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Original article

Synthesis and pharmacological evaluation of a novel series of 5-(substituted) aryl-3-(3-coumarinyl)-1-phenyl-2-pyrazolines as novel anti-inflammatory and analgesic agents

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ABSTRACT

A novel series of 5-(substituted)aryl-3-(3-coumarinyl)-1-phenyl-2-pyrazolines (**3a–l**) were synthesized by reacting various substituted 3-aryl-1-(3-coumarinyl)propan-1-ones (**2a–l**) with phenylhydrazine in the presence of hot pyridine. Structures of all new synthesized compounds were characterized on the basis of elemental analysis and spectral data (IR, ^{1}H NMR and ^{13}C NMR). The title compounds were screened for in vivo anti-inflammatory and analgesic activities at a dose of 200 mg/kg b.w. Among the 12 prepared compounds, Compounds **3d**, **e**, **i** and **j** exhibited significant anti-inflammatory activity in model of acute inflammation such as carrageenan-induced rat edema paw while compounds **3d** and **e** showed considerable activity in model of chronic inflammation such as adjuvant-induced arthritis and were compared with diclofenac (13.5 mg/kg b.w.) as a standard drug. These compounds were also found to have significant analgesic activity in the acetic acid induced writhing model and antipyretic activity in yeast-induced pyrexia model along with minimum ulcerogenic index.

1. Introduction

Cyclooxygenase (COX), the rate limiting enzyme of the prostanoid biosynthetic pathway catalyzes the conversion of arachidonic acid to important inflammatory mediators such as prostaglandins (PGs), prostacyclins and thromboxanes [1]. The existence of enzyme cyclooxygenase in its two distinct isoforms and thus non-selective action of classical non-steroidal anti-inflammatory drugs (NSAIDs) results in certain mechanism based side effects including dyspepsia, gastrointestinal ulcerations, bleeding and nephrotoxicity [2]. Both the isoforms differ in their regulation and expression. The constitutive COX-1 is responsible for the biosynthesis of PGs, which involves the cytoprotection of gastrointestinal tract and platelet aggregation [3]. COX-2 is induced by pro-inflammatory molecules such as interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α), lipopolysaccharide (LPS), carrageenan, etc. that leads to inflammation [4]. COX-2 levels are undetectable in most tissues under normal physiological conditions, but are significantly elevated in acute and chronic inflammations. Inhibition of both isoforms by classical NSAIDs with preferential binding affinity for enzyme COX-1 causes serious side effects.

The association of COX-2 with induced inflammation has led to the hypothesis that selective inhibition of COX-2 over COX-1 might provide good anti-inflammatory agents with reduced side effects than classical NSAIDs. Therefore, selective COX-2 inhibitors (coxibs) with better safety profile have been marketed as a new generation NSAIDs [5,6]. But careful prospective examination of coxibs has revealed unexpected cardiovascular adverse effect [7]. Therefore, development of novel compounds having anti-inflammatory and analgesic activities with an improved safety profile is still a necessity. In addition, inflammation is known not only as a symptom of great deal of common diseases but also as an early phase of some life-threatening diseases such as cancer, heart vascular diseases and Alzheimer's dementia. Thus the discovery of novel anti-inflammatory agents has been attracting a lot of interests.

The synthesis of coumarins and their derivatives has engrossed substantial attention from organic and medicinal chemists for many years as they belong to a class of compounds with proven utility in medicinal chemistry. Coumarin is an important scaffold since several coumarin derivatives are known to be associated with multiple biological activities [8–12] and especially anti-inflammatory/antioxidant activities. Recently the synthesis and in

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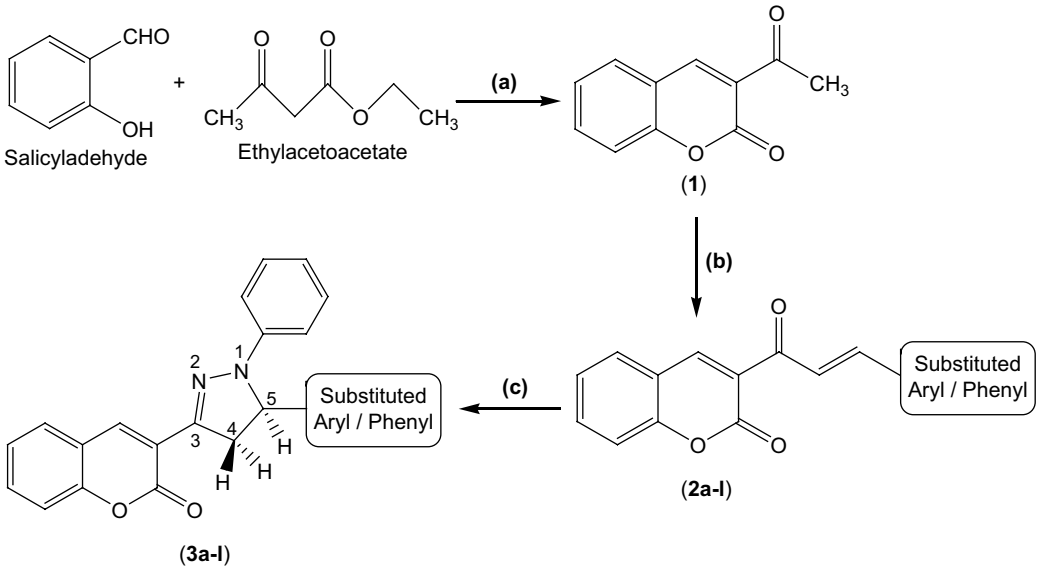


Scheme 1. Synthesis of 5-(substituted)aryl-3-(3-coumarinyl)-1-phenyl-2-pyrazolines (**3a**–**l**). Reagents and conditions: (a) Piperidine, stir, rt, 20 min; (b) Ar-CHO, Piperidine/*n*-butanol, reflux, 4 h; (c) Phenyl hydrazine, Pyridine, reflux, 6 h.

vivo/in vitro anti-inflammatory/antioxidant activities of several new coumarin derivatives with a 7-azomethine linkage have been reported [13].

