



## SYNTHESIS, BIOLOGICAL EVALUATION AND IN SILICO ADME PREDICTIONS OF SOME NEW COUMARIN-ACETAMIDE DERIVATIVES AS POTENT ANTI-INFLAMMATORY AGENTS

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*A new series of 4-methyl-7-methoxycoumarin derivatives linked with three amide groups were prepared starting from 2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy) acetohydrazide, compound 4. TLC was used to ensure the purity of all new compounds, and IR, <sup>1</sup>H NMR, and mass spectrometry, as well as elemental microanalyses, were used to confirm their structures. All of the target compounds were tested for anti-inflammatory activity. The results showed that most of the tested compounds exhibited significant anti-inflammatory activity in comparison to indomethacin (INM) as a reference drug. The results revealed that compound 8g was the most active one exceeding the activity of the reference INM. Moreover, compounds 8f and 8k showed approximately the same results as INM after 5 hr. In silico ADME prediction investigations also forecasting the drug-like characters of these compounds.*

### INTRODUCTION

Coumarins (2H-1-benzopyran-2-ones) are important oxygen containing fused heterocycles used in preparation of pharmaceutical compounds and dyes<sup>1</sup>. They are a large class of lactones with a benzopyrone structure that have been isolated from plants as well as fully synthesized in the laboratory<sup>2</sup>. Natural coumarins have been shown antidiabetic activity<sup>3</sup>, anabolic, antioxidant and hepatoprotective activities<sup>4</sup>. Substituted coumarin derivatives have been reported to have variety of biological activities including anticoagulant<sup>5</sup>, HIV protease inhibition<sup>6</sup>, CNS depressant<sup>7</sup>, analgesic and anti-inflammatory activities<sup>8,9</sup>. The potent antibiotics like

novobiocin<sup>10</sup>, coumarmycin<sup>11</sup> and chlorobiocin<sup>12</sup> are coumarin derivatives. Among the various coumarin derivatives, 4-methylcoumarin derivatives were present in various naturally occurring compounds which known to exhibit a wide range of biological and pharmaceutical activities<sup>13</sup>. Also, 7-substituted coumarins are an important class of chemicals with a variety of bioactivities and uses<sup>14</sup>. Moreover, 7-amino 4-methyl coumarin is also used as laser dye<sup>15</sup> and intermediate for the synthesis of bioactive compounds<sup>16</sup>.

Previous studies have shown that combining the coumarin backbone with some nitrogen-containing heterocyclic moieties could significantly broaden the spectrum of activity of these compounds and increase their anti-

inflammatory, antimicrobial or anti-fungal efficiency<sup>17-19</sup>.

Recent reports showed an amide moiety (i.e.-CONHAr) was thought to provide a better pharmacokinetic properties rather than an ester (i.e.-OCOCH<sub>3</sub>) moiety due to a higher chemical stability in both acidic and basic mediums. Chimenti *et al.*, pointed out that, a carboxamides function in the coumarin ring might play an important role leading to various biological activities<sup>20</sup>. This prompted us to synthesize and study the anti-inflammatory activity of some new coumarin derivatives linked to amide groups to get the synergistic effect, Figure 1<sup>20</sup>.

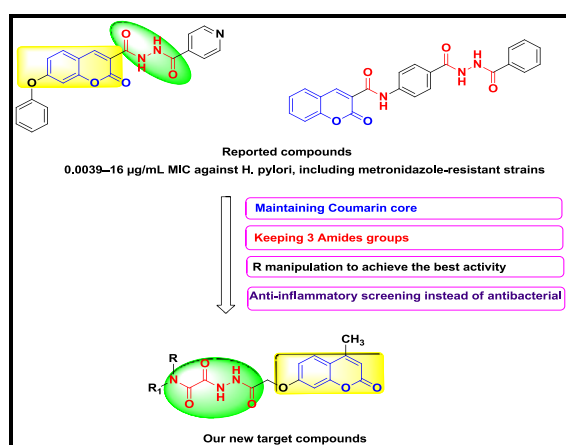


Fig. 1: Our design strategy

## MATERIALS AND METHODS

### Chemistry

Melting points were determined on an electro thermal melting point apparatus [Stuart Scientific, model SMP3, England, UK], and were uncorrected. A pre-coated silica gel plate (kieselgel 0.25 mm, 60 G F<sub>254</sub>, Merck, Germany) was used for TLC monitoring of reactions. IR spectra (KBr discs) were recorded on a Thermo Nicolet FT-IR spectrometer model 4700/6700 (Madison, USA) at faculty of science, Assiut University, Assiut, Egypt. <sup>1</sup>H-NMR spectra were scanned on a Varian EM-360L NMR spectrophotometer (60 MHz, Varian, USA) at Faculty of Pharmacy, JNM-LA series FT-NMR system (400 MHz, JEOL, Tokyo, Japan) at Unit of Trace Analysis, Assiut University, Assiut, Egypt using DMSO-*d*<sub>6</sub> as solvent. Chemical shifts are expressed in δ-value (ppm) relative to TMS as internal standard, and deuterium oxide was used for the detection of exchangeable protons. Mass

spectra were recorded with Gas Chromatography Mass, Quadruple-2010 Plus (Shimadzu, Kyoto, Japan) at the unit of Microanalysis, Faculty of Science, Cairo University, Elemental microanalyses were performed on a Vario elemental analyzer III (Vario, Hanau, Germany) at the unit of Microanalysis, Faculty of Science, Cairo University.

Reagents used for synthesis were purchased from Sigma-Aldrich and Merck. All solvents were obtained from commercial suppliers and used without further purification.

The starting materials 7-hydroxy-4-methyl coumarin 2<sup>21</sup>, ethyl 2-(4-methyl-2-oxo-2*H*-chromen-7-yl)oxy) acetate 3<sup>22</sup>, 2-(4-methyl-2-oxo-2*H*-chromen-7-yl)oxy) acetohydrazide 4<sup>23</sup> were synthesized according to reported procedures.

### Synthesis of ethyl 2-(2-(2-((4-methyl-2-oxo-2*H*-chromen-7-yl)oxy)acetyl)hydrazinyl)-2-oxoacetate (5).

To a suspension of (2-(4-methyl-2-oxo-2*H*-chromen-7-yl)oxy)aceto-hydrazide (4) (2.48 g, 10 mmol) in anhydrous acetonitrile (30 mL), anhydrous pyridine (1.2 mL, 15 mmol) was added. The suspension was stirred for 10 min at room temperature, and then cooled to 0°C before addition of ethyl oxalyl chloride (1 mL, 12 mmol) dropwise over approximately 15 mins. The reaction mixture was stirred at room temperature for 8 hrs under nitrogen atmosphere, and then poured into ice cooled water. The separated yellowish white precipitate was filtered, washed with water, dried and crystallized from ethanol to afford compound (5). Yields, m.p. and elemental analyses are listed in Tables 1 while IR, <sup>1</sup>H NMR and mass spectral data are listed in Tables 2.

