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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of pyranochromene and pyranopyrimidine derivatives from substituted natural coumarin isolated from *Ammi majus* L. and their biological evaluation

N. H. Ouf · Y. A. Selim · M. I. Sakran · A. S. Badr El-din

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Abstract A series of novel coumarin derivatives were synthesized from 6-hydroxy-7-methoxy-4-methyl coumarin which was isolated from the aerial parts of the Egyptian medicinal plant *Ammi majus* L. (Apiaceae). The key intermediate 3-amino-5-methoxy-1-(4-methoxyphenyl)-10-methyl-8-oxo-1,8-dihydropyrano[3,2-*f*]chromene-2-carbonitrile (**3c**) was obtained in one-pot synthesis by treating α-cyanocinnamonitrile (**1-c**) with the natural compound: 6-hydroxy-7-methoxy-4-methyl coumarin (**2**). Chemical, elemental and spectroscopic evidences confirmed the structures of the synthesized compounds. Some of the newly synthesized compounds exhibited better anti-inflammatory activities at low concentrations compared with indomethacin as positive control.

Keywords Coumarin · Pyranochromene · Pyranopyrimidinochromene · Anti-inflammatory

N. H. Ouf · M. I. Sakran · A. S. Badr El-din
Department of Chemistry and Biochemistry, Faculty of Science, Tabuk University, Tabuk, Saudi Arabia Kingdom

N. H. Ouf
Department of Chemistry, Faculty of Science, Zagazig University, Zagazig 44519, Egypt

Y. A. Selim (✉)
Faculty of Specific Education, Zagazig University, Zagazig 44519, Egypt
e-mail: y2selem@yahoo.com

M. I. Sakran · A. S. Badr El-din
Department of Chemistry, Faculty of Science, Tanta University, Tanta 31527, Egypt

Introduction

Coumarin is a plant flavonoid widely distributed in nature. In previous studies, pyran and fused 4H-pyran derivatives have attracted great interest owing to their antimicrobial activity (El-Agrody *et al.,* 2000, 2001; Bedair *et al*., 2000) inhibition of influenza, virus sialidases (Taylor *et al*., 1998), mutagenic activity (Hirmoto *et al*., 1997), antiviral (Martinez-Grau and Marco, 1997), antiproliferaction agents (Dell and Smith, 1993), sex-hormones (Bianchi and Tava, 1987), antitumor (Eiden and Denk, 1991), and anti-inflammatory agents (Shishoo *et al*., 1981). Moreover, pyrane derivatives are well known for their antihistaminic activity. Recent studies have shown the syntheses of some pyrimidines and their fused derivatives occurring as antibacterial and antitumor agents as well as in agrochemical and veterinary products (Ismail *et al*., 2008; El-Gaby *et al*., 2006; Prikazchikova *et al*., 1975; Brown *et al*., 1984). The significance of pyranopyrimidine derivatives is recognized because of their occurrence in the structure of various natural products, their biological activity, and their synthetic potential (Hren *et al*., 2009; Yu and Wang, 2005). We believed it would be of interest to combine the above mentioned heterocyclic compounds in a molecular framework to investigate a possible additive effect of these rings regarding biological activity. In this study, new coumarin, 6-hydroxy-7-methoxy-4 methyl coumarin 2, is a natural product isolated from *Ammi majus* L. (Apiaceae), and was found to have anti-inflammatory and antiviral activity (Selim and Ouf, 2012). We have synthesized some new heterocyclic-fused system containing coumarin nuclei, and tested their anti-inflammatory activities in comparison with indomethacin as positive control. Cytotoxicity and some biochemical parameters were also determined.

Experimental

Materials and instrumentation

All melting points were measured on Electrothermal IA 9000 series digital melting point apparatus. Elemental analysis data were obtained from the micro-analytical unit, Cairo University, Cairo, Egypt, and the results were in favorable agreement with the calculated values. The UV spectra were recorded on Bye-Unicam SP-1800 spectrometer. The IR spectra (KBr) were recorded on a Pye-Unicam spectrophotometer. The ^{1}H- and ^{13}C NMR spectra were measured in DMSO and recorded at 400 and 100 MHz on a PerkinElmer R12B Spectrometer using TMS as an internal standard. All reactions were followed by TLC (silica gel, aluminum sheets 60 F_{254}, Merck), and spots were visualized by UV Lamb.

Reaction of **1**(**a–f**) with 6-hydroxy-7-methoxy-4-methyl coumarin

These compounds were prepared by a solution of **1a–f** (0.01 mol) in ethanol (30 ml), and were treated with 6-hydroxy-7-methoxy-4-methyl coumarin **2** (0.01 mol) and piperidine (0.5 ml). The reaction mixture was heated until complete precipitation (reaction times: 15 min for **1**(**a–c**); 120 min for **1**(**d–f**). The solid product that formed was collected by filtration and recrystallized from a suitable solvent to give new synthesized pyrano chromene derivatives **3**(**a–f**). See (Scheme 1).

*3-amino-5-methoxy-10-methyl-8-oxo-1-phenyl-1,8-dihydropyrano[3,2-f]chromene-2-carbonitrile (**3a**)*

It was obtained as colorless crystals from benzene, mp 240 °C, yield (89 %). UV (CH_3OH) λ_{max} (log ε): 275(2.83). IR (KBr)1700(C=O of coumarin), 2195(CN), 2833(CH-stretching), 2927, 2966, 3317(NH_2), 3400, cm^{-1}. ^{1}H NMR(DMSO-d_6 400 MHz) δ ppm: 2.2(3H, s, CH_3), 3.8 (3H, s, OCH_3), 5.3(1H, s, H1), 6.2(1H, s, H9), 6.7(1H, s, H6), 7.1(2H, br, NH_2 cancelled by D_2O), 7.5–8(5H, m, Ar–H2′, 3′, 4′, 5′, 6′). ^{13}C NMR (DMSO-d_6 100 MHz) δ ppm: 19.8(CH_3), 56.9(OCH_3), 104(C-6),110(C-1), 112(C-2), 113(C-9), 116.3(CN), 119(C-1′), 121.4(C-2′), 125.3(C-4′, 6′), 127.6(C-3′, 5′), 143(C-10a), 146.2(C-6a), 147.5(C-4a, 10b), 174.1(C-3), 152(C-5,10), 162(C-8). MS: *m/z* [M+1]$^{+}$ 360.1. Anal. Calcd. for $C_{21}H_{16}N_2O_4$: C, 70.00; H, 4.48; N, 7.77; O 17.76. Found: C, 70.05; H, 4.37; N, 7.72; O 17.69.

