Bioorganic & Medicinal Chemistry Letters 19 (2009) 255–258

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and antiinflammatory activity of some new 1,3,5-trisubstituted pyrazolines bearing benzene sulfonamide

I. G. Rathish [a], Kalim Javed [a,*], Shamim Ahmad [a], Sameena Bano [a], M. S. Alam [a], K. K. Pillai [b], Surender Singh [c], Vivek Bagchi [d]

[a] Department of Chemistry, Faculty of Science, Jamia Hamdard (Hamdard University), New Delhi 110 062, India
[b] Department of Pharmacology, Faculty of Pharmacy, Jamia Hamdard (Hamdard University), New Delhi 110 062, India
[c] Department of Pharmacology, All India Institute of Medical Sciences, New Delhi 110 029, India
[d] Department of Chemistry, Indian Institute of Technology, New Delhi 110 016, India

ARTICLE INFO

Article history:
Received 27 February 2008
Revised 11 October 2008
Accepted 20 October 2008
Available online 30 October 2008

Keywords:
2-Pyrazolines
Celecoxib
Anti-inflammatory activity
COX-1
COX-2

ABSTRACT

Nineteen new 2-pyrazoline bearing benzenesulfonamide derivatives were synthesized by condensing chalcones with 4-hydrazinonbenzenesulfonamide hydrochloride. Their chemical structures were proved by means of IR, ^{1}H NMR, ^{13}C NMR, mass spectroscopic and elemental analyses data. These compounds were tested at dose of 20 mg/kg for their anti-inflammatory activity in carrageenan-induced rat paw edema model and volume of paw edema was measured at 0, 3 and 5 h. Two compounds **3k** and **3l** were found to be more active than celecoxib throughout the study (at 3 and 5 h). While two other compounds **3m** and **3n** showed more potent activity than celecoxib at 5 h. They are devoid of ulcerogenic potential when administered orally at a dose of 60 mg/kg. Compounds (**3k**–**m**) showed COX-1 and COX-2 inhibitory activity at 0.05 μM.

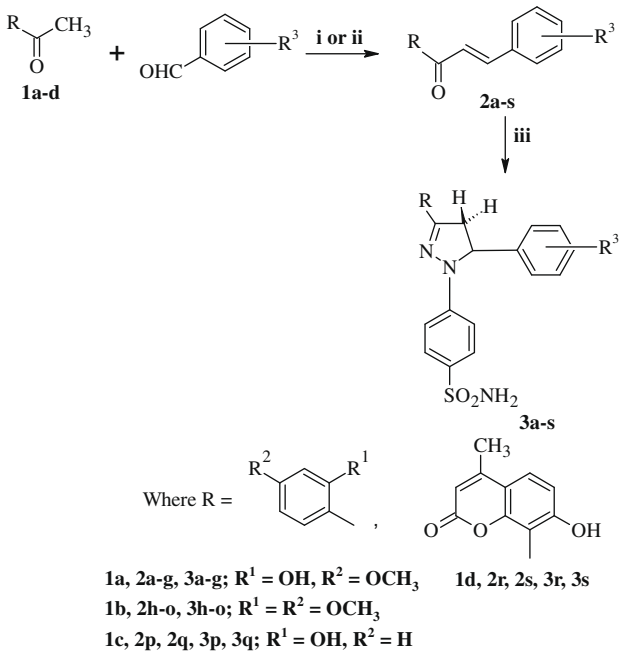
Cyclooxygenases (COXs) are key enzymes in the synthesis of prostaglandin H_2 which is a precursor for the biosynthesis of prostaglandins, thromboxanes, and prostacyclins.[1] COX enzymes exist in two isoforms: cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2).[2] The COX-1 enzyme is constitutively expressed and is critical for protection of gastric mucosa, platelet aggregation, and renal blood flow whereas the COX-2 enzyme is inducible and expressed during inflammation, pain, and oncogenesis.[3] Since COX-2 is involved in inflammation and pain, molecules that inhibit it's enzymatic activity would be of therapeutic value. Many non-steroidal anti-inflammatory drugs (NSAIDs) were found to interact with these enzymes and inhibit their enzymatic activity.[4] These molecules include aspirin and indomethacin which are non-selective and inhibit both COX-1 and COX-2. Aspirin inhibits COX-1 more strongly than COX-2[4] and inhibition of COX-1 by aspirin reduces the production of PGE_2 and PGI_2 which has an adverse ulcerogenic effect.[5] For this reason, in recent years, selective COX-2 inhibitors, that achieve the same anti-inflammatory efficacy as traditional NSAIDs but minimize the risk of unwanted side effects, have been developed. However, clinical studies have suggested that selective COX-2 inhibitors could cause typical COX-mediated side effects such as gastrointestinal injury, increased systemic blood pressure, and hypersensitivity.[6] Furthermore, concerns have been raised about the cardiovascular safety of selective COX-2 inhibitors and some of them, like Vioxx® or Bextra®, have been withdrawn from the market.

In our quest for synthesis of novel NSAID with similar or greater efficacy than other NSAIDs and with little or no gastric side effects, we synthesized a series of 2-pyrazoline-bearing benzene sulfonamide moiety and evaluated their ability to inhibit carrageenan-induced paw edema in rats and COX-1 and COX-2 enzyme in vitro using recombinant enzymes.