### Synthesis of 2-(2-(2-((4-methyl-2-oxo-2*H*-chromen-7-yl)oxy)acetyl)-hydrazinyl)-2-oxoacetic acid (6).

To a suspension of ethyl 2-(2-(2-((4-methyl-2-oxo-2*H*-chromen-7-yl)oxy)-acetyl)hydrazinyl)-2-oxoacetate (5) (3.48 g, 10 mmol) in ethanol, 10% sodium hydroxide (0.8 g, 20 mmol) was added and the reaction mixture was stirred for 4 hrs at 40°C. After removal of the solvent under reduced pressure; the residue was dissolved in H<sub>2</sub>O and acidified

with 2N HCl. The formed precipitate was filtered, washed with H<sub>2</sub>O and dried to afford (6). Yields, m.p., and elemental analyses are listed in Table 1, while IR, <sup>1</sup>H NMR and mass spectral data are listed in Table 2.

**Synthesis of N-substituted-2-(2-(2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy)-acetyl)hydrazinyl)-2-oxoacetamide (8 a-o).**

**General procedure**

To a suspension of 2-(2-(2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy)-acetyl)hydrazinyl)-2-oxoacetic acid (6) (2 mmol) in dry DCM (10 mL) with 2 drops of DMF, thionyl chloride (1 mL) was added and the mixture refluxed for 3 hrs. The resulting suspension was evaporated to dryness under reduced pressure, and the residue of crude (7) was dispersed in DCM (10 mL), then the solvent was eliminated under reduced pressure. Dispersion in DCM and solvent elimination was repeated twice...

To a suspension of the residue in dry DCM (10 mL), appropriate amine (2.4 mmol) was added and the reaction mixture was stirred for further 6 hr at room temperature.

After removal of the DCM under reduced pressure, the residue was washed with 5% HCl then with water repeatedly, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated under high vacuum. The obtained crude product was purified by column chromatography (60-120 mesh, 0.5//9.5 methanol/chloroform) to furnish the corresponding amides (8 a-o) in good to excellent yield. Yields, m.p., and elemental analyses are listed in Table 1, while IR, <sup>1</sup>H NMR and mass spectral data are listed in Table 2.

**Anti-inflammatory screening**

Male adult albino rats (120-150 g) were obtained from the animal house (Faculty of Medicine, Assiut University, Egypt). Indomethacin (Liometacin<sup>®</sup> vial, Nile company, Egypt), carrageenan (Sigma, USA), sodium carboxymethylcellulose (NaCMC) (El Nasr pharm. Company, Egypt) and normal saline (Almhenpharma company, Egypt).

Animals were housed in separate cages three animals each, in room temperature at 25±2°C. Animals were allowed free access to

rodent chow and water and maintained at a 12 hrs light/dark cycle. Work was conducted in accordance with the internationally accepted principles for laboratory animal's use and care as found in the European Community Guidelines<sup>24</sup>.

The test compounds and the reference drug were suspended in 1% NaCMC in normal saline. Suspensions of the test compounds, reference drug and 1% NaCMC-saline solution (negative control) were injected i.p. (1 mL each).

The anti-inflammatory activity of the test compounds was evaluated according to the carrageenan induced paw edema method<sup>25</sup> in comparison to indomethacin as a reference drug. The test is based on pedal inflammation in rat paws induced by subplantar injection of carrageenan suspension (0.2 mL of 1% solution in normal saline) into the right hind paw of the rats. Male adult albino rats were divided into groups of four animals each. The rat paw thickness was measured with a Vernier caliper (SMIEC, Shanghai, China) before and 1 hr after carrageenan injection to detect the carrageenan induced inflammation. The test compounds and indomethacin, at a dose of 28 μmol/kg, were injected i.p. to all different groups of rats 1 hr after carrageenan injection. In addition, a control group received the vehicle 1% NaCMC solution in normal saline. The difference between the thicknesses of the two paws was taken as a measure of edema inhibition. The measurement was carried out at 0.5, 1, 2, 3, 4 and 5 hrs after injection of the test compounds, reference drug and control. The results are listed in Table 3.

The percentages of edema inhibition were calculated according to the following equation.

$$\% \text{Edema} = \frac{[(V_R - V_L)_{\text{control}} - (V_R - V_L)_{\text{treated}}]}{(V_R - V_L)_{\text{control}}} \times 100$$

V<sub>R</sub>: Average right paw thickness, V<sub>L</sub>: Average left paw thickness.