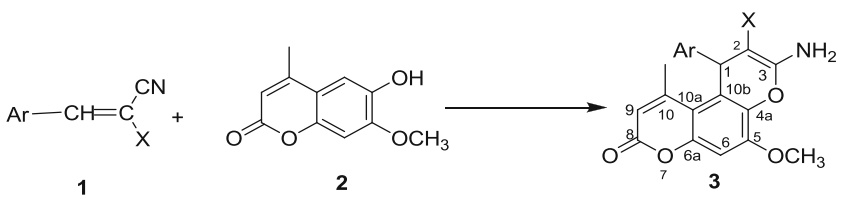
*3-amino-1-(4-chlorophenyl)-5-methoxy-10-methyl-8-oxo-1,8-dihydropyrano[3,2-f] chromene-2-carbonitrile (**3b**)*

It was obtained as yellow crystals from benzene mp 265 °C yield (90 %). UV (CH_3OH) λ_{max} (log ε): 275(2.81). IR (KBr)1700(C=O of coumarin), 2200(CN), 2840(CH-stretching), 2930, 2960, 3319(NH_2), 3448 cm^{-1}. ^{1}H NMR(DMSO-d_6 400 MHz) δ ppm: 2.1(3H, s, CH_3), 3.84(3H, s, OCH_3), 5.3(1H, s, H1),6.2(1H, s, H9), 5.3(1H, s, H1) ppm 6.7(1H, s, H6), 7.3(2H, br, NH_2 cancelled by D_2O), 7.5–8(4H, m, Ar-H2′, 3′, 5′, 6′). ^{13}C NMR(DMSO-d_6 100 MHz) δ ppm: 19.8(CH3), 57.9(OCH_3), 104.7(C-6), 110.1(C-1), 112(C-2), 114.5(C-9), 116.1(CN), 119.2(C-1′), 121.4(C-2′), 125.3(C-6′),127.6(C-3′, 5′), 131.2(C-4′),143(C-10a), 146.2(C-6a), 147.2(C-4a, 10b), 173.1(C-3), 152.4(C-5, 10), 162.5(C-8). MS: *m/z* [M+1]$^{+}$ 394.06. Anal. Calcd. for $C_{21}H_{15}ClN_2O_4$: C, 63.89; H, 3.83; Cl, 8.98; N, 7.10; O, 16.22. Found: C, 63.98; H, 3.81; Cl 8.88, N, 7.10; O, 16.19.

*3-amino-5-methoxy-1-(4-methoxyphenyl)-10-methyl-8-oxo-1,8-dihydropyrano[3,2f] chromene-2-carbonitrile (**3c**)*

It was obtained as colorless crystals from benzene, mp 250 °C, yield (80 %). UV (CH_3OH) λ_{max} (log ε): 275(2.85). IR (KBr)1700(C=O of coumarin), 3411, 3317(NH_2), 2969, 2927,2833(CH-stretching), 2195(CN)cm^{-1}. ^{1}H NMR(DMSO-d_6) δ ppm: 2.1(3H, s, CH_3), 3.85 (6H, s, 2OCH_3), 5.2(1H, s, H1), 6.2(1H, s, H9), 6.7(1H, s, H6), 7.1(2H, br, NH_2 cancelled by D_2O), 7.5–8(4H, m, Ar-H2′, 3′, 5′, 6′). ^{13}C NMR(DMSO-d_6 100 MHz) δ ppm: 19.8(CH3), 56.9, 59.5(2OCH_3), 104(C-6),

Scheme 1 Synthesis of pyranochromene derivatives



1 2 3

a: Ar = C_6H_5 X = CN
b: Ar = C_6H_4Cl-p, X = CN
c: Ar = C_6H_4OMe-p, X = CN
d: Ar = C_6H_5, X = CO_2Et
e: Ar = C_6H_4Cl-p, X = CO_2Et
f: Ar = C_6H_4OMe-p, X = CO_2Et

110(C-1), 112(C-2), 114.1(C-9), 116.3(CN), 119(C-1′), 121.4 (C-2′), 125.3(C-6′), 127.6(C-3′, 5′), 143(C-10a), 146.2(C-6a), 147.5(C-4a, 10b), 174.1(C-3), 152(C-5, 10, 4′), 162.5(C-8). MS: *m/z* $[M+1]^+$ 390.11. Anal. Calcd. for $C_{22}H_{18}N_2O_5$: C, 67.69; H, 4.65; N, 7.18; O, 20.46. Found: C, 68.01; H, 4.64; N, 7.18; O, 20.22.

