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Original article

Antioxidant and antibacterial studies of arylazopyrazoles and arylhydrazonopyrazolones containing coumarin moiety

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ABSTRACT

A series of novel 1-(4-methylcoumarinyl-7-oxyacetyl)-3,5-dimethyl-4(arylazo)pyrazoles and 1-(4-methylcoumarinyl-7-oxyacetyl)-3-methyl-4-(substituted phenyl) hydrazono-2-pyrazolin-5-ones were synthesised and evaluated for antibacterial and antioxidant activities. Compounds **3b**, **3g**, **5b**, **5d** and **5g** showed good antibacterial activity and compound **5e** was found to be the most active antioxidant in the series, and thus represent a new class of promising lead compounds.

1. Introduction

The active interest in coumarin compounds can be attributed to their potent gram-positive antibacterial activity and especially the sensitivity of methicillin-resistant strains of *Staphylococcus* to coumarin antibiotics [1]. The coumarin nucleus incorporates the styryl carbonyl moiety into a rigid framework, the presence of which in coumarin is expected to affect the scavenging of reactive substances derived from oxygen and influence the process involving free radical mediated injury [2].

Pyrazoles gained much attention as antimicrobial agents after the discovery of natural pyrazole C-glycoside pyrazofurin which showed broad spectrum antimicrobial activity [3]. Oxidised LDLs play a prominent role in the preliminary stages of atherosclerosis [4]. Literature review reveals the importance of pyrazoles as antioxidants by inhibiting the oxidation of LDL [5].

Pyrazolin-5-one derivative, 3-methyl-1-phenyl-2-pyrazolin-5-one has been reported to be a promising candidate for the treatment of neonatal hypoxic-ischemic encephalopathy [6] and paraquat poisoning [7] due to its free radical scavenging activity. Pyrazolones are associated with broad spectrum of biological activities including antimicrobial activity [8]. Incorporation of hydrazono and azo group has been reported to enhance the biological activity of heterocyclic compounds [9].

Keeping in view the biological importance of arylhydrazonogroup, arylazogroup, pyrazolones, pyrazoles and coumarin nucleus, design of arylazopyrazoles and arylhydrazonopyrazolones containing coumarin moiety was carried out. This was followed by the synthesis of target compounds and their *in vitro* antibacterial and antioxidant screening. The results of antibacterial and antioxidant activities are discussed in this paper.

2. Chemistry

4-Methylcoumarinyl-7-oxyaceticacid hydrazide (**1**) was prepared from 4-methyl-7-hydroxy coumarin according to the literature method [10]. 1,3-Diketo-1,3-dimethyl-2-(arylazo)propane **2a–g** was prepared from substituted phenyl diazonium salts and acetylacetone by Japp–Klingermann reaction [11]. Ethyl-2-(substituted phenyl)hydrazono-3-oxobutyrate **4a–g** was prepared from substituted phenyl diazonium salts and ethylacetoacetate by Japp–Klingermann reaction [9,12]. 4-Methylcoumarinyl-7-oxyaceticacid hydrazide (**1**) when treated with **2a–g** and **4a–g** in glacial acetic acid produced 1-(4-methylcoumarinyl-7-oxyacetyl)-3,5-dimethyl-4 (arylazo)pyrazoles **3a–g** and 1-(4-methylcoumarinyl-7-oxyacetyl)-3-methyl-4-(substituted phenyl) hydrazono-2-pyrazolin-5-ones **5a–g** respectively .The reaction sequences are outlined in Scheme 1.

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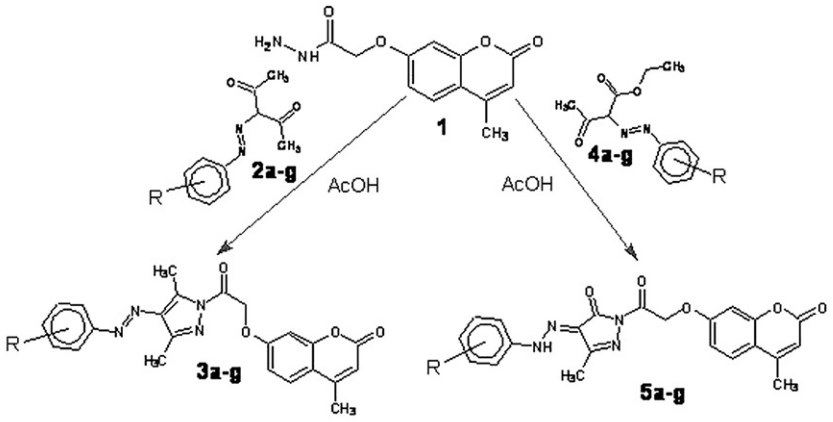

Scheme 1.