**Table 1:** Physicochemical properties of compounds 5, 6 and 8 a-o.

Compd. No.	Yield (%)	M.p. (°C)	M.F. (M.Wt.)	Microanalyses (calculated/found)		
				C%	H%	N%
5	(80%)	164-166°C	C <sub>16</sub> H <sub>16</sub> N <sub>2</sub> O <sub>7</sub> (348.31)	55.17	4.63	8.04
				55.41	4.72	8.17
6	(90%)	113-116°C	C <sub>14</sub> H <sub>12</sub> N <sub>2</sub> O <sub>7</sub> (320.25)	52.51	3.78	8.75
				52.74	3.81	8.89
8 a	(65%)	195-197°C	C <sub>20</sub> H <sub>17</sub> N <sub>3</sub> O <sub>6</sub> (395.37)	60.76	4.33	10.63
				60.89	4.39	10.84
8 b	(62%)	189-191°C	C <sub>21</sub> H <sub>19</sub> N <sub>3</sub> O <sub>6</sub> (409.39)	61.61	4.68	10.26
				61.94	4.73	10.34
8 c	(72%)	180-182°C	C <sub>22</sub> H <sub>21</sub> N <sub>3</sub> O <sub>6</sub> (423.42)	62.41	5.00	9.92
				62.58	5.12	10.06
8 d	(60%)	263-265°C	C <sub>21</sub> H <sub>19</sub> N <sub>3</sub> O <sub>6</sub> (409.39)	61.61	4.68	10.26
				61.73	4.74	10.42
8 e	(81%)	221-223°C	C <sub>21</sub> H <sub>19</sub> N <sub>3</sub> O <sub>6</sub> (409.39)	61.61	4.68	10.26
				61.70	4.71	10.47
8 f	(68%)	241-243°C	C <sub>21</sub> H <sub>19</sub> N <sub>3</sub> O <sub>6</sub> (409.39)	61.61	4.68	10.26
				61.76	4.73	10.44
8 g	(55%)	179-181°C	C <sub>21</sub> H <sub>19</sub> N <sub>3</sub> O <sub>7</sub> (425.39)	59.29	4.50	9.88
				59.43	4.55	9.95
8 h	(64%)	186-188°C	C <sub>21</sub> H <sub>19</sub> N <sub>3</sub> O <sub>7</sub> (425.39)	59.29	4.50	9.88
				59.40	4.58	10.01
8 i	(58%)	215-217°C	C <sub>20</sub> H <sub>16</sub> FN <sub>3</sub> O <sub>6</sub> (413.36)	58.11	3.90	10.17
				58.34	3.97	10.32
8 j	(63%)	251-253°C	C <sub>20</sub> H <sub>16</sub> BrN <sub>3</sub> O <sub>6</sub> (474.26)	50.65	3.40	8.86
				50.81	3.42	8.94
8 k	(59%)	207-209°C	C <sub>20</sub> H <sub>17</sub> N <sub>3</sub> O <sub>7</sub> (411.36)	58.39	4.17	10.21
				58.52	4.23	10.43
8 l	(72%)	247-249°C	C <sub>20</sub> H <sub>16</sub> N <sub>4</sub> O <sub>8</sub> (440.36)	54.55	3.66	12.72
				54.67	3.63	12.89
8 m	(79%)	255-257°C	C <sub>18</sub> H <sub>19</sub> N <sub>3</sub> O <sub>6</sub> (373.36)	57.90	5.13	11.25
				58.19	5.14	11.39
8 n	(89%)	260-261°C	C <sub>19</sub> H <sub>21</sub> N <sub>3</sub> O <sub>6</sub> (387.39)	58.91	5.46	10.85
				59.12	5.52	11.01
8 o	(85%)	249-251°C	C <sub>18</sub> H <sub>19</sub> N <sub>3</sub> O <sub>7</sub> (389.36)	55.53	4.92	10.79
				55.81	4.95	10.94

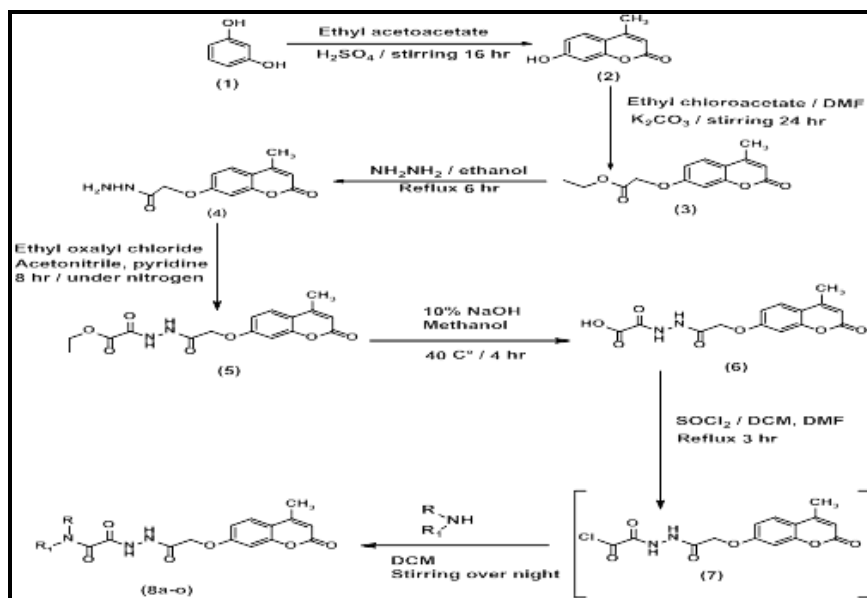
**Table 2:** Spectral characterization of compounds 5, 6 and 8 a-o.

