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Synthesis of some new 1,3,5-trisubstituted pyrazolines bearing benzene sulfonamide as anticancer and anti-inflammatory agents

Rafia Bashir^a, Syed Ovais^a, Shafiya Yaseen^a, Hinna Hamid^a, M. S. Alam^a, Mohammad Samim^a, Surender Singh^b, Kalim Javed^{a,*}

^a Department of Chemistry, Faculty of Science, Jamia Hamdard (Hamdard University), New Delhi 110 062, India

^b Department of Pharmacology, All India Institute of Medical Sciences, New Delhi 110 029, India

ARTICLE INFO

Article history:

Received 27 December 2010

Revised 11 May 2011

Accepted 18 May 2011

Available online 26 May 2011

Keywords:

2-Pyrazolines

Anti-inflammatory activity

Ulcerogenic activity

Cyclooxygenase activity

Celecoxib

ABSTRACT

Thirteen new 2-pyrazoline derivatives bearing benzenesulfonamide moiety (**2a–m**) were synthesized by condensing appropriate chalcones with 4-hydrazinobenzenesulfonamide hydrochloride and tested for anticancer and anti-inflammatory actions. According to the protocol of the National Cancer Institute (NCI) in vitro disease-oriented human cells screening panel assay compounds **2b**, **2c**, **2e**, **2f** and **2g** exhibited considerable antitumor activities against the entire tested tumor cell lines and showed effective growth inhibition GI₅₀ (MG-MID) values of 2.63, 2.57, 6.61, 3.31 and 2.57 μ M, respectively, beside a cyclostatic activity TGI (MG-MID) 9.54, 8.51, 24.0, 19.9 and 8.71 μ M, respectively. Two compounds **2g** and **2k** showed more potent anti-inflammatory activity than celecoxib at 5 h in carrageenan-induced rat paw edema bioassay. These compounds (**2g** and **2k**) proved to have superior gastrointestinal safety profiles as compared to celecoxib, when tested for their ulcerogenic effects. Compounds **2g** and **2k** showed no inhibition against the enzymatic activity of bovine COX-2 (in vitro).

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The sulfonamides constitute an important class of drugs, with several types possessing a host of biological properties including antibacterial,¹ anti-carbonic anhydrase,^{2,3} diuretic,^{2,4} hypoglycemic,⁵ antithyroid,⁶ and antiproteases activities.^{7–9} A large number of structurally novel sulfonamide derivatives have recently been reported to show substantial antitumor activity, both in vitro and/or in vivo. Although they have a common chemical motif of aromatic/heterocyclic sulfonamide, there are a variety of mechanisms of their antitumor action, most of them poorly understood at this moment. Some of these derivatives are currently being evaluated in clinical trials, and there is much optimism that they may lead to novel alternative anticancer drugs, devoid of the side effects of the presently available pharmacological agents.¹⁰ Many selective COX-2 inhibitors belong to a group of compounds which have a central tri-substituted planar ring (five-membered heterocyclic ring) attach to pendent benzene rings and a lipophilic group. One of the phenyl rings is attached to SO₂NH₂. The SO₂NH₂ pharmacophore is believed to induce COX-2 selectivity.¹¹ Pyrazolines have been reported to show a wide range of biological activity, including anti-inflammatory,^{12–15} antidepressant,¹⁶ anticancer,^{16,17} antibacterial,¹⁶ antitubercular^{18,19} and analgesic^{18,19} activities.

Due to great potential of both moieties (benzenesulfonamide and pyrazoline), the synthesis of pyrazolines bearing benzenesul-

fonamide was carried out to evaluate their anticancer and anti-inflammatory activities.

The synthesis of novel pyrazoline derivatives has been carried out according to the steps shown in Figure 1. In the initial step chalcones (**1a–m**) were synthesised by reacting 5-chloro-2-hydroxy-4-methylacetophenone/5-chloro-2-methoxy-4-methylacetophenone with appropriate aldehyde in the presence of a base by conventional Claisen–Schmidt condensation.²⁰ Reaction between synthesized chalcones and 4-hydrazinobenzenesulfonamide hydrochloride in ethanol led to synthesis of novel pyrazolines (**2a–m**).²¹ Purity of the compounds was checked on TLC plates (silica gel G) which were visualized by exposing to iodine vapours.

Structure of the synthesized compounds (**2a–m**) was established on the basis of elemental analysis and spectral data (IR, ¹H NMR, ¹³C NMR and MS). The IR spectra of the compounds **2a–m** showed absorption bands in the regions 1494–1628 cm^{–1} corresponding to C=N stretching bands because of ring closure. Also, in infrared spectra revealed NH₂ peak at 3258–3380 and 2923–3375 cm^{–1} and for SO₂NH₂ peak at 1305–1372 and 1102–1163 cm^{–1}.

In the ¹H NMR spectra of 2-pyrazolines, the three hydrogen atoms attached to the C-4 and C-5 carbon atoms of the heterocyclic ring gave an ABX spin system. Measured chemical shift and coupling constant values (cf. Experimental Section) equivocally prove the 2-pyrazoline structure. NH proton of the SO₂NH₂ group was observed at 5.83–7.13 ppm generally as broad band. The protons of methyl and aromatic protons were observed at expected ppm. In the ¹³C NMR spectra of these compounds (**2a–m**), the

* Corresponding author. Tel.: +91 9873463272.

E-mail address: kjaved@jamiahmdard.ac.in (K. Javed).