3. Pharmacology

3.1. Antibacterial studies

The newly synthesised compounds **3a–g** and **5a–g** were screened for their antibacterial activity against *Staphylococcus aureus* (NCIM 5021) and *Bacillus subtilis* (NCIM 2010) by disc diffusion method [13,14]. A standard inoculum ($1–2 \times 10^7$ c.f.u./ml 0.5 McFarland standards) was introduced on to the surface of sterile agar plates, and a sterile cotton swab was used for even distribution of the inoculum. The discs measuring 6.25 mm in diameter were prepared from Whatman no. 1 filter paper and sterilized by dry heat at 140 °C for 1 h. The sterile discs previously soaked in a known concentration of the test compounds were placed in nutrient agar medium. Solvent and growth controls were kept. The plates were inverted and incubated for 24 h at 37 °C. Ciprofloxacin was used as a standard drug. Inhibition zones were measured and compared with the controls. The bacterial zones of inhibition values are given in Table 1.

The results of antibacterial screening showed compounds **3b**, **5b** and **5g** to be moderatively active against *S. aureus* at 250 mcg/disc concentration. Compounds **3g** and **5b** were found to be moderatively active and **3b**, **5d** and **5g** to be significantly active against *B. subtilis* at 250 mcg/disc concentration. Rest of the compounds did not show antibacterial activity.

3.1.1. Structure–activity relationship

Substitution of bromo group at the para position of phenyl ring imparted moderate gram-positive antibacterial activity to the resulting pyrazole (**3b**) and pyrazolone (**5b**) against *S. aureus*; with increased lipophilicity of the substituent (i.e., dimethyl compounds **3a** and **5a**) this activity was lost. The presence of *o*-carboxy substituent favoured antibacterial activity for resulting pyrazolone (compound **5g**) against *B. subtilis*; an increase in the lipophilicity and polarisability of substituent (i.e., bromo compound **5b**) resulted in drastic decrease in this activity. On the other hand, replacement of *p*-bromosubstituent (compound **3b**) by *o*-carboxy substituent (compound **3g**) displayed a opposite effect on antibacterial activity of resulting pyrazoles against *B. subtilis*. The importance of stereochemistry of substituents in determining the antibacterial activity against *B. subtilis* was evident from the activity exhibited by *o*-carboxyphenyl derivatives (compounds **3g** and **5g**) where as the *p*-carboxyphenyl derivatives (compounds **3f** and **5f**) were devoid of this activity.

3.2. Scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical [2,15]

1.5 ml methanolic solution of the synthesized compounds (0.2 mM) was added to 1.5 ml (0.2 mM) solution of DPPH radical in methanol (final concentration of DPPH and synthesized compounds was 0.1 mM). The mixture was shaken vigorously and allowed to stand for 30 min. After this, the absorbance at 517 nm was determined and the percentage of scavenging activity was calculated using the formula shown below. Ascorbic acid was used as the reference compound. All tests and analyses were undertaken on three replicates and the results were averaged. The results are given in Table 1.

$$\text{Scavengingactivity}(\%) = \{[(\text{Ab} + \text{As}) - \text{Am}]/\text{Ab}\} \times 100\%$$

Ab: absorbance of 0.1 mM methanolic solution of DPPH at 517 nm, As: absorbance of 0.1 mM methanolic solution of test compound at 517 nm, Am: absorbance of methanolic mixture of the drug and DPPH at 517 nm.

Among the compounds from the pyrazole series: **3b** showed moderate antioxidant activity. Among the compounds from the pyrazolin-5-one series, **5a**, **5d** and **5e** exhibited moderate antioxidant activity. The activity exhibited by the compound **5e** was the highest.