Compd. No.	IR (KBr, cm <sup>-1</sup> )	<sup>1</sup> H-NMR ( DMSO- <i>d</i> <sub>6</sub> )	MS ( <i>m/z</i> )
5	3467, 3206 (NH), 1741, 1712, 1663 (C=O), 1161 (C-O-C).	δ 1.29 (t, <i>J</i> = 6.9 Hz, 3H, OCH <sub>2</sub> CH <sub>3</sub> ), 2.41 (s, 3H, CH <sub>3</sub> ), 4.29 (q, <i>J</i> = 7.1 Hz, 2H, OCH <sub>2</sub> CH <sub>3</sub> ), 4.79 (s, 2H, OCH <sub>2</sub> ), 6.24 (s, 1H, coumarin-H), 7.00 (d, <i>J</i> = 2.4 Hz, 1H, coumarin-H), 7.05 (dd, <i>J</i> = 8.8, 2.5 Hz, 1H, coumarin-H), 7.73 (d, <i>J</i> = 8.8 Hz, 1H, coumarin-H), 10.37 (s, 1H, NHCOCH <sub>2</sub> , D <sub>2</sub> O exchangeable), 10.84 (s, 1H, COCONH, D <sub>2</sub> O exchangeable).	348.22 (M <sup>+</sup> , 3.69%), 103.10 (100%).
6	2700-3600 (OH), 1705, 1663 (C=O), 1160 (C-O-C)	δ 2.4 (s, 3H, CH <sub>3</sub> ), 4.9 (s, 2H, OCH <sub>2</sub> ), 6.2 (s, 1H, coumarin-H), 7-7.3 (m, 2H, coumarin-H), 7.6-7.8 (d, 1H, coumarin-H), 10.4 (s, 2H, CONHNHCO, exchangeable with D <sub>2</sub> O), 10.9 (s, 1H, OH, exchangeable with D <sub>2</sub> O)	-
8 a	3272 (NH), 1728, 1671 (C=O), 1447 (C=C Ar), 700, 748	δ 2.4 (s, 3H, CH <sub>3</sub> ), 5 (s, 2H, OCH <sub>2</sub> ), 6.4 (s, 1H, coumarin-H), 7.2-8 (m, 8H, Ar-H), 10.4 (s, 2H, CONHNHCO), 10.6 (s, 1H, Ph-NHCO).	-
8 b	34990, 3319, 3262 (NH), 1714, 1686, 1665 (C=O), 1453 (C=C Ar), 700, 748.	δ 2.3 (s, 3H, CH <sub>3</sub> ), 4.3 (s, 2H, NCH <sub>2</sub> ), 4.8 (s, 2H, OCH <sub>2</sub> ), 6.2 (s, 1H, coumarin-H), 6.9-7.1 (m, 2H, coumarin-H), 7.2-7.5 (m, 5H, Ar-H), 7.7 (d, 1H, coumarin-H), 9.4 (s, 1H, NHCH <sub>2</sub> ), 10.3 (s, 1H, CH <sub>2</sub> -CO-NH), 10.4 (s, 1H, COCONHNH).	-
8 c	3260, 3228, 3170 (NH), 1704, 1624 (C=O), 1466 (C=C Ar), 746, 698.	δ 2.4 (s, 3H, CH <sub>3</sub> ), 2.81 (t, <i>J</i> = 7.4 Hz, 2H, NHCH <sub>2</sub> CH <sub>2</sub> ), 3.40 (t, <i>J</i> = 5.5 Hz, 2H, NHCH <sub>2</sub> CH <sub>2</sub> ), 4.76 (s, 2H, OCH <sub>2</sub> ), 6.25 (s, 1H, coumarin-H), 7.00 (d, <i>J</i> = 2.4 Hz, 1H, coumarin-H), 7.05 (dd, <i>J</i> = 8.8, 2.5 Hz, 1H, coumarin-H), 7.18-7.33 (m, 5H, Ar-H), 7.71 (d, <i>J</i> = 8.8 Hz, 1H, coumarin-H), 8.95 (s, 1H, CH <sub>2</sub> NHCO, D <sub>2</sub> O exchangeable), 10.29 (s, 1H, NHCOCH <sub>2</sub> , D <sub>2</sub> O exchangeable), 10.59 (s, 1H, COCONHNH, D <sub>2</sub> O exchangeable).	423.26 (M <sup>+</sup> , 0.86%), 275.19 (100%)
8 d	3428, 3231 (NH), 2922 (C-H), 1713, 1664 (C=O), 1441 (C=C Ar), 746, 700	δ 2.4 (s, 3H, CH <sub>3</sub> ), 3.4 (s, 3H, CH <sub>3</sub> N), 4.8 (s, 2H, OCH <sub>2</sub> ), 6.1 (s, 1H, coumarin-H), 7.0 - 7.2 (m, 2H, coumarin-H), 7.3-7.5 (m, 5H, Ar-H), 7.7 (d, 1H, coumarin-H), 10.3 (s, 2H, CONHNHCO).	-
8 e	3292 (NH), 2918 (C-H), 1693, 1666 (C=O), 1427 (C=C Ar), 817.	δ 2.3 (s, 3H, CH <sub>3</sub> ), 2.4 (s, 3H, coumarin-CH <sub>3</sub> ), 4.8 (s, 2H, OCH <sub>2</sub> ), 6.3 (s, 1H, coumarin-H), 7.1-7.8 (m, 7H, Ar-H), 10.5 (s, 2H, CONHNHCO), 10.8 (s, 1H, CONH-Ar).	-
8 f	3223 (NH), 2930 (C-H), 1701, 1670 (C=O), 1450 (C=C Ar), 757.	δ 2.2 (s, 3H, CH <sub>3</sub> ), 2.4 (s, 3H, CH <sub>3</sub> -coumarin), 4.8 (s, 2H, OCH <sub>2</sub> ), 6.3 (s, 1H, coumarin-H), 7.1-7.8 (m, 7H, Ar-H), 10.3 (s, 2H, CONHNHCO), 10.7 (s, 1H, CONH-Ar).	-

**Table 2:** Continued

8 g	3292 (NH), 2994 (C-H), 1697, 1657 (C=O), 1438 (C=C Ar), 1143 (C-O-C), 831.	$\delta$ 2.42 (s, 3H, CH <sub>3</sub> ), 3.75 (s, 3H, OCH <sub>3</sub> ), 4.80 (s, 2H, OCH <sub>2</sub> ), 6.25 (s, 1H, coumarin-H), 6.94 (d, $J$ = 2.4 Hz, 2H, Ar-H), 7.01(d, $J$ = 2.5 Hz, 1H, coumarin-H), 7.05 (dd, $J$ = 8.8, 2.5 Hz, 1H, coumarin-H), 7.65-7.78 (m, 3H, Ar-H), 10.39 (s, 1H, CH <sub>2</sub> CONH, D <sub>2</sub> O exchangeable), 10.68 (s, 1H, COCONHNH, D <sub>2</sub> O exchangeable), 10.83 (s, 1H, CONH-Ar, D <sub>2</sub> O exchangeable)	425.12 (M <sup>+</sup> , 13.15%), 149.10 (100%)
8 h	3280, 3217 (NH), 2928 (C-H), 1730, 1635 (C=O), 1463 (C=C Ar), 1156 (C-O-C), 756	$\delta$ 2.4 (s, 3H, CH <sub>3</sub> ), 3.8 (s, 3H, OCH <sub>3</sub> ), 4.8 (s, 2H, OCH <sub>2</sub> ), 6.3 (s, 1H, coumarin-H), 6.9-7.8 (m, 7H, Ar-H), 9.8 (s, 1H, CONH-Ar), 10.3 (s, 2H, CONHNHCO).	-
8 i	3223 (NH), 1697, 1640 (C=O), 1163 (C=C Ar), 835	$\delta$ 2.4 (s, 3H, CH <sub>3</sub> ), 5 (s, 2H, OCH <sub>2</sub> ), 6.3 (s, 1H, coumarin-H), 7.0-7.8 (m, 7H, Ar-H), 10.3 (s, 2H, CONHNHCO), 10.5 (s, 1H, CONH-Ar).	-
8 j	3280, (NH), 1697, 1650 (C=O), 1437 (C=C Ar), 826.	$\delta$ 2.4 (s, 3H, CH <sub>3</sub> ), 4.8 (s, 2H, OCH <sub>2</sub> ), 6.1 (s, 1H, coumarin-H), 6.9-7.8 (m, 7H, Ar-H), 10.2 (s, 1H, CONH-Ar), 10.4 (s, 2H, CONHNHCO).	-
8 k	3400-3100 (OH), 3219 (NH), 1713, 1658 (C=O), 1428 (C=C Ar), 1078 (C-O), 843	$\delta$ 2.4 (s, 3H, CH <sub>3</sub> ), 5 (s, 2H, OCH <sub>2</sub> ), 6.2 (s, 1H, coumarin-H), 6.9-7.8 (m, 7H, Ar-H), 10.3 (s, 2H, CONHNHCO), 10.5 (s, 1H, CONH-Ar), 11.4 (s, 1H, OH).	-
8 l	3481, 3360 (NH), 1709, 1655 (C=O), 1505, 1391 (NO <sub>2</sub> ), 1445 (C=C Ar), 756.	$\delta$ 2.4 (s, 3H, CH <sub>3</sub> ), 4.7 (s, 2H, OCH <sub>2</sub> ), 6.2 (s, 1H, coumarin-H), 6.9-7.8 (m, 7H, Ar-H), 10.3 (s, 2H, CONHNHCO), 11.1 (s, 1H, CONH-Ar).	-
8 m	3487, 3233 (NH), 2978 (C-H), 1704, 1644 (C=O), 1080 (C-N).	$\delta$ 1.85 (tq, $J$ = 13.1, 6.6 Hz, 4H, (CH <sub>2</sub> ) <sub>2</sub> ), 2.41 (s, 3H, CH <sub>3</sub> ), 3.31 (m, 4H, N(CH <sub>2</sub> ) <sub>2</sub> ), 4.77 (s, 2H, OCH <sub>2</sub> ), 6.25 (s, 1H, coumarin-H), 7.01 (d, $J$ = 2.4 Hz, 1H, coumarin-H), 7.05 (dd, $J$ = 8.8, 2.4 Hz, 1H, coumarin-H), 7.73 (d, $J$ = 8.8 Hz, 1H, coumarin-H), 10.30 (s, 1H, NHCOCH <sub>2</sub> , D <sub>2</sub> O exchangeable), 10.53 (s, 1H, COCONH, D <sub>2</sub> O exchangeable)	373.44 (M <sup>+</sup> , 0.90%), 98.09 (100%).
8 n	3269 (NH), 2951 (C-H), 1720, 1660 (C=O), 1079 (C-N).	$\delta$ 1.3-1.6 (m, 6H, (CH <sub>2</sub> ) <sub>3</sub> ), 2.3 (s, 3H, CH <sub>3</sub> ), 3.3-3.6 (m, 4H, N(CH <sub>2</sub> ) <sub>2</sub> ), 4.6 (s, 2H, OCH <sub>2</sub> ), 6.1 (s, 1H, coumarin-H), 6.8-7.1 (m, 2H, coumarin-H), 7.6 (d, 1H, coumarin-H), 10.3 (s, 2H, CONHNHCO).	-
8 o	3259 (NH), 2926 (C-H), 1716, 1666 (C=O), 1114 (C-O-C), 1079 (C-N).	$\delta$ 2.4 (s, 3H, CH <sub>3</sub> ), 3.2-3.8 (m, 8H, morpholine-H), 4.7 (s, 2H, OCH <sub>2</sub> ), 6.1 (s, 1H, coumarin-H), 6.9-7.1 (m, 2H, coumarin-H), 7.6 (d, 1H, coumarin-H), 10.3 (s, 2H, CONHNHCO).	-

