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Full Paper

Synthesis and Biological Evaluation of New Phthalazinone Derivatives as Anti-Inflammatory and Anti-Proliferative Agents

Alhamzah Dh. Hameed[1], Syed Ovais[1], Raed Yaseen[1], Pooja Rathore[1], Mohammed Samim[1], Surender Singh[2], Kalicharan Sharma[3], Mymona Akhtar[3], and Kalim Javed[1]

[1] Department of Chemistry, Faculty of Science, Jamia Hamdard (Hamdard University), New Delhi, India
[2] Department of Pharmacology, All India Institute of Medical Science, New Delhi, India
[3] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Drug Design and Medicinal Chemistry Laboratory, Jamia Hamdard (Hamdard University), New Delhi, India

The chemistry of phthalazine derivatives has been of increasing interest since many of these compounds have found many chemotherapeutic applications. So this study aims to synthesize a library of phthalazine derivatives and to investigate their anti-inflammatory and anti-proliferative activities. Sixteen new phthalazinone derivatives (**2a–p**) were synthesized and tested for their *in vitro* antiproliferative and *in vivo* anti-inflammatory activities. All the synthesized compounds were identified and characterized by IR, ^{1}H NMR, ^{13}C NMR spectroscopy, and MS. Two compounds, **2b** and **2i**, showed significant anti-inflammatory activity comparable with that of the standard drug etoricoxib in the carrageenan-induced rat paw edema model at 3 and 5 h, respectively. Three compounds (**2h**, **2j**, and **2g**) showed moderate sensitivity toward the renal cancer cell line UO-31.

Keywords: Anti-inflammatory activity / Anti-proliferative activity / COX-2 / LOX-5 inhibitors / Phthalazone

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Introduction

Cyclooxygenase (COX) and lipoxygenase (LOX) produce two groups of arachidonic acid (ARA) metabolites, prostaglandins (PGs) and leucotrienes (LTs), that play a key role in inflammation. Traditional non-steroidal anti-inflammatory drugs (NSAIDs) act via the inhibition of the COX-1 isoenzyme or the combined inhibition of COX-1 and COX-2 isoenzymes. For example, acetylsalicylate (aspirin) is a COX-1 selective inhibitor, whereas indomethacin (indocin) and naproxen (naprosyn) are COX-1/COX-2 inhibitors. Because COX-1 is mainly responsible for mucus formation in the gastrointestinal (GI) tract, COX-1 inhibition is blamed for inducing GI irritation, the main undesired side effect of such drugs [1]. Another side effect of selective COX-1 inhibitors, mild bleeding diathesis, also results from the inhibition of the COX-1 catalyzed synthesis of the platelet aggregation factor, thromboxan (TXA2) [2].

Because COX-2 isoenzyme was found to be overexpressed during inflammation, drug investigation was focused on selective COX-2 inhibition, hoping to prevent inflammation by sidestepping the undesired side effect of COX-1 inhibitors [3, 4]. Consequently, selective COX-2 inhibitors (coxibs) based on a diarylheterocyclic ring template were developed. These agents were characterized by the presence of a *para*-sulfonamide (SO_2NH_2) or a *para*-methanesulfonyl (SO_2Me) pharmacophore present on one of the aryl rings. Crystal structure studies supported the hypothesis that the *p*-SO_2NH_2 or *p*-SO_2Me pharmacophore was conferring COX-2 selectivity by orienting in a secondary pocket accessible only in the COX-2 active site [5, 6]. However, these drugs (COX-2

Correspondence: Dr. Kalim Javed, Department of Chemistry, Faculty of Science, Jamia Hamdard (Hamdard University), New Delhi 110 062, India.
E-mail: kjaved@jamiahamdard.ac.in, kjavedchem@yahoo.co.in
Fax: +91 011 26059663

inhibitors) were associated with increased risk of myocardial infarction and cardiovascular thrombotic events. The thrombotic effect of these drugs seems to be due to the decrease of the level of the prostacyclin (PGI_2), a molecule with vasodilatory and antiaggregatory properties, synthesized via the action of COX-2. In addition, COX-2 was found to exhibit a protective role in asthma [7] and GI tract irritation.

Leukotrienes (LTs) are synthesized via 5-LOX pathway and are involved in the pathogenesis of bronchial asthma and edema formation [8]. It is also believed that they play a role in the damage of gastric mucosa [9–11]. The inhibition of this metabolic pathway led to the development of new therapeutic treatments for pathologies such as asthma, allergies, and other inflammatory disorders.

The failure of COX-1 and COX-2 selective inhibitors evoked the concept that inflammation should be considered as a multifactorial process and all biochemical pathways should be taken into account, including the LOX pathway. Compounds that combine COX and LOX inhibition may present multiple advantages because they synergistically block metabolic pathways of arachidonic acid cascade and thrombosis and possess a wide range of anti-inflammatory activities [12].

In addition, both COX-2 and 5-LOX enzymes have been involved in the development and progression of numerous types of cancer [13–19]. So, the use of dual inhibitors opens up new perspectives in the prophylactic treatment of this dreadful disease.

Phthalazine derivatives have been attracting increasing attention over the years because of their broad spectrum biological activities and strong efficacy. Phthalazines form the structural profile for several biologically active compounds and hence they are considered important key compounds (Fig. 1). These derivatives have been reported to possess antihypertensive [20, 21], anticonvulsant [22, 23], antidiabetic [24, 25], antimicrobial [26], antitrypanosomal [27], anti-inflammatory [28–30], and PDE4 inhibitory activities [31].

The phthalazinone nucleus has been proven to be a versatile system in medicinal chemistry. Some of the phthalazinone derivatives like hydralazine [32], budralazine [33], azelastine [34], ponalrestat [35], and zopolrestat [36] have found application in clinical medicine. Azelastine has also been reported to have moderate 5-LOX inhibitory activity in intact murine peritoneal cells and in chopped guinea pig liver [37].

In view of the aforementioned facts, it was thought worthwhile to incorporate the *para*-methanesulfonylphenyl moiety at the 2 position of some 4-arylphthalazone derivatives in the hope to yield safe and potent compounds with anti-inflammatory and anticancer potential. Therefore, this paper describes the synthesis, *in vivo* anti-inflammatory and *in vitro* COX, 5-LOX, and anti-proliferative activities of some new 4-arylphthalazone compounds (**2a–p**). Compounds showing maximum anti-inflammatory activity were also evaluated for ulcerogenic potential in rats.