The synthetic route used to synthesize title compounds is outlined in Scheme 1. The aromatic ketones (**1a**, **1b**, **1c**, and **1d**) required for the synthesis of chalcones were obtained through reported methods[7,8] or purchased from Loba chemie Pvt. Ltd. The intermediate chalcones **(2a–s)** were prepared by base catalyzed Claisen–Schmidt condensation of the aromatic ketones with different aromatic aldehydes.[8,9] Finally 3,5-diaryl-2-pyrazolines bearing benzene sulfonamide moiety **(3a–s)** were obtained by the condensation of appropriate chalcones and 4-hydrazinobenzenesulfonamide hydrochloride in ethanol. The chemical structures of these compounds were determined on the basis of spectral data analysis; such as IR, ^{1}H NMR, ^{13}C NMR, and Mass spectral data.[10]

* Corresponding author. Tel.: +91 11 26059688.
E-mail addresses: kjavedchem@yahoo.co.in, kjaved@jamiahamdard.ac.in (K. Javed).

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.10.105



Scheme 1. Reagent and conditions: (i) 30% aq NaOH, EtOH, 0–5 °C, 12 h; (ii) piperidine, EtOH, reflux 2–3 h; (iii) 4-hydrazinobenzenesulfonamide hydrochloride, EtOH, reflux 12–18 h.

Anti-inflammatory activity was evaluated using the well-known carrageenan-induced rat paw edema.[11] Overnight fasted Wistar rats (16 h) of either sex weighing 150–175 g were divided into groups of six animals each. Test compounds (20 mg/kg bw) and celecoxib (20 mg/kg) were administered orally. After 30 min, all animals were injected with 0.1 ml of 1% carrageenan solution (prepared in normal saline) in the subplantar aponeurosis of left hind paw and the volume of paw was measured by using plethysmometer at 0, 3, and 5 h post-carrageenan treatment. All pyrazoline derivatives except **3b**, **3c**, and **3f** showed significant activity (Table 1). Two pyrazolines (**3k** and **3l**) were found to be more potent than celecoxcib throughout the study. The other two pyrazolines (**3m** and **3n**) showed more potent anti-inflammatory activity than celecoxcib at 5th hour.

Table 2
In vitro COX-1 and COX-2 enzyme inhibition assay data for test compounds **3k–n**.

Test compounds	% Inhibition (0.05 μm)	
	COX-1	**COX-2**
3k	30.04	38.57
3l	60.90	50.00
3m	73.25	85.71
3n	58.84	42.85

Pyrazoline derivatives (**3k–n**) showing anti-inflammatory activity greater than celecoxib were evaluated for acute gastric ulcerogenic effect in Wistar rats. Six hours after the treatment at a dose of 60 mg/kg bw (three times of the dose) they were sacrificed under deep ether anesthesia and their stomachs were removed and opened through great curvature for examining lesions or bleedings. These compounds did not cause any gastric ulceration.

Compounds (**3k–n**) were tested for their ability to inhibit COX-1 and COX-2. Cyclooxygenase activity of ovine COX-1 and COX-2 was assayed using COX inhibitory screening assay kit (Cayman chemicals) by method of Gierse et al.[12] All assays were conducted in duplicate at a concentration of 0.05 μM of test compounds (**3k–n**). All four compounds inhibit both the COX-1 and COX-2 (Table 2).

In summary four derivatives (**3k–n**) out of novel nineteen synthesized compounds exert potent anti-inflammatory activity that is mainly related to the inhibition of COX-1 and COX-2. They (**3k–n)** did not cause any gastric ulceration and these may possibly be used as lead compounds for developing new anti-inflammatory agents.

Crystallographic data for compound **3k** has been deposited with Cambridge Crystallographic Data Centre as supplementary publication number CCDC 703851. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/data_request/cif.

Table 1
Results of anti-inflammatory effect of 2-pyrazoline derivatives against carrageenan induced rat paw edema model in rats.

Test compounds	Treatment	Increase in paw volume ml ± SEM after carrageenan administration	
		3 h	5 h
	Control	0.75 ± 0.0026	0.75 ± 0.0045
	Celecoxib	0.15 ± 0.0031 (79.5%)	0.45 ± 0.0031 (40%)
R^1 = OH, R^2 = OCH_3, R^3 = 4-OCH_3	**3a**	0.55 ± 0.0023 (27%)	0.47 ± 0.0023 (37.3%)
R^1 = OH, R^2 = OCH_3, R^3 = 3,4-$(OCH_3)_2$	**3b**	0.68 ± 0.0036 (9%)	0.65 ± 0.0040 (13%)
R^1 = OH, R^2 = OCH_3, R^3 = H	**3c**	0.75 ± 0.0022 (0%)	0.75 ± 0.0021 (0%)
R^1 = OH, R^2 = OCH_3, R^3 = 2-Cl	**3d**	0.30 ± 0.0033 (60%)	0.48 ± 0.0036 (35.3%)
R^1 = OH, R^2 = OCH_3, R^3 = 3-OH	**3e**	0.5 ± 0.0023 (33.3%)	0.69 ± 0.0031 (8.3%)
R^1 = OH, R^2 = OCH_3, R^3 = 3,4,5-$(OCH_3)_2$	**3f**	0.75 ± 0.0021 (0%)	0.75 ± 0.0031 (0%)
R^1 = OH, R^2 = OCH_3, R^3 = 4-Cl	**3g**	0.53 ± 0.0040 (28.6%)	0.40 ± 0.0021 (46.3%)
R^1 = R^2 = OCH_3, R^3 = 2-Cl	**3h**	0.57 ± 0.0021 (26.3%)	0.40 ± 0.0034 (46.3%)
R^1 = R^2 = OCH_3, R^3 = 4-Cl	**3i**	0.56 ± 0.0021 (25%)	0.7 ± 0.0040 (6.6%)
R^1 = R^2 = OCH_3, R^3 = 4-OCH_3	**3j**	0.38 ± 0.0021 (49.3%)	0.67 ± 0.0026 (10.6%)
R^1 = R^2 = OCH_3, R^3 = 3,4,5-$(OCH_3)_2$	**3k**	0.13 ± 0.0031 (83.3%)	0.18 ± 0.0026 (76.6%)
R^1 = R^2 = OCH_3, R^3 = 4-N,$N(CH_3)_2$	**3l**	0.15 ± 0.0040 (79.6%)	0.15 ± 0.0021 (79.6%)
R^1 = R^2 = OCH_3, R^3 = H	**3m**	0.23 ± 0.0021 (70%)	0.25 ± 0.0022 (66.5%)
R^1 = R^2 = OCH_3, R^3 = 3-NO_2	**3n**	0.23 ± 0.0026 (68.8%)	0.32 ± 0.0021 (57.6%)
R^1 = R^2 = OCH_3, R^3 = 3-OH	**3o**	0.55 ± 0.0042 (26.6%)	0.71 ± 0.0033 (5.3%)
R^1 = OH, R^2 = H, R^3 = 4-OCH_3	**3p**	0.38 ± 0.0038 (50.0%)	0.44 ± 0.0031 (41.3%)
R^1 = OH, R^2 = H, R^3 = 3,4-$(OCH_3)_2$	**3q**	0.36 ± 0.0021 (52.0%)	0.67 ± 0.0022 (10.6%)
R^3 = 4-OCH_3	**3r**	0.60 ± 0.0026 (20.0%)	0.67 ± 0.0033 (10.6%)
R^3 = 3-OH	**3s**	0.60 ± 0.0026 (20.0%)	0.52 ± 0.0033 (30.6%)