Pyrazoline is an important nitrogenous five-membered heterocyclic component of the drugs. Literature survey revealed that numerous pyrazoline derivatives have found their clinical application as NSAIDs. Antipyrine, 2,3-dimethyl-1-phenyl-3-pyrazolin-5-one, was the first pyrazolone derivative used in the management of pain and inflammation. Several analogues of pyrazolidin-3,5-diones, pyrazolin-3-ones and pyrazolin-5-ones are also available as NSAIDs; examples are felcobuzone, mefobutazone, morazone, famprofazone, and ramifenazone [14]. Besides these, many pyrazoline derivatives are also reported in literature as having potent anti-inflammatory activity [15–18].

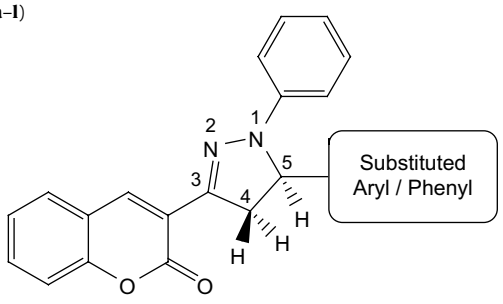
Therefore, both the coumarins and the pyrazolines possess worthy and imperative bioactivities, which render them useful substances in drug research. On this basis, Levai et al. [19] have recently described only the synthesis of 1-substituted-5-aryl-3-(3-coumarinyl)-2-pyrazolines by the reaction of (3-coumarinyl)-chalcones and hydrazines. In view of these observations and in continuation of our research programme on the synthesis of heterocyclic compounds [20–23], we report herein the synthesis of some new 2-pyrazoline derivatives, which have been found to possess an interesting profile of anti-inflammatory, analgesic and antipyretic activities along with significantly less ulcerogenic potential.

2. Chemistry

2-Pyrazolines are the most frequently studied compounds and various methods have been worked out for their synthesis [24]. Since the milestone work of Fischer and Knovenagel published in late 19th century, the reaction of α,β-unsaturated aldehydes and ketones with hydrazines became a generally used, simple and convenient procedure for the synthesis of 2-pyrazolines [25].

Synthetic methods for the preparation of 5-(substituted)aryl-3-(3-coumarinyl)-1-phenyl-2-pyrazoline derivatives (**3a**–**l**) are summarized in Scheme 1. It is apparent from the scheme that the new heterocyclic compounds possess both a coumarinyl moiety and a 2-pyrazoline unit. Reaction of (3-coumarinyl)-chalcones with phenyl hydrazines seemed to be a convenient route to fulfil this aim. Numerous synthetic routes to 3-substituted coumarins from 2-hydroxyarylaldehydes or 2-hydroxyarylketones have been reported including syntheses requiring the use of noxious phosphorylating agents such as $POCl_3$, bases such as piperidine or solvents such as DMF [26,27]. Synthesis of 3-acetyl coumarin (**1**) was carried out by reacting salicylaldehyde with ethylacetoacetate in the presence of catalytic amount of piperidine at room temperature. The reaction is an example of the Knoevenagel reaction [28], in which the active methylene compound reacts with 2-hydroxybenzaldehyde, has

Table 1
Physicochemical data of 5-(substituted)aryl-3-(3-coumarinyl)-1-phenyl-2-pyrazolines (**3a**–**l**)



(**3a-l**)

Compound	Aryl/phenyl	% Yield	Solvent[a]	Melting point[b]	R_f[c]	Molecular formula
3a	C_6H_5	68	Ethanol	180–182	0.48	$C_{24}H_{18}N_2O_2$
3b	4-OMe-C_6H_4	65	Ethanol	166–168	0.53	$C_{25}H_{20}N_2O_3$
3c	–CH=CH–C_6H_5	46	Ethanol	186–188	0.64	$C_{26}H_{20}N_2O_2$
3d	4-Cl-C_6H_4	69	Methanol	150–152	0.48	$C_{24}H_{17}ClN_2O_2$
3e	2,4-$(Cl)_2$-C_6H_3	65	Ethanol	178–180	0.90	$C_{24}H_{16}Cl_2N_2O_2$
3f	4-NMe_2-C_6H_4	55	Ethanol	134–136	0.56	$C_{26}H_{23}N_3O_2$
3g	3-NO_2-C_6H_4	71	Methanol	182–184	0.50	$C_{24}H_{17}N_3O_4$
3h	4-Me-C_6H_4	70	Ethanol	193–194	0.81	$C_{25}H_{20}N_2O_2$
3i	3-OMe-C_6H_4	68	Ethanol	174–176	0.63	$C_{25}H_{20}N_2O_3$
3j	4-F-C_6H_4	56	Methanol	108–110	0.76	$C_{24}H_{17}FN_2O_2$
3k	2-NO_2-C_6H_4	61	Methanol	160–162	0.62	$C_{24}H_{17}N_3O_4$
3l	4-OH-C_6H_4	59	Methanol	150–152	0.72	$C_{24}H_{18}N_2O_3$

[a] Recrystallisation solvent.
[b] Melting point in °C.
[c] Benzene:petroleum ether:methanol 9:1:0.1 as a mobile phase and iodine vapours as visualizing agent.

been extensively used as the first step in the synthesis of 3-acetylcoumarins. 3-Aryl-1-(3-coumarinyl)propan-1-ones (**2a–l**) were synthesized by the reaction of 3-acetyl coumarin and various substituted aromatic aldehydes in the presence of mixture of piperidine and glacial acetic acid. 5-(Substituted)aryl-3-(3-coumarinyl)-1-phenyl-2-pyrazoline compounds (**3a–l**) were obtained in good yields by reacting 3-aryl-1-(3-coumarinyl)propan-1-ones (**2a–l**) with phenylhydrazine in hot pyridine [19].