*Ethyl-3-amino-5-methoxy-10-methyl-8-oxo-1-phenyl-1, 8-dihydropyrano [3,2-f] chromene-2-carboxylate (**3d**)*

It was obtained as colorless needles from benzene, mp 190 °C, yield (85 %). UV (CH_3OH) λ_{max} (log ε): 275(2.82). IR (KBr)1700(C=O of coumarin), 1749(C=O of ester), 2847(CH-stretching), 2925, 2966, 3320(NH_2), 3440 cm^{-1}. ^1H NMR (DMSO-d_6 400 MHz) δ ppm : 1.25(3H, t, $J = 7.05$ Hz, CH_2CH_3), 2.2(3H, s, CH_3), 3.8. (3H, s, OCH_3), 4.15(2H, q, $J = 7.2$ Hz, CH_2CH_3), 5.3(1H, s, H1), 6.2(1H, s, H9), 6.7 (1H, s, Ar-H6), 7.1(2H, br, NH_2 cancelled by D_2O), 7.5–8(5H, m, Ar-H2′, 3′, 4′, 5′, 6′). MS: *m/z* $[M+1]^+$ 407.14. Anal. Calcd. for $C_{23}H_{21}NO_6$: C, 67.80; H, 3.44; N, 7.18; O, 23.56. Found: C, 67.80; H, 5.20; N, 3.43; O, 23.42.

*Ethyl-3-amino-1-(4-chlorophenyl)-5-methoxy-10-methyl-8-oxo-1, 8-dihydropyrano [3, 2-f] chromene-2-carboxylate (**3e**)*

It was obtained as colorless needles from benzene, mp 180 °C, yield (80 %). UV (CH_3OH) λ_{max} (log ε): 275(2.86). IR (KBr)1700(C=O of coumarin), 1740(C=O of ester), 2930, 2966, 2844(CH-stretching), 3320(NH_2), 3400 cm^{-1}. ^1H NMR(DMSO-d_6 400 MHz) δ ppm: 1.26 (3H, t, $J = 7.05$ Hz, CH_2CH_3), 2.2(3H, s, CH_3), 3.8(3H, s, OCH_3), 4.11(2H, q, $J = 7.1$ Hz, CH_2CH_3), 5.3(1H, s, H1), 6.2(1H, s, H9), 6.68(1H, s, H6), 7.1(2H, br, NH_2 cancelled by D_2O), 7.5–8.2(m, 4H, Ar-H2′, 3′, 5′, 6′), 4.15 (q, 7.2 Hz, CH_2). MS: *m/z* $[M+1]^+$ 441.11. Anal. Calcd. for $C_{23}H_{20}ClNO_6$: C, 62.52; H, 4.56; Cl, 8.03; N, 3.17; O, 21.76. Found: C, 52.83; H, 4.46; Cl, 7.93; N, 3.09; O, 21.24.

*Ethyl-3-amino-5-methoxy-1-(4-methoxyphenyl)-10-methyl-8-oxo-1,8-dihydropyrano [3, 2-f] chromene-2-carboxylate (**3f**)*

It was obtained as colorless needles from benzene, mp 200 °C, yield (80 %). UV (CH_3OH) λ_{max} (log ε): 275(2.85). IR (KBr)1700(C=O of coumarin), 1744(C=O of ester), 2840(CH-stretching), 2925, 2966, 3320(NH_2), 3400 cm^{-1}. ^1H NMR(DMSO-d_6 400 MHz) δ ppm: 1.25 (3H, t, $J = 7.05$ Hz, CH_2CH_3), 2.2(3H, s, CH_3), 3.85(6H, s, OCH_3), 4.15(2H, q, $J = 7.2$ Hz, CH_2CH_3), 5.3(1H, s, H1), 6.2(1H, s, H9), 6.7(1H, s, H6), 7.11(2H, br, NH_2 cancelled by D_2O), 7.5–8.2(4H, m, Ar-H2′, 3′, 5′, 6′). ^{13}C NMR(DMSO-d_6 100 MHz) δ ppm: 19.8, 28.2($2CH_3$), 56.9,58.9 ($2OCH_3$), 61.7(CH_2CH_3), 104(C-6), 109(C-1), 112.5(C-2), 114.1(C-9), 119(C-1′), 121.5(C-2′), 125.3(C-6′), 127.6(C-3′, 5′), 143(C-10a), 146.2(C-6a), 147.5(C-4a, 10b), 174.1(C-3), 152(C-5, 10, 4′), 162,5(C-8), 192.7 ($COOC_2H_5$). MS: *m/z* $[M+1]^+$ 437.15. Anal. Calcd. for $C_{24}H_{23}NO_7$: C, 65.90; H, 5.30; N, 3.20; O, 25.60. Found: C, 65.96; H, 5.22; N, 3.10; O, 25.24.

Synthesis of **4(a–c)**

These compounds were prepared by a mixture of 3c (0.01 mol), benzaldhyde, *p*-chlorobenzaldhyde, *p*-anisaldhyde (0.01 mol), DMSO (20 ml), and piperidine (0.5 ml), and were refluxed for 4 h to give new synthesized pyrano derivatives **4a–c** (Scheme 2).