Acknowledgment

Thanks are due to SAIF, CDRI, Lucknow, for providing Mass spectra.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.10.105.

References and notes

1. Hamberg, M.; Samuelsson, B. *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *70*, 899.
2. Fu, J. Y.; Masferrer, J. L.; Seibert, K.; Raz, A.; Needleman, P. *J. Biol. Chem.* **1990**, *265*, 16737.
3. Smith, W. L.; DeWitt, D. L. *Adv. Immunol.* **1996**, *62*, 167; Herschman, H. R. *Biochim. Biophys. Acta* **1996**, *1299*, 125.
4. Meade, E. A.; Smith, W. L.; DeWitt, D. L. *J. Biol. Chem.* **1993**, *268*, 6610.
5. Allison, M. C.; Howatson, A. G.; Torrance, C. J.; Lee, F. D.; Russell, R. I. G. *N. Engl. J. Med.* **1992**, *327*, 749.
6. De Gaetano, G.; Donati, M. B.; Cerletti, C. *Trends Pharmacol. Sci.* **2003**, *24*, 245.
7. Ahluwalia, V. K.; Bhagat, P.; Aggarwal, R.; Chandra, R. *Intermediates in Organic Synthesis*; I.K. International Pvt. Ltd.: New Delhi, Bangalore, Mumbai, 2005. pp. 116–117.
8. Khan, M. S. Y.; Sharma, P. *Indian J. Chem.* **1993**, *32B*, 374.
9. Andotra, C. S.; Khajuria, J.; Singh, G. B.; Singh, S. *J. Indian Chem. Soc.* **1993**, *70*, 266.
10. *Spectral data:* Compound **3a**: Mp 220 °C; 35% yield; IR (υ_{max}, cm^{-1}, KBr): 3351 and 3265 (NH_2), 1596 (C=N), 1339 and 1156 ($SO_2N<$); ^1H NMR (300 MHz, $CDCl_3$, δ, ppm): 3.27 [1H, dd, *J* = 5.9, 17.3 Hz, H-4 *trans* (pyrazoline)], 3.78 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 3.94 [1H, dd, J = 12.0, 17.2 Hz, H-4 *cis* (pyrazoline)], 4.60 (2H, s, SO_2NH_2), 5.25 [1H, dd, J = 5.8, 11.9 Hz, H-5 (pyrazoline)], 6.49 (1H, dd, *J* = 1.8, 8.6 Hz, Ar-H), 6.60 (1H, d, *J* = 1.8 Hz, Ar-H), 6.88 (2H, d, *J* = 8.5 Hz, Ar-H), 6.95 (2H, d, *J* = 8.7 Hz, Ar-H), 7.06 (1H, d, *J* = 1.8 Hz, 8.7 Hz, Ar-H), 7.18 (2H, d, *J* = 8.5 Hz, Ar-H), δ 7.71 (2H, d, *J* = 8.7 Hz, Ar-H), 10.65 (1H, s, phenolic OH); ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 43.83 (CH_2 of pyrazoline), 54.98 (O-CH_3), 55.11 (O-CH_3), 61.21 (CH of pyrazoline), 151.60 (C=N of pyrazoline), 158.66 (Ar C-OH), 159.0 (Ar *C*—O—C), 161.90 (Ar C—O—C); FAB-MS (*m*/*z*): 453 [M^+], 454 [M+1]. *Compound* ***3b****:* **M**p 210 °C; yield 30%; IR (υ_{max}, cm^{-1}, KBr): 3319 and 3253 (NH_2), 1599 (C=N), 1338 and 1155 ($SO_2N<$); ^1H NMR (300 MHz, $CDCl_3$, δ, ppm): 3.28 [1H, dd, *J* = 6.4, 17.4 Hz, H-4 *trans* (pyrazoline)], 3.83 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 3.86 (3H, s, OCH_3), 3.97 [1H, dd, *J* = 12.0, 17.3 Hz, H-4 *cis* (pyrazoline)], 5.22 [1H, dd, *J* = 6.5, 11.9 Hz, H-5 (pyrazoline)], 5.72 (2H, s, SO_2NH_2), 6.50 (1H, dd, *J* = 1.8 Hz, 8.5 Hz, Ar-H), 6.61 (1H, d, *J* = 1.8 Hz, Ar-H), 6.75 (1H, s, Ar-H), 6.84 (2H, s, Ar-H), 6.95 (2H, d, *J* = 