(-): Not determined



Comp. No.	R	R <sub>1</sub>	Comp. No.	R	R <sub>1</sub>
8 a	H	C <sub>6</sub> H <sub>5</sub>	8i	H	<i>p</i> -F-C <sub>6</sub> H <sub>4</sub>
8 b	H	CH <sub>2</sub> -C <sub>6</sub> H <sub>5</sub>	8j	H	<i>p</i> -Br-C <sub>6</sub> H <sub>4</sub>
8c	H	CH <sub>2</sub> CH <sub>2</sub> -C <sub>6</sub> H <sub>5</sub>	8k	H	<i>p</i> -OH-C <sub>6</sub> H <sub>4</sub>
8 d	CH <sub>3</sub>	C <sub>6</sub> H <sub>5</sub>	8l	H	<i>p</i> -NO <sub>2</sub> -C <sub>6</sub> H <sub>4</sub>
8 e	H	<i>p</i> -CH <sub>3</sub> -C <sub>6</sub> H <sub>4</sub>	8m	pyrrolidine	
8 f	H	<i>o</i> -CH <sub>3</sub> -C <sub>6</sub> H <sub>4</sub>	8n	piperidine	
8 g	H	<i>p</i> -OCH <sub>3</sub> -C <sub>6</sub> H <sub>4</sub>	8o	morpholine	
8 h	H	<i>o</i> -OCH <sub>3</sub> -C <sub>6</sub> H <sub>4</sub>			

**Scheme 1:** Synthetic route of compounds 8a-o

## RESULTS AND DISCUSSION

### Chemistry

The starting 2-(4-methyl-2-oxo-2H-chromen-7-yloxy) acetohydrazide 4 was prepared by refluxing ethyl 2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy) acetate 3 with hydrazine hydrate in ethanol<sup>23</sup>. Structure of compound 4 was confirmed by comparison its physical and spectral data with the reported ones<sup>9</sup>.

The new intermediate, compound 5 was prepared by treating compound 4 with ethyl oxalyl chloride using acetonitrile as solvent in presence of pyridine to afford ethyl 2-(2-(2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy)acetate)hydrazinyl)-2-oxoacetate 5 in an excellent yield.

IR spectrum of compound 5 showed two bands at 3467 and 3206 cm<sup>-1</sup> for two (NH) groups and one band at 1161 cm<sup>-1</sup> for (C-O-C), in addition to three bands at 1741, 1712 and 1663 cm<sup>-1</sup> represent (C=O) groups.

$^1\text{H}$  NMR spectrum of compound 5 displayed a triplet signal at  $\delta$  1.29 ppm and quartet at  $\delta$  4.29 ppm corresponding to ethyl group. Moreover, the spectrum illustrated two singlet signals at  $\delta$  10.36 and 10.80 ppm which exchangeable with  $\text{D}_2\text{O}$  represent both (NH), in addition to characteristic patterns of coumarin aromatic protons. The structure of 5 has been also confirmed from EI-MS, the spectrum showed the molecular ion peak  $\text{M}^+$  at  $m/z$  348.22 (3.69%) corresponding to its relative molecular mass (348.10) and a base peak at  $m/z$  103.10 (100%). It is further confirmed by elemental analysis.

Hydrolysis of compound 5 using 10% NaOH in methanol afford another new intermediate 2-(2-(2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy)acetyl)hydrazinyl)-2-oxoacetic acid 6 in 90% yield.

IR spectrum of 6 characterized by a broad band at  $2700\text{-}3600\text{ cm}^{-1}$  for acidic (OH), and a strong absorption band at  $1705\text{ cm}^{-1}$  corresponding to (C=O) carboxylic.

Moreover,  $^1\text{H}$  NMR data also confirmed the hydrolysis of ester; disappearance of ethyl group characteristic protons, instead new signal for acidic (OH) appeared which a convincing evidence of acid formation.