3.2.1. Structure–activity relationship

The effect of various substituents on the phenyl ring of pyrazoles (**3a–g**) in producing antioxidant activity in the descending order were found to be: *p*-Br (**3b**) > *m*-NO_2 (**3e**) > *p*-NO_2 (**3c**), *p*-Cl (**3d**), *p*-COOH (**3f**) > *o*-COOH (**3g**) > *o*,*p*-$(CH_3)_2$ (**3a**). Structure activity relationship studies shows that among pyrazoles having ring deactivating substituents, the highest antioxidant activity was obtained with substituent having highest lipophilicity, lowest electron withdrawing power and highest polarisability (i.e., bromo compound **3b**). Among pyrazolones (**5a–g**), substituents exerting antioxidant activity in descending order of potency were found to be: *m*-NO_2 (**5e**) > *o*,*p*-$(CH_3)_2$ (**5a**) > *p*-Cl (**5d**) > *p*-NO_2 (**5c**) > *o*-COOH (**5g**). Among *o*-substituted phenyl derivatives and *o*,*p*-disubstituted phenyl derivatives of pyrazolones, highest antioxidant activity was observed with substituent possessing highest lipophilicity (i.e., dimethyl compound **5a**). Considering the effect of orientation of nitro group in the phenyl ring of pyrazoles and pyrazolones on antioxidant activity, it was observed that compounds

Table 1
Zone of inhibition (mm) and scavenging activity (%) of compounds (**3a–g** and **5a–g**).[a]

Comp.	*Staphylococcus aureus*	*Bacillus subtilis*	Scavenging activity (%)
3a	–	–	24
3b	12.7 ± 0.6	27.0 ± 1.7	55
3c	–	–	32
3d	–	–	32
3e	–	–	38
3f	–	–	32
3g	–	16.7 ± 1.5	28
5a	–	–	57
5b	13.7 ± 0.6	14.0 ± 1.0	–
5c	–	–	31
5d	11.3 ± 1.5	24.3 ± 1.5	53
5e	–	–	69
5f	–	–	–
5g	12.3 ± 0.6	26.7 ± 1.5	20
Std[b]	30.0 ± 1.7	33.7 ± 1.2	–
Std[c]	–	–	96

[a] Data are means of three different experiments.
[b] Ciprofloxacin is used as standard.
[c] Ascorbic acid is used as standard;-Indicated compounds are inactive.

having nitro group in meta orientation (**3e** and **5e**) gave better activity than *o*-isomer (**3c** and **5c**). Among carboxy derivatives of pyrazoles, *p*-isomer (**3f**) gave better antioxidant activity in comparison with *o*-isomer (**3g**), were as with pyrazolones this effect was reversed.

4. Results and discussion

The IR spectrum of compound **3a** showed absorption band at 2927 cm^{-1} due to aliphatic CH stretch. The absorption band for pyrazolyl C=N was observed at 1610 cm^{-1}. The other prominent absorption bands in IR spectrum were observed at 1693 (C=O), 1510 (N=N) and 1287 (C–O–C) cm^{-1}.

The ^{1}H NMR spectrum of **3a** showed a singlet at δ 2.42 corresponding to CH_3 protons of coumarin. The CH_3 protons of 2,4-dimethylphenyl moiety resonated between δ 2.50 and 2.51. The protons of two methyl groups of pyrazole showed ^{1}H NMR signal at δ 3.33 and δ 3.58. The OCH_2 protons resonated as a singlet at δ 4.78. A singlet at δ 6.30 integrating for one proton was attributable to the C_3 proton of coumarin moiety. Aromatic protons resonated as multiplet at δ 6.98–7.74.

Further evidence for the formation of pyrazole (**3a**) was obtained by recording its mass spectrum. The mass spectrum of the compound (**3a**) showed molecular ion peak at *m*/*z* 444 (M^+), in conformity with the molecular formula $C_{25}H_{24}N_4O_4$ .The other fragmentation peaks observed were at *m*/*z* 217 (10%), 189.1 (12%), 137.1 (55%) and 123.2 (100%). The formation of pyrazoles was further supported by recording the ^{13}C NMR spectrum of compound **3a** which showed signal at δ 18.12 for coumarinyl CH_3, δ 38.59–40.25 for methyl carbons attached to pyrazole moiety and aromatic ring, δ 66.16 for OCH_2 carbon, δ 101.62–110.56 corresponding to aromatic carbons, C_3, C_5 to C_{10} of coumarin, δ 113.68 for pyrazole C_4, 126.49 for pyrazole C_5, δ 153.28 for pyrazole C_3, δ 154.45 for coumarin C_4, δ 160.54 for coumarin C_2 and δ 166.16 for >NC=O.