3. Pharmacological evaluation

The acute toxicity test was carried out according to the Organization for Economic Co-operation and Development (OECD) guidelines [29] to establish the effective dose of all the synthesized compounds. The in vivo acute anti-inflammatory activity for all test compounds was evaluated on male albino rats using carrageenan-induced rat paw edema model by adopting the earlier reported method of Winter et al. [30]. The compounds **3d**, **e**, **i** and **j** were evaluated for their ability to inhibit edema formation in chronic anti-inflammatory model using adjuvant-induced arthritis method by following the method of Newbould [31]. Further, analgesic activity, antipyretic activity and ulcerogenic studies were carried out for compounds **3d**, **e**, **i** and **j** by following the method of Koster et al. [32], Loux et al. [33] and Vogel [34], respectively.

4. Results and discussion

4.1. Synthetic and spectral studies

We have synthesized a series of 5-(substituted)aryl-3-(3-coumarinyl)-1-phenyl-2-pyrazoline derivatives by reacting appropriately substituted 3-aryl-1-(3-coumarinyl)propan-1-ones with phenylhydrazine in hot pyridine as illustrated in Scheme 1. Structure of the synthesized compounds (**3a–l**) was established on the basis of physicochemical, elemental analysis and spectral data (IR, ^{1}H NMR and ^{13}C NMR), which are summarized in Tables 1 and 2, respectively.

Table 2
Spectral and elemental analyses data of 5-(substituted)aryl-3-(3-coumarinyl)-1-phenyl-2-pyrazolines (**3a–l**)