Refluxing compound 6 with thionyl chloride in DCM for 3 hrs afforded the acid chloride 7 which used directly in the next step without further purification due its sensitivity against light and moisture. The acid chloride 7 was stirred overnight with an appropriate amine to afford the corresponding carboxamides 8 a-o.

IR spectra of the new compounds 8 a-o displayed no absorption bands belonging to (OH) group, instead new bands for aryl substitutions, reappearance of (NH) bands at  $3233\text{-}3487\text{ cm}^{-1}$ , in addition to bands of C=O groups at  $1720\text{-}1644\text{ cm}^{-1}$ .

$^1\text{H}$  NMR spectra also confirmed the disappearance of OH signal and displaying an exchangeable signal around  $\delta$  10 ppm for NH protons in addition to the introduced of aromatic moiety protons at  $\delta$  6.8-8 ppm.

As a representative example,  $^1\text{H}$  NMR spectrum of compound 8 c showed two characteristic triplet signals at  $\delta$  2.81 and 3.40 ppm corresponding to  $\text{CH}_2\text{CH}_2\text{N}$ , a multiplet signal at  $\delta$  7.18-7.33 ppm integrated for

aromatic 5H, in addition to the signals of the original nucleus.

Moreover, EI-MS of 8 c showed a weak molecular ion peak  $\text{M}^+$  at  $m/z$  423.26 (0.86%) corresponding to its relative molecular mass (423.14) and a base peak at  $m/z$  275.19 (100%).

In another example,  $^1\text{H}$  NMR data also confirm compound 8 g structure, it showed two singlets at  $\delta$  3.75 and 4.80 ppm corresponding to  $\text{OCH}_3$  and  $\text{OCH}_2$  respectively, a pair of doublets at  $\delta$  7.01 and 7.70 ppm integrating for four protons corresponding to *p*-disubstituted benzene moiety, and three amidic protons at  $\delta$  10.39, 10.68 and 10.83 ppm exchangeable with  $\text{D}_2\text{O}$

Furthermore, EI-MS analysis also confirms the suggested structures of compound 8 g. The spectrum showed a molecular ion peak  $\text{M}^+$  at  $m/z$  425.12 (13.15%) corresponding to its relative molecular mass (425.12) and a base peak at  $m/z$  149.10 (100%) which approved the structure.

#### Anti-inflammatory activity

Results of anti-inflammatory activities of compounds 5, 6, 8 a-o, Table 3, showed gradual increasing in activity up to maximum 4-5 h except compound 8 c and 8 m which showed their maximum effects after 3 hrs.

Intermediates 5 and 6 displayed moderate to good activities relative to INM as they showed 59 and 76% of the anti-inflammatory of INM respectively after 4 hrs, while they showed 63 and 70% respectively after 5 hrs.

Compounds 8 a, 8 b, 8 l and 8 o showed as significance activity achieving 84-94% of indomethacin activity at different time interval. Also, compound 8 g exceeding the activity of INM at 5 hrs interval; achieving 113% comparing with the reference drug. Moreover, compounds 8 f and 8 k showed approximately the same results as INM after 5 hrs.



**Table 3:** Percentage of edema inhibition of compounds **5, 6, 8 a-o** and indomethacin.

Comp. No.	Percentage of edema inhibition $\pm$ SE					
	30 mins	1hr	2hr	3hr	4hr	5hr
Control	-	-	-	-	-	-
IND	22.22 $\pm$ 0.29	33.33 $\pm$ 0.22	67.39 $\pm$ 0.23	78.72 $\pm$ 0.14	78.72 $\pm$ 0.20	79.16 $\pm$ 0.22
5	4.88 $\pm$ 0.25	14.66 $\pm$ 0.14	17.39 $\pm$ 1.10	27.65 $\pm$ 0.45	46.17 $\pm$ 0.22	49.79 $\pm$ 0.14
6	13.35 $\pm$ 0.36	20.68 $\pm$ 0.15	30.43 $\pm$ .018	51.06 $\pm$ 0.65	59.57 $\pm$ 0.20	55.20 $\pm$ 0.72
8 a	18.01 $\pm$ 0.54	23.78 $\pm$ 0.24	36.73 $\pm$ 1.05	31.48 $\pm$ 0.25	66.59 $\pm$ 0.28	60.41 $\pm$ 0.84
8 b	22.44 $\pm$ 0.54	29.11 $\pm$ 0.65	57.82 $\pm$ 0.45	67.02 $\pm$ 0.65	74.46 $\pm$ 0.84	54.16 $\pm$ 0.28
8 c	11.56 $\pm$ 0.78	21.12 $\pm$ 0.74	56.53 $\pm$ 0.65	56.59 $\pm$ 0.46	46.59 $\pm$ 0.75	47.91 $\pm$ 0.29
8 d	22.66 $\pm$ 0.28	28.23 $\pm$ 0.54	58.70 $\pm$ 1.23	59.57 $\pm$ 0.15	66.17 $\pm$ 0.42	54.16 $\pm$ 0.14
8 e	22.00 $\pm$ 0.47	30.18 $\pm$ 0.68	40.21 $\pm$ 1.36	54.25 $\pm$ 0.35	61.70 $\pm$ 0.13	58.33 $\pm$ 0.29
8 f	20.88 $\pm$ 0.69	28.89 $\pm$ 0.14	41.30 $\pm$ 0.47	57.44 $\pm$ 0.95	68.08 $\pm$ 0.16	79.16 $\pm$ 0.29
8 g	29.33 $\pm$ 0.96	52.12 $\pm$ 0.27	57.17 $\pm$ 0.63	63.19 $\pm$ 0.76	83.19 $\pm$ 0.54	89.16 $\pm$ 1.20
8 h	29.36 $\pm$ 1.25	22.15 $\pm$ 0.22	19.34 $\pm$ 0.54	21.27 $\pm$ 0.81	24.46 $\pm$ 0.47	25.12 $\pm$ 0.25
8 i	1.56 $\pm$ 0.57	17.72 $\pm$ 0.22	22.60 $\pm$ 0.03	20.00 $\pm$ 0.73	51.06 $\pm$ 0.96	50.00 $\pm$ 0.67
8 j	8.22 $\pm$ 1.06	16.14 $\pm$ 0.26	16.73 $\pm$ 0.17	41.48 $\pm$ 0.84	42.76 $\pm$ 0.54	58.33 $\pm$ 0.61
8 k	28.73 $\pm$ 1.04	57.30 $\pm$ 0.24	61.95 $\pm$ 0.62	70.20 $\pm$ 0.15	78.72 $\pm$ 0.69	79.17 $\pm$ 0.36
8 l	17.87 $\pm$ 0.23	45.07 $\pm$ 0.34	56.08 $\pm$ 0.48	58.93 $\pm$ 0.12	71.27 $\pm$ 0.47	75.83 $\pm$ 0.46
8 m	19.43 $\pm$ 0.38	36.67 $\pm$ 0.16	52.82 $\pm$ 0.74	63.82 $\pm$ 0.13	60.85 $\pm$ 0.52	60.41 $\pm$ 0.94
8 n	16.47 $\pm$ 0.27	27.67 $\pm$ 0.84	49.78 $\pm$ 1.7	60.42 $\pm$ 0.52	67.65 $\pm$ 0.82	67.29 $\pm$ 0.31
8 o	24.23 $\pm$ 0.65	25.00 $\pm$ 0.78	48.04 $\pm$ 0.65	54.04 $\pm$ 0.17	74.47 $\pm$ 0.93	46.66 $\pm$ 0.18