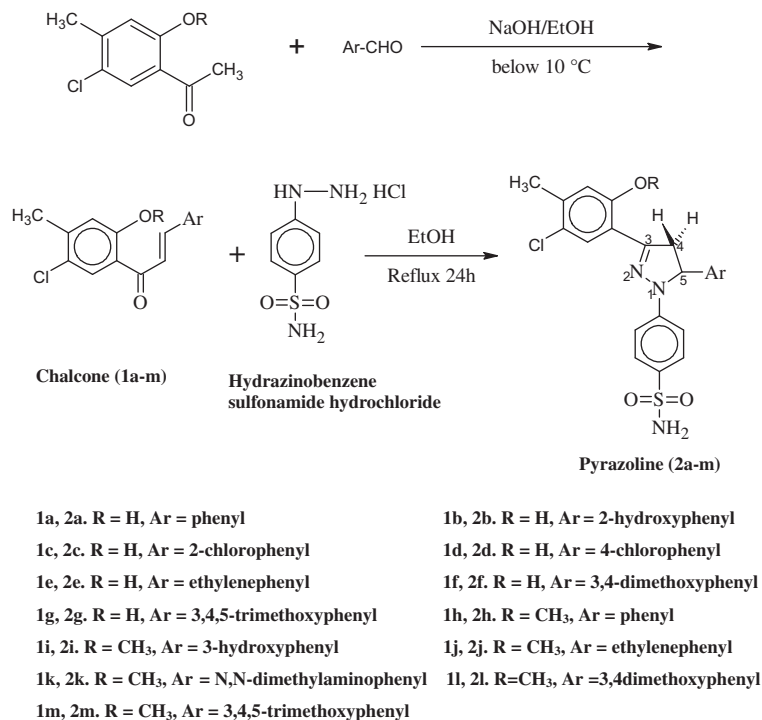


Figure 1. Synthesis of pyrazolines bearing benzenesulfonamide moiety.

chemical shift values of carbon atoms C-3 (147.88–151.65), C-4 (31.20–46.65) and C-5 (57.15–64.06).

Primary in vitro one-dose (10^{-5} M) anticancer assay was performed using full panel of about 60 human tumor cell lines in accordance with the protocol of the Drug Evaluation Branch, National Cancer Institute (NCI), Bethesda, and described elsewhere.^{22–24} The human tumor cell lines were derived from nine different cancer types: leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate, and breast cancers. Out of the newly synthesized ten compounds (**2b–f**, **2h** and **2j–m**), six compounds (**2b**, **2c**, **2e**, **2f**, **2g** and **2l**) were selected by the National Cancer Institute (NCI). The compound **2l** displayed mild sensitivity towards prostate cell line PC-3 (growth 52%) Table S1. The compounds **2b**, **2c**, **2e**, **2f** and **2g** possessed considerable activity and they were selected for an advanced assay against a full panel (approximately 60 cell lines) at five concentrations (100, 10, 1, 0.1 and 0.001 μ M). Three response parameters (GI_{50} , TGI, and LC_{50}) were calculated. The GI_{50} value (growth inhibitory activity) corresponds to concentration of the compounds causing 50% decrease in net cell growth, the TGI value (cytostatic activity) is the concentration of the compounds resulting in total growth inhibition, and the LC_{50} value (cytotoxic activity) is the concentration of the compounds causing net 50% loss of initial cells at the end of the incubation period (48 h). Values were calculated for each of these parameters if the level of activity was reached; however, if the effect was not reached or was exceeded, the value was expressed as greater or less than the maximum or minimum concentration tested. Furthermore mean graph midpoints (MG-MID) were calculated for each of the parameters, giving an average activity parameter over all cell lines for each compound.

For the calculation of the MG-MID, insensitive cell lines are included with the highest concentration tested.

In this Letter, **2b**, **2c**, **2e**, **2f** and **2g** exhibited considerable anti-tumor activities against the entire tested tumor cell lines. They (**2b**, **2c**, **2e**, **2f**, and **2g**) showed effective growth inhibition GI_{50} (MG-MID) values of 2.63, 2.57, 6.61, 3.31 and 2.57 μ M, respectively

(Table S2), beside a cyclostatic activity TGI (MG-MID) 9.54, 8.51, 24.0, 19.9 and 8.71 μ M, respectively (Table S3).

The ratio obtained by dividing the full panel MG-MID (μ M) of the compounds by their individual subpanel MG-MID (μ M) is considered as a measure of compound selectivity. Ratio of 3–6 refer to moderate selectivity, ratios greater than six indicate high selectivity towards the corresponding cell line, while compounds not meeting either of these criteria are rated as nonselective.²⁵ In this context, the active compounds in the present study were found to be nonselective with broad spectrum antitumor activity against the nine tumor subpanels tested with selectivity ratios ranges of 0.54–1.53 and 0.10–2.37 at the GI_{50} and TGI levels respectively.

Table S4 presents LC_{50} of compounds **2b**, **2c**, **2e**, **2f** and **2g** on each cell lines while Tables S5–S7 showed mean value of GI_{50} (MG-MID), TGI (MG-MID) and LC_{50} .

Carrageenan induced hind paw edema method was used for evaluating anti-inflammatory activity.²⁶ Wistar rats (either sex) weighing 150–175 g were procured from Central Animal House facility of Jamia Hamdard, New Delhi (Registration No. 173/CPCSEA). The experiments were performed in accordance with the guidelines for the care and use of laboratory animals, laid down by the Committee for the Purpose of Control and Supervision of Experiments in Animals (CPCSEA), Ministry of Social Justice and Empowerment, Govt. of India, Jan. 2000. Overnight fasted rats (16 h) were divided into groups of 6 animals each. One group of rats, which served as control was given vehicle (1% CMC in water in a volume of 10 mL/kg) only. Test compounds (20 mg/kg b.w.) and celecoxib (20 mg/kg b.w.) suspended in vehicle (10 mL/kg) were administered orally to respective groups. 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 to induce inflammation and the volume of injected paw was measured by using plethysmometer immediately (at 0 h). The paw volume was again measured after 3 and 5 h. The average paw volume in a group of treated rats was compared with vehicle