The IR spectrum of compound **5a** showed absorption bands at 3172 cm^{-1} (NH stretch), 3052 cm^{-1} (aromatic CH stretch), 2971 cm^{-1} (aliphatic CH stretch), 1722 cm^{-1} (C=O stretch), 1510 cm^{-1} (NH–N=C) and 1243 cm^{-1} (C–O–C).

The type of protons and number of protons in compound **5a** were ascertained with the help of ^{1}H NMR spectrum which showed a singlet at δ 2.41 corresponding to CH_3 protons of coumarin. The CH_3 protons of 2,4-dimethylphenyl moiety resonated at δ 2.49–2.51. A singlet at δ 3.36 represented methyl protons of pyrazolone ring where as singlet at δ 4.78 and δ 6.25 could be attributed to OCH_2 protons and C_4 proton of coumarin respectively. A multiplet at δ 7.00–7.74 indicated the presence of aromatic protons and singlet at δ 10.32 indicated the presence of NH proton.

Formation of pyrazolin-5-one was confirmed from mass spectrum of compound **5a** which showed molecular ion peak at *m*/*z* 445.3 $(M-1)^+$ in conformity with the molecular formula $C_{24}H_{22}N_4O_5$. The other fragment ions obtained were *m*/*z* 287.2 (40%), 219.2 (84%) and 175.4 (100%).

Further confirmation for pyrazolin-5-one formation was reached from ^{13}C NMR spectrum which showed signals at δ 18.12 for CH_3 of coumarin, δ 38.62–40.29 for methyl carbons attached to pyrazolin-5-one and aromatic ring, δ 66.19 for OCH_2 carbon, δ 101.62 for coumarin C_8, δ 111.46–126.49 for aromatic carbons, C_3, C_5 to C_7, C_9 and C_{10} of coumarin and pyrazolone C_4, δ 153.36 for pyrazolone C_3, δ 154.46 for coumarin C_4, δ 160.03 for coumarin C_2, δ 160.55 for pyrazolone C_5 and δ 166.14 for >N–C=O. The characterisation data of pyrazoles and pyrazolin-5-ones (**3** and **5**) are given in Table 2.

5. Conclusion

The present work comprises the synthesis of seven coumarinyl pyrazoles incorporated with arylazo substituent at C_4 of pyrazoles and seven coumarinyl pyrazolin-5-ones incorporated with arylhydrazono substituent at C_5 of pyrazolone. Among the pyrazoles, compound with 4-bromosubstituent in the phenyl ring exhibited highest antibacterial activity. From pyrazolin-5-ones, compound with 4-bromo substituent in the phenyl ring exhibited highest antibacterial activity against *S. aureus*, where as compound with 2-carboxy substituent in the phenyl ring showed highest antibacterial activity against *B. subtilis*. Compound with 4-bromophenylsubstituent in the phenyl ring exhibited highest antioxidant activity from pyrazole series and pyrazolin-5-one bearing 3-nitro-substituent in the phenyl ring exhibited enhanced antioxidant activity from the respective series.

6. Experiment protocols

6.1. Chemistry

Melting points were determined by open capillary method and are uncorrected. The IR spectra (in KBr pellets) were recorded on a Jasco FT/IR-410 spectrophotometer. ^{13}C NMR and ^{1}H NMR spectra were recorded on a Bruker 300 MHz NMR spectrometer. Mass spectra were recorded on LC-MS/MS (API-4000), MDS SCIEX.

Micro analytical data were obtained from Quest Research and Training Institute, Bangalore 560050, India. The purity of compounds was checked by thin layer chromatography on silica gel plate using mixture of toluene and methanol.

Compounds **1** [10], **2a–g** [11] and **4a–g** [9,12] were prepared according to the literature method.