Results and discussion

Chemistry

The synthetic path which is used to synthesize the desired compounds (**2a–p**) is outlined in Scheme 1. The β-aroylbenzoic acids (**1a–p**) which are required for the synthesis of phthalazinones derivatives were obtained from Friedel–Crafts acylation through reported method wherein appropriate aromatic hydrocarbon was mixed with phthalic anhydride using anhydrous $AlCl_3$ as catalyst in nitrobenzene. The cyclization to the phthalazinone derivatives bearing *p*-(methanesulfonyl)phenyl moiety was afforded by the condensation of appropriate aroylbenzoic acid (**1a–p**) and *p*-(methanesulfonyl)phenylhydrazine in absolute alcohol.

The structures of the synthesized compounds were determined on the basis of elemental analysis and by various spectroscopic methods such as 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. IR spectra of the compounds **2a–p** showed four bands characteristic of phthalazone moiety out of which one band for C=O of phthalazone moiety at 1718–1648 cm^{-1}, one band for C=N of phthalazone ring (1584–1496 cm^{-1}), and two bands for SO_2C (1342–1310 and 1168–1142 cm^{-1}) were observed. In the ^{1}H NMR spectra of **2a–p** a three-proton singlet for $–SO_2CH_3$ was observed in a range of δ 3.20–3.24. Phthalazinone, 4-aryl, and *N*-phenyl ring protons appeared in aromatic region (δ 7.22–8.68).

Pharmacology

The *in vivo* anti-inflammatory activity of all synthesized compounds **2a–p** (0.05 mmol/kg) doses was evaluated by using carrageenan-induced rat paw edema bioassay by the method of Winter et al. [38]. Results were compared with etoricoxib as it has some structural resemblance with the synthesized compounds. Two compounds (**2b** and **2i**) exhibited significant anti-inflammatory activity and therefore, they were further evaluated for their ulcerogenic potential in rats according to Daidone et al. [39]. An *in vitro* anti-proliferative assay of **2h**, **2j**, and **2g** was performed using a full panel of about 60 human tumor cell lines, with respect to the protocol of the Drug Evaluation Branch, National Cancer Institute (NCI), Bethesda [40–42]. Compounds **2b**, **2i**, **2j**, and **2k** were also evaluated for their ability to inhibit COX-2 and 5-LOX by *in vitro* an enzyme immuno-assay and an enzyme immune according to previously reported methods [43, 44], respectively.

Anti-inflammatory activity

Phthalazinone derivatives **2a–p** showed moderate to strong anti-inflammatory activity (20.27–65.96 and 23.52–69.32% at 3 and 5 h, respectively). Among them, two compounds **2b** (66.97 and 68.16) and **2i** (65.96 and 69.32) have higher anti-inflammatory activity compared to that of standard drug etoricoxib (64.69 and 67.14) (Table 1). With regard to structural–activity relationship (SAR), it was observed that introduction of chlorine atom at (C-4′) position of

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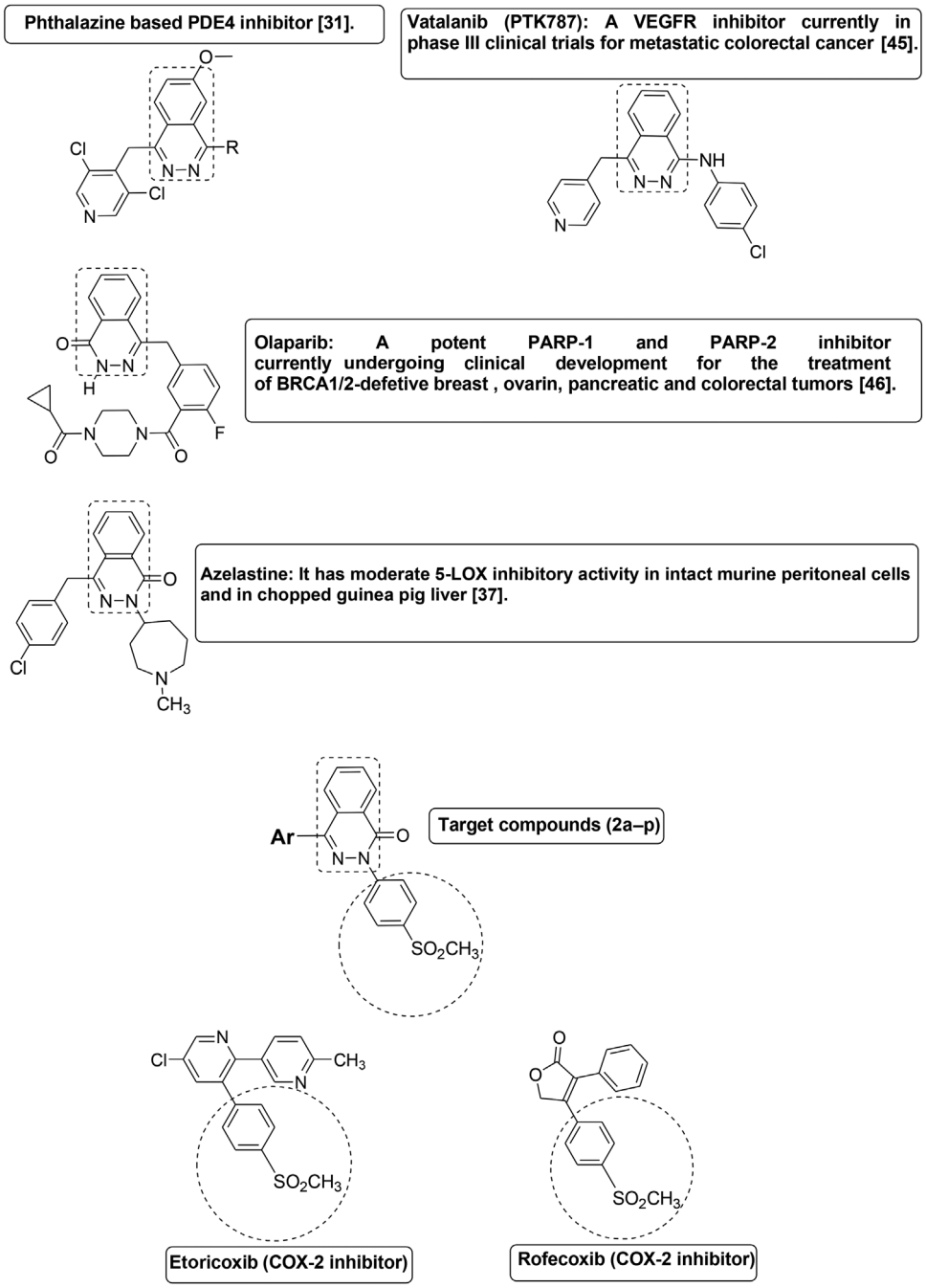
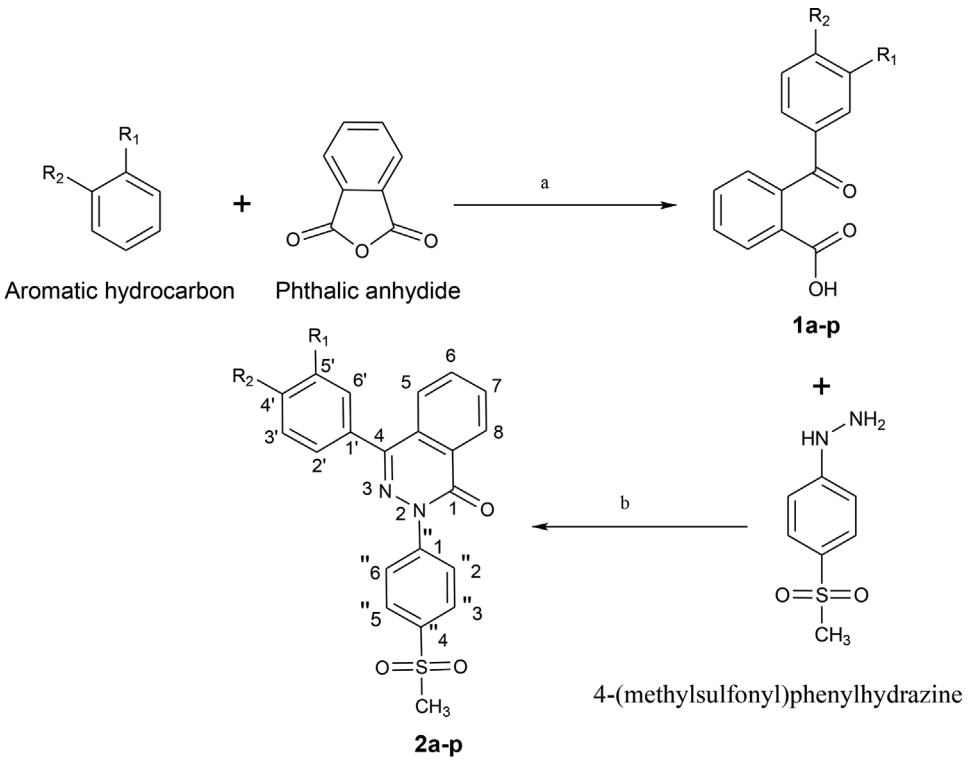