8.7 Hz, Ar-H), 7.08 (1H, d, *J* = 8.6 Hz, Ar-H), 7.72 (2H, d, *J* = 8.7 Hz, Ar-H), 10.69 (1H, s, phenolic OH); ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 43.30 (CH_2 of pyrazoline), 54.52 (O—CH_3), 55.03 (O-CH_3), 61.05 (CH of pyrazoline), 147.77 (Ar *C*-O-C), 148.76 (Ar *C*-O-C), 151.16 (C=N of pyrazoline), 158.0 (Ar C-OH), 161.23 (Ar *C*-O-C); **FAB-MS** (**m**/**z**): 483 [M^+], 484 [M+1]. **3c)** m.p. 238 °C; yield 28%; **IR** (υ_{max}, cm^{-1}, **KBr**): 3392 & 3282 (NH_2), 1595 (C=N), 1333 & 1153 ($SO_2N<$); ^1H **NMR** (**300 MHz**, $CDCl_3$, δ, **ppm**): 3.30 [1H, dd, *J* = 5.9, 17.4 Hz, H-4 *trans* (pyrazoline)], 3.84 (3H, s, OCH_3), 4.05 [1H, dd, *J* = 12.0, 17.4 Hz, H-4 *cis* (pyrazoline)], 5.33 [1H, dd, *J* = 5.9, 12.0 Hz, H-5 (pyrazoline)], 6.37 (2H, s, SO_2NH_2), 6.49 (1H, dd, *J* = 1.8 Hz, 8.5 Hz, Ar-H), 6.58 (1H, d, *J* = *J* = 1.8 Hz, Ar-H), 6.92 (2H, d, *J* = 8.6 Hz, Ar-H), 7.10 (1H, d, *J* = 8.6 Hz, Ar-H), 7.27-7.36 (5H, m, Ar-H), 7.71 (2H, d, *J* = 8.6 Hz, Ar-H), 10.66 (1H, s, phenolic OH); ^{13}C **NMR** (**75 MHz**, $CDCl_3$, δ, **ppm**): 44.01 (CH_2 of pyrazoline), 55.16(O-CH_3), 61.06 (CH of pyrazoline), 151.85 (C=N of pyrazoline), 158.08 (Ar C-OH), 161.64 (Ar *C*-O-C); **FAB-MS** (**m**/**z**): 423 [M^+], 424 [M+1]. **3d)** m.p. 220 °C; yield 25%; **IR** (υ_{max}, cm^{-1}, **KBr**): 3386 & 3284 (NH_2), 1597 (C=N), 1332 & 1149 ($SO_2N<$); ^1H **NMR** (**300 MHz**, ***DMSO-d*$_6$**, δ, **ppm**): 3.22 [1H, dd, *J* = 5.2, 17.0 Hz, H-4 *trans* (pyrazoline)], 3.84 (3H, s, OCH_3), 4.12 [1H, dd, *J* = 13.0, 17.4 Hz, H-4 *cis* (pyrazoline)], 5.66 [1H, dd, *J* = 5.5, 12.3 Hz, H-5 (pyrazoline)], 6.36 (2H, s, SO_2NH_2), 6.49 (1H, dd, *J* = 1.8 Hz, *J* = 8.6 Hz, Ar-H), 6.58 (1H, s, Ar-H), 6.86 (2H, d, *J* = 7.8 Hz, Ar-H), 7.10–7.49 (5H, m, Ar-H), 7.74 (2H, d, *J* = 7.7 Hz, Ar-H), 10.61 (1H, s, phenolic OH); ^{13}C **NMR** (**75 MHz**, ***DMSO-d*$_6$**, δ, **ppm**): 43.00 (CH_2 of pyrazoline), 55.39 (O-CH_3), 58.82 (CH of pyrazoline), 152.06 (C=N of pyrazoline), 158.01 (Ar C-OH), 161.80 (Ar *C*-O-C); **FAB-MS** (**m**/**z**): 457 [M^+], 459 [M+2], 461[M+4], 425, 391. **3e)** m.p. 236 °C; yield 60%; **IR** (υ_{max}, cm^{-1}, **KBr**): 3302 & 3196 (NH_2), 1596 (C=N), 1342 & 1154 ($SO_2N<$); ^1H **NMR** (**300 MHz**, ***DMSO-d*$_6$**, δ, **ppm**): 3.78 (3H, s, OCH_3), 4.02 [1H, dd, *J* = 12.1, 17.7 Hz, H-4 *cis* (pyrazoline)], 5.48 [1H, dd, *J* = 4.8, 11.9 Hz, H-5 (pyrazoline)], 6.52–7.62 (13 H, m, Ar-H and SO_2NH_2), 9.45 (1 H, brs, phenolic OH), 10.48 (1 H, brs, phenolic OH); ^{13}C **NMR** (**75 MHz**, ***DMSO-d*$_6$**, δ, **ppm**): 44.09 (CH_2 of pyrazoline), 55.36 (O-CH_3), 61.0 (CH of pyrazoline), 152.07 (C=N of pyrazoline), 158.0 (2 x Ar C-OH), 161.73 (Ar *C*-O-C); **FAB-MS** (**m**/**z**): 441 [M+2], 439 [M^+], 427, 391, 359, 346. **3f)** m.p. 194 °C; yield 27%; **IR** (υ_{max}, cm^{-1}, **KBr**): 1594 (C=N), 1334 & 1159 ($SO_2N<$); ^1H **NMR** (**300 MHz**, $CDCl_3$, δ, **ppm**): 3.29 [1H, dd, *J* = 7.0, 17.6 Hz, H-4 *trans* (pyrazoline)], 3.75 (9H, s, 3xOCH_3), 3.85 (3H, s, OCH_3), 4.01 [1H, dd, *J* = 12.0, 17.7 Hz, H-4 *cis* (pyrazoline)], 5.20 [1H, dd, *J* = 7.1, 11.9 Hz, H-5 (pyrazoline)], 6.39 (2H, s, SO_2NH_2), 6.50 (3H, m, Ar-H), 6.58 (1H, d, *J* = 2.4 Hz, Ar-H), 6.95 (2H, d, *J* = 8.8 Hz, Ar-H), 7.10 (1H, d, *J* = 8.6 Hz, Ar-H), 7.24 (2H, d, *J* = 8.7 Hz, Ar-H), 10.68 (1H, s, phenolic OH); ^{13}C **NMR** (**75 MHz**, $CDCl_3$,δ, **ppm**): 43.75 (CH_2 of pyrazoline), 54.94 (O-CH_3), 55.66 (2 x O-CH_3), 60.20 (O-CH_3), 62.08 (CH of pyrazoline), 136.3 (Ar C-O-C), 151.6 (C=N of pyrazoline), 153.45 (2 x Ar *C*-O-C), 158.48 (Ar C-OH), 161.75 (Ar *C*-O-C); **FAB-MS** (**m**/**z**): 513 [M^+], 514 [M+1]. **3 g)** m.p. 216 °C; yield 15%; **IR** (υ_{max}, cm^{-1}, **KBr**): 3392 (OH), 3321 & 3286 (NH_2), 1595 (C=N), 1335 & 1156 ($SO_2N<$); ^1H **NMR** (**300 MHz**, ***DMSO-d*$_6$**, δ, **ppm**): 3.78 (3H, s, OCH_3), 4.05 [1H, m, H-4 *cis* (pyrazoline)], 5.59 [1H, m, H-5 (pyrazoline)], 6.56 (2H, s, SO_2NH_2), 6.96–7.62 (11 H, m, Ar-H); ^{13}C **NMR** (**75 MHz**, ***DMSO-d*$_6$**, δ, **ppm**): 44.05 (CH_2 of pyrazoline), 55.40 (O-CH_3), 60.41 (CH of pyrazoline), 152.0 (C=N of pyrazoline), 158.04 (Ar C-OH), 161.79 (Ar C-O-C); **FAB-MS** (**m**/**z**): 457 [M^+], 459, 391. **3 h)** m.p. 216 °C; yield 42%; **IR** (υ_{max}, cm^{-1}, **KBr**): 3379 & 3254 (NH_2), 1599 (C=N), 1338 & 1147 ($SO_2N<$); ^1H **NMR** (**300 MHz**, ***DMSO-d*$_6$**, δ, **ppm**): 3.15 [1H, dd, J = 5.3, 18.4 Hz, H-4 *trans* (pyrazoline)], 3.78 (3H, s, OCH_3), 3.79 (3H, s, OCH_3), 4.05 [1H, dd, J = 12.1, 18.3 Hz, H-4 *cis* (pyrazoline)], 5.63 [1H, dd, J = 5.2, 12.0 Hz, H-5 (pyrazoline)], 6.53 (2H, m, Ar-H), 6.89 (4H, m, SO_2NH_2 and Ar-H), 7.01 (1H, d, J = 6.65 Hz, Ar-H), 7.21 (2H, m, Ar-H), 7.45 (1H, d, J = 7.51 Hz, Ar-H) 7.59 (2H, d, J = 8.7 Hz, Ar-H), 7.87 (1H, d, J = 8.6 Hz, Ar-H); ^{13}C **NMR** (**75 MHz**, ***DMSO-d*$_6$**, δ, **ppm**): 44.89 (CH_2 of pyrazoline), 55.43 (O-CH_3), 55.74 (O-CH_3), 59.58 (CH of pyrazoline), 149.06 (C=N of pyrazoline), 158.87 (Ar C-O-C), 161.89 (Ar C-O-C); **FAB-MS** (**m**/**z**): 471 [M^+], 473 [M+2], 460, 455, 443, 428. **3i)** m.p. 110 °C, yield 26%; **IR** (υ_{max}, cm^{-1}, **KBr**): 3381 & 3262 (NH_2), 1592 (C=N), 1332 & 1156 ($SO_2N<$); ^1H **NMR** (**300 MHz**, $CDCl_3$, δ, **ppm**): 3.29 [1H, dd, J = 5.8, 18.2 Hz, H-4 *trans* (pyrazoline)], 3.79 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 4.00 [1H, dd, J = 12.1, 18.2 Hz, H-4 *cis* (pyrazoline)], 4.61 (2H, s, SO_2NH_2), 5.22 [1H, dd, J = 5.8, 12.0 Hz, H-5 (pyrazoline)], 6.46 (1H, d, J = 2.0 Hz, Ar-H), 6.57 (1H, dd, J = 2.0 Hz, 8.7 Hz, Ar-H), 7.0 (2H, d, J = 8.8 Hz, Ar-H), 7.19 (2H, d, J = 8.3 Hz, Ar-H), 7.31 (2H, d, J = 8.3 Hz, Ar-H), 7.68 (2H, d, J = 8.8 Hz, Ar-H), 7.95 (1H, d, J = 8.6 Hz, Ar-H); ^{13}C **NMR** (**75 MHz**, $CDCl_3$, δ, **ppm**): 46.75 (CH_2 of pyrazoline), 55.42 (2 x O-CH_3), 62.68 (CH of pyrazoline), 149.50 (C=N of pyrazoline), 159.02 (Ar *C*-O-C), 162.19 (Ar *C*-O-C); FAB-MS (m/z): 471 [M^+], 475 [M+4], 473 [M+2], 470 [M-1], 457 and 441. **3j**) m.p. 122 °C; yield 20%; IR (υ_{max}, cm^{-1}, **KBr**): 3367 & 3272 (NH_2), 1596 (C=N), 1336 & 1156 ($SO_2N<$); ^1H **NMR** (**300 MHz**, $CDCl_3$,δ, **ppm**): 3.31 [1H, dd, J = 5.8, 18.1 Hz, H-4 *trans* (pyrazoline)], 3.77 (3H, s, OCH_3), 3.79 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 3.97 [1H, dd, J = 12.0, 18.2 Hz, H-4 *cis* (pyrazoline)], 4.61 (2H, s, SO_2NH_2), 5.20 [1H, dd, J = 5.8, 11.9 Hz, H-5 (pyrazoline)], 6.45 (1H, s, Ar-H), 6.56 (1H, d, J = 7.8 Hz, Ar-H), 6.85 (2H, d, J = 8.6 Hz, Ar-H), 7.04 (2H, d, J = 8.4 Hz, Ar-H), 7.17 (2H, d, J = 8.1 Hz, Ar-H), 7.65 (2H, d, J = 8.4 Hz, Ar-H), 7.95 (1H, d, J = 8.3 Hz, Ar-H); ^{13}C **NMR** (**75 MHz**, $CDCl_3$, δ): 46.94 (CH_2 of pyrazoline), 55.40 (3 x O-CH_3), 62.85 (CH of pyrazoline), 149.60 (C=N of pyrazoline), 159.04 (Ar *C*-O-C), 162.07 (Ar *C*-O-C), **FAB-MS** (**m**/**z**): 467[M^+], 469 [M+2], 451, 436, 387, 360. **3k**) m.p. 160 °C; yield 47%; **IR** (υ_{max}, cm^{-1}, **KBr**): 3299 & 3086 (NH_2), 1592 (C=N), 1321 & 1143 ($SO_2N<$); ^1H **NMR** (**300 MHz**, ***DMSO-d*$_6$**, δ, **ppm**): 3.17 [1H, m, H-4 *trans* (pyrazoline)], 3.79 (3H, s, OCH_3), 3.81 (3H, s, OCH_3), 3.98 [1H, m, H-4 *cis* (pyrazoline)], 5.40 [1H, m, H-5 (pyrazoline)], 6.62 (2H, s, SO_2NH_2), 7.00-7.34 (9H, m, Ar-H), 7.55 (2H, d, J = 8.3 Hz, Ar-H), 7.85 (1H, d, J = 8.2 Hz, Ar-H); ^{13}C **NMR** (**75 MHz**, ***DMSO-d*$_6$**, δ, **ppm**): 46.40 (CH_2 of pyrazoline), 55.45 (O-CH_3), 55.72 (O-CH_3), 62.08 (CH of pyrazoline), 148.92 (C=N of pyrazoline), 158.89 (Ar C-O-C), 161.84 (Ar C-O-C); **FAB-MS** (**m**/**z**): 437[M^+], 439 [M+2]. **3l)** m.p. 220 °C; yield 48%; **IR** (υ_{max}, cm^{-1}, **KBr**): 3329 & 3235 (NH_2), 1594 (C=N), 1338 & 1157 ($SO_2N<$); ^1H **NMR** (**300 MHz**, $CDCl_3$, δ, **ppm**): 3.31 [1H, dd, J = 6.5, 18.2 Hz, H-4 *trans* (pyrazoline)], 3.81-3.82 (12H, s, 4xOCH_3), 3.86 (3H, s, OCH_3), 4.00 [1H, dd, J = 11.9, 18.0 Hz, H-4 *cis* (pyrazoline)], 5.14 [1H, dd, J = 6.6, 11.8 Hz, H-5 (pyrazoline)], 6.11 (2H, s, SO_2NH_2), 6.48 (3H, s, Ar-H), 6.57 (1H, d, J = 8.6 Hz, Ar-H), 7.04 (2H, d, J = 8.3 Hz, Ar-H), 