Full Paper

Synthesis and Biological Evaluation of 4-Arylphthalazones Bearing Benzenesulfonamide as Anti-Inflammatory and Anti-Cancer Agents

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Nine 4-arylphthalazones bearing benzenesulfonamide (**2a–i**) were synthesized by the condensation of the appropriate 2-aroylbenzoic acid (**1a–i**) and 4-hydrazinobenzenesulfonamide in ethanol. The structures of these compounds were elucidated by elemental analysis, IR, ^{1}H NMR, ^{13}C NMR, and MS spectroscopy. Two compounds, **2b** and **2i**, showed significant anti-inflammatory activity comparable to that of the standard drug celecoxib in the carrageenan-induced rat paw edema model. These compounds (**2b** and **2i**) had selective inhibitory activity towards the COX-2 enzyme. Compound **2b** had a better selectivity ratio (COX-1/COX-2) compared to that of celecoxib and can be used as a novel template for the design of selective COX-2 inhibitors. Compounds **2d** and **2i** were screened for their antiproliferative activity towards 60 human cancer cell lines by the National Cancer Institute (USA). The compounds **2d** and **2i** displayed mild activity toward the renal cancer cell line UO-31.

Keywords: Anti-cancer / Anti-inflammatory / COX-2 inhibitor / Phthalazone / Sulfonamides

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Introduction

Inflammation is the first response of the body to infection, irritation or other injuries. It is an essential process to neutralize aggressor agents and to repair damaged tissues, assuring this way the survival of the host. Unfortunately, the available anti-inflammatory treatments are not always sufficiently effective and frequently present numerous and severe side effects especially in long-term use. Most of the current non-steroidal anti-inflammatory drugs (NSAIDs) show serious side effects including gastrointestinal disorders and kidney damage.

NSAIDs exert their side effects by inhibition of the COX-1 enzyme. Since the COX-1 isoform is the constitutive one that is responsible for regulation of physiological processes and the COX-2 isoform is discovered to be the enzyme induced by inflammatory stimuli, selective inhibition of COX-2 provides a rationale for developing anti-inflammatory and analgesic agents that lack the GI liabilities. Unfortunately, some NSAIDS including selective COX-2 inhibitors have been shown to be associated with cardiovascular events, thus it has become a challenge to explore novel NSAIDs with reduced gastrointestinal tract and cardiovascular side effects.

In the last few years, attention was oriented towards the synthesis and biological evaluation of phthalazine derivatives as they exhibit a broad spectrum of biological activities (Fig. 1). These derivatives have been reported to possess antihypertensive [1, 2], anticonvulsant [3, 4], antidiabetic [5, 6], antimicrobial [7], antitrypanosomal [8], anti-inflammatory [9–11], and PDE4 inhibitory activities [12]. The phthalazinone nucleus has been proven to be a versatile system in medicinal chemistry. Some of the phthalazinone derivatives like hydralazine [13], budralazine [14], azelastine [15], ponalrestat [16], and zopolrestat [17] have found application in clinical medicine. Recently, several groups have studied the structure-activity relationship of novel series of 4-arylsubstituted phthalazinones and showed that the presence of 4-aryl substituted on the phthalazinone nucleus contributes to good anti-inflammatory and antinociceptive activities [18].