Compound	UV (CH_3OH)	IR (KBr, cm^{-1})	^{1}H NMR ($CDCl_3$/DMSO)	^{13}C NMR ($CDCl_3$/DMSO)	Elemental (CHN) analysis
3a	λ_{max} 294 (ε 26 554)	1729.84 (lactone of coumarin), 1599.06 (C=N), 1533.98 (C=C)	δ 3.42 (dd, 1H, J = 7.1, 17.9 Hz, 4-H$_{trans}$), 4.05 (dd, 1H, J = 12.5, 18.5 Hz, 4-H$_{cis}$), 5.34 (dd, 1H, 5-H of pyrazoline), 6.85–7.70 (m, 14H, Ar-H), 8.44 (s, 1H, 4-H of coumarin)	δ 45.5, 65.1, 113.4, 116.1, 117.8, 120.1, 124.6, 125.3, 127.7, 128.4, 129.1, 131.1, 131.8, 132.5, 137.2, 141.8, 144.1, 154.1, 160.3	Anal. Calcd for $C_{24}H_{18}N_2O_2C$, 78.67; H, 4.95; N, 7.65. Found: C, 78.75; H, 4.90; N, 7.68
3b	λ_{max} 297 (ε 24 500)	1728.01 (lactone of coumarin), 1599.13 (C=N), 1536.14 (C=C)	δ 3.37 (dd, 1H, J = 7.2, 18.3 Hz, 4-H$_{trans}$), 3.82 (s, 3H, OCH_3), 4.09 (dd, 1H, J = 12.4, 18.4 Hz, 4-H$_{cis}$), 5.33 (dd, 1H, 5-H of pyrazoline), 6.9–7.6 (m, 13H, Ar-H), 8.39 (s, 1H, 4-H of coumarin)	δ 45.2, 55.6, 64.1, 113.2, 115.0, 116.7, 119.8, 124.5, 126.6, 127.9, 128.8, 131.3, 134.5, 137.6, 142.9, 144.3, 152.7, 160.6	Anal. Calcd for $C_{25}H_{20}N_2O_3C$, 75.75; H, 5.08; N, 7.07. Found: C, 75.78; H, 5.12; N, 7.11
3c	λ_{max} 257 (ε 31 544)	1723.46 (lactone of coumarin), 1599.89 (C=N), 1495.01 (C=C)	δ 3.34 (dd, 1H, J = 7.0, 17.8 Hz, 4-H$_{trans}$), 4.15 (dd, 1H, J = 12.8, 18.2 Hz, 4-H$_{cis}$), 5.40 (dd, 1H, 5-H of pyrazoline), 6.63–7.86 (m, 16H, Ar-H), 8.48 (s, 1H, 4-H of coumarin)	δ 40.2, 62.7, 111.9, 113.2, 114.2, 117.0, 120.5, 124.5, 126.6, 127.9, 128.8, 131.3, 133.1, 133.9, 138.6, 142.9, 143.6, 151.5, 155.3, 159.6	Anal. Calcd for $C_{26}H_{20}N_2O_2C$, 79.56; H, 5.14; N, 7.15. Found: C, 79.67; H, 5.11; N, 7.19
3d	λ_{max} 294 (ε 31 416)	1727.09 (lactone of coumarin), 1596.14 (C=N), 1566.07 (C=C)	δ 3.32 (dd, 1H, J = 7.4, 18.3 Hz, 4-H$_{trans}$), 4.06 (dd, 1H, J = 12.2, 18.2 Hz, 4-H$_{cis}$), 5.28 (dd, 1H, 5-H of pyrazoline), 6.82–7.60 (m, 13H, Ar-H), 8.42 (s, 1H, 4-H of coumarin)	δ 44.9, 64.6, 113.6, 116.2, 120.0, 120.6, 121.3, 125.4, 127.5, 128.2, 129.1, 129.5, 131.7, 133.3, 138.0, 140.9, 144.4, 152.9, 160.2	Anal. Calcd for $C_{24}H_{17}ClN_2O_2$ C, 71.92; H, 4.25; N, 6.99. Found: C, 71.69; H, 4.22; N, 7.08
3e	λ_{max} 292 (ε 28 327)	1725.13 (lactone of coumarin), 1595.47 (C=N), 1534.59 (C=C)	δ 3.29 (dd, 1H, J = 7.2, 18.6 Hz, 4-H$_{trans}$), 4.19 (dd, J = 12.8, 18.6 Hz, 4-H$_{cis}$), 5.65 (dd, 1H, 5-H of pyrazoline), 6.88–7.61 (m, 12H, Ar-H), 8.40 (s, 1H, 4-H of coumarin)	δ 44.8, 63.9, 113.5, 116.9, 120.7, 121.3, 122.3, 123.8, 125.4, 126.5, 128.2, 129.4, 129.8, 131.7, 133.3, 141.8, 143.4, 151.3, 158.5	Anal. Calcd for $C_{24}H_{16}Cl_2N_2O_2C$, 66.22; H, 3.71; N, 6.44. Found: C, 66.29; H, 3.82; N, 6.38
3f	λ_{max} 340 (ε 30 188)	1721.24 (lactone of coumarin), 1601.51 (C=N), 1493.18 (C=C)	δ 2.73 (s, 6H, N(CH_3)$_2$), 3.43 (dd, 1H, J = 7.3, 18.5 Hz, 4-H$_{trans}$), 4.08 (dd, J = 12.1, 18.7 Hz, 4-H$_{cis}$), 5.55 (dd, 1H, 5-H of pyrazoline), 6.94–7.58 (m, 13H, Ar-H), 8.47 (s, 1H, 4-H of coumarin)	δ 38.5, 43.7, 64.4, 113.7, 114.6, 117.2, 121.3, 122.3, 123.8, 125.4, 126.5, 128.2, 129.1, 129.7, 132.8, 133.4, 142.8, 146.4, 150.9, 160.8	Anal. Calcd for $C_{26}H_{23}N_3O_2C$, 76.26; H, 5.66; N, 10.26. Found: C, 76.19; H, 5.78; N, 10.33
3g	λ_{max} 268 (ε 33 496)	1723.16 (lactone of coumarin), 1600.04 (C=N), 1532.62 (C=C)	δ 3.37 (dd, 1H, J = 7.4, 18.2 Hz, 4-H$_{trans}$), 4.03 (dd, 1H, J = 12.3, 18.1 Hz, 4-H$_{cis}$), 5.41 (dd, 1H, 5-H of pyrazoline), 6.84–7.63 (m, 13H, Ar-H), 8.48 (s, 1H, 4-H of coumarin)	δ 45.1, 61.9, 113.4, 117.0, 118.9, 121.2, 124.3, 126.8, 127.6, 128.3, 132.3, 133.5, 134.7, 139.6, 143.9, 145.6, 151.8, 155.6, 162.3	Anal. Calcd for $C_{24}H_{17}N_3O_4C$, 70.08; H, 4.15; N, 10.22. Found: C, 70.13; H, 4.25; N, 10.28.
3h	λ_{max} 296 (ε 23 499)	1725.71 (lactone of coumarin), 1601.31 (C=N), 1496.34 (C=C)	δ 2.25 (s, 3H, CH_3), 3.33 (dd, 1H, J = 7.0, 18.3 Hz, 4-H$_{trans}$), 4.15 (dd, J = 12.4, 18.8 Hz, 4-H$_{cis}$), 5.48 (dd, 1H, 5-H of pyrazoline), 6.76–7.38 (m, 13H, Ar-H), 8.36 (s, 1H, 4-H of coumarin).	δ 22.4, 46.1, 64.8, 110.2, 113.2, 116.7, 120.1, 124.6, 125.4, 126.5, 127.7, 128.5, 128.9, 129.7, 132.3, 137.1, 138.0, 140.3, 160.1	Anal. Calcd for $C_{25}H_{20}N_2O_2$ C, 78.93; H, 5.28; N, 7.37. Found: C, 78.99; H, 5.25; N, 7.38
3i	λ_{max} 294 (ε 20 968)	1726.54 (lactone of coumarin), 1599.02 (C=N), 1536.03 (C=C)	δ 3.41 (dd, 1H, J = 7.6, 18.2 Hz, 4-H$_{trans}$), 3.81 (s, 3H, OCH_3), 4.13 (dd, 1H, J = 12.8, 18.4 Hz, 4-H$_{cis}$), 5.35 (dd, 1H, 5-H of pyrazoline), 6.85–7.51 (m, 13H, Ar-H), 8.42 (s, 1H, 4-H of coumarin)	δ 45.0, 55.3, 64.7, 110.9, 112.6, 114.1, 116.4, 118.2, 119.8, 120.6, 123.5, 124.5, 125.4, 127.9, 128.9, 130.3, 131.5, 137.6, 142.9, 143.3, 145.1, 154.2, 161.1	Anal. Calcd for $C_{25}H_{20}N_2O_3C$, 75.75; H, 5.08; N, 7.08. Found: C, 75.83; H, 5.14; N, 7.11
3j	λ_{max} 305 (ε 32 474)	1727.93 (lactone of coumarin), 1599.02 (C=N), 1533.99 (C=C)	δ 3.36 (dd, 1H, J = 7.4, 18.6 Hz, 4-H$_{trans}$), 4.08 (dd, 1H, J = 12.6, 18.6 Hz, 4-H$_{cis}$), 5.36 (dd, 1H, 5-H of pyrazoline), 6.72–7.65 (m, 13H, Ar-H), 8.5 (s, 1H, 4-H of coumarin)	δ 45.4, 64.1, 113.9, 115.6, 116.1, 117.4, 119.8, 120.3, 121.0, 121.9, 124.5, 127.7, 128.3, 129.3, 131.5, 137.8, 138.9, 143.5, 154.0, 159.3	Anal. Calcd for $C_{24}H_{17}FN_2O_2C$, 74.99; H, 4.47; N, 7.29. Found: C, 75.05; H, 4.44; N, 7.32
3k	λ_{max} 343 (ε 27 426)	1723.10 (lactone of coumarin), 1599.28 (C=N), 1531.95 (C=C)	δ 3.44 (dd, 1H, J = 7.1, 17.9 Hz, 4-H$_{trans}$), 4.13 (dd, 1H, J = 12.6, 18.6 Hz, 4-H$_{cis}$), 5.47 (dd, 1H, 5-H of pyrazoline), 6.74–7.53 (m, 13H, Ar-H), 8.39 (s, 1H, 4-H of coumarin)	δ 45.1, 62.3, 113.7, 117.2, 119.1, 120.8, 123.7, 126.3, 127.3, 128.8, 132.5, 133.9, 135.1, 142.6, 144.0, 146.1, 151.8, 154.5, 161.8	Anal. Calcd for $C_{24}H_{17}N_3O_4C$, 70.07; H, 4.16; N, 10.21. Found: C, 70.13; H, 4.24; N, 10.15
3l	λ_{max} 278 (ε 27 148)	1727.06 (lactone of coumarin), 1611.54 (C=N), 1501.60 (C=C)	δ 3.35 (dd, 1H, J = 7.8, 18.8 Hz, 4-H$_{trans}$), 4.09 (dd, 1H, J = 12.7, 18.7 Hz, 4-H$_{cis}$), 5.15 (s, 1H, –OH), 5.33 (dd, 1H, 5-H of pyrazoline), 6.66–7.93 (m, 13H, Ar-H), 8.4 (s, 1H, 4-H of coumarin)	δ 45.6, 62.5, 113.5, 117.1, 120.1, 120.9, 123.7, 126.3, 127.8, 128.5, 131.9, 133.7, 135.4, 142.1, 144.8, 145.9, 153.2, 156.2, 159.5, 160.4	Anal. Calcd for $C_{24}H_{18}N_2O_3C$, 75.38; H, 4.74; N, 7.33. Found: C, 75.43; H, 4.74; N, 7.35