Figure 1. Structures of some biologically active pharmacophores and the target compounds. See Refs. [37, 45, 46] for further information on azelastine, vatalanib, and olaparib, respectively.

phthalazinone nucleus increases the activity whereas introduction of bromine and fluorine atoms at the same position decreases the activity at both 3 and 5 h (**2f** vs. **2g** and **2f** vs. **2h**, respectively). Introduction of biphenyl group showed better activity when compared to the biphenyl ether (**2b** vs. **2j**) and introduction of hexyl group showed increase in activity comparing to butyl, *n*-propyl, and *tert*-butyl groups (**2m** vs. **2p** and **2m** vs. **2e** and **2m** vs. **2l**, respectively). Tetralin-substituted compound showed significant activity as compared to other compounds at 3 and 5 h.



1a,2a: $R_1 = R_2 = H$

1b,2b: $R_1 = H$, R_2 = Phenyl

1c,2c: R_1 and R_2 linked with $-CH_2-CH_2-CH_2-$

1d,2d: R_1 =H, R_2 =Isopropyl

1e,2e: $R_1 = H$, R_2 = Propyl

1f,2f: $R_1 = H$, R_2 = Chloro

1g,2g: $R_1 = H$, R_2 = Fluoro

1h,2h: $R_1 = H$, R_2 = Bromo

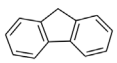
1i,2i: R_1 and R_2 linked with $-CH_2-CH_2-CH_2-CH_2-$

1j,2j: $R_1 = H$, R_2 = Phenoxy

1k,2k: R_1=F, R_2 =F

1l,2l: R_1 =H, R_2 =Tert-butyl

1m,2m: $R_1 = H$, R_2 = Hexyl

1n,2n: $R_1 = H$, R_2 = 

1o,2o: $R_1 = H$, R_2 = Ethyl

1p,2p: Ar = Butyl

Scheme 1. Synthesis of 4-arylphthalazones **2a–p**. Reagents and conditions: (a) anhydrous $AlCl_3$, room temperature; (b) absolute alcohol, reflux.

Ulcerogenic activity

The compounds **2b** and **2i** were found safe as they do not cause 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 [39].

In vitro cyclooxygenase/lipoxygenase inhibition studies

The ability of the test compounds to inhibit ovine COX-1 and human recombinant COX-2 were determined using an enzyme immune-assay according to previously reported method [43], while the ability of these compounds to inhibit potato 5-LOX were determined using an enzyme immune assay according to previously reported method [44]. The IC_{50} values were determined following the instruction given in the kit manual (Table 2).

Anti-proliferative activity

The structures of the phthalazinone products (**2a–p**) were submitted to National Cancer Institute (NCI), 20 Bethesda,

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Table 1. Effect of phthalazinone derivatives (2a–p) at 0.05 mmol/kg in carageenan-induced rats paw edema assay.

