PLP score
1	89.740	-80.935	-50.807
2	113.891	-16.452	-48.405
3	79.758	-66.700	-43.160
4	72.199	-50.382	-45.052
5	110.529	-26.695	-38.935
6	82.732	-47.947	-45.546
7	62.198	-23.745	-44.521
8	81.586	-75.398	-47.567
9	93.643	-83.473	-47.708
10	65.716	-29.311	-44.518
11	107.585	-81.291	-43.571
12	71.504	-57.481	-44.010
Naproxen	53.506	-599.180	-39.945

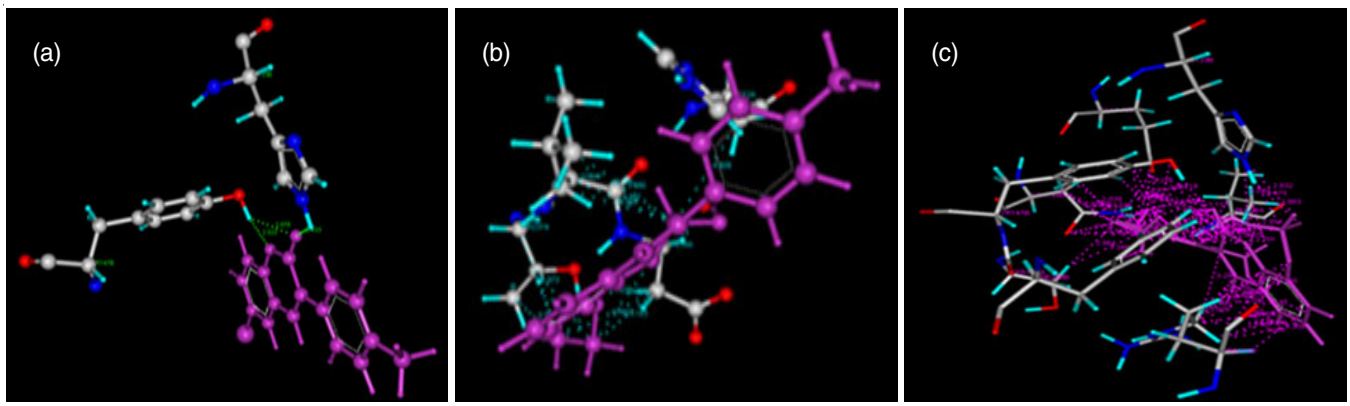


Fig. 1. (a) Hydrogen bond interactions in compound **10**, (b) hydrophobic interaction of compound **12** and (c) van der Waals interaction of compound **1**

TABLE-2
RESULTS OF ANALGESIC ACTIVITY STUDY OF EDDY'S HOT PLATE TECHNIQUE

Compound	Dose (µg/mL)	Basal reaction time (s)	15 min	30 min	60 min	90 min
Pentazocine		1.83 ± 0.13	2.42 ± 0.11**	3.43 ± 0.06**	4.96 ± 0.10**	5.52 ± 0.09**
10	50	2.79 ± 0.132	3.03 ± 0.05**	3.51 ± 0.14**	4.22 ± 0.18**	3.84 ± 0.23**
	100	2.01 ± 0.21	1.86 ± 0.34**	3.69 ± 0.29**	4.08 ± 0.22**	3.75 ± 0.17**
	150	1.86 ± 0.17	2.50 ± 0.15**	3.00 ± 0.15**	4.28 ± 0.25**	1.04 ± 0.21**
11	50	1.93 ± 0.09	2.22 ± 0.10**	2.69 ± 0.11**	3.16 ± 0.12**	2.01 ± 0.13**
	100	1.90 ± 0.11	2.38 ± 0.12**	2.72 ± 0.13**	3.54 ± 0.13**	2.22 ± 0.08**
	150	2.06 ± 0.41	2.43 ± 0.39**	2.84 ± 0.5**	3.60 ± 0.07**	2.65 ± 0.84**
2	50	1.81 ± 0.09	2.36 ± 0.05**	2.83 ± 0.06**	3.78 ± 0.06**	4.40 ± 0.05**
	100	1.86 ± 0.17	2.40 ± 0.18**	3.08 ± 0.16**	4.39 ± 0.27**	5.35 ± 0.25**
	150	1.68 ± 0.19	2.31 ± 0.17**	4.43 ± 0.25**	5.29 ± 0.12**	5.16 ± 0.25**

Antioxidant activity

DPPH Assay method: Out of 12 compounds, compounds **9**, **4**, **1** and **3** show significant activity (68.58, 66.77, 66.26, 66.77%, respectively) as compared to standard (ascorbic acid 69.51%). Compounds **2**, **6**, **8**, **11** and **12** have shown moderate activity with percentage inhibition ranging from 51% to 61%. Whereas compounds **5**, **7** and **10** showed the low percentage inhibition ranging from 47.02 to 49.76% (Table-3). Thus, it is concluded that substitution like Cl, CH_3, O-C_2H_5, OCH_3, OH, Br, NO_2 groups played an important role for the moderate antioxidant activity. Substitutions like OH, Cl, Br, CH_3, *o*-C_2H_5 and OCH_3 are the most important groups for excellent antioxidant activity, whereas substitutions like Cl and CH_3 on phenyl ring played very important role in antioxidant activity.