6.2. Synthesis of 1-(4-methylcoumarinyl-7-oxyacetyl)-3,5-dimethyl-4-(arylazo)pyrazoles (*3a–g*)

A mixture of 1,3-diketo-1,3-dimethyl-2-(arylazo)propane (**2a–g**) (0.001 mol) and 4-methylcoumarinyl-7-oxyacetic acid hydrazide **1** (0.248 g, 0.001 mol) in glacial acetic acid (10 ml) was refluxed for 10 h. The resultant solution was cooled and allowed to stand overnight. The resultant solid thrown out was collected by filtration, purified by repeated washings with acetic acid and recrystallised from acetic acid. The purity of all the compounds was established by

Table 2
Characterisation data of compounds (**3a**–**g** and **5a**–**g**).

Comp.	R	Mol. formula	M.p [°C]	Yield [%]	Analysis. (%) found (calc.)		
					C	H	N
3a	2,4-$(CH_3)_2$	$C_{25}H_{24}N_4O_4$	208–210	54	67.45(67.55)	5.48(5.44)	12.59(12.60)
3b	4-Br	$C_{23}H_{19}BrN_4O_4$	258–260	66	55.82(55.77)	3.85(3.87)	11.26(11.31)
3c	4-NO_2	$C_{23}H_{19}N_5O_6$	218–220	65	59.92(59.87)	4.18(4.15)	15.13(15.18)
3d	4-Cl	$C_{23}H_{19}ClN_4O_4$	266–268	70	61.32(61.27)	4.22(4.25)	12.38(12.43)
3e	3-NO_2	$C_{23}H_{19}N_5O_6$	268–270	62	59.92(59.87)	4.13(4.15)	15.29(15.18)
3f	4-COOH	$C_{24}H_{20}N_4O_6$	242–244	64	62.37(62.60)	4.32(4.38)	12.08(12.17)
3g	2-COOH	$C_{24}H_{20}N_4O_6$	216–218	62	62.37(62.60)	4.47(4.38)	11.99(12.17)
5a	2,4-$(CH_3)_2$	$C_{24}H_{22}N_4O_5$	254–256	48	64.23(64.57)	4.88(4.97)	12.34(12.55)
5b	4-Br	$C_{22}H_{17}BrN_4O_5$	195–197	62	52.94(53.13)	3.39(3.45)	11.18(11.27)
5c	4-NO_2	$C_{22}H_{17}N_5O_7$	190–192	58	56.99(57.02)	3.68(3.70)	15.25(15.11)
5d	4-Cl	$C_{22}H_{17}ClN_4O_5$	248–250	65	57.96(58.35)	3.76(3.78)	7.94(7.83)
5e	3-NO_2	$C_{22}H_{17}N_5O_7$	250–253	55	56.96(57.02)	3.83(3.70)	15.02(15.11)
5f	4-COOH	$C_{23}H_{18}N_4O_7$	185–188	64	59.64(59.74)	3.83(3.92)	12.26(12.12)
5g	2-COOH	$C_{23}H_{18}N_4O_7$	175–177	45	59.68(59.74)	3.95(3.92)	12.01(12.12)

single spot on the TLC plates. The solvent system used was toluene:methanol (3:7).

Compound ***3a***: IR (KBr, ν cm^{-1}): 2927 (CH), 1693 (C═O), 1610 (C═N), 1287 (C–O–C); ^{1}H NMR (DMSO-d_6) δ ppm: 2.42 (s, 3H, coumarin CH_3), 2.50 (s, 3H, aromatic $C_{2'}$–CH_3), 2.51 (s, 3H, aromatic $C_{4'}$–CH_3), 3.33 (s, 3H, pyrazole C_3–CH_3), 3.58 (s, 3H, pyrazole C_5–CH_3), 4.78 (s, 2H, OCH_2), 6.30 (s, 1H, CH), 6.98–7.74 (m, 6H, Ar-H); ^{13}C NMR (DMSO-d_6) δ ppm: 18.12 (coumarin CH_3), 38.59 (CH_3 of pyrazole C_5), 39.42 (CH_3 of pyrazole C_3), 39.70 (CH_3 of aromatic $C_{2'}$), 40.25 (CH_3 of aromatic $C_{4'}$), 66.16 (OCH_2), 101.62 (coumarin C_8), 102.25 (coumarin C_6), 102.87 (coumarin C_3), 104.12 (coumarin C_{10}), 105.37 (aromatic $C_{1'}$), 106.11 (aromatic $C_{5'}$), 107.25 (coumarin C_5), 107.87 (aromatic $C_{6'}$), 108.49 (aromatic $C_{3'}$), 109.12 (aromatic $C_{2'}$), 109.81 (aromatic $C_{4'}$), 110.10 (coumarin C_9), 110.56 (coumarin C_7), 113.68 (pyrazole C_4), 126.49 (pyrazole C_5), 153.28 (pyrazole C_3), 154.45 (coumarin C_4), 160.54 (coumarin C_2), 166.16 (>NC═O); mass (%): M^+ 444.0 (13), 217.0 (10), 189.1 (12), 137.1 (55), 123.2 (100).