7.69 (2H, d, J = 8.3 Hz, Ar-H), 7.94 (1H, d, J = 8.6 Hz, Ar-H); ^{13}C **NMR** (**75 MHz**, $CDCl_3$, δ, **ppm**): 46.48 (CH_2 of pyrazoline), 55.01 (2 x O-CH_3), 55.69 (2 x O-CH_3), 60.24 (O-CH_3), 63.47 (CH of pyrazoline), 137.40 (Ar C-O-C), 148.95 (C=N of pyrazoline), 153.34 (2 x Ar *C*-O-C), 158.58 (Ar *C*-O-C), 161.75 (Ar C-O-C); **FAB-MS** (**m**/**z**): 527[M^+], 529 [M+2], 512, 495, 463, 447. **3m)** m.p. 204 °C; yield 37%; **IR** (υ_{max}, cm^{-1}, **KBr**): 3342 & 3282 (NH_2), 1593 (C=N), 1534 & 1350 (NO_2), 1321 & 1158 ($SO_2N<$); ^1H **NMR** (**300 MHz**, $CDCl_3$, δ, **ppm**): 3.32 [1H, dd, J = 5.7, 18.3 Hz, H-4 *trans* (pyrazoline)], 3.81 (3H, s, OCH_3), 3.86 (3H, s, OCH_3), 4.09 [1H, dd, J = 12.4, 18.2 Hz, H-4 *cis* (pyrazoline)], 5.39 [1H, dd, J = 5.7, 12.2 Hz, H-5 (pyrazoline)], 5.89 (2H, s, SO_2NH_2), 6.46 (1H, s, Ar-H), 6.58 (1H, d, J = 8.5 Hz, Ar-H), 7.00 (2H, d, J = 8.5 Hz, Ar-H), 7.56 (2H, m, Ar-H), 7.69 (2H, d, J = 8.5 Hz, Ar-H), 7.97 (1H, d, J = 8.6 Hz, Ar-H), 8.14 (2H, m, Ar-H); ^{13}C **NMR** (**75 MHz**, $CDCl_3$, δ, **ppm**): 45.80 (CH_2 of pyrazoline), 55.46 (2 x O-CH_3), 61.64 (CH of pyrazoline), 147.91 (Ar C-NO_2), 148.24 (C=N of pyrazoline), 158.2 (Ar *C*-O-C), 162.42 (Ar *C*-O-C); **FAB-MS** (**m**/**z**): 482 [M^+], 484 [M+2], 460. **3n)** m.p. 192 °C; 52% yield; **IR** (υ_{max}, cm^{-1}, **KBr**): 3318, 3158 & 3069 [NH_2 and $N(CH_3)_2$], 1594 (C=N), 1338 & 1157 ($SO_2N<$); ^1H **NMR** (**300 MHz**, $CDCl_3$, δ, **ppm**): 2.96 (6H, s, 2xCH_3), 3.17 [1H, dd, J = 4.7, 18.2 Hz, H-4 *trans* (pyrazoline)], 3.79 (3H, s, OCH_3), 3.82 (3H, s, OCH_3), 3.95 [1H, dd, J = 11.9, 18.1 Hz, H-4 *cis* (pyrazoline)], 5.48 [1H, m, H-5 (pyrazoline)], 6.62-7.22 (10H, m, Ar-H and SO_2NH_2), 7.55 (2H, d, J = 8.7 Hz, Ar-H), 7.87 (1H, d, J = 9.2 Hz, Ar-H); ^{13}C **NMR** (**75 MHz**, $CDCl_3$, δ, **ppm**): 44.42 [$N(CH_3)_2$] of pyrazoline), 46.16 (CH_2 of pyrazoline), 55.39 (O-CH_3), 55.69 (O-CH_3), 61.26 (CH of pyrazoline), 148.8 (C=N of pyrazoline), 158.8 (Ar C-O-C), 161.78 (Ar *C*-O-C); **FAB-MS** (**m**/**z**): 480 [M^+]. **3o)** m.p. 138 °C; yield 32%; **IR** (υ_{max}, cm^{-1}, **KBr**): 3486 (OH), 3281 & 3107 (NH_2), 1591 (C=N), 1324 & 1143 ($SO_2N<$); ^1H **NMR** (**300 MHz**, ***DMSO-d*$_6$**, δ, **ppm**): 3.17 [1H, dd, J = 4.7, 18.2 Hz, H-4 *trans* (pyrazoline)], 3.80 (3H, s, OCH_3), 3.82 (3H, s, OCH_3), 3.95 [1H, dd, J = 11.9, 18.2 Hz, H-4 *cis* (pyrazoline)], 5.40 [1H, dd, J = 4.7, 11.8 Hz, H-5 (pyrazoline)], 6.59–6.70 (5H, m, Ar-H and SO_2NH_2), 6.98–7.16 (5H, m, Ar-H), 7.56 (2H, d, J = 8.6 Hz, Ar-H), 7.86 (1H, d, J = 9.2 Hz, Ar-H), 9.44 (1H, s, phenolic OH); ^{13}C **NMR** (**75 MHz**, ***DMSO-d*$_6$**, δ, **ppm**): 46.33 (CH_2 of pyrazoline), 55.44