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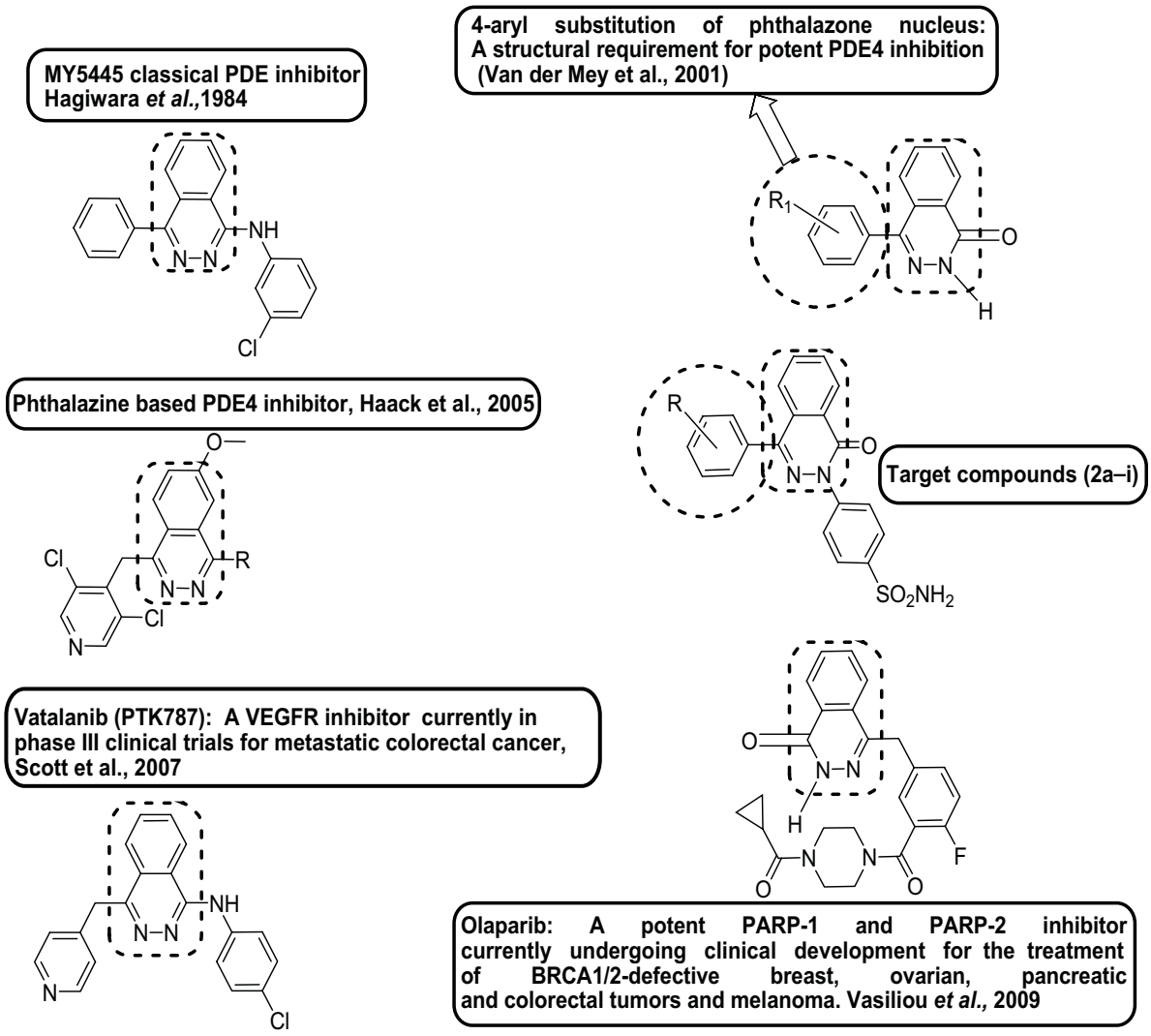


Figure 1. Structures of some biologically active phthalazine pharmacophores and the target compounds [18, 22, 23, 46, 47].

Substitution with acetamide derivative on the 2 position (the lactam nitrogen) in the 4-arylphthalazinone derivatives resulted in compounds having considerably high antinociceptive and anti-inflammatory activities [9, 19]. A large number of phthalazine derivatives have been studied for their antitumor activities [20–22]. Vatalanib (PTK-787), substituted phthalazine, is a VEGFR (vascular endothelial growth factor receptor) inhibitor. It is currently in phase III clinical trials for metastatic colorectal cancer [23].

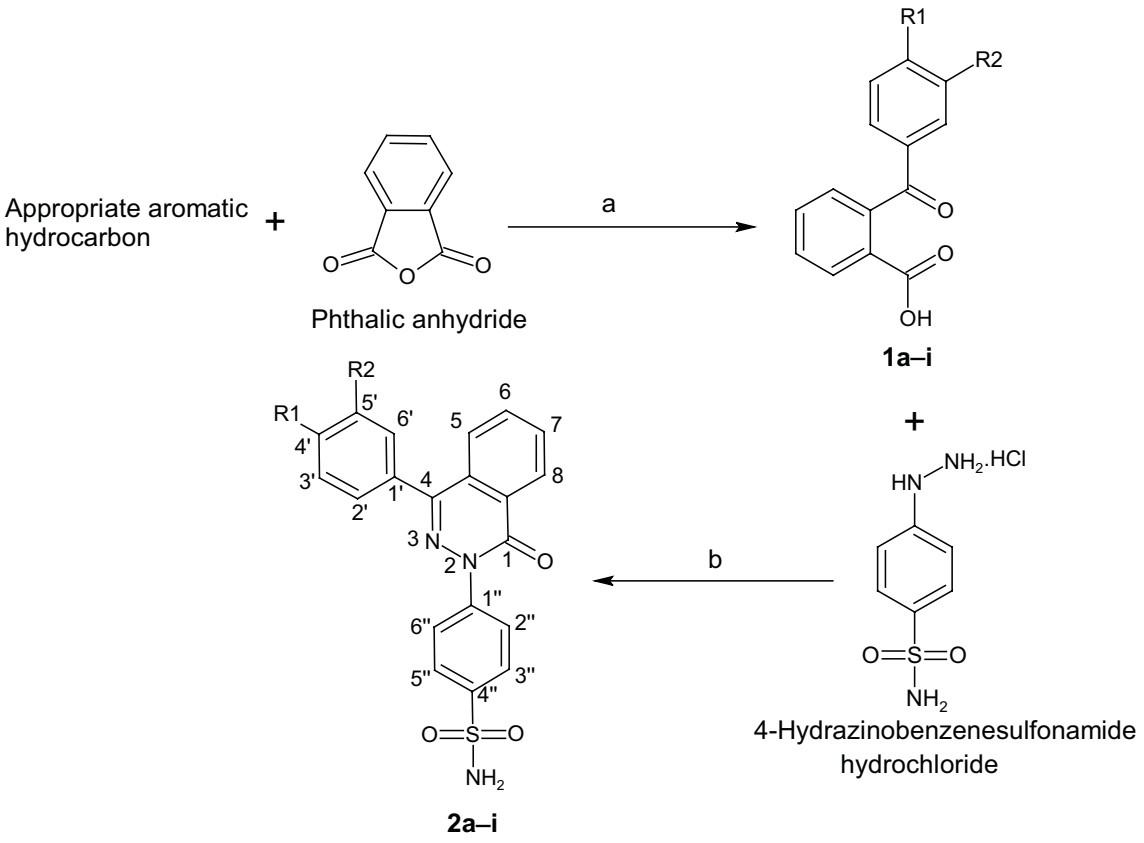
The sulfonamides constitute an important class of drugs, with several types possessing a number of biological properties including antibacterial [24], anti-carbonic anhydrase [25, 26], diuretic [25, 27], hypoglycemic [28], antithyroid [29], and antiprotons activities [30–32]. The SO_2NH_2 pharmacophore is believed to induce COX-2 selectivity [33].