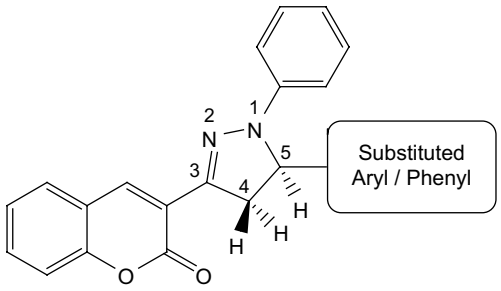
4.2. Acute toxicity study

From the preliminary toxicity studies, it was observed that, all the test compounds have revealed good safety profile till the uppermost dose (2000 mg/kg). No mortality of animals observed even after 24 h but there were few changes in the behavioral response like alertness, touch response and restlessness. Therefore, 1/10th of the maximum tolerated dose i.e. 200 mg/kg b.w. was chosen for the various pharmacological evaluations.

4.3. Acute anti-inflammatory activity

Table 3 reveals the in vivo acute anti-inflammatory activity of a novel series of 5-(substituted)aryl-3-(3-coumarinyl)-1-phenyl-2-pyrazolines (**3a–l**) at a dose of 200 mg/kg in carrageenan-induced paw edema method. Carrageenan-induced edema is a non-specific inflammation resulting from a complex of diverse mediators. Since edema of this type is highly sensitive to NSAIDs, carrageenan has been accepted as a useful agent for studying new anti-inflammatory agents. This model reliably predicts the anti-inflammatory efficacy of the NSAIDs, and during the second phase it detects compounds that are anti-inflammatory agents as a result of inhibition of prostaglandin amplification [35]. As shown in Table 3, the entire investigated compounds exhibited moderate to good anti-inflammatory activity with the percentage inhibition of edema formation ranged from 26.6 to 67.5, while the reference drug diclofenac (13.5 mg/kg) showed 78.7% inhibition at fourth hour. Compound **3d** (39.2 and 64.7%) and **3i** (38.3 and 61.2%) showed good inhibitory activity at second and fourth hour, respectively, while the most active compounds **3e** (56.7 and 67.5%) and **3j** (45.0 and 66.7%) among the series, exhibited the excellent inhibitory activity at second and fourth hour, respectively. The anti-inflammatory activity of these compounds was comparable with that of the standard drug diclofenac, although not equal. Compounds that showed significant anti-inflammatory activity profile were further tested for chronic anti-inflammatory, analgesic, antipyretic and ulcerogenic activities.

Table 3
In vivo acute anti-inflammatory activity of 5-(substituted)aryl-3-(3-coumarinyl)-1-phenyl-2-pyrazolines (**3a–l**) in carrageenan-induced paw edema



(**3a-l**)

Compound	Aryl/phenyl	Anti-inflammatory activity[a]	
		% Inhibition after 2 h (±SEM)	% Inhibition after 4 h (±SEM)
3a	C_6H_5	27.2 (±0.02)	32.7 (±0.01)
3b	4-OMe-C_6H_4	24.8 (±0.005)	36.2 (±0.006)
3c	–CH=CH–C_6H_5	26.7 (±0.006)	42.5 (±0.003)*
3d	4-Cl-C_6H_4	39.2 (±0.026)*	64.7 (±0.021)*
3e	2,4-$(Cl)_2$-C_6H_3	56.7 (±0.030)**	67.5 (±0.024)**
3f	4-NMe_2-C_6H_4	28.4 (±0.040)	40.1 (±0.044)*
3g	3-NO_2-C_6H_4	26.1 (±0.13)	31.2 (±0.006)
3h	4-Me-C_6H_4	30.1 (±0.017)	38.0 (±0.013)
3i	3-OMe-C_6H_4	38.3 (±0.030)*	61.5 (±0.013)**
3j	4-F-C_6H_4	44.9 (±0.023)**	66.7 (±0.011)**
3k	2-NO_2-C_6H_4	24.7 (±0.046)	26.6 (±0.037)
3l	4-OH-C_6H_4	28.8 (±0.035)	34.6 (±0.013)
Diclofenac		63.7 (±0.017)***	78.7 (±0.013)***

The results are expressed as mean ± SEM ($n = 6$). Significance was calculated by using one-way ANOVA with Dunnet's *t*- test. The difference in results was considered significant when $p < 0.05$. *$p < 0.05$ vs control at 200 mg/kg b.w; **$p < 0.01$ vs control at 200 mg/kg b.w; ***$p < 0.001$ vs control at 13.5 mg/kg b.w.