Compound ***3b***: IR (KBr, ν cm^{-1}): 2921 (CH), 1720 (C═O), 1621 (C═N), 1282 (C–O–C); ^{1}H NMR (DMSO-d_6) δ ppm: 2.40 (s, 3H, coumarin CH_3), 2.50 (s, 3H, pyrazole C_3–CH_3), 3.30 (s, 3H, pyrazole C_5–CH_3), 4.78 (s, 2H, OCH_2), 6.25 (s, 1H, CH), 6.86–7.74 (m, 7H, Ar-H); ^{13}C NMR (DMSO-d_6) δ ppm: 18.12 (coumarin CH_3), 38.62 (CH_3 at C_5 of pyrazole), 40.29 (CH_3 at C_3 of pyrazole), 66.19 (OCH_2), 101.62 (coumarin C_8), 102.25 (coumarinC_6), 102.87 (coumarin C_3), 104.12 (coumarin C_{10}), 105.37 (aromatic $C_{4'}$), 106.62 (aromatic $C_{1'}$), 107.24 (coumarin C_5), 107.87 (aromatic $C_{2'}$,$C_{6'}$), 109.12 (aromatic $C_{3'}$,$C_{5'}$), 111.47 (coumarin C_9), 112.55 (coumarin C_7), 113.67 (pyrazole C_4), 126.49 (pyrazole C_5), 153.37 (pyrazole C_3), 154.46 (coumarin C_4), 160.55 (coumarin C_2), 166.15 (>NC═O); mass (%): $(MH+2)^+$ 497.6 (15), MH^+ 495.9 (17), 291.1 (86), 189.0 (42), 137.5 (62), 121.2 (100).

Compound ***3c***: IR (KBr, ν cm^{-1}): 2925 (CH), 1672 (C═O), 1596 (C═N), 1262 (C–O–C); ^{1}H NMR (DMSO-d_6) δ ppm: 2.24 (s, 3H, coumarin CH_3), 2.48 (s, 3H, pyrazole C_3–CH_3), 3.44 (s, 3H, pyrazole C_5–CH_3), 4.87 (s, 2H, OCH_2), 6.22 (s, 1H, CH), 6.93–8.36 (m, 7H, Ar-H); mass (%): MH^+ 462.4 (20), 257.2 (90), 175.4 (34), 137.0 (58), 121.4 (100).

Compound ***3d***: IR (KBr, ν cm^{-1}): 2810 (CH), 1681 (C═O), 1604 (C═N), 1286 (C–O–C), 1078 (Ar–Cl); ^{1}H NMR (DMSO-d_6) δ ppm: 2.41 (s, 3H, coumarin CH_3), 2.51 (s, 3H, pyrazole C_3–CH_3), 3.37 (s, 3H, pyrazole C_5–CH_3), 4.67 (s, 2H, OCH_2), 6.25 (s, 1H, CH), 7.01–7.79 (m, 7H, Ar-H); mass (%): $(MH+2)^+$ 453.1 (7), MH^+ 450.9 (22), 246.2 (80), 189.3 (16), 123.2 (100).

Compound ***3e***: IR (KBr, ν cm^{-1}): 2945 (CH), 1598 (C═N), 1287 (C–O–C); ^{1}H NMR (DMSO-d_6) δ ppm: 2.36 (s, 3H, coumarin CH_3), 2.51 (s, 3H, pyrazole C_3–CH_3), 3.37 (s, 3H, pyrazole C_5–CH_3), 4.78 (s, 2H, OCH_2), 6.25 (s, 1H, CH), 6.90–8.43 (m, 7H, Ar-H); mass (%): MH^+ 462.2 (22), 257.6 (75), 175.4 (36), 137.0 (62), 121.2 (100).