Table 1

Effect of 2-pyrazolines derivatives bearing benzene sulfonamide moiety on carrageenan induced hind paw edema in rats

Group No.	Treatment	Dose per kg b.w.	Increase in paw volume ml \pm SEM after carrageenan administration	
			3 h	5 h
I	Vehicle	10 ml/kg	0.38 \pm 0.0307	0.42 \pm 0.0401
II	Celecoxib	20 mg	0.066 \pm 0.0210 (82.6%)	0.06 \pm 0.02 (85.7%)
III	2a	20 mg	0.2 \pm 0.036 (47.4%)	0.166 \pm 0.056 (60.3%)
IV	2b	20 mg	0.1 \pm 0.0365 (73.6%)	0.17 \pm 0.0422 (59.5%)
V	2c	20 mg	0.17 \pm 0.0333 (55.3%)	0.12 \pm 0.0167 (71.4%)
VI	2d	20 mg	0.13 \pm 0.0333 (65.8%)	0.12 \pm 0.0167 (71.4%)
VII	2e	20 mg	0.08 \pm 0.0307 (78.9%)	0.12 \pm 0.0307 (71.4%)
VIII	2f	20 mg	0.15 \pm 0.0429 (60.5%)	0.133 \pm 0.0494 (68.3%)
IX	2g	20 mg	0.12 \pm 0.0307 (68.4%)	0.05 \pm 0.0342 (88.1%)
X	2h	20 mg	0.17 \pm 0.0211 (55.3%)	0.17 \pm 0.0333 (59.5%)
XI	2i	20 mg	0.23 \pm 0.0615 (39.5%)	0.35 \pm 0.0224 (16.7%)
XII	2j	20 mg	0.17 \pm 0.0494 (55.3%)	0.2 \pm 0.0365 (52.4%)
XIII	2k	20 mg	0.08 \pm 0.0167 (78.9%)	0.02 \pm 0.0167 (95.2%)
XIV	2l	20 mg	0.26 \pm 0.0422 (31.6%)	0.22 \pm 0.0703 (47.6%)
XV	2m	20 mg	0.25 \pm 0.0342 (34.2%)	0.33 \pm 0.0558 (21.4%)

* $P < 0.05$ compared to control (one-way ANOVA followed by Dunnett's test). Values are presented as mean \pm SEM ($n = 6$). Values in parentheses represent percent inhibitions.

(control group) and the percentage inhibition of edema was calculated by using the formula:

$$\text{Percent inhibition} = (1 - V_t/V_c) \times 100$$

where V_t is the mean paw volume of the test drug treated rats and V_c is the mean paw volume of the control.

The results (Table 1) show that all compounds except **2i**, **2l** and **2m** caused more than 50% reduction of oedema at 3 or 5 h. the most pronounced anti-inflammatory activity among the compounds studied was observed for **2g** and **2k**. The efficacy of **2g** and **2k** was higher than that of celecoxib at 5 h.

Structure–activity relationship showed that introduction of electron donating groups in 5-aryl ring of pyrazolines (**2a–g**) synthesised from appropriate 2'-hydroxychalcone (**1a–g**) enhanced the anti-inflammatory activity. On the other hand, introduction of electron donating group in the 5-aryl ring of pyrazolines (**2h–m**) synthesised from appropriate 2'-methoxy chalcones (**1h–m**) reduced the activity.

Acute gastric ulcerogenic effect of the compound **2g** and **2k** were evaluated in Wistar rats.²⁷ Albino rats of Wistar strain (160–220 g) fasted over 24 h were randomly allotted into three groups of six animals each. The animals of one group were given vehicle 10 mL/kg (CMC 1% w/v in distilled water) orally. The compound **2g** (60 mg/kg) and **2k** (60 mg/kg) suspended in vehicle were administered orally in a volume of 10 mL/kg to the animals of two different groups, respectively. They were scarified under deep ether anaesthesia after six hours of the treatment at a dose of 60 mg/kg (three times). Their stomach were removed and opened through greater curvature for examining lesions or bleedings. Compounds **2g** and **2k** showed no ulcerogenic activity.

The compounds (**2g** and **2k**) were also tested for their ability to inhibit bovine COX-1 and COX-2 enzymes in vitro. Cyclooxygenase activity was assayed using COX inhibitory screening kit (Cayman chemicals) by the method of Gierse et al.²⁸ These compounds were found fail to inhibit the activity of COX-2. However, they (**2g** and **2k**) showed very mild inhibition against the enzymatic activity of COX-1. On the basis of these observations it may be assumed that these derivatives inhibit enzymatic activity of 5-lipo-oxygenase which like COX-2 is responsible for modification of arachidonic acid to produce proinflammatory molecules prostanoids and leukotrienes.

In conclusion, the present study describes the synthesis of pyrazolines bearing benzene sulfonamide moiety (**2a–m**). The cytotoxicity of six compounds (**2b**, **2c**, **2e**, **2f**, **2g** and **2l**) was evaluated in vitro in a screen panel consisting of 60 human tumor

cell lines. The results of this assay indicated an activity in the micro-molar ranged linked particularly five compounds (**2b**, **2c**, **2e**, **2f** and **2g**). The synthesized compounds were also evaluated for their anti-inflammatory activity in carrageenan-induced rat paw edema bioassay. Two compounds **2g** and **2k** showed more potent anti-inflammatory activity than celecoxib at 5 h. These compounds (**2g** and **2k**) proved to have superior gastrointestinal safety profiles as compared to celecoxib, when tested for their ulcerogenic effects. Compounds **2g** and **2k** showed no inhibition against the enzymatic activity of bovine COX-2 (in vitro).