[a] Inhibitory activity in carrageenan-induced rat paw edema.

Table 4
In vivo chronic anti-inflammatory activity of selected compounds in adjuvant-induced arthritis model

Compound	Anti-inflammatory activity[a]		
	Paw edema volume (mean ± SEM)		% Inhibition after treatment (on day 19)
	Day 15[b]	Day 19[c]	
Control	0.92 ± 0.08	0.87 ± 0.02	05.5 ± 0.24
3d	0.87 ± 0.12	0.53 ± 0.07	39.1 ± 0.81*
3e	0.86 ± 0.07	0.47 ± 0.06	45.4 ± 1.63*
3i	0.81 ± 0.05	0.64 ± 0.09	20.9 ± 1.25
3j	0.89 ± 0.04	0.53 ± 0.03	40.5 ± 1.80*
Diclofenac	0.83 ± 0.09	0.39 ± 0.05	53.0 ± 1.92**

The results are expressed as mean ± SEM ($n = 6$). Significance was calculated by using one-way ANOVA with Dunnet's *t*- test. The difference in results was considered significant when $p < 0.05$. *$p < 0.05$ vs control at 200 mg/kg b.w.; **$p < 0.01$ vs control at 13.5 mg/kg b.w.

[a] Inhibitory activity in Freund's adjuvant-induced rat paw edema.

[b] Paw edema volume before treatment.

[c] Paw edema volume after treatment.

4.4. Chronic anti-inflammatory activity

Table 4 explains the in vivo chronic anti-inflammatory activity of compounds **3d**, **e**, **i** and **j** in adjuvant-induced arthritis in rats. Injection of Freund's complete adjuvant to the hind footpad in rats produced arthritis and rats with developed arthritis were chosen for the study. On day 15, there were not many variations in the edema volumes of different groups. Administration of **3d**, **e**, **i** and **j** at a dose of 200 mg/kg and diclofenac (13.5 mg/kg) for four days (from day 16 to day 19) in rats has produced significant reduction in paw volume (by day 19) when compared to the arthritic control group. Compounds **3d** (39.1 ± 0.81), **3e** (45.4 ± 1.63) and **3j** (40.5 ± 1.80) were found to possess a substantial inhibition of edema formation when compared to **3i** (20.9 ± 1.25) while diclofenac (53.0 ± 1.92) exhibited significant anti-inflammatory activity.

4.5. Analgesic activity

Abdominal constriction response induced by acetic acid is a sensitive procedure to establish efficacy of peripherally acting analgesics. The compounds **3d**, **e**, **i** and **j** were tested for analgesic activity at 200 mg/kg b.w. in mice. The results of analgesic activity indicated that all test compounds exhibited moderate to good

Table 5
Analgesic activity of compounds **3d**, **e**, **i** and **j** by acetic acid induced writhing method

Compound	No. of wriths in 15 min (mean ± SEM)	% Protection
Control	66.9 ± 0.21	–
3d	38.2 ± 0.29*	41.0
3e	27.1 ± 0.36**	59.0
3i	43.3 ± 0.45*	35.0
3j	30.5 ± 0.46**	55.0
Acetylsalicylic acid	22.4 ± 0.57***	67.0

The results are expressed as mean ± SEM ($n = 6$). Significance was calculated by using one-way ANOVA with Dunnet's *t*- test. The difference in results was considered significant when $p < 0.05$. *$p < 0.05$ vs control at 200 mg/kg b.w.; **$p < 0.01$ vs control at 200 mg/kg b.w.; ***$p < 0.001$ vs control at 135 mg/kg b.w.

Table 6
Antipyretic activity of compounds **3d**, **e**, **i** and **j** by yeast-induced pyrexia

Serial no.	Compound	Mean temperature in °C at intervals[b]						TI[c]
		0[a]	1	2	3	4	5	
1	Control	39.5	39.4	39.2	38.9	38.7	38.2	−3.1
2	**3d**	39.3	39.1	38.9	38.5	38.1*	38.0	−3.9
3	**3e**	39.2	38.9*	38.6*	38.1**	37.8**	37.2**	−5.4
4	**3i**	39.6	39.4	38.8	38.6	38.2*	37.9	−5.1
5	**3j**	39.4	39.2	38.3**	37.9***	37.7***	37.1**	−6.6
6	*p*-Acetaminophenol	39.0	38.4**	38.0***	37.6***	37.3***	37.0**	−6.7

The results are expressed as mean values ($n = 6$). Significance was calculated by using one-way ANOVA with Dunnet's *t*- test. The difference in results was considered significant when $p < 0.05$. $^{*}p < 0.05$ vs control; $^{**}p < 0.01$ vs control; $^{***}p < 0.001$ vs control.
[a] Eighteenth hour after yeast injection was considered as 0 h.
[b] Temperature was recorded hourly from 0 to 5 h after dosing.
[c] Temperature index (TI) is the sum of mean temperature changes from the 0 h.

analgesic activity (Table 5). Compounds **3e** (59%) and **3j** (55%) have shown nearly the comparable activity to that of reference drug acetylsalicylic acid (67%) in peripheral analgesic activity model.

4.6. Antipyretic activity

Furthermore, the encouraging results from analgesic activity prompted us to carry out the antipyretic activity in yeast-induced pyrexia for compounds **3d**, **e**, **i** and **j** at a dose of 200 mg/kg b.w. in rats. Results of antipyretic activity are given in Table 6, perusal of data suggests that compounds **3e**, **i** and **j** possess reasonable to good antipyretic activity as compared to standard drug *p*-acetaminophenol. However, compound **3d** was found to be less active as antipyretic among the tested compounds.

4.7. Ulcerogenic activity

The major drawback of NSAIDs is their gastric ulcer formation due to gastric irritation. The extent of ulcerogenic effect was evaluated for compounds **3d**, **e**, **i** and **j** in rat stress model at the therapeutic dose (i.e. 200 mg/kg b.w.). The gastric ulcerogenic potential was evaluated by calculating the ulcer index in treated and control animals. Results are given in Table 7 that indicates these four compounds (ulcer index ranges from 1.6 to 2.7) cause less gastric ulceration and disruption of gastric epithelial cells at the above-mentioned oral dose as compared to acetylsalicylic acid (ulcer index 4.3). Hence gastric tolerance to these compounds was better than that of standard drug.