Compound ***3f***: IR (KBr, ν cm^{-1}): 3422 (OH), 2817 (CH), 1681 (C═O), 1594 (C═N), 1206 (C–O–C); ^{1}H NMR (DMSO-d_6) δ ppm: 2.12 (s, 3H, coumarin CH_3), 2.25 (s, 3H, pyrazole C_3–CH_3), 3.14 (s, 3H, pyrazole C_5–CH_3), 4.51 (s, 2H, OCH_2), 6.01 (s, 1H, CH), 7.41–7.85 (m, 7H, Ar-H), 13.45 (COOH); mass (%): MH^+ 461.3 (16), 256.3 (72), 219.4 (100), 136.8 (15), 123.2 (20).

Compound ***3g***: IR (KBr, ν cm^{-1}): 3446 (OH), 2922 (CH), 1650 (C═O), 1587 (C═N), 1247 (C–O–C); ^{1}H NMR (DMSO-d_6) δ ppm: 2.27 (s, 3H, coumarin CH_3), 2.51 (s, 3H, pyrazole C_3–CH_3), 3.37 (s, 3H, pyrazole C_5–CH_3), 4.78 (s, 2H, OCH_2), 6.25 (s, 1H, CH), 6.97–8.04 (m, 7H, Ar-H), 10.32 (COOH); mass (%): MH^+ 461.3 (18), 256.3 (65), 219.2 (100), 136.8 (22), 121.8 (26).

*6.3. Synthesis of 1-(4-methylcoumarinyl-7-oxyacetyl)-3-methyl-4-(substituted phenyl) hydrazono-2-pyrazolin-5-one (**5a**–**g**)*

Ethyl-2-(substituted phenyl) hydrazono-3-oxobutyrate (**4a**–**g**) (0.02 mol) was dissolved in glacial acetic acid (20 ml) acid and 4-methylcoumarinyl-7-oxyacetic acid hydrazide **1** (0.496 g, 0.002 mol) in glacial acetic acid (20 ml) was added and the mixture was refluxed for 4 h, cooled and then allowed to stand overnight. The resultant solid was dried and then recrystallised from ethanol .The purity of all the compounds was established by single spot on the TLC plates. The solvent system used was methanol:toluene (7:3).

Compound ***5a***: IR (KBr, ν cm^{-1}): 3172 (NH), 3053 (Ar.CH), 2971 (Aliph.CH), 1722 (C═O), 1510 (NH–N═C), 1243 (C–O–C); ^{1}H NMR (DMSO-d_6) δ ppm: 2.41 (s, 3H, coumarin CH_3), 2.49 (s, 3H, aromatic $C_{2'}$–CH_3), 2.51 (s, 3H, aromatic $C_{4'}$–CH_3), 3.36 (s, 3H, pyrazolone CH_3), 4.78 (s, 2H, OCH_2), 6.25 (s, 1H, CH), 7.00–7.74 (m, 6H, Ar-H), 10.32 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ ppm: 18.12 (coumarin CH_3), 38.90 (pyrazolone CH_3), 39.73 (CH_3 of aromatic $C_{2'}$), 40.29 (CH_3 of aromatic $C_{4'}$), 66.19 (OCH_2), 101.62 (coumarin C_8), 111.46 (coumarin C_6), 112.58 (coumarin C_3), 112.94 (coumarin C_{10}), 113.52 (aromatic $C_{6'}$), 113.59 (aromatic $C_{5'}$), 113.66 (coumarin C_5), 114.45 (aromatic $C_{4'}$), 114.48 (pyrazole C_4), 115.92 (aromatic $C_{2'}$), 116.14 (aromatic $C_{3'}$), 116.97 (aromatic $C_{1'}$), 117.36 (coumarin C_9), 126.49 (coumarin C_7), 153.36 (pyrazolone C_3), 154.46 (coumarin C_4), 160.03 (coumarin C_2), 160.55 (pyrazolone C_5) 166.14 (>NC═O); mass (%): $(M\text{-}1)^+$ 445.3 (15), 287.2 (40), 219.2 (84) and 175.4 (100).