In view of the aforementioned facts, it was thought worthwhile to incorporate the benzenesulfonamide moiety in the 2 position of some 4-arylphthalazone derivatives in the hope to yield safe and potent compounds with anti-inflammatory and anti-cancer potential. This paper describes the synthesis, *in vivo* anti-inflammatory and *in vitro* anticancer activities of some new 4-arylphthalazone molecules **(2a–i)**. Compounds showing maximum anti-inflammatory activity were also evaluated for *in vitro* COX activity and ulcerogenic potential in rats.

Results and discussion

Chemistry

The synthesis of the title compounds (**2a–i**) was carried out by the condensation of appropriate 2-aroylbenzoic acid



1a, 2a: R1 = R2 = H

1b, 2b: R1 = Methyl, R2 = H

1c, 2c: R1 = Ethyl, R2 = H

1d, 2d: R1 = Chloro, R2 = H

1e, 2e: R1 = Bromo, R2 = H

1f, 2f: R1 = Phenyl, R2 = H

1g, 2g: R1 = Methoxy, R2 = H

1h, 2h: R1 = Phenoxy, R2 = H

1i, 2i: R1 = R2 = Methoxy

Reagents and conditions: (a) anhydrous $AlCl_3$, room temperature; (b) absolute alcohol, reflux.

Scheme 1. Synthesis of 4-arylphthalazones bearing benzenesulfonamide, **2a–i**.

(**1a–i**) with 4-hydrazinobenzenesulfonamide in ethanol (Scheme 1). The intermediates (**1a–i**) were prepared by Friedel–Crafts method wherein the appropriate aromatic hydrocarbon was reacted with phthalic anhydride using anhydrous $AlCl_3$ as catalyst in nitrobenzene [34, 35]. A literature survey revealed that **2a**, **2g**, and **2i** were registered with CAS numbers 847734-10-5, 735324-06-8, and 852904-89-3, respectively. But no reference was

available for work on its method of synthesis and biological activity.

The structures of the synthesized compounds were confirmed by elemental analysis and spectral data (IR, ^{1}H NMR, ^{13}C NMR and mass). Elemental analysis (C, H, N and S) data were within ±0.4% of the theoretical values. In the IR spectra of **2a–i**, six bands characteristic of the phthalazone moiety out of which two bands for NH (3339–3162 cm^{-1}, 3173–3052 cm^{-1}), one band for the carbonyl group of the phthalazone moiety at 1714–1635 cm^{-1}, one band for C=N of the phthalazone ring (1591–1520 cm^{-1}) and two bands for SO_2N (1380–1338 cm^{-1} and 1191–1143 cm^{-1}) were observed. In the ^{1}H NMR spectra of **2a–i** a two-proton singlet for $-SO_2NH_2$ was observed at δ 4.93–7.47. Phthalazinone, 4-aryl and *N*-phenyl ring protons appeared in the aromatic region (δ 7.10–8.63).