Treatment	Increase in paw volume (mL) after carageenan administration[a)]	
	3 h	5 h
Vehicle	1.62 ± 0.021	1.70 ± 0.023
Etoricoxib	0.707 ± 0.22 (64.69%)[b)]	0.657 ± 0.019 (67.14%)
2a	1.36 ± 0.019 (20.27%)	1.22 ± 0.020 (23.52%)
2b	0.643 ± 0.017 (64.97%)	0.59 ± 0.013 (68.16%)
2c	0.92 ± 0.012 (49.16%)	0.95 ± 0.024 (48.09%)
2d	1.16 ± 0.592 (34.52%)	1.11 ± 0.0145 (41.22%)
2e	1.22 ± 0.019 (28.34%)	1.12 ± 0.011 (37.38%)
2f	1.04 ± 0.024 (55.62%)	1.04 ± 0.024 (42.69%)
2g	1.036 ± 0.021 (42.06%)	0.27 ± 0.07 (36.40%)
2h	1.22 ± 0.019 (28.34%)	1.16 ± 0.014 (33.34%)
2i	0.661 ± 0.019 (65.96%)	0.572 ± 0.012 (69.32%)
2j	0.7145 ± 0.019 (61.12%)	0.716 ± 0.021 (58.19%)
2k	0.81 ± 0.022 (56.92%)	0.79 ± 0.012 (59.17%)
2l	1.18 ± 0.6146 (33.70%)	1.12 ± 0.018 (39.25%)
2m	0.98 ± 0.18 (47.03%)	1.04 ± 0.024 (43.59%)
2n	1.22 ± 0.018 (28.13%)	1.26 ± 0.020 (27.52%)
2o	1.29 ± 0.018 (25.22%)	1.31 ± 0.020 (23.42%)
2p	1.053 ± 0.026 (44.03%)	0.25 ± 0.04 (37.63%)

All data are markedly different compared to respective control values, $p < 0.05$.
[a)]Values are expressed as mean. [b)] Values in parenthesis (percent inhibitions).

Maryland, USA, and the three compounds **2h**, **2j**, and **2g** were selected on the basis of degree of structural variation and computer modeling techniques for evaluation of their antiproliferative activity. These compounds (**2h**, **2j**, and **2g**) were granted NSC codes, viz, NSC 776884, NSC 776885, and NSC 776886, respectively. The elected compounds were submitted to *in vitro* anticancer assay against tumor cells in a full panel of 59 or 60 cell lines taken from different tissues (blood, lung, colon, CNS, skin, ovary, kidney, prostate, and breast). The compounds were tested at a single dose concentration of 10 μM, and the percentages of growth inhibition over the 60 tested cell lines were determined [40–42]. The compounds **2h**, **2j**, and **2g** displayed mild sensitivity toward a renal cancer cell line UO-31 (Table 3) which is comparable to 5-fluorouracil (5-FU), a clinically used anticancer drug.

Conclusion

Condensation of *p*-(methanesulfonyl)phenylhydrazine with aroyl benzoic acids yielded the target compounds **2a–p**. The structures of the synthesized compounds (**2a–p**) are well supported by spectroscopic data (IR, ^{1}H NMR, ^{13}C NMR, and mass). Two compounds, **2b** and **2i**, showed significant anti-

Table 2. *In vitro* COX-1, COX-2, and 5-LOX enzyme inhibition assay data for compounds 2b, 2j, 2i, and 2k.

Compound	COX-1 IC_{50}[a)] (μM)	COX-2 IC_{50}[a)] (μM)	COX-2 selectivity index (COX-1 IC_{50}/COX-2 IC_{50})	5-LOX IC_{50}[a)] (μM)
2b	14.74	1.00	14.74	6.2
2j	12.30	0.78	15.76	5.3
2i	10.20	0.72	14.17	8.0
2k	20.30	0.34	59.7	6.0
Etoricoxib	21.10	0.18	116.5	–
Caffeic acid	–	–	–	3.5

Values were obtained using an enzyme inhibition assay (EIA) kit [catalog no. (560131 and 760700), respectively, Cyman Chemical Inc., Ann Arbor, MI].
[a)]IC_{50} are means of two determinations and the deviation from the mean is >10% of the mean value.

Table 3. Anticancer screening data for 2h, 2j, and 2g at concentration of 10^{-5} M.

Compound	Code	Mean growth (%)	Most sensitive cell line	Growth of most sensitive cell line (%)
2h	NSC: 776884	97.40	UO-31 (renal cancer)	67.89
2j	NSC: 776885	93.38	UO-31 (renal cancer)	61.40
2g	NSC: 776886	95.79	UO-31 (renal cancer)	65.44
5-Fluorouracil (5-FU)	NSC: 19893	43.22	UO-31 (renal cancer)	41.3

inflammatory activity which is comparable to that of standard drug etoricoxib. The data of these synthesized derivatives were subjected to NCI, USA for antiproliferative activity and three compounds, **2h**, **2j**, and **2g**, were selected. These compounds showed a moderate sensitivity toward renal cancer cell line UO-31. Compounds **2b** and **2i** can act as lead for the medicinal chemists to develop effective and safer anti-inflammatory drugs.

Experimental

Chemistry

Melting points were determined by open capillary tubes and are uncorrected. All the Fourier transform infra-red (FTIR) spectra were recorded on a Brukers Vector 22 spectrophotometer in film; υ_{max} values are given in cm^{-1}. ^1H NMR spectra were recorded on a Bruker Spectrospin DPX 300/400 MHz spectrometer using deuterated chloroform as solvent. Chemical shifts are given in δ (ppm) scale and coupling constants (*J*-values) are expressed in hertz. 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 chloroform as solvent. Purity of the compounds was checked on TLC plates (silica gel G) which were visualized by exposing to iodine vapors.

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

To liquid aromatic hydrocarbon (30 mL), anhydrous aluminum chloride (16.6 g, 0.125 mole) was added. It was stirred on magnetic stirrer at room temperature for 30 min to get a colored complex. To it, phthalic anhydride (5 g, 0.05 mole) 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 precipitated solid was dissolved in 5% sodium carbonate solution and was then filtered. The filtrate so obtained was acidified with dilute HCl to give solid mass. It was then filtered, washed with cold water, dried, and crystallized from appropriate solvent to give 2-aroylbenzoic acids.

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

A mixture of the appropriate aroylbenzoic acid (**1a–p**) (0.001 mole) and (*p*-(methanesulfonyl)phenylhydrazine) (0.001 mol) in absolute alcohol (20–30 mL) was refluxed for 18–22 h. The volume of the reaction mixture was reduced to one-third by evaporating the solvent and left at room temperature when a solid separated out. The crude product was filtered off and was stirred with 5% sodium bicarbonate solution (25 mL). It was filtered and washed with 5% HCl and then with water. The product was dried and crystallized from methanol or acetone to give pure compound (**2a–p**). The solvent system used for TLC was toluene/ethyl acetate/formic acid (5:4:1). Please see the Supporting Information for the InChI codes of compounds **2a–p**.