Acknowledgments

The authors are very grateful to the staff members of Department of Health and Human Services, National Cancer Institute (NCI), Bethesda, Maryland, USA, for carrying out in vitro anti cancer screening of the newly synthesized compounds. This work was supported by Grant No. 32-228/2006 (SR) from the University Grants Commission, New Delhi, India. One of the authors, Rafia Bashir is thankful to UGC for fellowship. 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.2011.05.061](https://doi.org/10.1016/j.bmcl.2011.05.061).

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20. General method for the synthesis of chalcones (**1a–m**): To a cold solution (below 10 °C) of acetophenone (0.01 mol) and desired aromatic aldehyde (0.01 mol) in ethanol (20 ml) was added a chilled solution of sodium hydroxide (10 ml of 30% solution). The reaction mixture was covered with a layer of petroleum ether (60–80 °C) and left at room temperature for a period of 12 h. It was diluted with water (100 ml) and acidified with concd HCl. The product obtained was filtered, washed with water and crystallized from ethanol.
21. General method for the synthesis of 3,5-diaryl-1-(*p*-sulfamylphenyl)-2-pyrazolines (**2a–m**): Appropriate chalcones (0.001 mol) and 4-hydrazinobenzenesulfonamide hydrochloride (0.001 mol) were dissolved in ethanol (25 ml) and refluxed for 24 h. After completion of the reaction, refluxing condenser was removed and the reaction solution was concentrated to 10–15 ml. It was left at room temperature to give crystalline compound. It was filtered and recrystallized to give pure compound. For preparing pyrazoline a small amount of NaOAc was added to refluxing alcohol.

3-(5'-Chloro-2'-hydroxy-4'-methylphenyl)-5-phenyl-1-(*p*-sulfamylphenyl)- Δ^2 -pyrazoline (**2a**): Recrystallized from methanol; yield = 48%; mp 217–219 °C; R_f = 0.57; (toluene/ethyl acetate/formic acid, 5:4:1); IR ν_{\max} (solvent, in cm^{-1}): 3376 (OH), 3323 and 3269 (NH_2), 1599 (C=N), 1305 and 1150 cm^{-1} ($\text{SO}_2\text{N}<$); ^1H NMR (200 MHz, DMSO, δ): 2.31 (3H, s, CH_3), 2.76 (1H, dd, J = 12.14 Hz, J = 12 Hz, H-4 cis (pyrazoline)), 5.25 (1H, d, J = 9.4 Hz, H-5 (pyrazoline)), 7.01 (1H, s, H-3'), 7.11 (2H, s, SO_2NH_2), 7.32 (2H, d, J = 8.8 Hz, H-3'', H-5''), 7.40–7.59 (5H, m, H-2, H-3, H-4, H-5, H-6), 7.68 (2H, d, J = 8.8 Hz, H-2'', H-6''), 8.00 (1H, s, H-3'), 9.89 (1H, s, OH); MALDI (m/z): 441 [M^+], 442 [$\text{M}+1$], 444, 464 [$\text{M}+\text{Na}$]. Anal. Calcd for $\text{C}_{22}\text{H}_{20}\text{ClN}_3\text{O}_5\text{S}$: C, 59.79; H, 4.56; N, 9.51; S, 7.26. Found: C, 59.74; H, 4.53; N, 9.50; S, 7.24.

3-(5'-Chloro-2'-hydroxy-4'-methylphenyl)-5-(2-hydroxyphenyl)-1-(*p*-sulfamylphenyl)- Δ^2 -pyrazoline (**2b**): Recrystallized from methanol; yield = 40%; mp 212–213 °C; R_f = 0.67, (toluene/ethyl acetate/formic acid, 5:4:1); IR ν_{\max} (solvent, in cm^{-1}): 3260 (OH), 1599 (C=N), 1338 and 1155 cm^{-1} ($\text{SO}_2\text{N}<$); ^1H NMR (400 MHz, DMSO, δ): 3.11 [1H, m, H-4 trans (pyrazoline)], 3.84 (3H, s, CH_3), 3.99 [3H, m, H-4 cis (pyrazoline)], 5.64 [3H, m, H-5 (pyrazoline)], 6.65 (1H, d, J = 7.20 Hz, H-3), 6.83 (2H, d, J = 8.00 Hz, H-3'', H-5''), 6.92–7.04 (6H, m, H-3', H-4, H-5, H-6', SO_2NH_2), 7.53–7.57 (3H, m, H-6, H-2'', H-6''), 9.92 (1H, s, OH), 10.32 (1H, s, OH); FAB-MS (m/z): 457 [M^+ , base peak], 456 [$\text{M}-1$], 393 [M^+-SO_2], 377 [$\text{M}^+-\text{SO}_2\text{NH}_2$], 364 [$\text{M}^+-5\text{-aryl ring}$], 271; ^{13}C NMR (DMSO, δ): 20.20 ($-\text{CH}_3$), 43.37 (C-4 pyrazoline), 57.15 (C-5 pyrazoline), 148.39 (C-3 pyrazoline), 154.68 (C-2'). Anal. Calcd for $\text{C}_{22}\text{H}_{20}\text{ClN}_3\text{O}_5\text{S}$: C, 57.70; H, 4.40; N, 9.18; S, 7.00. Found: C, 57.68; H, 3.93; N, 9.17; S, 6.99.