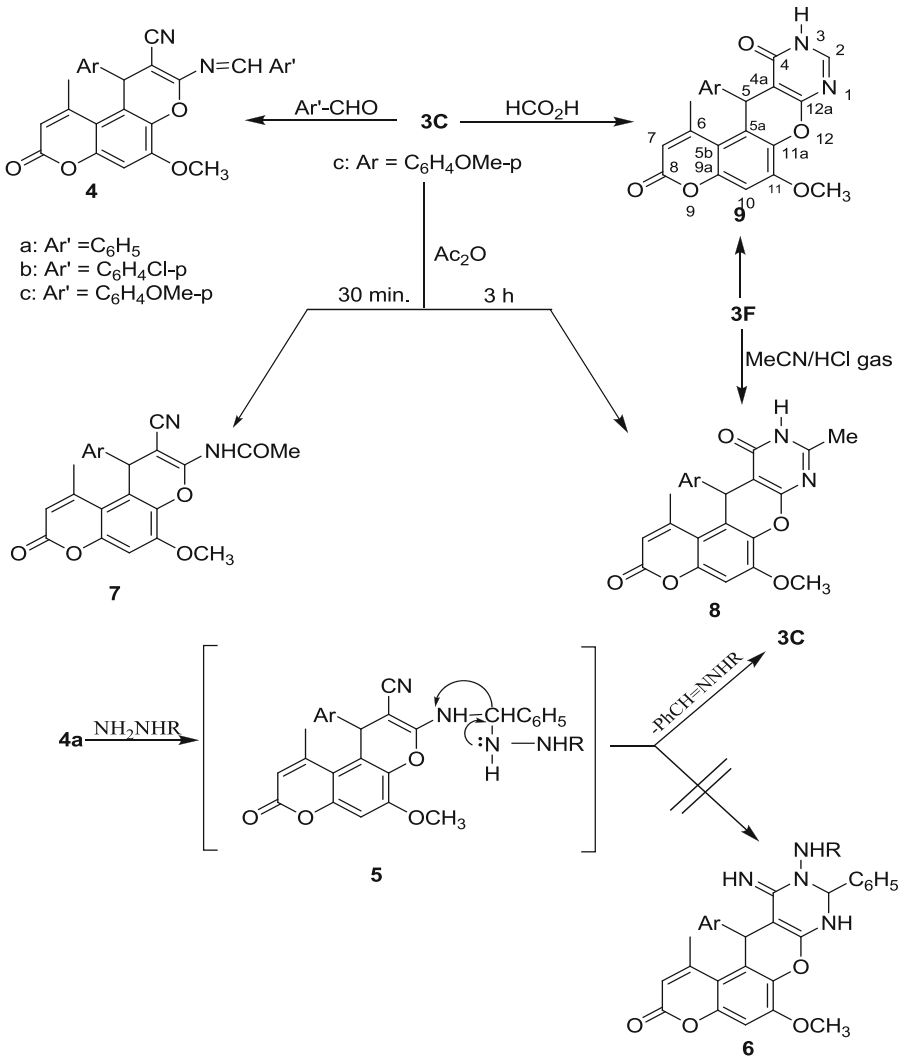
*(E)-3-(benzylideneamino)-5-methoxy-1-(4-methoxyphenyl)-10-methyl-8-oxo-1, 8-dihydro pyrano [3,2-f]chromene-2-carbonitrile (**4a**)*

It was obtained as yellow crystals from benzene, mp 280 °C, yield (82 %). IR (KBr)1641(C=NH), 1700(C=O of coumarin), 2210(CN), 2833(CH-stretching), 2925, 3074 cm^{-1}. ^1H NMR(DMSO-d_6 400 MHz) δ ppm: 2.2(3H, s, CH_3), 3.8(6H, s, $2OCH_3$), 5.3(1H, s, H1), 6.2(1H, s, H9), 6.7(1H, s, H6), 7.5–8.2(9H, m, Ar-H2′, 3′, 5′, 6′, 2″, 3″, 4″, 5″, 6″), 9.06(1H, s, N=CH). ^{13}C NMR(DMSO-d_6 100 MHz) δ ppm: 19.8(CH_3), 56.9, 59.9($2OCH_3$), 104(C-6), 110.2(C-1), 112.9(C-2), 114.5(C-9), 116.3(CN), 119(C-1′), 121.4(C-2′, 2″), 125.3(C-6′, 4″, 6″), 127.6(C-3′, 5, 3″, 5″), 133.7(c-1″), 143(C-10a), 143.5(N=CH), 146.2(C-6a), 147.5(C-4a, 10b), 174.1(C-3), 152(C-5, 10, 4′), 162(C-8). MS: *m/z* $[M+1]^+$ 478.45. Anal. Calcd. for $C_{29}H_{22}N_2O_5$: C, 72.79; H, 4.63; N, 5.85; O, 16.72. Found: C, 71.96; H, 4.21; N, 5.13; O, 16.21.

*(E)-3-(4-chlorobenzylideneamino)-5-methoxy-1-(4-methoxyphenyl)-10-methyl-8-oxo-1, 8-dihydropyrano [3,2-f] chromene-2-carbonitrile (**4b**)*

It was obtained as yellow crystals from benzene, mp 310 °C, yield (88 %). IR (KBr)1641(C=N)1700(C=O of coumarin), 2211(CN), 2833 (CH-stretching), 2925,3074 cm^{-1}. ^1H NMR (DMSO-d_6 400 MHz) δ ppm: 2.2(3H, s, CH_3), 3.83(6H, s, $2OCH_3$), 5.3(1H, s, H1), 6.2(1H, s, H9), 6.7 (1H, s, H6),7.5–8.1(8H, m, Ar-H2′, 3′, 5′, 6′, 2″, 3″, 5″, 6″), 9.06(1H, s, N=CH). MS: *m/z* $[M+1]^+$ 512.11. Anal.

Scheme 2 Synthesis of pyranochromene and pyranopyrimidine derivatives



Calcd. for $C_{29}H_{21}ClN_2O_5$: C, 67.90; H, 4.13; Cl, 6.90; N, 5.46; O, 15.60. Found: C, 67.92; H, 4.10; Cl 6.54; N, 5.47; O, 15.21.

*(E)-5-methoxy-3-(4-methoxybenzylideneamino)-1-(4-methoxyphenyl)-10-methyl-8-oxo-1, 8-dihydropyrano [3,2-f] chromene-2-carbonitrile (**4c**)*

It was obtained as yellow crystals from benzene, mp 272 °C, yield (90 %). IR (KBr) 1641(C=N), 1710(C=O of coumarin), 2211(CN), 2833(CH-stretching), 2929, 3174, cm^{-1}. ^1H NMR (DMSO-d_6 400 MHz) δ ppm: 2.2(3H, s, CH_3), 3.86(9H, s, $3OCH_3$), 5.3(1H, s, H1), 6.2(1H, s, H9), 6.7(1H, s, H6), 7.5–8.2(8H, m, Ar-H2′, 3′, 5′, 6′, 2″, 3″, 5″, 6″), 9.16(1H, s, N=CH) ppm. MS: *m/z* $[M+1]^+$ 508.16. Anal. Calcd. for $C_{30}H_{24}N_2O_6$: C, 70.86; H, 4.76; N, 5.51; O, 18.85. Found: C, 71.10; H, 4.68; N, 5.47; O, 18.42.

