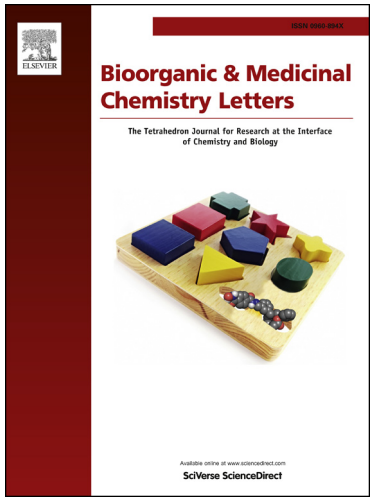

Synthesis and biological evaluation of novel formyl-pyrazoles bearing coumarin moiety as potent antimicrobial and antioxidant agents

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Synthesis and biological evaluation of novel formyl-pyrazoles bearing coumarin moiety as potent antimicrobial and antioxidant agents

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Abstract

A series of coumarin appended formyl-pyrazoles **14-18** were synthesized by a simple and accessible approach. The reaction of 8-acetyl-4-methyl-7-hydroxy coumarin **3** and phenyl hydrazine hydrochlorides **4-8** produces the intermediate compounds 8-acetyl-4-methyl-7-hydroxy coumarin hydrazones **9-13**. The reaction of compounds **9-13** and DMF in the presence of $POCl_3$ yielded formyl-pyrazoles bearing coumarin moiety **14-18** in good yield. The synthesized new compounds **14-18** and the intermediates 8-acetyl-4-methyl-7-hydroxy coumarin hydrazones **9-13** prepared were screened in vitro for their antibacterial, antifungal antioxidant activities. The compounds **12** and **17** having chloro substitution exhibited promising antifungal and antibacterial activity against the different organisms tested. The compound **17** showed remarkable DPPH radical scavenging ability.

Key words: Antibacterial, antifungal, antioxidant, heterocycles, MIC.

Coumarins are chemically known as 2*H*-1-benzopyran-2-ones and were first identified in 1820's as an oxygen heterocycle. Alternariol is chemically a 3,7,9-trihydroxy-1-methyl-6*H*-benzo[*c*]chromen-6-one, a toxic metabolite of *Alternaria* fungi and is an important contaminant in cereals and fruits exhibiting antifungal and phytotoxic activity.[1] Coumarins are widely distributed in plants, for example, umbelliferone (7-hydroxy coumarin) was found in *Apiaceae*, osthole (7-methoxy-8-(3-methylbut-2-en-1-yl)coumarin) was found in *Cnidium monnieri* and

scoparone (6,7-dimethoxy coumarin) was found in *Artemisia scoparia*.[2] In the recent years coumarins have attracted great attention because of their synthetic utility as building blocks for the synthesis of biologically potent molecules. Coumarin derivatives exhibit enormous amount of biological activities such as antioxidant, antimicrobial, anti-HIV, antibiotic, anticancer, muscle relaxant, anti-inflammatory and anti-coagulant activity.[3] Further they are widely used as perfumes, additives in food, chemical, laser dyes, optical brightening agents and cosmetics.

The discovery of antipyretic action of a pyrazole derivative in man by Knorr in 1884 created interest in the researchers. Pyrazoles represent a key motif in heterocyclic chemistry and occupy a prime place in medicinal chemistry due to their competence to exhibit a wide range of pharmacological activities such as antimicrobial,[4] anticancer,[5] anti-inflammatory,[6] anticonvulsant,[7] antipyretic activities.[8] Pyrazoles having a functional group like aldehyde or carboxylate C-4 position have shown promising antimicrobial properties.[9] Pyrazole incorporated with coumarin was synthesized and observed in the enhancement of pharmacological effect.[10] When one biodynamic heterocyclic system was coupled with another heterocyclic system, enhanced biological activity was observed.