Biological results and discussion

The *in vivo* anti-inflammatory activities for all synthesized compounds **2a–i** (at 0.05 mmol/kg dose) were evaluated using carrageenan-induced rat paw edema model by adopting the reported method of Winter et al. [36]. These derivatives (**2a–i**) showed mild to strong anti-inflammatory activity (inhibition percentages are 20.0–85.8% and 36.4–85.8% at 3 and 5 h, respectively). Among them, two compounds **2b** and **2i** have significant activity, which is comparable to that of celecoxib (Table 1). Introduction of chloro or phenyl group at *para* position (C-4′) of phenyl ring attached with C-4 of phthalazinone nucleus leads to significant increase in the activity at 3 h (**2a** vs. **2d** and **2a** vs. **2f**, respectively). On the other hand, introduction of bromo, ethyl or phenoxy group at C-4′ leads to significant reduction in the activity (**2a** vs. **2c**, **2a** vs. **2e**, **2a** vs. **2h**, respectively). Introduction of methyl (at C-4′) or two methoxyl groups (at C-4′ and C-3′) significantly influenced the anti-inflammatory activity (**2a** vs. **2b**, **2a** vs. **2i**) at both study times 3 and 5 h.

Two compounds (**2b** and **2i**) showing maximum anti-inflammatory activity were evaluated for their ulcerogenic potential in rats according to Daidone et al. [37]. The compounds (**2b** and **2i**) did not show any ulceration or harmful effects on the stomach at a dose of 0.25 mmol/kg po, when administered twice at 2 h interval in fasted rats [32].

These two compounds (**2b** and **2i**) were also evaluated for their ability to inhibit COX-2 and COX-1 by *in vitro* colorimetric COX (ovine) inhibitor assay method [38], which utilizes the peroxidase component of cyclooxygenase. The peroxidase activity is assayed colorimetrically by monitoring the appearance of oxidized *N*,*N*,*N*′*N*′-tetramethyl-*p*-phenylene diamine (TMPD) during the reduction of PGG_2 to PGH_2, at 590 nm. The IC_{50} values for **2b** and **2i** were calculated using non-linear regression analysis. The results of the *in vitro* COX enzyme inhibition assay are summarized in Table 2. The results showed that both compounds (**2b** and **2i**) exhibited effective inhibition against COX-2, compared to the inhibition of COX-1. To assess the selectivity profiles of the tested compounds, their approximate selectivity ratios (COX-1/COX-2) were compared to that of the standard COX-2 selective inhibitor, celecoxib. The compound **2b** showed better selectivity compared to that of celecoxib.

The structures of the synthesized compounds (**2a–i**) were submitted to the National Cancer Institute (NCI), Bethesda, Maryland, USA, for their anti-proliferative activity. Only two compounds (**2d** and **2i**) were selected by NCI on the basis of degree of structural variation and computer modeling techniques. These compounds (**2d** and **2i**) were granted NSC codes, viz. NSC 750151 and NSC 750152, respectively. The selected compounds were subjected to *in vitro* anticancer assay against tumor cells in a full panel of 60 cell lines taken from nine different tissues (blood, lung, colon, CNS, skin, ovary, kidney, prostate, and breast) at a concentration

Table 1. Effect of phthalazone derivatives (**2a–i**) at 0.05 mmol/kg in carageenan-induced rat paw edema assay.

Treatment	Increase in paw volume (mL) after carageenan administration[a)]	
	3 h	5 h
Vehicle	0.325 ± 0.05	0.425 ± 0.05
Celecoxib	0.06 ± 0.02 (81.5%)[b)]	0.083 ± 0.02 (80.4%)
2a	0.13 ± 0.03 (60.0%)	0.16 ± 0.03 (62.3%)
2b	0.05 ± 0.02 (84.6%)	0.06 ± 0.02 (85.8%)
2c	0.18 ± 0.05 (44.6%)	0.18 ± 0.05 (57.6%)
2d	0.07 ± 0.02 (78.4%)	0.13 ± 0.03 (69.4%)
2e	0.20 ± 0.06 (38.4%)	0.27 ± 0.07 (36.4%)
2f	0.07 ± 0.07 (78.4%)	0.15 ± 0.05 (64.7%)
2g	0.12 ± 0.06 (63.0%)	0.17 ± 0.05 (60.0%)
2h	0.26 ± 0.06 (20.0%)	0.22 ± 0.02 (48.2%)
2i	0.046 ± 0.025 (85.8%)	0.08 ± 0.04 (81.1%)

All data are significantly different compared to respective control values, $p < 0.05$.
[a)] Values are expressed as mean ± SEM and analyzed by ANOVA.
[b)] Values in parenthesis (percent inhibition).

Table 2. *In vitro* COX enzyme inhibition data for compounds **2b** and **2i**.

Compound	IC50 (μM)[a)]		Selectivity index
	COX-1	COX-2	COX-1/COX-2
2b	6.0	0.018	333.33
2i	7.0	0.068	102.94
Celecoxib	6.5	0.020	325.00

[a)] Values are acquired using an ovine COXs assay kit (Catalog No. 760111, Cyman Chemical Inc., Ann Arbor, MI). Experiments were carried out in duplicate and have <10% error.