*2-[4-(Methylsulfonyl)phenyl]-4-phthalazin-1(2H)-one (**2a**)*

Yield = 58%, m.p. 217–219°C, Rf = 0.74 (toluene/ethyl acetate/formic acid, 5:4:1). IR υ_{max} (KBr): 1652 cm^{-1} (C=O), 1566 cm^{-1} (C=N), 1151 and 1325 cm^{-1} (SO_2C). ^1H NMR (400 MHz, $CDCl_3$, δ): 3.22 (3H, s, SO_2CH_3), 7.26–7.33 (2H, m, J = 7.2 Hz, H-3′, H-5′), 7.60–7.71 (2H, m, J = 7.8 Hz, H-2′, H-6′), 8.06–8.11 (4H, m, H-2″, H-3″, H-5″, H-6″), 7.84–7.91 (2H, m, H-6, H-7), 8.53–8.66 (1H, m, H-5), 7.75 (1H, m, H-8). ESI-MS (*m/z*): 377 [M+1]. Anal. calcd. for $C_{21}H_{16}N_2O_3S$: C 67.00, H 4.28, N 7.44, S 8.52. Found: C 67.13, H 4.25, N 7.41, S 8.55.

*4-(4-Biphenyl)-2-[4-(methylsulfonyl)phenyl]phthalazin-1(2H)-one (**2b**)*

Yield = 53%, m.p. 260–264°C, Rf = 0.68 (toluene/ethyl acetate/formic acid, 5:4:1). IR υ_{max} (KBr): 1688 cm^{-1} (C=O), 1558 cm^{-1} (C=N), 1148 and 1316 cm^{-1} (SO_2C). ^1H NMR (400 MHz, $CDCl_3$, δ): 3.21 (3H, s, SO_2CH_3), 7.45–7.52 (5H, m, H-2′, H-3′, H-4′, H-5′, H-6′), 7.76–7.79 (3H, m, H-6, H-7,H-8), 8.09–8.11 (4H, m, *N*-phenyl protons), 8.66 (1H, m, H-5). ^{13}C NMR (300 MHz, $CDCl_3$, δ): 44.67, 126.1, 127.1, 127.4, 127.9, 128.0, 128.7, 128.9, 129.4, 129.8, 132.4, 133.7, 138.8, 142.5, 146.3, 148.4, 158.9. ESI-MS (*m/z*): 453 [M+1]. Anal. calcd. for $C_{27}H_{20}N_2O_3S$: C 71.66, H 4.45, N 6.19, S 7.09. Found: C 71.63, H 4.37, N 6.22, S 8.13.

*4-(2,3-Dihydro-1H-inden-5-yl)-2-[4-(methylsulfonyl)-phenyl]phthalazin-1(2H)-one (**2c**)*

Yield = 60%, m.p. 212–214°C, Rf = 0.72 (toluene/ethyl acetate/formic acid, 5:4:1). IR υ_{max} (KBr): 1662 cm^{-1} (C=O), 1496 cm^{-1}

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(C=N), 1158 and 1310 cm^{-1} (SO_2C). ^1H NMR (300 MHz, $CDCl_3$, δ): 3.22 (3H, s, SO_2CH_3), 2.94 (4H, t, H-4′, H-6′), 2.01–2.04 (2H, t, H-5′), 7.41 (2H, d, H-3′, H-7′), 7.50 (1H, s, H-2′), 7.72–7.81 (3H, m, H-6, H-7, H-8), 8.10–8.13 (4H, m, *N*-phenyl protons), 8.61–8.73 (1H, m, H-5). ^{13}C NMR (300 MHz, $CDCl_3$, δ): 25.50, 32.8, 44.68, 124.5, 125.3, 126.1, 127.4, 127.9, 128.7, 129.2, 131.9, 132.4, 133.6, 138.6, 145.2, 146.1, 146.4, 149.2, 159.0. ESI-MS (*m/z*): 417 [M+1]. Anal. calcd. for $C_{24}H_{20}N_2O_3S$: C 69.26, H 4.65, N 6.59, S 7.05. Found: C 69.53, H 4.77, N 6.72, S 7.12.

*4-(4-Isopropyl-phenyl)-2-[4-(methylsulfonyl)phenyl]-phthalazin-1(2H)-one (**2d**)*
Yield = 48%, m.p. 214–216°C, Rf = 0.74 (toluene/ethyl acetate/formic acid, 5:4:1). IR υ_{max} (KBr): 1655 cm^{-1} (C=O), 1536 cm^{-1} (C=N), 1167 and 1323 cm^{-1} (SO_2C). ^1H NMR (300 MHz, $CDCl_3$, δ):1.41 (6H, d, PhCH($\underline{CH_3}$)$_2$), 3.10 (1H, m, Ph$\underline{CH}$(CH_3)$_2$), 3.21 (3H, s, SO_2CH_3), 7.37–7.40 (2H, d, J = 8.1 Hz, H-3′, H-5′), 7.59 (2H, d, J = 8.1 Hz, H-2′, H-6′), 8.05 (2H, d, J = 9.0 Hz, H-3″, H-5″), 8.12 (2H, d, J = 8.70 Hz, H-2″, H-6″), 7.82–7.91 (3H, m, H-6, H-7, H-8), 8.63 (1H, m, H-5). ^{13}C NMR (300 MHz, $CDCl_3$, δ): 25.21, 31.82, 44.65, 126.1, 127.3, 127.8, 128.0, 128.7, 128.3, 129.1, 129.3, 131.9, 132.0, 133.6, 138.7, 144.4, 146.3, 148.8, 158.9. ESI-MS (*m/z*): 417 [M–1]. Anal. calcd. for $C_{24}H_{22}N_2O_3S$: C 68.88, H 5.30, N 6.69, S 7.66. Found: C 68.57, H 5.71, N 6.94, S 7.32.