Synthesis of N-(2-cyano-5-methoxy-1-(4-methoxyphenyl)-10-methyl-8-oxo-1,8-dihydro-pyrano [3,2-f]chromen-3-yl)acetamide, (**7**)

This new pyrano compound was prepared by a solution of 3c (0.01 mol) in acetic anhydride (20 ml) and was heated under reflux for 30 min. The solid product formed was filtered, washed with cold ethanol, dried, and recrystallized from ethanol. It was obtained as colorless needles, mp 240 °C, yield(81 %).IR (KBr)1700(C=O of coumarin), 2206(CN), 2916(CH-stretching), 3200(NH), 3046, ^1H NMR (DMSO-d_6 400 MHz)δ ppm: 2.2(3H, s, CH_3), 3.2(3H, s, $COCH_3$), 3.8(6H, s, $2OCH_3$), 5.13(1H, s, H1), 6.21(1H, s, H9), 6.17(1H, s, H6),7.5–8.2(4H, m, Ar-H2′, 3′, 5′, 6′), 11–12(1H, br, NH). ^{13}C NMR(DMSO-d_6 100 MHz) δ ppm: 17.2 (2CH3), 57.2, 59.6($2OCH_3$), 106.2(C-6),111.3(C-1), 112.3 (C-2), 114.6(C-9), 117.2(CN),119.2(C-1′), 121.4(C-2′), 126.3(C-6′), 129.2(C-3′, 5′), 143.2(C-10a), 148.1(C-6a),

149.1(C-4a, 10b), 177.1(C-3), 152.4(C-5, 10, 4′), 162.5(C-8), 169.3($\underline{C}OCH_3$). MS: *m/z* $[M+1]^+$ 423.13. Anal. Calcd. for $C_{24}H_{20}N_2O_6$: C, 66.66; H, 4.49; N, 6.35; O, 22.25. Found: C, 66.76; H, 4.49; N, 6.35; O, 21.99.

Synthesis of 2, 6-dimethyl-4, 8-dioxo-5-(4-methoxy phenyl)-11-methoxy-5, 8-dihydro pyrano [3, 2-f]-3, 4-dihydropyrimidino [4, 5-i] chromene (**8**)

Method (a)

This new pyrano pyrimidine compound was prepared by a solution of 3c(0.01 mol) in acetic anhydride (20 ml) and was heated under reflux for 3 h. The solid product formed was filtered, washed with cold ethanol, dried, and recrystallized from ethanol to give **8** as colorless needles, mp 170 °C, yield (67 %).IR (KBr)1700(C=O of coumarin), 2916(CH-stretching), 3200(NH) 3046, cm^{-1}. ^1H NMR(DMSO-d_6 400 MHz) δ ppm: 2.3(6H, s, $2CH_3$),3.8(6H, s, $2OCH_3$), 5.3(1H, s, H5), 6.2(1H, s, H10), 6.7(1H, s, H7),7.2–9.07(5H, m, Ar-H2′, 3′, 5′, 6′ + NH). ^{13}C NMR (DMSO-d_6 100 MHz) δ ppm: 19.8(2CH3), 55.2, 57.1($2OCH_3$), 108.2(C-10), 111.9(C-4a), 113.3(C-5), 115.1(C-7), 119(C-1′), 123.6 (C-2′), 126.3(C-6′),127.2(C-2),129.6 (C-3′,5′), 131.5(C-4′),143(C-5b), 146.2(C-9a), 148.3 (C-5a, 11a, 12a), 152.4(C-6,11), 160.2(C-4), 164,5(C-8). MS: *m/z* $[M+1]^+$ 423.23. Anal. Calcd. for $C_{24}H_{20}N_2O_6$: C, 66.61; H, 4.46; N, 6.35; O, 22.22. Found: C, 66.71; H, 4.49; N, 6.33; O, 21.89.

Method (b)

This compound was prepared by a stream of dry HCl gas and was passed through a mixture of **3f** (0.01 mol) and acetonitrile (30 ml) for 5–8 h. The reaction mixture was poured into ice water and basified with 10 % ammonium hydroxide to give **8** with yield (80 %).

Synthesis of 6-methyl-4, 8-dioxo-5-(4-methoxy phenyl)-11-methoxy-5, 8-dihydro pyrano[3, 2-f]-3, 4-dihydropyrimidino[4, 5-i] chromene (**9**)

Method (a)

This new pyrano pyrimidine compound was prepared by a solution of **3c** (0.01 mol) in formic acid (20 ml) was heated under reflux for 30 min. The solid product formed was filtered, washed with cold ethanol, dried, and recrystallized from ethanol. It was obtained as needles, mp 240 °C, yield(81 %).IR (KBr)1700(C=O of coumarin), 2916(CH-stretching), 3200(NH), 3046 cm^{-1}. ^1H NMR(DMSO-d_6 400 MHz) δ ppm: 2.3(3H, s, CH_3),3.8(6H, s, OCH_3),5.3(1H, s, H5), 6.22(1H, s, H10), 6.71(1H, s, H7),7.2–9.07(5H, m, Ar-H2, 2′, 3′ ,5′ , 6′ + NH),8.3(1H, s, H3). ^{13}C NMR(DMSO-d_6 100 MHz) δ ppm: 19.8(CH3), 56.1,59.2($2OCH_3$), 104.7(C-10), 112.5(C-4a), 113(C-5), 114.0(C-7), 119(C-1′), 121.4(C-2′), 125.3(C-6′),126(C-2),127.6(C-3′,5′), 131.2(C-4′), 143(C-5b),146.2(C-9a), 147.5(C-5a, 11a, 12a), 152.0(C-6,11), 159(C-4), 162,5(C-8). MS: *m/z* $[M+1]^+$ 418.23. Anal. Calcd. for $C_{23}H_{18}N_2O_6$: C, 66.02; H, 4.34; N, 6.70; O, 22.92. Found: C, 66.52; H, 4.25; N, 6.66; O, 22.58.