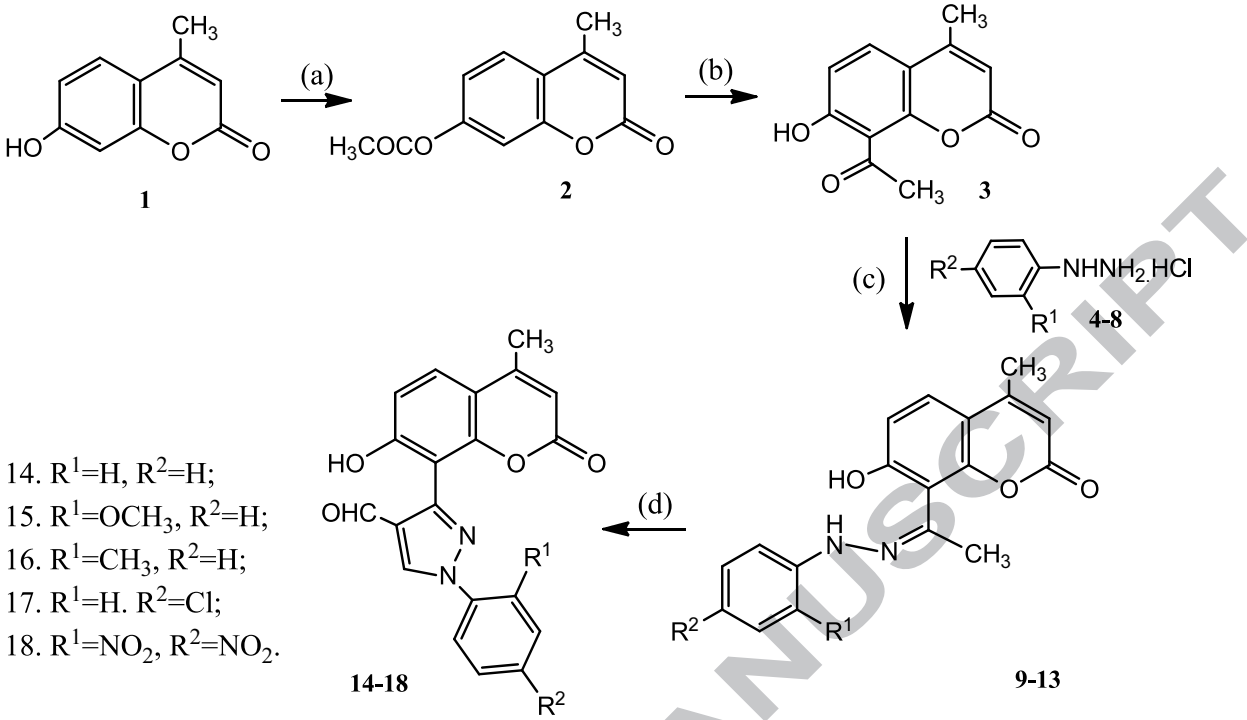
With this in view, this project was undertaken to synthesize a series of new pyrazoles bearing coumarin nucleus. We herein report the synthesis of series of novel heterocyclic scaffolds formyl-pyrazoles bearing coumarin nucleus and in vitro screening of the synthesized compounds for their antibacterial, antifungal and antioxidant activities.

In this work we intended to introduce the pyrazole fragment to the coumarin skeleton in order to build a novel family of bioactive molecules. Thus a series of pyrazole derivatives **14-18** were synthesized starting from 4-methyl-7-hydroxy coumarin **1**. Pechmann reaction has been widely used for the synthesis of coumarins because of its preparative simplicity and inexpensive

starting material.[11] 4-Methyl-7-hydroxy coumarin **1** was prepared by the resorcinol with ethyl acetoacetate in the presence of sulfuric acid as a catalyst.[12]