*4-(4-Propyl-phenyl)-2-[4-(methylsulfonyl)phenyl]-phthalazin-1-one (**2e**)*
Yield = 40.6%, m.p. 210–212°C, Rf = 0.74 (toluene/ethyl acetate/formic acid, 5:4:1). IR υ_{max} (KBr): 1668 cm^{-1} (C=O), 1528 cm^{-1} (C=N), 1164 and 1318 cm^{-1} (SO_2C). ^1H NMR (300 MHz, $CDCl_3$, δ): 1.1 (3H, t, $PhCH_2CH_2\underline{CH_3}$), 1.78 (2H, m, $PhCH_2\underline{CH_2}CH_3$), 2.76 (2H, t, $Ph\underline{CH_2}CH_2CH_3$), 3.24 (3H, s, SO_2CH_3), 7.37–7.40 (2H, d, J = 7.5 Hz, H-3′, H-5′), 7.60 (2H, d, J = 7.8 Hz, H-2′, H-6′), 8.06 (2H, d, J = 8.4 Hz, H-3″, H-5″), 8.14 (2H, d, J = 8.4.0 Hz, H-2″, H-6″), 7.86 (3H, m, H-6, H-7, H-8), 8.63 (1H, m, H-5). ^{13}C NMR (300 MHz, $CDCl_3$, δ): 13.86, 24.46, 37.89, 44.69, 126.1, 127.3, 127.8, 128.0, 128.7, 128.4, 129.1, 129.3, 131.9, 132.0, 133.6, 138.7, 144.4, 146.3, 148.8, 158.9. ESI-MS (*m/z*): 419 [M+1]. Anal. calcd. for $C_{24}H_{22}N_2O_3S$: C 68.88, H 5.30, N 6.69, S 7.66. Found: C 68.67, H 5.61, N 6.74, S 7.42.

*4-(4-Chlorophenyl)-2-[4-(methylsulfonyl)phenyl]-phthalazin-1(2H)-one (**2f**)*
Yield = 50.4%, m.p. 242–243°C, Rf = 0.77 (toluene/ethyl acetate/formic acid, 5:4:1). IR υ_{max} (KBr): 1648 cm^{-1} (C=O), 1554 cm^{-1} (C=N), 1168 and 1322 cm^{-1} (SO_2C). ^1H NMR (400 MHz, $CDCl_3$, δ): 3.12 (3H, s, SO_2CH_3), 7.56–7.60 (2H, m, J = 7.4 Hz, H-3′, H-5′), 7.66–7.70 (2H, m, J = 7.4 Hz, H-2′, H-6′), 8.04–8.08 (4H, m, H-2″, H-3″, H-5″, H-6″), 7.85–7.91 (2H, m, H-6, H-7), 8.51–8.63 (1H, m, H-5), 7.78 (1H, m, H-8). ^{13}C NMR (300 MHz, $CDCl_3$, δ): 44.65, 125.2, 126.5, 127.7, 128.3, 129.6, 131.4, 132.2, 132.5, 133.5, 138.6, 145.1, 149.2, 158.2, 158.6. ESI-MS (*m/z*): 411 [M+1]. Anal. calcd. for $C_{21}H_{15}ClN_2O_3S$: C 61.39, H 3.68, N 6.82, S 7.80. Found: C 61.53, H 3.81, N 6.97, S 7.51.

*4-(4-Fluorophenyl)-2-[4-(methylsulfonyl)phenyl]-phthalazin-1(2H)-one (**2g**)*
Yield = 58.7%, m.p. 244–245°C, Rf = 0.76 (toluene/ethyl acetate/formic acid, 5:4:1). IR υ_{max} (KBr): 1656 cm^{-1} (C=O), 1562 cm^{-1} (C=N), 1155 and 1332 cm^{-1} (SO_2C). ^1H NMR (400 MHz, $CDCl_3$, δ): 3.20 (3H, s, SO_2CH_3), 7.28–7.32 (2H, m, J = 7.2 Hz, H-3′, H-5′), 7.63–7.75 (2H, m, J = 7.8 Hz, H-2′, H-6′), 8.08–8.10 (4H, m, H-2″, H-3″, H-5″, H-6″), 7.87–7.92 (2H, m, H-6, H-7), 8.52–8.64 (1H, m, H-5), 7.76 (1H, m, H-8). ^{13}C NMR (300 MHz, $CDCl_3$, δ): 44.68, 124.2, 126.5, 126.7, 128.3, 128.6, 131.4, 132.2, 132.5, 133.7, 138.7, 146.2, 147.7, 157.4, 158.6. ESI-MS (*m/z*): 395 [M+1]. Anal. calcd. for $C_{21}H_{15}FN_2O_3S$: C 63.95, H 3.83, N 7.10, S 8.13. Found: C 63.63, H 3.75, N 7.22, S 8.31.

*4-(4-Bromophenyl)-2-[4-(methylsulfonyl)phenyl]-phthalazin-1(2H)-one (**2h**)*
Yield = 45.3%, m.p. 246–248°C, Rf = 0.75 (toluene/ethyl acetate/formic acid, 5:4:1). IR υ_{max} (KBr): 1664 cm^{-1} (C=O), 1581 cm^{-1} (C=N), 1152 and 1341 cm^{-1} (SO_2C). ^1H NMR (400 MHz, $CDCl_3$, δ): 3.23 (3H, s, SO_2CH_3), 7.58–7.62 (2H, d, J = 7.6 Hz, H-3′, H-5′), 7.68–7.74 (2H, d, J = 7.7 Hz, H-2′, H-6′), 8.06–8.09 (4H, m, H-2″, H-3″, H-5″, H-6″), 7.83–7.92 (2H, m, H-6, H-7), 8.64–8.68 (1H, m, H-5), 7.80 (1H, m, H-8). ^{13}C NMR (300 MHz, $CDCl_3$, δ): 44.66, 124.0, 126.0, 126.7, 128.0, 128.7, 131.0, 132.2, 133.5, 138.9, 146.1, 147.6, 157.2, 158.8. ESI-MS (*m/z*): 457 [M+2]. Anal. calcd. for $C_{21}H_{15}BrN_2O_3S$: C 55.39, H 3.32, N 6.15, S 7.04. Found: C 55.63, H 3.51, N 6.47, S 7.24.