TABLE-3
RESULTS OF ANTIOXIDANT ACTIVITY STUDIES BY DPPH ASSAY METHOD

Compound	Absorbance at 517 nm		Inhibition (%)	
	100 µg/mL	50 µg/mL	100 µg/mL	50 µg/mL
Control	0.4975	–	–	–
1	0.1672	0.4303	66.25	13.19
2	0.2152	0.3769	56.58	23.96
3	0.1647	0.3682	66.77	25.72
4	0.1647	0.2241	66.77	54.79
5	0.2496	0.3717	49.64	25.01
6	0.2181	0.4116	56.01	16.96
7	0.2535	0.4222	48.86	14.86
8	0.2387	0.4423	57.84	10.77
9	0.1556	0.2625	68.58	47.04
10	0.2490	0.3701	49.76	25.33
11	0.1936	0.3100	60.94	37.46
12	0.2271	0.3329	54.18	32.84
Ascorbic acid	0.1228	0.2511	69.51	49.34

Nitric oxide scavenging method: In nitric oxide method, Compounds **5** and **7** have shown excellent scavenging potential 63.00% and 62.83%, respectively due to the presence of Br group. Compound **8** (60.32%) has exhibited an excellent activity due to the presence of Br & NO_2 groups. Whereas other compounds **1**, **4**, **6**, **9**, **10** and **12** also show moderate scavenging potential (40.72 to 59.47 %) against nitric oxide radical due to presence of Cl, OC_2H_5, OCH_3, NO_2 groups (Table-4). Thus, the antioxidant assay results shows that synthesized 3-phenyl substituted coumarin derivatives has good potential to scavenge DPPH and nitric oxide radical and hence may be used as new nucleus/basic moiety for synthesis of potential antioxidant drug.

Conclusion

In this work, substituted aromatic aldehyde was reacted with phenylacetic acid to obtain a series of 3-phenyl substituted coumarin derivatives. Among the synthesized derivaties, compounds **2**, **10** and **11** shown strong analgesic efficacy; compounds **1**, **3**, **4** and **9** demonstrated strong antioxidant potential in the DPPH method, while compounds **5** and **7** exhibited the superior scavenging capability to ascorbic acid in the nitric oxide scavenging method.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. P.M. Kamau, H. Li, Z. Yao, Y. Han, A. Luo, H. Zhang, C. Boonyarat, C. Yenjai, J. Mwangi, L. Zeng, S. Yang, R. Lai and L. Luo, *Biomed. Pharmacother.*, **153**, 113310 (2022); https://doi.org/10.1016/j.biopha.2022.113310
2. Y.K. Al-Majedy, A.A.H. Kadhum, A.A. Al-Amiery and A.B. Mohamad, *Syst. Rev. Pharm.*, **8**, 62 (2017); https://doi.org/10.5530/srp.2017.1.11
3. I. Bjarnason and K.D. Rainsford, *Inflammopharmacology*, **29**, 1 (2021); https://doi.org/10.1007/s10787-020-00766-8

TABLE-4
RESULTS OF ANTIOXIDANT ACTIVITY STUDIES BY NITRIC OXIDE SCAVENGING METHOD

Compound	Conc. (µg/mL)	Inhibition (%)	Compound	Conc. (µg/mL)	Inhibition (%)	Compound	Conc. (µg/mL)	Inhibition (%)
	100	44.54		100	63.00		100	56.12
1	50	31.81	**5**	50	35.12	**9**	50	30.21
	25	16.90		25	15.75		25	12.97
	100	19.25		100	44.45		100	49.76
2	50	11.68	**6**	50	26.46	**10**	50	28.09
	25	4.61		25	15.44		25	115.40
	100	24.23		100	62.83		100	59.47
3	50	14.71	**7**	50	34.15	**11**	50	34.39
	25	9.54		25	18.19		25	18.14
	100	59.31		100	60.32		100	49.16
4	50	32.16	**8**	50	28.47	**12**	50	35.48
	25	14.82		25	17.62		25	12.40
							100	68.31
						Ascorbic acid	50	52.76
							25	37.11