4-Methyl-7-hydroxy coumarin **1** was converted to an ester **2** with acetic anhydride by a standard procedure; then the ester was subjected to Fries rearrangement at 140-160 ºC to get 8-acetyl-4-methyl-7-hydroxy coumarin **3**, the product characteristics are in good agreement with the reported results.[13] The condensation of the compound **3** with substituted phenylhydrazines **4-8** in ethyl alcohol and a catalytic amount of acetic acid at water bath reflux conditions, produced the corresponding hydrazones **9-13**.[14]

The hydrazones **9-13** (0.0032 mol) were added to the Vilsmeier-Haack reagent prepared by drop-wise addition of $POCl_3$ (1.2 ml) in ice cooled DMF (10 ml). The mixture was stirred at 60-65 ^oC for 6 hours. The progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was poured into ice cold water, neutralized with $NaHCO_3$, the solid separated was filtered, washed with water and recrystallized from ethanol to obtain target molecules 1-aryl-3-(7-hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1*H*-pyrazole-4-carbaldehydes **14-18** in good yield.[15] The reaction pathway is illustrated in Scheme 1.



14. R^1=H, R^2=H;
15. R^1=OCH_3, R^2=H;
16. R^1=CH_3, R^2=H;
17. R^1=H. R^2=Cl;
18. R^1=NO_2, R^2=NO_2.

Scheme 1. Synthesis of compounds **9-13** and **14-18**. Reagents and conditions: (a) Ac_2O; (b) Anh. $AlCl_3$, 140-160 ^oC, reflux, 2h; (c) CH_3COOH, C_2H_5OH; (d) DMF, $POCl_3$, 60-65 ^oC, 5-6 h.

Designed series of molecules **14-18** were characterized by spectral and CHN analysis[15] before being evaluated for *in vitro* antimicrobial and antioxidant activities. For instance, all new compounds showed a sharp and strong absorption band in the region 1660-1671 cm^{-1} in the IR spectrum, which is due to C=O str. of newly formed -CHO functional group. ^1H NMR spectrum of the compounds showed singlet in the region δ 10.70-10.82 ppm. These results supported the presence of -CHO functional group in the products. Furthermore, all compounds showed signals due to aromatic, substituent protons in the expected region. MS results and elemental analysis data confirmed the formation of these compounds.

Minimum inhibitory concentrations (MICs) of the synthesized compounds **9-18** against different bacterial and fungal strains were determined by broth dilution technique. Gram-negative bacteria species *Escherichia coli, Pseudomonas aeruginosa,* Gram-positive bacteria species *Staphylococcus aureus*, *Streptococcus pyogenes* were used as bacterial strains and

Cryptococcus neoformans, Aspergillus nigar, Aspergillus flavus, Candila albicans were used as fungal strains. The antibiotics Ciprofloxacin and Amphotericin B were used as standard drugs against bacteria and fungi species respectively. The experiments were carried out in triplicate; the results were taken as a mean of three determinations. The results of MIC's of the synthesized compounds against bacteria species are summarized in Table 1; and against fungal species in Table 2.

Table 1: MIC's of the test compounds (9-18) against bacteria species

Compound	Minimum inhibitory concentration (MIC's) in µg/mL			
	Staphylococcus aureus	*Streptococcus pyogenes*	*Escherichia coli*	*Pseudomonas aeruginosa*
09	40	-	-	25
10	30	60	50	-
11	40	50	75	100
12	20	50	50	25
13	50	75	-	-
14	-	-	30	50
15	30	50	75	25
16	30	50	40	50
17	15	35	30	15
18	40	75	40	20
Ciprofloxacin	25	50	25	12.5

All the synthesized compounds hydrazones and formyl-pyrazoles exerted a wide range of modest *in vitro* antibacterial activity against all the tested organisms. However, the compound **9** failed to inhibit the growth of *S. pyogenes* and *E. coli* even at a higher concentration of 200 µg/mL. Similarly, compound **10** failed to inhibit *P. aeruginosa*; **13** failed to inhibit *E. coli* and *P. aeruginosa*; and **14** failed to inhibit *S. aureus* and *S. pyogenes* organisms.