*2-(4-Methanesulfonyl-phenyl)-4-(5,6,7,8-tetrahydro-naphthalen-2-yl)-phthalazin-1-one (**2i**)*
Yield = 66.3%, m.p. 282–284°C, Rf = 0.74 (toluene/ethyl acetate/formic acid, 5:4:1). IR υ_{max} (KBr): 1665 cm^{-1} (C=O), 1584 cm^{-1} (C=N), 1147 and 1325 cm^{-1} (SO_2C). ^1H NMR (300 MHz, $CDCl_3$, δ): 3.12 (3H, s, SO_2CH_3), 1.94 (4H, t, H-5′, H-6′), 2.85 (4H, t, H-4′, H-7′), 7.38 (2H, m, H-3′, H-8′), 7.25 (1H, d, H-2′), 7.82–7.85 (3H, m, H-6, H-7, H-8), 8.04 (2H, d, J = 8.7 Hz, H-3″, H-5″), 8.12 (2H, d, J = 8.3 Hz, H-2″, H-6″), 8.45–8.47 (1H, m, H-5). ^{13}C NMR (300 MHz, $CDCl_3$, δ): 28.50, 37.84, 44.69, 113.1, 124.7, 125.5, 125.9, 126.1, 126.5, 127.6, 128.0, 129.8, 132.9, 133.6, 144.4, 146.6, 148.2, 154.9, 159.7. ESI-MS (*m/z*): 431 [M+1]. Anal. calcd. for $C_{25}H_{22}N_2O_3S$: C 69.75, H 5.15, N 6.51, S 7.45. Found: C 69.57, H 5.63, N 6.77, S 7.71.

*4-(4-Methanesulfonyl-phenyl)-4-(4-phenoxy-phenyl)-phthalazin-1-one (**2j**)*
Yield = 30.1%, m.p. 284–286°C, Rf = 0.75 (toluene/ethyl acetate/formic acid, 5:4:1). IR υ_{max} (KBr): 1679 cm^{-1} (C=O), 1566 cm^{-1} (C=N), 1145 and 1315 cm^{-1} (SO_2C). ^1H NMR (400 MHz, $CDCl_3$, δ): 3.14 (3H, s, SO_2CH_3), 7.11–7.21 (5H, m, H-3‴, H-5‴, H-2‴, H-6‴, and H-4‴), 7.39–7.43 (2H, m, H-3′, H-5′), 7.61 (2H, d, J = 8.7 Hz, H-2′, H-6′), 7.85–7.91 (3H, m, H-6, H-7, and H-8), 8.09–8.11 (4H, m, *N*-phenyl protons), 8.61–8.63 (1H, m, H-5). ^{13}C NMR (300 MHz, $CDCl_3$, δ): 44.69, 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, 158.3. FAB-MS (*m/z*): 491 [M+Na], 300 [M–Ph–O–Ph]. Anal. calcd. for

$C_{27}H_{20}N_2O_4S$: C 69.22, H 4.30, N 5.98, S 6.84. Found: C 69.58, H 4.611, N 5.79, S 6.59.

*4-(3,4-Difluorophenyl)-2-[4-(methylsulfonyl)phenyl]-phthalazin-1(2H)-one (**2k**)*
Yield = 52.2%, m.p. 246–247°C, Rf = 0.56 (toluene/ethyl acetate/formic acid, 5:4:1). IR υ_{max} (KBr): 1684 cm^{-1} (C=O), 1568 cm^{-1} (C=N), 1142 and 1320 cm^{-1} (SO_2C). ^1H NMR (400 MHz, $CDCl_3$, δ): 3.23 (3H, s, SO_2CH_3), 7.37–7.42 (2H, m, H-3′, H-5′), 7.62 (2H, d, J = 8.6 Hz, H-2′, H-6′), 7.86–7.92 (3H, m, H-6, H-7, and H-8), 8.12–8.15 (4H, m, *N*-phenyl protons), 8.60–8.64 (1H, m, H-5). ^{13}C NMR (300 MHz, $CDCl_3$, δ): 44.62, 117.6, 117.9, 118.6, 118.9, 125.9, 126.0, 126.5, 128.1, 128.5, 131.4, 132.4, 133.9, 139.0, 146.0, 146.5, 158.7. FAB-MS (*m/z*): 411 [M–1]. Anal. calcd. for $C_{21}H_{14}F_2N_2O_3S$: C 61.16, H 3.42, N 6.69, S 7.78. Found: C 61.42, H 3.71, N 6.88, S 7.66.

*4-(4-tert-Butyl-phenyl)-2-[4-(methylsulfonyl)phenyl]-phthalazin-1-one (**2l**)*
Yield = 60.2%, m.p. 232–234°C, Rf = 0.74 (toluene/ethyl acetate/formic acid, 5:4:1). IR υ_{max} (KBr): 1656 cm^{-1} (C=O), 1547 cm^{-1} (C=N), 1130 and 1342 cm^{-1} (SO_2C). ^1H NMR (400 MHz, $CDCl_3$, δ): 1.4 (9H, s, Ph($\underline{CH_3}$)$_3$), 3.15 (3H, s, SO_2CH_3), 7.58–7.62 (4H, m, J = 7.4 Hz, H-3′, H-5′, H-2′, H-6′), 7.50–7.61 (2H, d, J = 7.3, H-2′, H-6′), 8.12 (2H, d, J = 9.0 Hz, H-3″, H-5″), 8.21 (2H, d, J = 8.7 Hz, H-2″, H-6″), 7.82–7.91 (3H, m, H-6, H-7, H-8), 8.60–8.72 (1H, m, H-5). ^{13}C NMR (300 MHz, $CDCl_3$, δ): 31.37, 34.86, 44.69, 125.7, 126.1, 127.3, 127.8, 128.0, 128.7, 129.1, 131.6, 132.0, 133.6, 138.7, 146.4, 148.7, 152.8, 159.0. ESI-MS (*m/z*): 431 [M–1]. Anal. calcd. for $C_{25}H_{24}N_2O_3S$: C 69.42, H 5.59, N 6.48, S 7.41. Found: C 69.73, H 5.83, N 6.78, S 7.71.