3-(5'-Chloro-2'-hydroxy-4'-methylphenyl)-5-(2-chlorophenyl)-1-(*p*-sulfamylphenyl)- Δ^2 -pyrazoline (**2c**): Recrystallized from methanol; yield = 30%; mp 221 °C; R_f = 0.71, (toluene/ethyl acetate/formic acid, 5:4:1); IR ν_{\max} (solvent, in cm^{-1}): 3364 (OH), 3317 & 3258 (NH_2), 1596 (C=N), 1311 & 1102 cm^{-1} ($\text{SO}_2\text{N}<$); ^1H NMR (400 MHz, DMSO, δ): 2.28 (3H, s, CH_3), 2.70 [1H, m, H-4 trans (pyrazoline)], 5.43 [1H, m, H-5 (pyrazoline)], 6.99 (1H, s, H-3'), 7.06 (2H, s, SO_2NH_2), 7.28 (2H, d, J = 7.6 Hz, H-3'', H-5''), 7.44 (2H, m, H-4, H-5), 7.52 (1H, d, J = 6.4 Hz, H-3), 7.64 (2H, d, J = 7.6 Hz, H-2'', H-6''), 7.72 (1H, d, H-6, J = 6.4 Hz), 7.98 (1H, s, H-6'), 9.86 (1H, s, OH); FAB-MS (m/z): 475 [M^+], 474 [$\text{M}-1$], 411 [M^+-SO_2], 395 [$\text{M}^+-\text{SO}_2\text{NH}_2$], 364 [$\text{M}^+-5\text{-aryl ring}$], 77 (base peak); ^{13}C NMR (DMSO, δ): 20.10 ($-\text{CH}_3$), 31.20 (C-4 pyrazoline), 74.17 (C-5 pyrazoline), 148.39 (C-3 pyrazoline), 154.68 (C-2'). Anal. Calcd for $\text{C}_{22}\text{H}_{19}\text{Cl}_2\text{N}_3\text{O}_5\text{S}$: C, 55.47; H, 4.02; N, 8.82; S, 6.73. Found: C, 55.41; H, 3.99; N, 8.82; S, 6.71.

3-(5'-Chloro-2'-hydroxy-4'-methylphenyl)-5-(4-chlorophenyl)-1-(*p*-sulfamylphenyl)- Δ^2 -pyrazoline (**2d**): Recrystallized from the mixture of acetone and methanol; yield = 34%; mp 236–238 °C; R_f = 0.41, (toluene/ethyl acetate/formic acid, 5:4:1); IR ν_{\max} (solvent, in cm^{-1}): 3450 (OH), 3326 and 3375 (NH_2), 1595 (C=N), 1319 and 1136 cm^{-1} ($\text{SO}_2\text{N}<$); ^1H NMR (300 MHz, DMSO, δ): 2.31 (3H, s, CH_3), 2.75 [1H, dd, J = 12.14 Hz, J = 16.74 Hz, H-4 cis (pyrazoline)], 5.28 [1H, d, H-5 (pyrazoline)], 7.02 (1H, s, H-3'), 7.10 (2H, s, SO_2NH_2), 7.33 (2H, d, J = 8.51 Hz, H-3'', H-5''), 7.53 (2H, d, J = 8.27 Hz, H-3, H-5), 7.60 (2H, d, J = 8.29 Hz, H-2, H-6), 7.68 (2H, d, J = 8.51 Hz, H-2'', H-6''), 7.99 (1H, s, H-6'), 9.90 (1H, s, OH); FAB-MS (m/z): 476 [$\text{M}^+ + 1$], 475 [M^+], 411 [M^+-SO_2], 395 [$\text{M}^+-\text{SO}_2\text{NH}_2$], 364 [$\text{M}^+-5\text{-aryl ring}$], 157; ^{13}C NMR (DMSO, δ): 20.09 ($-\text{CH}_3$), 32.19 (C-4 pyrazoline), 76.29 (C-5 pyrazoline), 148.45 (C-3 pyrazoline), 154.66 (C-2'). Anal. Calcd for $\text{C}_{22}\text{H}_{19}\text{Cl}_2\text{N}_3\text{O}_5\text{S}$: C, 55.47; H, 4.02; N, 8.82; S, 6.73. Found: C, 55.42; H, 3.99; N, 8.81; S, 6.72.

3-(5'-Chloro-2'-hydroxy-4'-methylphenyl)-5-(ethylenephyl)-1-(*p*-sulfamylphenyl)- Δ^2 -pyrazoline (**2e**): Recrystallized from acetone; yield = 64%; mp 267–268 °C; R_f = 0.47, (toluene/ethyl acetate/formic acid, 7.5:2:0.5); IR ν_{\max} (solvent, in cm^{-1}): 3451 (OH), 3323 & 3342 (NH_2), 1628 (C=N), 1327 and 1144 cm^{-1} ($\text{SO}_2\text{N}<$); ^1H NMR (300 MHz, DMSO, δ): 2.31 (3H, s, CH_3), 3.85 [1H, m, H-4 cis (pyrazoline)], 5.23 [1H, m, H-5 (pyrazoline)], 6.32 (1H, q, $-\text{CH}=\text{CH}-$), 6.73 (1H, d, J = 15.82 Hz, $-\text{CH}=\text{CH}-$), 6.99 (1H, s, H-3'), 7.07 (2H, s, SO_2NH_2), 7.18–7.30 (5H, m, H-2, H-3, H-4, H-5, H-6), 7.42 (2H, d, J = 7.82 Hz, *N*-phenyl protons), 7.57 (1H, s, H-6'), 7.67 (2H, d, 7.82 Hz, *N*-phenyl protons), 10.30 (1H, s, OH-2'); FAB-MS (m/z): 467 [M^+ , base peak], 466 [M^+-1], 387 [$\text{M}^+-\text{SO}_2\text{NH}_2$], 157; ^{13}C NMR (DMSO, δ): 20.22 ($-\text{CH}_3$), 41.48 (C-4 pyrazoline), 60.89 (C-5 pyrazoline), 128.15 (C- α), 134.05 (C- β), 151.65 (C-3 pyrazoline), 155.31 (C-2'). Anal. Calcd for $\text{C}_{24}\text{H}_{22}\text{ClN}_3\text{O}_5\text{S}$: C, 61.60; H, 4.74; N, 8.98; S, 6.85. Found: C, 61.54; H, 4.70; N, 8.97; S, 6.83.