Table 3. Anticancer screening data for **2d** and **2i** on 60 cell lines at a concentration of 10^{-5} M.

Compound	Code	Mean growth (%)	Most sensitive cell line	Growth of most sensitive cell line (%)
2d	NSC: 750151	97.72	UO-31 (renal cancer)	52.56
2i	NSC: 750152	102.57	UO-31 (renal cancer)	58.88

of 10^{-5} M. These two compounds **2d** and **2i** displayed mild sensitivity towards the renal cancer cell line UO-31 (Table 3).

Conclusion

Cyclocondensation of 4-hydrazinobenzenesulfonamide hydrochloride with aroyl benzoic acids yielded respective phthalazone derivatives (**2a–i**). The structures proposed to the synthesized compounds (**2a–i**) are well supported by elemental analysis and spectroscopic data (IR, ^{1}H NMR, ^{13}C NMR and mass). Two compounds **2b** and **2i** showed significant anti-inflammatory activity, which is comparable to that of the standard drug celecoxib. These compounds (**2b** and **2i**) had selective inhibitory activity towards the COX-2 enzyme. One of these derivatives (**2b**) had a better selectivity ratio (COX-1/COX-2) compared to that of celecoxib and can be used as a new template for the design of selective COX-2 inhibitors. The compounds **2d** and **2i** displayed mild sensitivity toward the renal cancer cell line UO-31.

Experimental

Chemistry

Melting points were determined by open capillary tubes and are uncorrected. All the Fourier transform infrared (FTIR) spectra were recorded on a Bruker Vector 22 spectrophotometer in KBr; υ_{max} values are given in cm^{-1}. ^{1}H NMR spectra were recorded on a Bruker Spectrospin DPX 300 MHz/400 MHz spectrometer using deuterated DMSO or deuterated chloroform as solvent and tetramethyl silane (TMS) as an internal standard. Chemical shifts are given in δ (ppm) scale and coupling constants (*J*-values) are expressed in Hz. Mass spectra (MS) were scanned by ESI Bruker Esquire 3000. The *m*/*z* values of the more intense peaks are mentioned. ^{13}C NMR spectra were recorded on Bruker spectrospin DPX 300 MHz using deuterated DMSO or deuterated chloroform as solvent and TMS as internal standard. Purity of the compounds was checked on TLC plates (silica gel G), which were visualized by exposing to iodine vapors. Elemental analysis was carried out on CHNS Elementar (Vario EL III).

General procedure for the synthesis of aroyl benzoic acids (1a–i)

To a slurry of anhydrous aluminum trichloride (13.2 g, 0.1 moles) in nitrobenzene (30 mL), liquid aromatic hydrocarbon (0.05 moles) was added in portions while stirring on magnetic stirrer at room temperature for 30 min to obtain a colored complex. To it, phthalic anhydride (4.9 g, 0.05 moles) was added in five portions with continuous stirring. Vigorous reaction started with evolution of HCl gas. Stirring was further continued for 6 h at room temperature. It was left at room temperature for 48 h and then decomposed by adding ice-cold hydrochloric acid (50%, 100 mL). The excess solvent was removed by steam distillation. It was allowed to cool to room temperature. The crude product was dissolved in 5% sodium carbonate solution and was then filtered. The filtrate so obtained was acidified with dilute HCl to give a precipitate. It was then filtered, washed with cold water, dried and crystallized from ethyl alcohol to give 2-aroylbenzoic acids (**1a–i**). The melting point of the compounds (**1a–i**) was compared to those already mentioned in the literature [34, 35].

General procedure for the preparation of 4-arylphthalazone derivatives (2a–i)

On complete dissolution of 4-hydrazinobenzenesulfonamide hydrochloride (0.001 mole) in absolute ethanol (25 mL), desired 2-aroylbenzoic acid was added (0.001 mole) and the mixture was heated to reflux for 18–24 h. The volume was reduced to half by evaporating the solvent and left at room temperature till the solid product separated out. It was filtered and then converted into fine powder, which was stirred with 5% sodium bicarbonate solution (25 mL). It was filtered, washed with 2% HCl and then with water. It was dried and crystallized with appropriate solvent to give TLC pure compound.

*4-(1-Oxo-4-phenylphthalazin-2(1H)-yl)-benzenesulfonamide **2a***

Yield = 53%, m.p. 262–263°C, Rf = 0.67 (toluene/ethyl acetate/formic acid, 5:4:1). IR υ_{max} (KBr): 3303 cm^{-1} and 3127 cm^{-1} (NH_2), 1691 cm^{-1} (C=O), 1552 cm^{-1} (C=N), 1343 cm^{-1} and 1173 cm^{-1} (SO_2N). ^{1}H NMR (400 MHz, DMSO, δ): 6.89 (2H, s, SO_2NH_2), 7.46–7.56 (5H, m, H-2′, H-3′, H-4′, H-5′, H-6′), 7.71–7.79 (3H, m, H-6, H-7, H-8), 7.83–7.95 (4H, m, *N*-phenyl protons), 8.46–8.48 (1H, m, H-5). ^{13}C NMR (300 MHz, DMSO, δ): 126.1, 126.3, 126.9, 127.1, 128.0, 128.5, 128.6, 129.4, 129.5, 132.4, 134.2, 134.4, 142.7, 144.2, 147.4, 158.1. ESI-MS (*m*/*z*): 376 [M−1], 400 [M+Na]. Anal. calcd. for $C_{20}H_{15}N_3O_3S$: C 63.66, H 4.01, N 11.13, S 8.50. Found: C 63.63, H 4.00, N 11.09, S 8.53.