Method (b)

This compound was prepared by a solution of **3f** (0.01 mol) in formamide (20 ml) and was heated under reflux for 6 h. The solid product formed was filtered, washed with cold ethanol, dried, and recrystallized from ethanol to give **9** (Yield 70 %)

Biology part

Experimental animals

Male albino mice weight around 150–200 g were purchased from Faculty of Science, Tanta University, Egypt. They were acclimatized to animal house conditions. Animals were provided with standard diet.

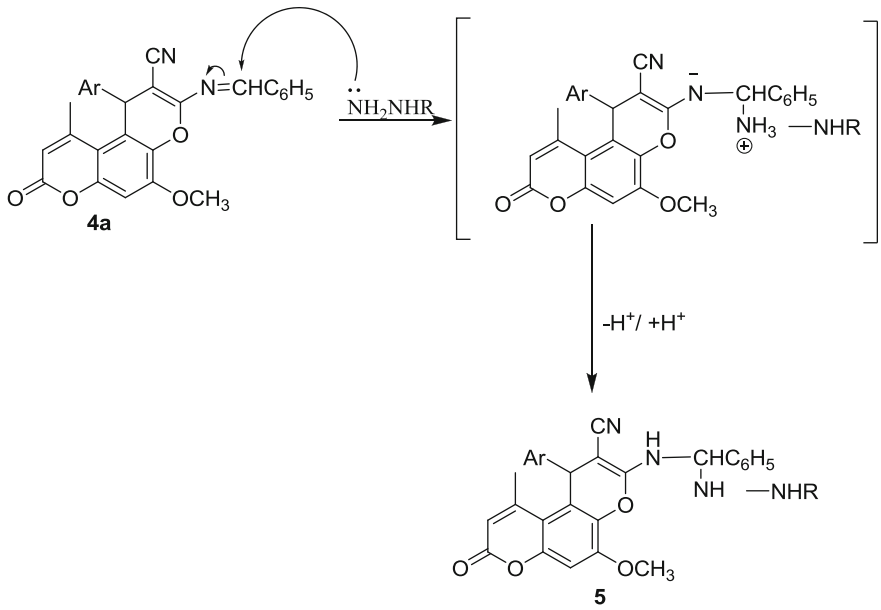
Anti-inflammatory activity

The anti-inflammatory activity was carried out following the method of Domenjoz (1952). Rats were divided into nine different groups each of ten animals. At the beginning, the thickness of the left paw was measured. They were treated orally with the tested compounds, at 40 mg/kg body weight or indomethacin 600 mg/kg as a reference standard. After 1 h of administration, the inflammation was induced by S.C. injection of 0.1 ml of 6 % formalin solution in normal saline. The right hind paw was injected with unequal volume of saline. The difference in thickness between the two paws gave the swelling induced by formalin. The anti-inflammatory efficacy was estimated by comparing the swelling of the treated one with the control. The difference in thickness was recorded after 0.5, 1, 1.5, and 2 h.

Acute toxicity studies (LD_{50})

Preliminary experiments were carried out on six main groups (10 mice/each dose/each group). Compounds **7, 8,** and **9** were injected in different doses to find out the range of doses which cause zero and 100 % mortality of animals. A range of doses was determined for each compound, and LD_{50} was determined. See (Table 2,3,4). The LD_{50} was evaluated by Spearman and Karber method (Finney, 1964)

Scheme 3 Mechanism of formation of compound 5 from 4a



on groups of mice, and the number of animals that died within 24 h was recorded.

The LD_{50} was then calculated by the application of the following formula:

$$LD_{50} = \frac{D_m - \Sigma(Z.d)}{n}$$

where Dm is the dose by which killed all the mice in the group; ZHalf the sum of the dead rats from two2 successive groups; dthe difference between 2 successive doses; Nnumber of animals in each group.

Biochemical studies

Experimental design

This experiment was carried out to examine the effect of anti-inflammatory compounds **7, 8,** and **9** on liver enzymes. A solution of 6 g % for compounds **7, 8,** and **9** in DMSO was prepared for intragastric intubation of male albino rats. Groups of animals each consisting of six rats in each were treated daily for 15 days as follows: group I, control (was given similar volume of saline), group II, Normal (was given similar volume of DMSO); group III, was treated with compound **7**(300 mg/kg. b.w.) dissolved in DMSO orally in a single daily dose; group IV, was treated with compound **8** (300 mg/kg. b.w.) dissolved in DMSO orally in a single daily dose; group V, was treated with compound **9** (300 mg/kg. b.w.) dissolved in DMSO orally in a single daily dose; and group VI, was treated with indomethacin (600 mg/kg. b.w.) dissolved in DMSO orally in a single daily dose (Lavergne *et al.,* 2005).After 10 days of treatment, animals were killed by cervical dislocation, and blood samples were withdrawn from each animal. The separated blood was used for the estimation of AST, ALT, and γ-GT, ALP, LDH, and MDA.

Biochemical parameters

Serum levels of aspartate transaminase (AST), alanine transaminase(ALT) (Reitman and Frankel, 1975), alkaline phosphatase (ALP) (King and Armstrong, 1988), gamma glutamyltransferase γ-GT(Fiala *et al.,* 1972), lactate dehydrogenase(Buhl and Jackson, 1978), and Malondiadhyde as TBARS in serum (Uchiyama and Mihara, 1978). Blood superoxide dismutase (SOD) (Marklund and Marklund, 1974), glutathione peroxidase (GPx) activities (Paglia and Valentine, 1967), reduced GSH levels (Hussein *et al.,* 2001), and blood hemoglobin concentration (Van Kampen and Zijlstra, 1961).