4. R.S. Cheke, H.M. Patel, V.M. Patil, I.A. Ansari, J.P. Ambhore, S.D. Shinde, A. Kadri, M. Snoussi, M. Adnan, P.S. Kharkar, V.R. Pasupuleti and P.K. Deshmukh, *Antibiotics*, **11**, 566 (2022); https://doi.org/10.3390/antibiotics11050566
5. T. Al-Warhi, A. Sabt, E.B. Elkaeed and W.M. Eldehna, *Bioorg. Chem.*, **103**, 104163 (2020); https://doi.org/10.1016/j.bioorg.2020.104163
6. S. Emami and S. Dadashpour, *Eur. J. Med. Chem.*, **102**, 611 (2015); https://doi.org/10.1016/j.ejmech.2015.08.033
7. Y. Mustafa, E. Mohammed and R. Khalil, *Egypt. J. Chem.*, **64**, 4461 (2021); https://doi.org/10.21608/ejchem.2021.73699.3641
8. G. Kumar, V. Siva Krishna, D. Sriram and S.M. Jachak, *Arch. Pharm.*, **353**, 2000077 (2020); https://doi.org/10.1002/ardp.202000077
9. S. Shukla, P. Gahlot, A. Khandekar, A. Agrawal and S. Pasricha, *Antiinfect. Agents*, **15**, 2 (2018); https://doi.org/10.2174/2211352515666171120142559
10. D. Hussein, S.B. Al-Juboory and A.A. Mahmood. M. Hussein, S.B. Al-Juboory and A.A. Razzak Mahmood, *Orient. J. Chem.*, **33**, 768 (2017); https://doi.org/10.13005/ojc/330224
11. J.B. Shaik, B.K. Palaka, M. Penumala, K. Kotapati, S.R. Devineni, M.M. Darla, S. Eadlapalli, D.R. Ampasala, R. Vadde and G.D. Amooru, *Eur. J. Med. Chem.*, **107**, 219 (2016); https://doi.org/10.1016/j.ejmech.2015.10.046
12. M. Mohammadi-Khanaposhtani, N. Ahangar, S. Sobhani, P.H. Masihi, A. Shakiba, M. Saeedi and T. Akbarzadeh, *Bioorg. Chem.*, **89**, 102989 (2019); https://doi.org/10.1016/j.bioorg.2019.102989
13. R. Elias, R.I. Benhamou, Q.Z. Jaber, O. Dorot, S.L. Zada, K. Oved, E. Pichinuk and M. Fridman, *Eur. J. Med. Chem.*, **179**, 779 (2019); https://doi.org/10.1016/j.ejmech.2019.07.003
14. P.P. Song, J. Zhao, Z.L. Liu, Y.B. Duan, Y.P. Hou, C.Q. Zhao, M. Wu, M. Wei, N.H. Wang, Y. Lv and Z.J. Han, *Pest Manag. Sci.*, **73**, 94 (2017); https://doi.org/10.1002/ps.4422
15. S. Sardari, Y. Mori, K. Horita, R.G. Micetich, M. Daneshtalab and S. Nishibe, *Bioorg. Med. Chem.*, **7**, 1933 (1999); https://doi.org/10.1016/S0968-0896(99)00138-8
16. T. Namba, O. Morita, S.L. Huang, K. Goshima, M. Hattori and N. Kakiuchi, *Planta Med.*, **54**, 277 (1988); https://doi.org/10.1055/s-2006-962432
17. I. Cazacu, C. Mogosan and F. Loghin, *Clujul Med.*, **88**, 128 (2015); https://doi.org/10.15386/cjmed-413
18. M.A. Musa, J.S. Cooperwood and M.O.F. Khan, *Curr. Med. Chem.*, **15**, 2664 (2008); https://doi.org/10.2174/092986708786242877
19. J. Dandriyal, R. Singla, M. Kumar and V. Jaitak, *Eur. J. Med. Chem.*, **119**, 141 (2016); https://doi.org/10.1016/j.ejmech.2016.03.087
20. A. Stefanachi, F. Leonetti, L. Pisani, M. Catto and A. Carotti, *Molecules*, **23**, 250 (2018); https://doi.org/10.3390/molecules23020250
21. D.H. Dawood, R.Z. Batran, T.A. Farghaly, M.A. Khedr and M.M. Abdulla, *Arch. Pharm.*, **348**, 875 (2015); https://doi.org/10.1002/ardp.201500274
22. C.A. Kontogiorgis, K. Savvoglou and D.J. Hadjipavlou-Litina, *J. Enzym. Inhib. Med. Chem*., **21**, 21 (2006); https://doi.org/10.1080/14756360500323022
23. H.M. Revankar, S.N.A. Bukhari, G.B. Kumar and H.-L. Qin, *Bioorg. Chem.*, **71**, 146 (2017); https://doi.org/10.1016/j.bioorg.2017.02.001
24. O. Trott and A.J. Olson, *J. Comput. Chem.*, **31**, 455 (2009); https://doi.org/10.1002/jcc.21334