*4-[4-(4-Methylphenyl)-1-oxophthalazin-2(1H)-yl]-benzenesulfonamide **2b***

Yield = 50%, m.p. 210–211°C, Rf = 0.70 (toluene/ethyl acetate/formic acid, 5:4:1). IR υ_{max} (KBr): 3277 cm^{-1} and 3052 cm^{-1} (NH_2), 1656 cm^{-1} (C=O), 1520 cm^{-1} (C=N), 1374 cm^{-1} and 1143 cm^{-1} (SO_2N). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 2.31 (3H, s, CH_3), 6.42 (2H, s, SO_2NH_2), 7.19 (2H, d, J = 7.8 Hz, H-3′, H-5′), 7.38 (2H, d, J = 7.8 Hz, H-2′, H-6′), 7.67–7.71 (3H, m, H-6, H-7, H-8), 7.79 (2H, d, J = 8.7 Hz, H-3″, H-5″), 7.87 (2H, d, J = 9.0 Hz, H-2‘‘, H-6″), 8.41–8.43 (1H, m, H-5). ESI-MS (*m*/*z*): 390 [M−1], 414

[M+Na]. Anal. calcd. for $C_{21}H_{17}N_3O_3S$: C 64.43, H 4.38, N 10.73, S 8.19. Found: C 64.32, H 4.42, N 10.89, S 8.12.

4-[4-(4-Ethylphenyl)-1-oxophthalazin-2(1H)-yl]-benzenesulfonamide ***2c***

Yield = 30.5%, m.p. 214–215°C, Rf = 0.78 (toluene/ethyl acetate/formic acid, 5:4:1). IR υ_{max} (KBr): 3217 cm^{-1} and 3136 cm^{-1} (NH_2), 1635 cm^{-1} (C=O), 1583 cm^{-1} (C=N), 1338 cm^{-1} and 1159 cm^{-1} (SO_2N). ^1H NMR (300 MHz, $CDCl_3$, δ): 1.31 (3H, t, CH_3), 2.76 (2H, q, CH_2), 5.05 (2H, s, SO_2NH_2), 7.37 (2H, d, J = 8.1 Hz, H-3′, H-5′), 7.56 (2H, d, J = 8.1 Hz, H-2′, H-6′), 7.80 (3H, m, H-6, H-7, H-8), 7.98–8.02 (4H, m, *N*-phenyl protons), 8.59–8.62 (1H, m, H-5). ESI-MS (*m*/*z*): 404 [M−1], 428 [M+Na]. Anal. calcd. for $C_{22}H_{19}N_3O_3S$: C 65.17, H 4.72, N 10.36, S 7.91. Found: C 65.09, H 4.81, N 10.42, S 7.89.

4-[4-(4-Chlorophenyl)-1-oxophthalazin-2(1H)-yl]-benzenesulfonamide ***2d***

Yield = 50.1%, m.p. 242–243°C, Rf = 0.77 (toluene/ethyl acetate/formic acid, 5:4:1). IR υ_{max} (KBr): 3315 cm^{-1} and 3123 cm^{-1} (NH_2), 1695 cm^{-1} (C=O), 1591 cm^{-1} (C=N), 1372 cm^{-1} and 1192 cm^{-1} (SO_2N). ^1H NMR (300 MHz, DMSO, δ): 7.46 (2H, s, SO_2NH_2), 7.53–7.76 (4H, m, H-2′, H-3′, H-5′, H-6′), 7.89–7.99 (7H, m, *N*-phenyl protons, H-6, H-7, H-8), 8.47 (1H, m, H-5). ^{13}C NMR (300 MHz, DMSO, δ): 126.2, 126.3, 126.8, 127.1, 128.0, 128.3, 128.7, 131.4, 132.5, 133.3, 134.3, 142.8, 144.1, 146.4, 158.1. ESI-MS (*m*/*z*): 410 [M−1]. Anal. calcd. for $C_{20}H_{14}ClN_3O_3S$: C 58.32, H 3.43, N 10.20, S 7.79. Found: C 58.23, H 3.49, N 10.14, S 7.73.