3-(5'-Chloro-2'-hydroxy-4'-methylphenyl)-5-(3,4-dimethoxyphenyl)-1-(*p*-sulfamylphenyl)- Δ^2 -pyrazoline (**2f**): Recrystallized from methanol; yield = 60%; mp 229–231 °C; R_f = 0.26, (Petrol/acetone 7:3); IR ν_{\max} (solvent, in cm^{-1}): 3253 (OH), 1592 (C=N), 1510, 1330 and 1155 cm^{-1} ($\text{SO}_2\text{N}<$); ^1H NMR (400 MHz, DMSO, δ): 2.26 (3H, s, CH_3), 3.65 (3H, s, OCH_3), 3.68 (3H, s, OCH_3), 3.97 [1H, dd, J = 12.40 Hz, 18.00 Hz, H-4 cis (pyrazoline)], 5.47 [1H, dd, J = 6.40 Hz, J = 10.80 Hz, H-5 (pyrazoline)], 6.64 (1H, d, J = 8.00 Hz, H-5), 6.83 (1H, d, J = 7.6 Hz, H-6), 6.87 (2H, d, J = 8.80 Hz, H-3'', H-5''), 6.90–7.02 (4H, m, H-2, H-2'', SO_2NH_2), 7.54 (2H, d, J = 8.80 Hz, H-2'', H-6''), 7.57 (1H, s, H-6'), 10.29 (1H, s, OH); FAB-MS (m/z): 501 [M^+ , base peak], 500 [$\text{M}-1$], 437 [M^+-SO_2], 421 [$\text{M}^+-\text{SO}_2\text{NH}_2$], 364 [$\text{M}^+-5\text{-aryl ring}$]; ^{13}C NMR (DMSO, δ): 20.19 ($-\text{CH}_3$), 44.79 (C-4 pyrazoline), 55.92 (OCH₃ at C-3, C-4), 62.03 (C-5 pyrazoline), 150.78 (C-3 pyrazoline), 155.28 (C-2'). Anal. Calcd for $\text{C}_{24}\text{H}_{24}\text{ClN}_3\text{O}_6\text{S}$: C, 57.42; H, 4.82; N, 8.37; S, 6.39. Found: C, 57.37; H, 4.79; N, 8.36; S, 6.37.

3-(5'-Chloro-2'-hydroxy-4'-methylphenyl)-5-(3,4,5-trimethoxyphenyl)-1-(*p*-sulfamylphenyl)- Δ^2 -pyrazoline (**2g**): Recrystallized from acetone; yield = 64%; mp 237–238 °C; R_f = 0.75, (petroleum ether (60–80)/toluene/ethyl acetate; 10:5:3); IR ν_{\max} (solvent, in cm^{-1}): 3409 (OH), 2923 (NH_2), 1592 (C=N), 1502, 1328 and 1124 cm^{-1} ($\text{SO}_2\text{N}<$); ^1H NMR (400 MHz, DMSO, δ): 2.27 (3H, s, CH_3), 3.59 (3H, s, OCH_3), 3.65 (6H, s, OCH_3), 4.05 [1H, m, H-4 trans (pyrazoline)], 5.42 [1H, dd, J = 5.6 Hz, J = 10.32 Hz, H-5 (pyrazoline)], 6.56 (2H, s, SO_2NH_2), 6.87 (1H, s, H-3'), 6.94–7.04 (4H, m, H-3'', H-5'', H-2, H-6), 7.54 (1H, s, H-6'), 7.62 (2H, d, H-2'', H-6''), 10.27 (1H, s, OH); FAB-MS (m/z): 531 [M^+ , base peak], 530 [M^+-1], 467 [M^+-SO_2], 451 [$\text{M}^+-\text{SO}_2\text{NH}_2$], 364 [$\text{M}^+-5\text{-aryl ring}$]; ^{13}C NMR (DMSO, δ): 20.15 ($-\text{CH}_3$), 44.89 (C-4 pyrazoline), 56.34 (OCH₃ at C-3, C-4), 60.41 (OCH₃ at C-5), 62.71 (C-5 pyrazoline), 150.91 (C-3 pyrazoline), 155.34 (C-2'). Anal. Calcd for $\text{C}_{25}\text{H}_{26}\text{ClN}_3\text{O}_6\text{S}$: C, 56.44; H, 4.93; N, 7.90; S, 6.03. Found: C, 56.39; H, 4.88; N, 7.89; S, 6.01.

3-(5'-Chloro-2'-methoxy-4'-methylphenyl)-5-(phenyl)-1-(*p*-sulfamylphenyl)- Δ^2 -pyrazoline (**2h**): Recrystallized from methanol; yield = 50%; mp 132–134 °C; R_f = 0.79, (toluene/ethyl acetate/formic acid, 5:4:1); IR ν_{\max} (solvent, in cm^{-1}): 3385 & 3261 (NH_2), 1591 (C=N), 1506, 1370 and 1154 cm^{-1} ($\text{SO}_2\text{N}<$); ^1H NMR (300 MHz, DMSO, δ): 2.33 (3H, s, CH_3), 3.25 [1H, dd, J = 6.20 Hz, J = 18.00 Hz, H-4 (trans)], 4.01 [1H, dd, J = 12.00 Hz, J = 18.10 Hz, H-4 (cis)], 3.77 (3H, s, OCH_3), 5.55 [1H, dd, J = 5.1 Hz, J = 12.00 Hz, H-5], 7.10 (1H, s, H-3'), 7.03–7.23 (5H, 2 m, phenyl protons), 7.32 (2H, d, J = 8.7 Hz, H-2'', H-6''), 7.54 (2H, d, J = 8.7 Hz, H-3'', H-5''), 7.88 (1H, s, H-6'), MALDI (m/z): 455 [M^+], 454 [$\text{M}-1$], 457 [$\text{M}+2$], 376; ^{13}C NMR (DMSO, δ): 20.39 ($-\text{CH}_3$), 46.48 (C-4 pyrazoline), 56.56 (OCH₃ at C-2'), 62.77 (C-5 pyrazoline), 147.89 (C-3 pyrazoline), 156.50 (C-2'). Anal. Calcd for $\text{C}_{24}\text{H}_{25}\text{N}_3\text{O}_5\text{S}$: C, 60.59; H, 4.86; N, 9.22; S, 7.03. Found: C, 60.53; H, 4.82; N, 9.21; S, 7.02.