Compound ***5b***: IR (KBr, ν cm^{-1}): 3164 (NH), 3058 (Ar.CH), 2971 (Aliph.CH), 1718 (C═O), 1517 (NH–N═C), 1260 (C–O–C); ^{1}H NMR (DMSO-d_6) δ ppm: 2.41 (s, 3H, coumarin CH_3), 2.51

(s, 3H, pyrazolone CH_3), 4.78 (s, 2H, OCH_2), 6.25 (s, 1H, CH), 7.00–7.73 (m, 7H, Ar-H), 10.32 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ ppm: 18.12 (coumarin CH_3), 38.90 (pyrazolone CH_3), 66.19 (OCH_2), 101.62 (Coumarin C_8), 111.47 (coumarin C_6), 112.55 (coumarin C_3), 112.74 (aromatic $C_{4'}$), 112.92 (coumarin C_{10}), 113.32 (aromatic $C_{2'}$,$C_{6'}$), 113.66 (coumarin C_5), 114.46 (pyrazolone C_4), 115.36 (aromatic $C_{3'}$,$C_{5'}$), 115.96 (aromatic $C_{1'}$), 117.32 (coumarin C_9), 126.48 (coumarin C_7), 153.36 (pyrazolone C_3), 154.46 (coumarin C_4), 160.03 (coumarin C_2), 160.54 (pyrazolone C_5) 166.15 (>NC=O); mass (%): $(MH+2)^+$ 499.8 (13), MH^+ 497.6 (12), 482.2 (40), 465.4 (100), 123.1 (55).

Compound ***5c***: IR (KBr, ν cm^{-1}): 3096 (Ar. CH), 2923 (Aliph. CH), 1459 (NH–N=C), 1283 (C–O–C); ^{1}H NMR (DMSO-d_6) δ ppm: 2.44 (s, 3H, coumarin CH_3), 2.50 (s, 3H, pyrazolone CH_3), 4.78 (s, 2H, OCH_2), 6.25 (s, 1H, CH), 6.96–8.30 (m, 8H, Aromatic and NH protons); mass (%): MH^+ 464.12 (18), 219.4 (78), 175.5 (100), 137.2 (36), 123.4 (45).

Compound ***5e***: IR (KBr, ν cm^{-1}): 3178 (NH), 3055 (Ar.CH), 2968 (Aliph. CH), 1722 (C=O), 1508 (NH–N=C), 1263 (C–O–C), 1348 (Symmetric NO_2 stretch); ^{1}H NMR (DMSO-d_6) δ ppm: 2.41 (s, 3H, coumarin CH_3), 2.49 (s, 3H, pyrazolone CH_3), 4.88 (s, 2H, OCH_2), 6.25 (s, 1H, CH), 6.99–7.74 (m, 7H, Aromatic-H), 10.32 (s, 1H, NH); mass (%): MH^+ 464.4 (24), 219.2 (64), 175.7 (100), 137.4 (42), 122.8 (34).

Compound ***5f***: IR (KBr, ν cm^{-1}): 3310 (OH), 3230 (Ar.CH), 2968 (Aliph. CH), 1725 (C=O), 1253 (C–O–C); ^{1}H NMR (DMSO-d_6) δ ppm: 2.41 (s, 3H, coumarin CH_3), 2.50 (s, 3H, pyrazolone CH_3), 4.78 (s, 2H, OCH_2), 6.25 (s, 1H, CH), 7.0–7.98 (m, 7H, Ar-H), 10.32 (s, 1H, NH), 11.66 (s, 1H, COOH); mass (%): MH^+ 463.2 (16), 219.4 (72), 175.2 (100), 136.2 (42), 123.8 (10).

Compound ***5g***: IR (KBr, ν cm^{-1}): 3422 (OH), 1719 (C=O), 1595 (NH–N=C), 1263 (C–O–C); ^{1}H NMR (DMSO-d_6) δ ppm: 2.41 (s, 3H, coumarin CH_3), 2.50 (s, 3H, pyrazolone CH_3), 4.78 (s, 2H, OCH_2), 6.25 (s, 1H, CH), 6.9–7.96 (m, 7H, Ar-H), 10.32 (s, 1H, NH), 11.44 (s, 1H, COOH); mass (%): MH^+ 463.4 (20), 218.6 (63), 174.9 (100), 136.4 (34), 122.9 (16).

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