4-[4-(4-Bromophenyl)-1-oxophthalazin-2(1H)-yl]-benzenesulfonamide ***2e***

Yield = 35.1%, m.p. 224–225°C, Rf = 0.57 (toluene/ethyl acetate/formic acid, 5:4:1). IR υ_{max} (KBr): 3337 cm^{-1} and 3123 cm^{-1} (NH_2), 1651 cm^{-1} (C=O), 1579 cm^{-1} (C=N), 1376 cm^{-1} and 1161 cm^{-1} (SO_2N). ^1H NMR (400 MHz, DMSO, δ): 7.19 (2H, s, SO_2NH_2), 7.46–7.63 (4H, m, H-2′, H-3′, H-5′ H-6′), 7.81–7.85 (5H, m, H-3″, H-5″, H-6, H-7, H-8), 7.91 (2H, d, J = 8.4 Hz, H-2″, H-6″), 8.42–8.44 (1H, m, H-5). ^{13}C NMR (300 MHz, DMSO, δ): 123.0, 126.2, 126.3, 127.1, 128.0, 128.7, 129.5, 131.7, 132.5, 133.7, 134.4, 142.8, 144.2, 146.4, 147.5, 158.1. ESI-MS (*m*/*z*): 453 [M−2], 478 [M+Na]. Anal. calcd. for $C_{20}H_{14}BrN_3O_3S$: C 52.64, H 3.09, N 9.21, S 7.03. Found: C 52.59, H 3.13, N 9.19, S 7.10.

4-[4-Biphenyl-1-oxophthalazin-2(1H)-yl]-benzenesulfonamide ***2f***

Yield = 50.1%, m.p. 255–256°C, Rf = 0.73 (toluene/ethyl acetate/formic acid, 5:4:1). IR υ_{max} (KBr): 3162 cm^{-1} (NH_2), 1652 cm^{-1} (C=O), 1583 cm^{-1} (C=N), 1341 cm^{-1} and 1161 cm^{-1} (SO_2N). ^1H NMR (400 MHz, DMSO, δ): 7.04 (2H, s, SO_2NH_2), 7.28–7.31 (1H, m, H-4‴), 7.37–7.41 (2H, m, H-3‴, H-5‴), 7.58 (2H, d, J = 7.6 Hz, H-2‴, H-6‴), 7.63–7.70 (4H, m, H-2′, H-3′, H-5′, H-6′), 7.79–7.80 (3H, m, H-6, H-7, H-8), 7.84 (2H, d, J = 8.8 Hz, H-2″, H-6″), 7.92 (2H, d, J = 8.4 Hz, H-3″, H-5″), 8.45–8.46 (1H, m, H-5). ESI-MS (*m*/*z*): 452 [M−1], 476 [M+Na]. Anal. calcd. for $C_{26}H_{19}N_3O_3S$: C 68.86, H 4.22, N 9.27, S 7.07. Found: C 68.95, H 4.13, N 9.19, S 7.15.

4-[4-(4-Methoxyphenyl)-1-oxophthalazin-2(1H)-yl]-benzenesulfonamide ***2g***

Yield = 19.1%, m.p. 248–249°C, Rf = 0.67 (toluene/ethyl acetate/formic acid, 5:4:1). IR υ_{max} (KBr): 3320 cm^{-1} and 3130 cm^{-1} (NH_2), 1689 cm^{-1} (C=O), 1529 cm^{-1} (C=N), 1367 cm^{-1} and 1191 cm^{-1} (SO_2N), 1046 cm^{-1} (OCH_3). ^1H NMR (300 MHz, DMSO, δ): 3.85 (3H, s, OCH_3), 7.13 (2H, d, J = 8.4 Hz, H-3′, H-5′), 7.46 (2H, s, SO_2NH_2), 7.64 (2H, d, J = 8.4 Hz, H-2′, H-6′), 7.79–7.99 (7H, m, H-2″, H-3″, H-5″, H-6″, H-6, H-7, H-8), 8.45–8.47 (1H, m, H-5). ^{13}C NMR (300 MHz, DMSO, δ): 54.4, 113.1, 124.7, 125.5, 125.9, 126.1, 126.5, 127.6, 128.0, 129.8, 130.9, 132.6, 141.4, 143.6, 146.9, 157.7, 159.4. ESI-MS (*m*/*z*): 406 [M−1], 430 [M+Na]. Anal. calcd. for $C_{21}H_{17}N_3O_4S$: C 61.90, H 4.21, N 10.31, S 7.87. Found: C 61.82, H 4.31, N 10.29, S 7.98.