Statistical analysis

All the grouped data were statistically evaluated using SPSS/7.5 software. Hypothesis testing methods included one-way analysis of variance (ANOVA) followed by least significant difference (LSD) test. *P* values of less than 0.05 were considered to indicate statistical significance. All the results were expressed as mean ± SD for 10 animals in each group.

Results and discussion

Chemistry

In continuation of previous studies (Ismail *et al.,* 2008; El-Gaby *et al.,* 2006; Prikazchikova *et al.,* 1975; Brown *et al.,*

Table 1 Anti-inflammatory activity of the biologically active compounds

Formalin induced rat paw oedema thickness (mm)/min					
Compound	Dose (mg/kg)	0.5 h	1 h	1.5 h	2 h
Control		9.66 ± 0.035	9.73 ± 0.06	9.55 ± 0.045	10.00 ± 0.0.08
7	30	6.44 ± 0.035**	6.17 ± 0.054**	6.00 ± 0.11**	5.90 ± 0.026**
8	30	6.11 ± 0.014**	6.00 ± 0.042**	5.98 ± 0.028**	5.98 ± 0.030**
9	30	8.67 ± 0.071*	8.45 ± 0.165*	8.10 ± 0.096*	7.80 ± 0.174**
Indomethacin#	600	6.5 ± 0.03**	7.33 ± 0.07*	7.39 ± 0.071*	6.7 ± 0.057**

Indomethacin is used as a reference

* Significant at $P < 0.05$

** Significant at $P < 0.01$

*** Significant at $P < 0.005$

Table 2 Determination of LD_{50} of compound (**7**) given i.p. in adult mice

Number	Dose (mg/kg)	Animals/ group	Dead animals	(*Z*)	(*d*)	(*Z.d*)
1	80	10	0	0.5	40	20
2	130	10	1	1.5	30	45
3	160	10	2	2.5	50	125
4	200	10	4	6	60	360
5	250	10	7	9	120	1080
6	295	10	10	0	00	00

LD_{50} = 216.91 mg/100 g b.w

Table 3 Determination of LD_{50} of compound (**8**) given i.p. in adult mice

Number	Dose (mg/kg)	Animals/ group	Dead animals	(*Z*)	(*d*)	(*Z.d*)
1	75	10	0	1	40	40
2	110	10	2	2.5	50	125
3	175	10	3	4	75	300
4	250	10	5	7	80	560
5	320	10	8	9	100	900
6	400	10	10	0	00	00

LD_{50} = 217.5 mg/100 g.b.w

Table 4 Determination of LD_{50} of compound (**9**) given i.p. in adult mice

Number	Dose (mg/kg)	Animals/ group	Dead animals	(*Z*)	(*d*)	(*Z.d*)
1	90	10	0	1 .5	20	30
2	140	10	1	2.5	40	100
3	190	10	2	4	60	240
4	240	10	5	7	80	560
5	290	10	8	9	100	900
6	390	10	10	0	00	00

LD_{50} = 207 mg/100 g b.w

1984; Hren *et al.,* 2009) on the synthesis of fused pyrans using enaminonitriles, we report here the synthesis of a variety of new heterocyclic compounds. Thus, condensation of various substituted α-cyanocinnamonitriles (**1a–f**) with the natural product 6-hydroxy-7-methoxy-4-methyl-coumarin **2** isolated from *Ammi majus L.* (Selim and Ouf, 2012) in ethanolic piperidine afforded 1:1 adducts (Ismail *et al.,* 2008; Prikazchikova *et al.,* 1975). Structure **3** (Scheme 1) was established on the basis of the ^{1}H NMR spectra which showed 1-H at ***δ*** 5.27–5.51 ppm (**3a–f**). The increased chemical shift for this signal, compared to the expected value δ 4.0–5.0 ppm for such protons, can be attributed to the deshielding effect of the diamagnetic current of the coumaryl, aryl, and allylic π-electrons. The UV spectrum of new synthesized d **(3a–f)** revealed a weak shoulder (Prikazchikova *et al.,* 1975; Yang *et al.,* 2012), characteristic for 4H-pyran, at λ_{max} (CH_3OH) 275 nm (log ε 2.80–2.86) (**3a–f**), respectively, (Scheme 1).

Interaction of 3-amino-5-methoxy-1-(4-methoxyphenyl)-10-methyl-8-oxo-1,8-dihydro pyrano [**3,2-f**]-chromene-2-carbonitrile (**3c**) with aromatic aldehydes in DMSO–piperidine under reflux gave the corresponding 3-aryl methylene amino new synthesized derivatives (**4a–c**) (Scheme 2). When (E)-3-(benzylideneamino)-5-methoxy-1-(4-methoxyphenyl)-10-methyl-8-oxo-1,8-dihydropyrano[**3,2-f**]-chromene-2-carbonitrile (**4a**) was treated with hydrazine hydrate or phenyl hydrazine in ethanol at room temperature or under reflux, an additional product formed **5**,from which elimination of benzaldehydehydrazone and benzaldehyde-phenylhydrazone, respectively, gave the enaminonitrile **3c** (Prikazchikova *et al.,* 1975; Domenjoz, 1952) instead of the pyrimidine derivative **6**. Treatment of **3c** with acetic anhydride for30 min afforded the new *N*-acetyl derivative **7**, while heating of **3c** with acetic anhydride under reflux for 3 h afforded 2,6-dimethyl-4,8-dioxo-5-

Table 5 Serum levels of AST, ALT, ALP, γ-GT, LDH and MDA in normal and experimental groups of rats