3-(5'-Chloro-2'-methoxy-4'-methylphenyl)-5-(3-hydroxyphenyl)-1-(*p*-sulfamylphenyl)- Δ^2 -pyrazoline (**2i**): Recrystallized from methanol; yield = 50%; mp 216–218 °C; R_f = 0.41, (toluene/ethyl acetate/formic acid, 7.5:2:0.5); IR ν_{\max} (solvent, in cm^{-1}): 3359 (NH_2), 1591 (C=N), 1506, 1370 and 1152 cm^{-1} ($\text{SO}_2\text{N}<$); ^1H NMR (300 MHz, DMSO, δ): 2.36 (3H, s, CH_3), 3.79 (3H, s, OCH_3), 3.97 [1H, m, H-4 trans (pyrazoline)], 5.45 [1H, m, H-5 (pyrazoline)], 6.58–6.70 (3H, m, H-3', H-4, H-5), 7.03–7.13 (6H, m, H-6, H-2, H-3'', H-5'', SO_2NH_2), 7.58 (2H, d, H-2'', H-6''), 7.89 (1H, s, H-6'), 9.47 (1H, s, OH-3); FAB-MS (m/z): 470 [M^+], 471 [$\text{M}^+ + 1$], 407 [M^+-SO_2], 391 [$\text{M}^+-\text{SO}_2\text{NH}_2$], 378 [$\text{M}^+-5\text{-aryl ring}$], 157; ^{13}C NMR (DMSO, δ): 20.20 ($-\text{CH}_3$), 46.43 (C-4 pyrazoline), 56.53 (OCH₃ at C-2'), 62.72 (C-5 pyrazoline), 147.91 (C-3 pyrazoline), 158.34 (C-2'). Anal. Calcd for $\text{C}_{23}\text{H}_{22}\text{ClN}_3\text{O}_5\text{S}$: C, 58.53; H, 4.70; N, 8.90; S, 6.79. Found: C, 58.48; H, 4.67; N, 8.90; S, 6.78.

3-(5'-Chloro-2'-methoxy-4'-methylphenyl)-5-(ethylenephyl)-1-(*p*-sulfamylphenyl)- Δ^2 -pyrazoline (**2j**): Recrystallized from methanol; yield = 53.6%; mp 222–226 °C; R_f = 0.27, (toluene/ethyl acetate/formic acid, 7.5:2:0.5); IR ν_{\max} (solvent, in cm^{-1}): 3258 (NH_2), 1590 (C=N), 1370, 1096 cm^{-1} (OCH₃) and 1153 cm^{-1} ($\text{SO}_2\text{N}<$); ^1H NMR (300 MHz, DMSO, δ): 2.35 (3H, s, CH_3), 3.31 [1H, d, H-4 trans (pyrazoline)], 3.75 [1H, d, H-4 cis (pyrazoline)], 3.82 (3H, s, OCH_3), 5.15 (1H, m, H-5), 6.30 (1H, d, H- α), 6.65 (1H, d, H- β), 7.02 (2H, s, SO_2NH_2), 7.12 (1H, s, H-3'), 7.22–7.28 (5H, m, cinnamal unit protons), 7.40 (2H, d, J = 8.6 Hz, H-3'', H-5''), 7.62 (2H, d, J = 8.7 Hz, H-2'', H-6''), 7.83 (1H, s, H-6'); FAB-MS (m/z): 481 [M^+], 482 [$\text{M}^+ + 1$, base peak], 401 [M^+-80], 157; ^{13}C NMR (DMSO, δ): 20.39 ($-\text{CH}_3$), 43.36 (C-4 pyrazoline), 56.59 (OCH₃ at C-2'), 61.64 (C-5 pyrazoline), 127.86 (C- α), 136.34 (C- β), 148.73 (C-3 pyrazoline). Anal. Calcd for $\text{C}_{25}\text{H}_{24}\text{ClN}_3\text{O}_5\text{S}$: C, 62.30; H, 5.02; N, 8.72; S, 6.65. Found: C, 62.24; H, 4.97; N, 8.71; S, 6.64.