4-[4-(4-Phenoxyphenyl)-1-oxophthalazin-2(1H)-yl]-benzenesulfonamide ***2h***

Yield = 30.1%, m.p. 185–186°C, Rf = 0.75 (toluene/ethyl acetate/formic acid, 5:4:1). IR υ_{max} (KBr): 3336 cm^{-1} and 3127 cm^{-1} (NH_2), 1691 cm^{-1} (C=O), 1533 cm^{-1} (C=N), 1380 cm^{-1} and 1180 cm^{-1} (SO_2N). ^1H NMR (400 MHz, $CDCl_3$, δ): 4.93 (2H, s, SO_2NH_2), 7.11–7.21 (5H, m, H-3′, H-5′, H-2‴, H-5‴ and H-4‴), 7.39–7.43 (2H, m, H-3‴, H-5‴), 7.61 (2H, d, J = 8.4 Hz, H-2′, H-6′), 7.85–7.90 (3H, m, H-6, H-7 and H-8), 8.00–8.06 (4H, m, *N*-phenyl protons), 8.61–8.63 (1H, m, H-5). ^{13}C NMR (300 MHz, DMSO, δ): 117.6, 118.8, 123.4, 125.1, 125.9, 126.4, 127.0, 127.9, 128.2, 128.5, 129.3, 130.4, 131.3, 133.0, 141.6, 143.9, 147.0, 155.5, 157.9, 158.1. FAB-MS (*m*/*z*): 492 [M+Na], 300 [M−Ph−O−Ph]. Anal. calcd. for $C_{26}H_{19}N_3O_4S$: C 66.51, H 4.08, N 8.95, S 6.83. Found: C 66.45, H 4.12, N 8.89, S 6.90.

4-[4-(3,4-Dimethoxyphenyl)-1-oxophthalazin-2(1H)-yl]-benzenesulfonamide ***2i***

Yield = 30.1%, m.p. 206–207°C, Rf = 0.54 (toluene/ethyl acetate/formic acid, 5:4:1). IR υ_{max} (KBr): 3339 cm^{-1} and 3173 cm^{-1} (NH_2), 1714 cm^{-1} (C=O), 1533 cm^{-1} (C=N), 1362 cm^{-1}, 1157 cm^{-1} (SO_2N) and 1049 cm^{-1} (OCH_3). ^1H NMR (300 MHz, DMSO, δ): 3.78 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 7.10–7.23 (3H, m, H-2′, H-5′, H-6′), 7.47 (2H, s, SO_2NH_2), 7.81–7.96 (7H, m, *N*-phenyl protons, H-6, H-7, H-8), 8.42–8.45 (1H, m, H-5). ^{13}C NMR (300 MHz, DMSO, δ): 55.6, 55.7, 111.6, 112.9, 122.2, 126.1, 126.3, 126.8, 127.0, 127.2, 127.9, 128.7, 132.3, 134.2, 142.7, 144.2, 147.4, 148.6, 149.7, 158.1. ESI-MS (*m*/*z*): 436 [M−1]. Anal. calcd. for $C_{22}H_{19}N_3O_5S$: C 60.40, H 4.38, N 9.61, S 7.33. Found: C 60.25, H 4.45, N 9.55, S 7.27.

Pharmacology

Anti-inflammatory activity

In the present work, rats of the Wistar strain (either sex) obtained from the central animal house of the university were used. 1% Tween-10 in distilled water was used as vehicle for dosing (10 mL/kg). All the treatments suspended in vehicle were given orally.

The experiment was conducted 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 on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Govt. of India.

The experiment was performed by the method of Winter *et al.* [36]. Overnight fasted rats (150–200 g) were taken and divided into groups of six animals each. Animals of group-I were administered orally with vehicle (1% Tween-10, 10 mL/kg) and served as control. Group II animals were fed with celecoxib (0.05 mmol/kg b.w.) and served as standard. Test groups were administered orally with respective test drugs (**2a–i**) in the dose of 0.05 mmol/kg b.w. After 30 min, all animals were injected with 0.1 mL of 1% carageenan solution (prepared in normal saline) in the sub plantar aponeurosis of left hind paw and the paw volume was measured by using a plethysmometer at 0, 3, and 5 h post-carageenan treatment.

The average paw volume in a group of treated rats was compared with vehicle (control group) and the percentage inhibition of edema was calculated by using the formula:

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

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.

Ulcerogenic activity

Two groups of six rats each weighing 180–200 g and fasted for 24 h were used. The compounds **2a** and **2i** were given orally at a dose of 0.25 mmol/ kg po, and administered twice at 2 h interval. Rats were sacrificed by ether inhalation 6 h after the first dose. Their stomachs were removed, opened along the greater curvature and examined for the presence of gastric ulcers or hyperemia [37].

In vitro cylcooxygenase inhibition studies

Two compounds (**2b** and **2i**) were tested for their ability to inhibit *in vitro* COX-1 and COX-2 using a colorimetric COX (ovine) inhibitor screening kit (Catalog No. 760 111, Cayman Chemicals Inc Ann Arbor, MI, USA) using the previously established method [38].

In vitro anticancer activity

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 [39–45]. The human tumor cell lines were derived from nine different cancer types: leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate, and breast cancers.

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The authors have declared no conflict of interest.

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