Significant difference from control group at  $p < 0.05$

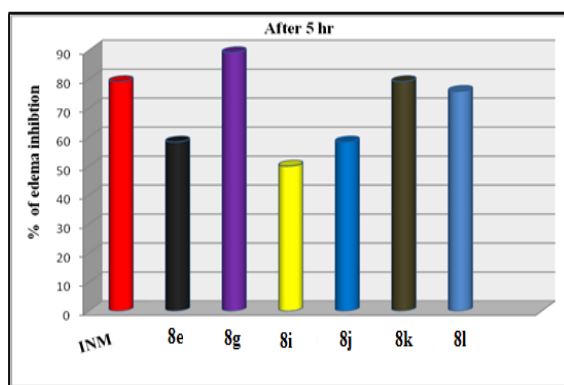
Moreover, by comparing the activities of different *N*-substitutions; compounds 8 a-o, the results revealed that the anti-inflammatory activity of compound 8 g ( $R=H$ ,  $R_1=p-OCH_3$ ) higher than that of other derivatives where  $R_1 = p-CH_3, p-NO_2, p-F, p-Br, p-OH$ , Figure 2.

Also, in comparing the anti-inflammatory activities of compounds 8 m, 8 n and 8 o with different saturated heterocyclic substitutions, their results showed that 8 o with morpholine moiety was the most active one with 95%

activity comparable to INM after 4 hrs, Figure3.

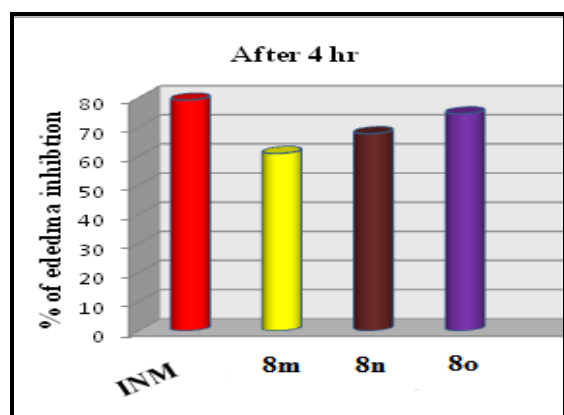
#### Structure activity relationship

Presence of substituted-phenyl part directly attached to amide moiety is seemed to enhance the anti-inflammatory activity than unsubstituted phenyl or benzyl group using



**Fig. 2:** Anti-inflammatory activity of compounds 8 e, 8 g, 8 i, 8 j, 8 k, 8 l and INM after 5 hr

Moreover, *para*-substitutions on the phenyl ring had proven a much greater anti-inflammatory effect over the *o*-substitutions; especially with oxygen containing functionalities, either electron donating groups (eg. hydroxyl or methoxy) or electron withdrawing groups (eg. NO<sub>2</sub>).



**Fig. 3:** Anti-inflammatory activity of 8 m, 8 n, 8 o and INM

### Computational analysis *In-silico* forecasting of physicochemical properties, pharmacokinetic profile, and drug-likeness profile

Clinical trials of new drugs are known to be extremely difficult due to improper ADME (absorption, delivery, metabolism, and excretion) properties, as well as the high costs of developing a new drug. As a result, determining the pharmacokinetic properties of a new drug is an important phase in the drug development process that can help focus lead optimization efforts on recovered analogues<sup>26</sup>. *In silico* ADME screens can now be used to identify the most promising compounds and reduce the possibility of late-stage drug attrition<sup>27</sup>. To achieve a desirable *in-vivo*

response, pharmacodynamic and pharmacokinetic properties must be in balance. Predictions of delivery volume, brain penetration, oral bioavailability, and clearance also provide more information about the regimen and medication dose<sup>28</sup>. A variety of parameters are investigated using virtual screening methods, including partition coefficient, drug likeness score, polar surface area PSA, human intestinal absorption HIA, and cell permeability. If the molecular weight is less than 500, LogP is less than 5, the number of hydrogen bond acceptors is less than 10, and the number of hydrogen bond donors is less than 5, an available orally drug is chosen in accordance with Lipinski's law<sup>29</sup>. The number of rotatable bonds is used to denote molecular stability, which is essential for oral bioavailability; if the molecule is flexible, it means the drug is not as effective when taken orally. Also, since polar surface area (PSA) is inversely proportional to percentage absorption (percent ABS), it has been suggested that the amount of hydrogen bonding groups be replaced with polar surface area (PSA) as a component and involved in the measurement of percentage absorption (percent ABS).

$$\%ABS = 109 - 0.345 \text{ PSA}$$

Oral bioavailability should be strong for compounds with a PSA of less than 140 A<sup>2</sup> and 10 or less rotatable bonds<sup>30</sup>.

For predicting the pharmacokinetic parameters of the most active compounds, we used the Molsoft<sup>31</sup>, Pre-ADMET<sup>32</sup>, SwissADME<sup>33</sup>, and Molinspiration<sup>34</sup> softwares.

From the results, compounds stratify according to Lipinski's law, with MW ranging from 389.36 to 425.39 (< 500), HBD ranging from 2 to 4 (< 5), Log P values ranging from 0.52 to 1.78 (< 5), and HBA ranging from 6 to 7 (< 10). As a result, they should have good oral absorption, and variations in bioactivity cannot be attributed to this property. Moreover, the compounds had numbers of rotatable bonds ranging from 8 to 9 (< 10) and topological PSA values of 126.74 and 146.97 A<sup>2</sup> (< 140 A<sup>2</sup>), respectively, suggesting good permeability, absorption, and transport across biological membranes.