3-(5'-Chloro-2'-methoxy-4'-methylphenyl)-5-(4-(*N,N*-dimethylamino)-phenyl)-1-(*p*-sulfamylphenyl)- Δ^2 -pyrazoline (**2k**): Recrystallized from methanol; yield = 74.5%; mp 210–211 °C; R_f = 0.67 (toluene/ethyl acetate/formic acid, 5:4:1); IR ν_{\max} (solvent, in cm^{-1}): 3380 (NH_2), 1591 (C=N), 1506, 1367 and 1153 cm^{-1} ($\text{SO}_2\text{N}<$); ^1H NMR (300 MHz, DMSO, δ): 2.34 (3H, s, CH_3), 2.86 [6H, s, $\text{N}(\text{CH}_3)_2$], 3.15 [1H, m, H-4 trans (pyrazoline)], 3.77 (3H, s, OCH_3), 3.92 [1H, m, H-4 cis (pyrazoline)], 5.44 [1H, m, H-5 (pyrazoline)], 6.78 (2H, d, J = 8.40 Hz, H-3, H-5), 7.03–7.09 (7H, m, five aromatic protons and SO_2NH_2), 7.55 (2H, d, H-2, H-6, J = 8.67 Hz), 7.87 (1H, s, H-6'); FAB-MS (m/z): 498 [M^+ , base peak], 497 [M^+-1], 434 [M^+-SO_2], 418 [$\text{M}^+-\text{SO}_2\text{NH}_2$], 378 [$\text{M}^+-5\text{-aryl ring}$]; ^{13}C NMR (DMSO, δ): 20.39 (CH_3), 41.96 (C-4 pyrazoline), 46.49 ($\text{N}(\text{CH}_3)_2$), 56.55 (OCH₃), 62.35 (C-5 pyrazoline), 147.88 (C-3 pyrazoline),

156.48 (C-2'). Anal. Calcd for $C_{25}H_{27}ClN_4O_3S$: C, 60.17; H, 5.45; N, 11.23; S, 6.43. Found: C, 60.11; H, 5.41; N, 11.22; S, 6.40.

3-(5'-Chloro-2'-methoxy-4'-methylphenyl)-5-(3,4-dimethoxyphenyl)-1-(p-sulfamylphenyl)-4²-pyrazoline (21): Recrystallized from methanol, yield = 61%; mp 223–225 °C; R_f = 0.57 (toluene/ethyl acetate/formic acid, 5:4:1); IR ν_{max} (solvent, in cm^{-1}): 3335 and 3342 (NH_2), 1588 ($C=N$), 1506, 1368 cm^{-1} and 1152 cm^{-1} ($SO_2N<$); 1H NMR (300 MHz, DMSO, δ): 2.40 (3H, s, CH_3-4'), 3.81 (3H, s, OCH_3), 3.34 [1H, dd, J = 6.21 Hz, J = 18.22 Hz, H-4 trans (pyrazoline)], 3.82 (3H, s, OCH_3), 3.85 (3H, s, OCH_3-4'), 3.97 [1H, dd, J = 12.36 Hz, J = 18.36 Hz, H-4 cis (pyrazoline)], 5.23 [1H, dd, J = 6.05 Hz, J = 11.57 Hz, H-5 (pyrazoline)], 5.83 (2H, s, SO_2NH_2), 6.74–6.82 (4H, m, H-3', H-2, H-5, H-6), 7.08 (2H, d, J = 8.56 Hz, H-3'', H-5''), 7.70 (2H, d, J = 8.57 Hz, H-2'', H-6''), 7.97 (1H, s, H-6'); FAB-MS (m/z): 515 [M^+], 516 [M^++1 , base peak], 451 [M^+-SO_2], 435 [$M^+-SO_2NH_2$], 378 [M^+-5 -aryl ring], 157; ^{13}C NMR (DMSO, δ): 20.39 ($-CH_3$), 46.54 (C-4 pyrazoline), 55.88 (OCH_3 at C-2'), 55.92 (OCH_3 at C-3), 56.57 (OCH_3 at C-4), 62.78 (C-5 pyrazoline), 149.50 (C-3 pyrazoline), 149.50 (C-2'). Anal. Calcd for $C_{25}H_{26}ClN_3O_5S$: C, 58.19; H, 5.08; N, 8.14; S, 6.21. Found: C, 58.13; H, 5.04; N, 8.14; S, 6.20.

3-(5'-Chloro-2'-methoxy-4'-methylphenyl)-5-(3,4,5-trimethoxyphenyl)-1-(p-sulfamylphenyl)-4²-pyrazoline (2m): Recrystallized from methanol; yield = 67%; mp 239–241 °C; R_f = 0.60 (toluene/ethyl acetate/formic acid, 5:4:1); IR ν_{max} (solvent, in cm^{-1}): 3374 (NH_2), 3269, 1591 ($C=N$), 1363 and 1152 ($SO_2N<$), 1097 cm^{-1} (OCH_3); 1H NMR (300 MHz, DMSO, δ): 2.40 (3H, s,

CH_3-4'), 3.32 [1H, dd, J = 12.13 Hz, J = 18.29 Hz, H-4 trans (pyrazoline)], 3.81 (12H, s, 4 \times OCH_3), 4.01 [1H, dd, J = 6.31 Hz, J = 11.92 Hz, H-4 cis (pyrazoline)], 5.19 (1H, m, H-5 pyrazoline), 6.28 (2H, s, SO_2NH_2), 6.47 (2H, s, H-2, H-6), 6.82 (1H, s, H-3'), 7.06 (2H, d, J = 8.22 Hz, H-3'', H-5''), 7.69 (2H, d, J = 8.23 Hz, H-2'', H-6''), 7.97 (1H, s, H-6'); FAB-MS (m/z): 545 [M^+ , base peak], 544 [M^+-1], 481 [M^+-SO_2], 465 [$M^+-SO_2NH_2$], 157; ^{13}C NMR (DMSO, δ): 20.50 ($-CH_3$), 46.65 (C-4 pyrazoline), 55.85 (OCH_3 at C-2'), 56.13 (OCH_3 at C-3, C-5), 60.63 (OCH_3 at C-4), 64.06 (C-5 pyrazoline), 147.99 (C-3 pyrazoline), 156.10 (C-2'). Anal. Calcd for $C_{26}H_{28}ClN_3O_6S$: C, 57.19; H, 5.17; N, 7.70; S, 5.87. Found: C, 57.13; H, 5.12; N, 7.69; S, 5.86.

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