5. Conclusion

In the present paper, we report the synthesis, spectral studies, and pharmacological evaluation of a novel series of 5-(substituted) aryl-3-(3-coumarinyl)-1-phenyl-2-pyrazolines (**3a**–**l**). These biheterocyclic compounds containing both coumarin and pyrazoline ring systems were prepared by the condensation process between 3-aryl-1-(3-coumarinyl)propan-1-ones (**2a**–**l**) and phenylhydrazine in hot pyridine. The examined compounds did not show toxic effects at doses upto 2000 mg/kg b.w. in acute toxicity experiments.

Table 7
Ulcerogenic activity of selected compounds in comparison with acetylsalicylic acid

Compound	Ulcer index (±SEM)
Control	1.33 (±0.28)
3d	2.33 (±0.25)
3e	2.67 (±0.28)*
3i	2.12 (±0.11)
3j	1.66 (±0.14)
Acetylsalicylic acid	4.33 (±0.63)**

The results are expressed as mean ± SEM ($n = 6$). Data analyzed by one-way ANOVA followed by Dunnett's *t*-test. $^{*}p < 0.05$ significant from control; $^{**}p < 0.01$ significant from control.

The preliminary in vivo biological activities of these novel compounds evidenced that the presence of chlorine, fluorine and methoxy groups in the aromatic ring of 5-position of the pyrazoline nucleus gave rise to an increased anti-inflammatory and analgesic activities. Among the 12 prepared compounds, compounds **3d**, **e**, **i** and **j** exhibited significant anti-inflammatory activity in model of acute inflammation such as carrageenan-induced rat edema paw while compounds **3d** and **e** showed considerable activity in model of chronic inflammation such as adjuvant-induced arthritis. These compounds were also found to have significant analgesic activity in the acetic acid induced writhing model and antipyretic activity in yeast-induced pyrexia model. In addition, the tested compounds were also found to possess less degree of ulcerogenic potential as compared to aspirin. Thus these compounds constitute an interesting template for the evaluation of new inflammatory inhibitors and may be helpful for the design of new therapeutic tools against inflammation.

6. Experimental

6.1. Chemistry protocols

All research chemicals were purchased from Sigma–Aldrich (St. Louis, Missouri, USA) or Lancaster Co. (Ward Hill, MA, USA) and used as such for the reactions. Solvents except laboratory reagent grade were dried and purified according to the literature when necessary. Reactions were monitored by thin-layer chromatography (TLC) on pre-coated silica gel plates from E. Merck and Co. (Darmstadt, Germany).

Melting points of synthesized compounds were determined in Thermonik (Mumbai, India) melting point apparatus and are uncorrected. UV spectra were recorded on Thermospectronic (Rochester, NY, USA) and IR spectra were recorded on Thermo Nicolet IR200 FT-IR Spectrometer (Madison, WI, USA) by using KBr pellets. The ^{1}H NMR and ^{13}C NMR were recorded on Bruker AVANCE 300 (Bruker, Rheinstetten/Karlsruhe, Germany) using $CDCl_3$/DMSO-d_6 as solvent. Chemical shifts are reported in δ ppm units with respect to TMS as internal standard. The elemental analysis (C, H, N) of the compounds was performed on Heraus CHN rapid analyzer. Results of elemental analysis were within ±0.4% of the theoretical values. Purity of the compounds was checked on TLC plates using silica gel G as stationary phase and iodine vapors as visualizing agent. The anti-inflammatory activity was carried out using digital plethysmometer (Ugo-Basile, Italy) and antipyretic activity was carried out using Elico telethermometer (Hyderabad, India).

6.2. Synthesis

6.2.1. General procedure for the synthesis of 3-acetyl coumarin (*1*)

To a cold mixture of salicylaldehyde (0.2 M) and ethylacetoacetate (0.2 M), 2 ml of piperidine was added by rapid stirring. After 20 min the yellowish solid separated was filtered off subsequently washed with ethanol and was recrystallised from water:ethanol (3:7), M.P. 120 °C and yield was 83.6%.

6.2.2. General procedure of preparation of 3-aryl-1-(3-coumarinyl)propan-1-ones (*2a–l*)

A mixture of 3-acetyl coumarin (**1**, 0.01 M) and the various substituted aromatic aldehydes (0.012 M) were dissolved in 10 ml of *n*-butanol under heating; then 0.3 ml of glacial acetic acid and the

same quantity of piperidine were added. The reaction mixture was refluxed for 4 h and then the solvent was removed in vacuum. The residue was triturated with 10 ml of ethanol until a precipitate formed. The precipitate was filtered off and crystallized from appropriate solvent.

*6.2.3. General procedure for the synthesis 5-(substituted)aryl-3-(3-coumarinyl)-1-phenyl-2-pyrazoline (**3a–l**)*

3-Aryl-1-(3-coumarinyl)propan-1-ones (**2a–l**, 0.05 M) and phenylhydrazine (0.2 M) were dissolved in pyridine (30 ml) and refluxed for 6 h. Reaction mixture was poured onto the crushed ice and neutralized with 2 N hydrochloric acid. The precipitated solid was filtered, dried and recrystallised from appropriated solvent to afford the title compounds (**3a–l**). The physicochemical, spectral and elemental analysis data of the synthesized compounds are depicted in Tables 1 and 2, respectively.

6.3. Pharmacological evaluation

6.3.1. Animals

Albino mice of either sex weighing 20–25 g were used for acute toxicity studies and analgesic activity. Healthy male albino adult rats weighing 150–230 g were used for various pharmacological screenings. Animals were procured from Venkateshwara Enterprises, Bangalore, India, (245/CPCSEA) and housed individually in polypropylene cages, maintained under standard conditions of alternating 12 h light and dark cycles at a constant temperature (25 ± 2 °C and 35–60% relative humidity). Animals were fed with standard rat pellet diet, (Hindustan Lever Ltd., Mumbai, India) and water ad libitum.