The presence of chloro substitution in compounds **12**, **17** influenced these molecules to exhibit inhibition to greater extent against the organisms tested. Nitro substitution present in compounds **13**, **18** retarded the inhibitory effect of these compounds against the organism tested, which may be due to electron withdrawing nature of this functional group. The presence of electron donating groups at ortho position or no substitution resulted with moderate activity.

Table 2: MIC's of the test compounds 9-18 against fungi species

Compound	Minimum inhibitory concentration (MIC) in µg/mL			
	Cryptococcus neoformans	*Aspergillus niger*	*Aspergillus flavus*	*Candila albicans*
09	50	75	75	50
10	40	50	40	30
11	40	75	-	-
12	25	60	50	30
13	**-**	**-**	**-**	**-**
14	50	100	-	-
15	25	50	25	50
16	30	60	-	-
17	15	40	60	25
18	-	-	-	-
Amphotericin B	25	50	50	25

All the synthesized compounds **9-18** exerted a moderate to good *in vitro* antibacterial activity against all the tested organisms, except **13**, **18** which failed to exhibit inhibition against all the organisms. However, the compounds **11**, **14**, **16** failed to inhibit the growth of *A. flavus* and *C. albicans* even at a higher concentration of 200 µg/mL. The compounds **12**, **17** having chloro substitution exhibited remarkable activity against all the organisms tested, while the remaining compounds exhibited moderate activity.

The antioxidant activity of synthesized compounds **14-18** was carried out by DPPH radical scavenging assay using butylated hydroxyl toluene (BHT) as standard antioxidant.[16] The experiments were carried out at five different concentrations in triplicates; the results are expressed as mean ± standard deviation (SD) and were summarised in Table 3.

Table 3: DPPH Radical Scavenging ability of the compounds **14-18** relative to the standard antioxidant BHT

Test Samples	% Radical Scavenging activity*				
	10 (µg/mL)	20 (µg/mL)	30 (µg/mL)	40 (µg/mL)	50 (µg/mL)
14	39.36 ± 0.81	42.12 ± 0.78	46.42 ± 0.85	50.12 ± 0.88	57.76 ± 0.94
15	11.10 ± 0.89	11.98 ± 0.89	13.33 ± 0.92	15.63 ± 0.86	17.36 ± 0.98
16	12.80 ± 0.81	13.81 ± 1.01	14.11 ± 0.68	15.12 ± 0.83	17.10 ± 0.74
17	48.86 ± 0.98	53.33 ± 0.78	58.15 ± 0.78	63.89 ± 0.98	69.35 ± 0.98
18	34.67 ± 0.71	41.93 ± 1.00	47.12 ± 1.00	51.07 ± 0.82	56.02 ± 1.00
Control	at a 0 µg/mL concentration		0.00 ± 0.00		

*Results are expressed as mean ± standard deviation (n=3)

The compounds **14-18** showed promising DPPH free radical scavenging ability, but of lesser activity compared with the standard antioxidant. The results reveal that, all exhibited poor radical scavenging ability at lower concentrations. However, the gradual increase in the activity in all the cases was observed with increase in the concentrations of the test compounds. Among the compounds tested, the compound **17** having chloro substituent in the aromatic ring exhibited promising radical scavenging ability. The compounds **14** and **18** having no and two nitro substituents in the aromatic ring showed moderate radical scavenging abilities; while **15** and **16** having methoxy and methyl substituent respectively in the aromatic ring showed poor radical scavenging abilities in comparison with the standard antioxidant BHT. The presence of electron

donating methoxy and methyl groups; stereo chemical factors due to *ortho* substitution might be the cause for lesser activity associated with the compounds **15** and **16**.