($O\text{-}CH_3$), 55.74 ($O\text{-}CH_3$), 61.98 (CH of pyrazoline), 148.90 (C=N of pyrazoline), 157.86 (Ar *C*-O-C), 158.86 (Ar *C* -O-C), 161.79 (Ar *C*-O-C); **FAB-MS** (**m/z**): 453 [M^+]. **3p)** m.p. 218 °C; yield 51%; **IR** (υ_{max}, **cm^{-1}**, **KBr**): 3381 & 3271 (NH_2), 1593 (C=N), 1331 & 1151 ($SO_2N<$); **^{1}H NMR** (**300 MHz**, **$CDCl_3$**,δ, **ppm**): 3.31 [1H, dd, J = 5.7, 17.4 Hz, H-4 *trans* (pyrazoline)], 3.79 (3H, s, OCH_3), 4.01 [1H, dd, J = 12.0, 17.4 Hz, H-4 *cis* (pyrazoline)], 5.33 [1H, dd, J = 5.8, 11.9 Hz, H-5 (pyrazoline)], 6.21 (2H, s, SO_2NH_2), 6.86-7.21 (9H, m, Ar-H), 7.44 (2H, m, Ar-H), 7.72 (2H, d, J = 8.3 Hz, Ar-H), 10.53 (1H, s, phenolic OH); **^{13}C NMR** (**75 MHz**, **$CDCl_3$**, δ, **ppm**): 43.41 (CH_2 of pyrazoline), 54.67 ($O\text{-}CH_3$), 61.10 (CH of pyrazoline), 151.17 (C=N of pyrazoline), 156.48 (Ar C-OH), 158.68 (Ar C-O-C); **FAB-MS** (**m/z**): 423 [M^+]. **3q)** m.p. 184 °C; yield 47%; **IR** (υ_{max}, **cm^{-1}**, **KBr**): 3338 & 3251 (NH_2), 1596 (C=N), 1332 & 1147 ($SO_2N<$); **^{1}H NMR** (**300 MHz**, **$CDCl_3$**, δ, **ppm**): 3.33 [1H, dd, J = 5.8, 17.3 Hz, H-4 *trans* (pyrazoline)], 3.83 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 4.05 [1H, dd, J = 12.0, 17.4 Hz, H-4 *cis* (pyrazoline)], 5.32 [1H, dd, J = 5.8, 11.9 Hz, H-5 (pyrazoline)], 6.54 (2H, s, SO_2NH_2), 6.80 (1H, s, Ar-H), 6.84-7.06 (6H, m, Ar-H), 7.22 (1H, d, J = 7.4 Hz, Ar-H), 7.32 (1H, m, Ar-H), 7.72 (2H, d, J = 8.7 Hz, Ar-H), 10.52 (1H, s, phenolic OH); **^{13}C NMR** (**75 MHz**, **$CDCl_3$**, δ, **ppm**): 44.28 (CH_2 of pyrazoline), 55.45 (2 x $O\text{-}CH_3$), 61.42 (CH of pyrazoline), 148.18 (Ar C-O-C), 149.11 (Ar C-O-C), 151.78 (C=N of pyrazoline), 156.28 (Ar C-OH); **FAB-MS** (**m/z**): 453 [M^+]. **3r)** m.p. 276 °C; yield 34%; **IR** (υ_{max}, **cm^{-1}**, **KBr**): 3376 & 3266 (NH_2), 1722 (C=O of coumarin), 1596 (C=N), 1326 & 1155 ($SO_2N<$); **^{1}H NMR** (**300 MHz**, **DMSO-d_6**, δ **ppm**): 2.41 (3H, s, CH_3), 3.78 (3H, s, OCH_3), 3.81 [1H, dd, J = 6.5, 18.9 Hz, H-4 *trans* (pyrazoline)], 4.47 [1H, dd, J = 12.4, 18.9 Hz, H-4 *cis* (pyrazoline)], 5.31 [1H, dd, J = 6.4, 12.2 Hz, H-5 (pyrazoline)], 5.92 (2H, s, SO_2NH_2), 6.10 (1H, s, coumarin-H), 6.87 (2H, d, J = 8.3 Hz, Ar-H), 6.95 (2H, d, J = 8.6 Hz, Ar-H), 7.02 (1H, d, J = 8.8 Hz, coumarin-H), 7.19 (2H, d, J = 8.3 Hz, Ar-H), 7.52 (1H, d, J = 8.8 Hz, H- coumarin-H '), 7.73 (2H, d, J = 8.6 Hz, Ar-H), 12.07 (1H, s, phenolic OH); **^{13}C NMR** (**75 MHz**, **DMSO-d_6**, δ, **ppm**): 18.45 (CH_3), 46.99 (CH_2 of pyrazoline), 55.07 ($O\text{-}CH_3$), 60.89 (CH of pyrazoline), 153.84 (C=N of pyrazoline), 158.72 (Ar C-O-C), 159.41 (C=O of coumarin), 159.87 (Ar C-OH); **FAB-MS** (**m/z**): 505 [M^+], 507 [M+2], 489, 460. **3s**) m.p. 208 °C, yield 61%. **IR** (υ_{max}, **cm^{-1}**, **KBr**): 3256, 1729 (C=O of coumarin), 1593 (C=N), 1329 & 1156 ($SO_2N<$); **^{1}H NMR** (**300 MHz**, **DMSO-d_6**, δ, **ppm**): 2.43 (3H, s, CH_3), 3.81 [1H, dd, J = 6.2, 18.9 Hz, H-4 *trans* (pyrazoline)], 4.47 [1H, dd, J = 12.4, 18.9 Hz, H-4 *cis* (pyrazoline)], 5.29 [1H, dd, J = 6.3, 12.2 Hz, H-5 (pyrazoline)], 6.10 (1H, s, coumarin-H), 6.27 (2H, s, SO_2NH_2), 6.74 (3H, m, Ar-H), 6.96 (2H, d, J = 8.63 Hz, Ar-H), 7.03 (1H, d, J = 8.89 Hz, coumarin-H), 7.16 (1H, m, Ar-H), 7.54 (1H, d, J = 8.83 Hz, coumarin-H), 7.74 (2H, d, J = 8.62 Hz, Ar-H), 9.07 (1H, s, phenolic OH), 12.07 (1H, s, phenolic OH); **^{13}C NMR** (**75 MHz**, **DMSO-d_6**, δ, **ppm**): 18.43 (CH_3), 46.92 (CH_2 of pyrazoline), 61.21 (CH of pyrazoline), 153.76 (C=N of pyrazoline), 157.89 (Ar C-OH), 159.32 (C=O of coumarin), 159.83 (Ar C-OH); **FAB-MS** (**m/z**): 491 [M^+], 493 [M+2], 489, 411.

11. Winter, C. A.; Risely, E. A.; Nus, G. N. *Proc. Soc. Exp. Biol.* **1962**, *111*, 544.
12. Gierse, J. K.; Koboldt, C. M.; Walker, M. C.; Siebert, K.; Isakson, P. C. *Biochem. J.* **1999**, *339*, 607.