In addition, the drug-likeness model score for compounds was calculated using Molsoft software (Table 5). Aqueous solubility can

affect absorption and distribution characteristics. In this case, these compounds met the requirements of the drug-likeness model. The higher the drug-likeness model ranking, the more likely it is to be a drug molecule. Compound 8 o (0.24) was predicted to have a positive model-score, while the other compounds were predicted to have a negative model-score (-0.09 to -0.40).

Pre-ADMET software was also used to conduct an in silico analysis of the following pharmacokinetic parameters: Human intestinal absorption (HIA), Caco2 (human colon adenocarcinoma) permeability coefficient, inhibition of cytochrome P4502D6 (CYP2D6), MDCK (Madin-Darby canine kidney cells) permeability coefficient, Blood brain barrier partition coefficient (BBB), and human plasma protein binding (PPB). The estimated ADME

parameters' results are shown in the table below (Table 6). The findings showed that the compounds had a medium CNS absorption range of 0.2 to 0.4 (0.1-2); In the CaCo-2 and MDCK models, the investigated compounds had cell permeability ranging from 2.90 to 18.21 nm/s and 0.36 to 30.85 nm/s, respectively. They were also non-inhibitors of the CYP2D6 enzyme, implying that they would have no interactions with CYP2D6 inhibitors and/or inducers.

Consequently, they showed strong human intestinal absorption values ranging from 80.00 to 90.25% ( $\leq 80\%$ ), suggesting very well-absorbed compounds, the examined compounds were found to be low bound to human plasma proteins, ranging from 40.32 to 81.83 percent ( $\geq 90\%$ ).

**Table. 4 :** Physicochemical and lipophilicity of the potent compounds using SwissADME and Molinspiration

Code	Lipophilicity Consensus log P	Physicochemical properties								
		MW <sup>a</sup> g/mol	Heavy atoms	Aromatic heavy atoms	Rotatable Bonds	H-bond acceptors	H-bond donors	MR <sup>b</sup>	TPSA <sup>c</sup> (Å <sup>2</sup> )	% ABS <sup>d</sup>
8 a	1.68	395.37	29	16	9	6	3	103.39	126.74	65.27
8 b	1.77	409.39	30	16	9	6	3	106.66	126.74	65.27
8 i	2.03	413.36	30	16	9	7	3	103.35	126.74	65.27
8 o	0.52	389.36	28	10	8	7	2	99.58	127.18	65.12
8 f	1.78	425.39	31	16	9	7	3	109.89	135.97	62.09
8 k	1.33	411.36	30	16	9	7	4	105.42	146.97	58.29

Abbreviation: <sup>a</sup>MW, molecular weight; <sup>b</sup>MR, molar refractivity; <sup>c</sup>TPSA, topological polar surface area; <sup>d</sup>%ABS: percentage of absorption

**Table. 5:** Lipinski drug likeness of the most active compounds using Molsoft and wissADME software.

Code	Drug likeness model score	Lipinski violations	Bioavailability score
8 a	-0.38	0	0.55
8 b	-0.18	0	0.55
8 i	-0.09	0	0.55
8 o	0.24	0	0.55
8 f	-0.40	0	0.55
8 k	-0.15	0	0.55

**Table 6:** ADME data of most active compounds calculated using preADMET software

Code	Pharmacokinetics					
	BBB <sup>a</sup>	Caco-2 <sup>b</sup>	HIA <sup>c</sup>	MDCK <sup>d</sup>	PPB <sup>e</sup>	CYP 2D6 <sup>f</sup>
8 a	0.30	9.93	89.71	16.59	79.28	None
8 b	0.30	9.58	90.25	30.85	81.83	None
8 i	0.30	18.21	89.75	0.36	80.55	None
8 o	0.40	2.90	84.23	11.08	40.32	None
8 f	0.20	14.62	88.85	0.58	72.16	None
8 k	0.40	15.13	80.00	0.38	71.41	None

<sup>a</sup>BBB: blood brain barrier penetration; <sup>b</sup>CACO-2: permeability through cells derived from human colon adenocarcinoma; <sup>c</sup>HIA: percentage human intestinal absorption; <sup>d</sup>MDCK: permeability through Madin-Darby canine kidney cells; <sup>e</sup>PPB: plasma protein binding; <sup>f</sup>CYP2D6: cytochrome P450 2D6

### Conclusion

In summary, a new series of coumarinyloxyacetamide derivatives with three synergistic amide groups was designed, synthesized in good yields, and structurally elucidated using IR, <sup>1</sup>HNMR and mass spectrometry along with elemental microanalyses. All new finals and intermediate compounds were tested for their possible anti-inflammatory activities using *in-vivo* carrageenan induced paw edema method and they showed a significance activity especially compound 8 g (R=H, R<sub>1</sub>=*p*-OCH<sub>3</sub>) which exceed the activity of the reference drug achieving 113% activity of indomethacin. Also compounds 8 f (R=H, R<sub>1</sub>=*o*-CH<sub>3</sub>) and 8 k (R=H, R<sub>1</sub>=*p*-OH) showed approximately the same results as indomethacin. Moreover, among different saturated heterocyclic substitutions; compound 8 o with morpholine moiety was the most active one with 95% activity comparable to indomethacin.

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## نشرة العلوم الصيدلانية جامعة أسيوط



### تخليق ودراسة الخواص الفيزيوكيميائية والتأثير البيولوجي لبعض مشتقات الكومارين - أسيتاميد الجديدة كمضات قوية للالتهاب

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تم عمل مجموعة جديدة من مشتقات الميثيل كومارين مرتبطة بثلاث مجموعات فعالة من جزئ الأמיד. أيضاً تم التأكد من التركيب البنائي لجميع المركبات المحضرة باستخدام العديد من تقنيات التعرف علي المركبات مثل الأشعة تحت الحمراء والتحليل النووي المغناطيسي ومطياف الكتلة والتحليل الدقيق للعناصر وغيرها. بالإضافة لذلك تم اخضاع المركبات المحضرة للتحليل كمضادات للالتهابات. هذا وقد أثبتت النتائج فاعلية مباشرة لهذه المركبات نسبة لعقار الاندوميثازين الذي تم استخدامه كدواء مرجعي لهذه الدراسة. علماً بأن المركب 8 g كان أقوى المركبات الفاعلة، متخطياً عقار الاندوميثازين. هذا وقد أحرز المركبين 8 f و 8 k نتائج مقاربة لنفس العقار. بالإضافة لعمل دراسة حاسوبية باستخدام بعض البرامج المتخصصة لتوقع الخواص البيولوجية لأقوي المركبات المحضرة. توطئة لإحتمالية استخدام هذه المركبات كأدوية.