The reactions described represent a simple access to the synthesis of coumarin based pyrazoles derivatives of potential interest from the readily available materials. The antibacterial, antifungal and antioxidant properties of the synthesized compounds show promising activity. However, the structure-activity relationship remains of interest. From the results of biological activity, it is concluded that, these molecules can be designed as potential drugs with a slight modification in the structure of the molecules.

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References

1. (a) Davis, V. M.; Stack, M. E. Appl. Environ. Microbiol. **1994**, 60, 3901. PMC 201908. ; (b) Brugger, E.M.; Wagner, J.; Schumacher, D.M.; Koch, K.; Podlech, J.; Metzler, M.; Lehmann,L. Toxicol. Lett. **2006**, 164, 221. doi:10.1016/j.toxlet.2006.01.001.
2. (a) Raistrick, H.; Stilkings, C. E.; Thomas, R. Biochemistry. **1953**, 55, 421; (b) Abaul, J.; Philogene, E.; Bourgeois, P. J. Nat. Prod. **1994**, 57, 846.
3. Murakami, A.; Gao, G.; Omura, M.; Yano, M.; Ito, C.; Furukawa, H.; Takahashi, D.; Koshimizu, K.; Ohigashi, H. Bioorg. Med. Chem. Lett. **2000**, 10, 59.
4. Gilbert, A. M.; Failli, A.; Shumsky, J.; Yang, Y.; Severin, A.; Singh, G.; Hu, W.; Keeney, D.; Petersen, P. J.; Katz, A. H. J. Med. Chem. **2006**, 49, 6027.
5. Magedov, I. V.; Manpadi, M.; Slambrouck, S. V.; Steelant, W. F. A.; Rozhkova, E.; Przheval'skii, N. M.; Rogelj, S.; Kornienko, A. J. Med. Chem. **2007**, 50, 5183.

6. (a) Szabo, G.; Fischer, J.; Kis-Varga, A.; Gyires, K. J. Med. Chem. **2008**, 51, 142; (b) Benaamane, N.; Nedjar-Kolli, B.; Bentarzi, Y.; Hammal, L.; Geron-ikaki, A.; Eleftheriou, P.; Langunin, A. Bioorg. Med. Chem. **2008**, 16, 3059.

7. Ozdemir, Z.; Kandilici, H. B.; Gumusel, B.; Calis, U.; Bilgin, A. A. Eur. J. Med. Chem. **2007**, 42, 373.

8. Sener, A.; Sener, M. K.; Bildmci, I.; Kasimogullari, R.; Akcamur, Y.; J. Heterocycl. Chem. **2002**, 39, 869.

9. Sridhar, R.; Perumal, P. T.; Etti, S.; Shanmugam, G.; Ponnuswamy, M. N.; Prabavathy, V. R.; Mathivanan, N. Bioorg. Med. Chem. Lett. **2004**, 14, 6035–6040.

10. Omaima, M. A.; Kamelia, M. A.; Rasha, Z. B.; Timothy, J. M.; Somaia, A. N.; Shalini, S. Bioorg. Med. Chem, **2010**, 18, 3378.

11. Sethna, S. M.; Phadke, R. Org. React. **1953**, 7, 1.

12. Bose, D. S.; Rudradas, A. P.; Babu, M. H. Tetrahedron Lett. **2002**, 43, 9195.

13. Valery, F. T. Molecules. **2004**, 9, 50.

14. [Crystallized from ethyl alcohol, **9**: yield 80%, mp 170-172 °C; **10**: yield 78%, mp 98-100 °C; **11**: yield 62%, mp 138-140°C; **12**: yield 74%; **13**: yield 70%, mp 116-118 °C].

15. [3-(7-Hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde **14**: Obtained as light yellow solid in 75% yield, m.p. 220-222 ºC. IR (Nujol): 1660, 1745, 3210 cm^{-1}. H^1 NMR (DMSO-d_6): δ 2.43 (s, 3H), 6.20 (s, 1H), 7.03 (d, 1H), 7.41 (d, 1H), 7.68-8.02 (m, 5H), 9.32 (s, 1H), 9.70 (s, 1H), 10.72 (s, 1H). MS (m/z): 347 (M+1), 319, 271, 250, 177 (base peak). Anal. Calcd. for $C_{20}H_{14}N_2O_4$: C, 69.36; H, 4.07; N, 8.09%; Found: C, 69.36; H, 4.15; N, 8.15%.