Group	AST U/l	ALT U/l	ALP U/l	γ–GT U/l	LDH U/l	MDA mmol/ml
Normal Saline	9.32 ± 1.82	33 ± 3.5	30.5 ± 2.8	4.5 ± 1.5	220.53 ± 10.17	4.20 ± 1.1
Control (DMSO)	10.8 ± 1.73	37.5 ± 3.7	33.5 ± 2.73	4.82 ± 0.43	237.5 ± 6.5	4.54 ± 0.95
Compound **7** (300 mg/kg.b.)	11.9 ± 1.14	38.4 ± 3.5	32.8 ± 3.6	4.95 ± 1.12	235.65 ± 5.5	4.4 5 ± 0.35
Compound **8** (300 mg/kg.b.)	12.11 ± 1.5	39.5 ± 3.9	35.8 ± 2.90	4.76 ± 0.53	233.2 ± 7.8	4.76 ± 0.42
Compound **9** (300 mg/kg.b.)	10.9 ± 1.1	36.75 ± 3.8	34.6 ± 3.56	4.86 ± 1.2	236.1 ± 7.51	4.32 ± 0.88

Compounds **7**, **8**, **9** and indomethacin were given orally as a single daily dose for 15 days. Control group was compared to normal group. Experimental groups were compared to control group. Values are given as mean ± SD for groups of six animals each

* Significantly different from control group at $p < 0.05$

Table 6 Level of reduced glutathione (GSH) and activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) in blood of normal and experimental groups of rats

Group	GSH (mg %)	SOD(U/g Hb)	GPx (U/g Hb)
Normal (Saline)	65.5 ± 2.30	14.22 ± 2.11	185.5 ± 4.50
Control (DMSO)	61.51 ± 2.50	12.50 ± 1.5	180.4 ± 5.76
Compound **7** (300 mg/kg. b.w)	63.40 ± 2.40	12.4 ± 2.51	179.51 ± 8.50
Compound **8** (300 mg/kg. b.w)	64.12 ± 2.31	12.87 ± 2.65	181.10 ± 5.33
Compound **9** (300 mg/kg. b.w)	63.60 ± 2.2	12.92 ± 1.53	180.32 ± 4.54
Indomethacin (600 mg/kg. b.w)	48.50 ± 3.50*	8.1 ± 2.1*	135.5 ± 9.12*

Compounds **7**, **8**, **9** and indomethacin were given orally as a single daily dose for 15 days. Control group was compared to normal group. Experimental groups were compared to control group. Values are given as mean ± SD for groups of six animals each

* Significantly different from control group at $p < 0.05$

(4-methoxy phenyl)-11-methoxy-5,8-dihydropyrano[3,2-f]-3,4-dihydropyrimidino[4,5-i]-chromene **8**. Structure of new derivative **8** is supported by an independent synthesis of the same product from **3f** and acetonitrile in the presence of HCl gas (23) (Scheme 2). Reaction of **3c** with formic acid 6-methyl-4,8-dioxo-5-(4-methoxy phenyl)-11-methoxy-5,8-dihydropyrano[3,2-f]-3,4-dihydropyrimidino[4,5-i] chromene **9**. The structure of new derivative **9** was supported by an independent synthesis from **3f** and formamide (Scheme 2). Also we suppose the mechanism of how compound **4a** was converted to compound **5** in Scheme 3, which is nucleophilic attack of $RNHNH_2$ to N=C, followed by proton transfer.

Biological activity

Anti-inflammatory activity

Data listed in Table 1 indicate that compounds **7**, **8**, and **9** have promising anti-inflammatory activity compared with the reference anti-inflammatory drug, indomethacin.

Acute toxicity (LD_{50}) studies

The i.p. injection of compound **7** in doses of 80,130, 160, 200, 250, and 295 mg/100 g b.w. resulted in mortalities of 0, 1, 2, 4, 7, and 10 mice, respectively. The dose of compound **7** that killed half of the mice (LD_{50}) was 216.91 mg/100 g b.w. See (Table 2).The results show that i.p. injection of compound **8** in doses of 75,110, 175, 250, 320, and 400 mg/100 g b.w. resulted in mortalities of 0, 2, 3, 5, 8, and 10, respectively. The dose of compound **8** that killed half of the mice (LD_{50}) was 217.5 mg/100 g b.w. (Table 3). The results are given in (Table 4) shows that i.p. injection of compound **9** in doses of 90, 140, 190, 240, 290, and 390 mg/100 g b.w. resulted in mortalities of 0, 1, 2, 5, 8, and 10, respectively. The dose of compound **9** that killed half of the mice (LD_{50}) was 207 mg/100 g b.w.

Biochemical studies

We showed that administration of compounds **7**, **8**, and **9** orally to the rats at dose of 300 mg/kg b.w. for 15 days revealed non-significant differences in liver enzymes AST, ALT, ALP, LDH, γ–GT, and serum MDA compared with the control group. See (Table 5). On the other hand, oral administration of indomethacin showed significant increase of serum AST, ALT, ALP, LDH, γ–GT, and TBARs compared with the control group. Table 6 shows the concentration of GSH, SOD, and GPx in blood of normal and experimental groups of rats. The levels of GSH, SOD, and GPx in treated rats were nonsignificantly changed

compared with the control group, but administration of indomethacin orally at dose of 600 mg/kg.b.w. revealed significant decrease of blood SOD, GPx, and GSH as compared with the control group. The result of the present study indicates that in the present case, a series of Coumarnyl derivatives were synthesized. The results indicate that Compounds **7**, **8**, and **9** exhibited anti-inflammatory activity, high LD_{50} value, and found to be more safe on liver enzymes. Further studies are in progress to investigate the effect of Compounds **7**, **8**, and **9** on different blood parameters to ensure their anti-inflammatory mechanism.

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