6.3.2. Acute toxicity

The acute toxicity test was carried out according to the Organization for Economic Co-operation and Development (OECD) guidelines to establish the effective dose of test compounds after obtaining ethical clearance from Animal Ethics Committee of KLES College of Pharmacy, Hubli (India). Albino mice of either sex weighing between 20 and 25 g were grouped into 12 groups of six animals each, starved for 24 h with water ad libitum prior to test. On the day of the experiment animals were administered with different compounds to different groups in an increasing dose of 10, 20, 100, 200, 1000 and 2000 mg/kg body weight orally. The animals were then observed continuously for 3 h for general behavioral, neurological, autonomic profiles and then every 30 min for next 3 h and finally for next 24 h or till death.

6.3.3. Acute anti-inflammatory activity

In vivo acute anti-inflammatory activity was evaluated using carrageenan-induced rat paw edema assay model of inflammation by adopting the method of Winter et al. [30] for the compounds listed in Table 3. Male albino rats (170–220 g) were fasted with free access to water at least 12 h prior to experiments and were divided randomly into 14 groups of six each. Control group received 1 ml of 0.5% sodium carboxymethyl cellulose (sodium CMC), standard group received 13.5 mg/kg of diclofenac and test groups received 200 mg/kg of synthesized compounds (**3a–l**). The rats were dosed orally, 1 h later; a subplantar injection of 0.05 ml of 1% solution of carrageenan in sterile distilled water was administered to the left hind footpad of each animal. The paw edema volume was measured with a digital plethysmometer at 0, 2, 4 h after carrageenan injection. Paw edema volume was compared with vehicle control group and percent reduction was calculated as 1−(edema volume in the drug treated group/edema volume in the control group) × 100.

6.3.4. Chronic anti-inflammatory activity

In vivo chronic anti-inflammatory activity was assessed using adjuvant-induced arthritis assay model of inflammation by utilizing the earlier reported method of Newbould [31]. Normally fed male albino rats weighing between 150 and 230 g were used for the experiment. On day 1, 0.1 ml of heat-killed *Mycobacterium tuberculosis* (Freund's adjuvant complete) was injected into the left hind footpad of each rat. The rats were kept in cages for 15 days. On day 15, all animals with 'developed' arthritis were used for the study and were divided into six groups of six rats each for various treatments as shown in Table 4. Control group received 1 ml of 0.5% sodium CMC, standard group received 13.5 mg/kg b.w. of diclofenac. Test groups received 200 mg/kg b.w. of selected compounds **3d**, **e**, **i** and **j**. All compounds were administered orally from day 16 to 19. The hind paw volumes, body weight and degree of secondary lesions were recorded daily from day 16 to 19. The decrease or increase, in mean paw volume per group per day was calculated as a percent inhibition from day 15 onwards.

6.3.5. Analgesic activity

Twenty-four hours prior to actual testing a large number of mice (20–25 g) received intraperitoneally 10 ml/kg 0.6% glacial acetic acid. Animals were observed for writhing movements. Only those showing one or other type of writhing movements (positive responders) were chosen for the test on the next day. On the test day the responders received compounds half an hour prior to glacial acetic acid challenge. Compounds **3d**, **e**, **i** and **j** were orally administered at a dose of 200 mg/kg as a suspension in 0.5% sodium CMC. Standard drug used was acetylsalicyclic acid at a dose of 30 mg/kg as a suspension in 0.5% sodium CMC. Each mouse was then observed for the total number of stretching episodes or writhings for 15 min following glacial acetic acid injection. The mean value for each group was calculated.

6.3.6. Antipyretic activity

Male albino rats weighing between 170 and 230 g were injected subcutaneously with 2.5 ml of 20% aqueous suspension of baker's yeast. Rectal temperature was recorded using telethermometer prior to and at 18 h after yeast injection. Rats developing satisfactory pyrexia (raise in body temperature 1.8–2.0 °C) were divided into six groups of six animals each. Thirty minutes after the eighteenth hour reading, animals of control group received orally 1 ml of 0.5% sodium CMC, standard group received 135 mg/kg of *p*-acetaminophenol and test groups received compounds **3d**, **e**, **i** and **j** at a dose of 200 mg/kg. Temperatures were recorded hourly up to fifth hour after dosing. The mean temperatures after the treatment were compared with those of eighteenth hour and expressed as a temperature index, which was the sum of mean temperature changes.

6.3.7. Ulcerogenic activity

Albino rats of either sex were divided into control, standard and different test groups of six animals each group (170–250 g). They were starved for 48 h (water ad libitum) prior to drug administration. Control group received only 0.5% sodium CMC solution, standard group was orally administered with acetylsalicylic acid in sodium CMC solution and test compounds **3d**, **e**, **i** and **j** were administered orally at the dose of 200 mg/kg b.w. All animals were sacrificed after 7 h of drug administration. Stomach was removed and placed on saline-soaked filter paper until inspection. A longitudinal incision along the greater curvature was made with fine scissors. The stomach was everted over the index finger and the presence or absence of gastric irritation was determined. The ulcer index for each group was determined according to a previously reported method [36] by counting the number of lesions (x) in each of five size classes (y). The classes were defined as $y = 1$ (pinpoint lesion), $y = 2$ (lesions <1 mm diameter), $y = 3$ (lesions 1–2 mm diameter), $y = 4$ (lesions 2–4 mm diameter) and $y = 5$ (lesions > 4 mm diameter). The ulcer index was calculated using $\Sigma 5_{i=1}\, x_i y_i$.

6.4. Statistical analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Dunnett's *t*-test for multiple comparisons of all compounds in various pharmacological assays. Data are expressed as mean ± SEM. The significance of difference was accepted at $p < 0.05$.

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