3-(7-Hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1-(2-methoxy-phenyl)-1*H*-pyrazole-4-carbaldehyde **15**: Obtained as orange solid in 78% yield; m.p. 108-110 ºC. IR (Nujol): 1671, 1740, 3228 cm^{-1}. H^1 NMR (DMSO-d_6): δ 2.49 (s, 3H), 3.46 (s, 3H), 6.26 (s, 1H), 7.09 (d, 1H), 7.40 (d, 1H), 7.52-7.83 (m, 4H), 9.36 (s, 1H), 9.70 (s, 1H), 10.75 (s, 1H). MS (m/z): 361 (M+1), 333, 271, 255, 177 (base peak). Anal. Calcd. for $C_{21}H_{16}N_2O_5$: C, 67.02; H, 4.28; N, 7.44%; Found: C, 67.08; H, 4.23; N, 7.35%.

3-(7-Hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1-(2-methylphenyl)-1*H*-pyrazole-4-carbaldehyde **16**: Obtained as light orange solid in 64% yield; m.p. 206-208 ºC. IR (Nujol): 1662, 1760, 3218 cm^{-1}. H^1 NMR (DMSO-d_6): δ 2.12 (s, 3H), 2.48 (s, 3H), 6.22 (s, 1H), 7.08 (d, 1H), 7.46 (d, 1H), 7.52-7.66 (m, 4H), 9.38 (s, 1H), 9.79 (s, 1H), 10.80 (s, 1H). Anal. Calcd. for $C_{21}H_{16}N_2O_4$: C, 69.99; H, 4.48; N, 7.77%; Found: C, 69.83; H, 4.28; N, 7.43%.

1-(4-Chloro-phenyl)-3-(7-hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1*H*-pyrazole-4-carbaldehyde **17**: Obtained as light orange solid in 60% yield. IR (Nujol): 1668, 1752, 3220 cm^{-1}. H^1 NMR (DMSO-d_6): δ 2.44 (s, 3H), 6.18 (s, 1H), 7.03 (d, 1H), 7.43 (d, 1H),7.56 (m, 4H), 9.35 (s, 1H), 9.71 (s, 1H), 10.77 (s, 1H). MS (m/z): 382 [M^+, ^{37}Cl], 380 [M^+, ^{35}Cl, 352, 270, 255, 177 (base peak). Anal. Calcd. for $C_{20}H_{13}ClN_2O_4$: C, 63.08; H, 3.44; N, 7.36%; Found: C, 63.03; H, 3.44; N, 7.26%.

1-(2,4-Dinitro-phenyl)-3-(7-hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)-1*H*-pyrazole-4-carbaldehyde **18**: Obtained as brown solid in 71% yield; m.p. 130-132 ºC. IR (Nujol): 1665, 1748, 3215 cm^{-1}. H^1 NMR (DMSO-d_6): δ 2.46 (s, 3H), 6.13 (s, 1H), 7.23 (d, 1H), 7.42 (d, 1H), 7.82 (d, 1H), 8.34 (d, 1H), 8.98 (s, 1H), 9.33 (s, 1H), 9.76 (s, 1H), 10.81 (s, 1H). Anal. Calcd. for $C_{20}H_{12}N_4O_8$: C, 55.05; H, 2.77; N, 12.84%; Found: C, 55.12; H, 2.68; N, 12.96%.]

16. Ajay Kumar, K.; Lokanatha Rai, K. M.; Vasanth Kumar, G.; Mylarappa, B. N. Int. J. Pharm. Pharm. Sci. **2012**, 4 Suppl 4, 564.

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