*4-(4-Hexyl-phenyl)-2-[4-(methylsulfonyl)phenyl]-phthalazin-1-one (**2m**)*
Yield = 54.4%, m.p. 220–222°C, Rf = 0.76 (toluene/ethyl acetate/formic acid, 5:4:1). IR υ_{max} (KBr): 1669 cm^{-1} (C=O), 1558 cm^{-1} (C=N), 1122 and 1334 cm^{-1} (SO_2C). ^1H NMR (400 MHz, $CDCl_3$, δ): 0.99 (3H, t, $PhCH_2CH_2CH_2CH_2CH_2\underline{CH_3}$), 1.3 (4H, m, $PhCH_2CH_2CH_2\underline{CH_2CH_2}CH_3$), 1.6 (2H, m, $PhCH_2CH_2\underline{CH_2}CH_2CH_2CH_3$), 1.72 (2H, m, $PhCH_2\underline{CH_2}CH_2CH_2CH_2CH_3$), 2.77 (2H, t, $Ph\underline{CH_2}CH_2CH_2CH_2CH_2CH_3$), 3.17 (3H, s, SO_2CH_3), 7.36–7.41 (2H, d, J = 7.1 Hz, H-3′, H-5′), 7.50–7.61 (2H, d, J = 7.3, H-2′, H-6′), 8.1 (2H, d, J = 8.7 Hz, H-3″, H-5″), 8.2 (2H, d, J = 8.7 Hz, H-2″, H-6″), 7.78–7.83 (3H, m, H-6, H-7, H-8), 8.61–8.72 (1H, m, H-5). ^{13}C NMR (300 MHz, $CDCl_3$, δ): 14.22, 24.11, 29.78, 32.66, 34.41, 36.89, 44.69, 126.1, 127.3, 127.8, 128.0, 128.7, 128.2, 129.1, 129.3, 131.9, 132.0, 133.6, 138.7, 144.4, 146.3, 148.8, 158. ESI-MS (*m/z*): 461 [M+1]. Anal. calcd. for $C_{27}H_{28}N_2O_3S$: C 70.41, H 6.13, N 6.08, S 6.96. Found: C 70.75, H 6.43, N 6.38, S 6.78.

*4-(9H-Fluoren-2-yl)-2-[4-(methylsulfonyl)phenyl]-phthalazin-1-one (**2n**)*
Yield = 40.6%, m.p. 278–280°C, Rf = 0.72 (toluene/ethyl acetate/formic acid, 5:4:1). IR υ_{max} (KBr): 1718 cm^{-1} (C=O), 1576 cm^{-1} (C=N), 1148 and 1339 cm^{-1} (SO_2C). ^1H NMR (300 MHz, $CDCl_3$, δ): 3.12 (3H, s, SO_2CH_3), 4.04 (2H, s, H-9′), 7.38–7.49 (3H, m, H-2′, H-3′, H-8′), 7.61–7.72 (4H, m, H-4′, H-5′, H-6′, H-7′), 7.91–8.11 (3H, m, H-6, H-7, H-8), 8.12 (2H, d, J = 9.0 Hz, H-3″, H-5″), 8.13 (2H, d, J = 8.7 Hz, H-2″, H-6″), 8.62–8.68 (1H, m, H-5). ^{13}C NMR (300 MHz, $CDCl_3$, δ): 33.45, 44.67, 126.5, 127.4, 128.4, 128.9, 129.0, 129.7, 129.9, 130.4, 131.8, 132.4, 135.33, 136.23, 135.7, 138.8, 144.5, 145.55, 147.21, 147.3, 149.4, 159.9. ESI-MS (*m/z*): 463 [M–1]. Anal. calcd. for $C_{28}H_{20}N_2O_3S$: C 72.39, H 4.34, N 6.03, S 6.90. Found: C 72.65, H 4.72, N 6.28, S 6.81.

*4-(4-Ethyl-phenyl)-2-[4-(methylsulfonyl)phenyl]-phthalazin-1-one (**2o**)*
Yield = 42.6%, m.p. 202–204°C, Rf = 0.78 (toluene/ethyl acetate/formic acid, 5:4:1). IR υ_{max} (KBr): 1658 cm^{-1} (C=O), 1536 cm^{-1} (C=N), 1161 and 1323 cm^{-1} (SO_2C). ^1H NMR (300 MHz, $CDCl_3$, δ): 1.3 (3H, t, $PhCH_2\underline{CH_3}$), 2.76 (2H, t, $Ph\underline{CH_2}CH_3$), 3.23 (3H, s, SO_2CH_3), 7.33–7.38 (2H, d, J = 7.5 Hz, H-3′, H-5′), 7.62 (2H, d, J = 7.8Hz, H-2′, H-6′), 8.05 (2H, d, J = 8.4 Hz, H-3″, H-5″), 8.11 (2H, d, J = 8.4.0 Hz, H-2″, H-6″), 7.85 (3H, m, H-6, H-7, H-8), 8.60 (1H, m, H-5). ^{13}C NMR (300 MHz, CDCl3, δ): 24.46, 37.89, 44.69, 126.1, 127.3, 127.8, 128.0, 128.7, 128.4, 129.1, 129.3, 131.9, 132.0, 133.6, 138.7, 144.2, 146.3, 148.8, 158.9. ESI-MS (*m/z*): 405 [M+1]. Anal. calcd. for $C_{24}H_{22}N_2O_3S$: C 68.55, H 5.75, N 6.66, S 7.63. Found: C 68.67, H 5.82, N 6.81, S 7.72.

*4-(4-Butyl-phenyl)-2-[4-(methylsulfonyl)phenyl]-phthalazin-1-one (**2p**)*
Yield = 44.4%, m.p. 225–227°C, Rf = 0.75 (toluene/ethyl acetate/formic acid, 5:4:1). IR υ_{max} (KBr): 1662 cm^{-1} (C=O), 1552 cm^{-1} (C=N), 1124 and 1335 cm^{-1} (SO_2C). ^1H NMR (400 MHz, $CDCl_3$, δ): 0.98 (3H, t, $PhCH_2CH_2CH_2\underline{CH_3}$), 1.8 (4H, m, $PhCH_2\underline{CH_2CH_2}CH_3$), 2.71 (2H, t, $Ph\underline{CH_2}CH_2CH_2CH_3$), 3.16 (3H, s, SO_2CH_3), 7.38–7.43 (2H, d, J = 7.1 Hz, H-3′, H-5′), 7.52–7.63 (2H, d, J = 7.3, H-2′, H-6′), 8.13 (2H, d, J = 8.7 Hz, H-3″, H-5″), 8.32 (2H, d, J = 8.7 Hz, H-2″, H-6″), 7.78–7.83 (3H, m, H-6, H-7, H-8), 8.63–8.74 (1H, m, H-5). ESI-MS (*m/z*): 433 [M+1]. Anal. calcd. for $C_{25}H_{24}N_2O_3S$: C 69.42, H 5.59, N 6.48, S 7.41. Found: C 69.45, H 5.56, N 6.44, S 7.45.

Pharmacology

Anti-inflammatory activity
Carrageenan-induced hind paw edema method was sued for evaluating anti-inflammatory activity of the synthesized compounds [38]. In the current study, rats of Wistar progeny (either sex) obtained from Central Animal House facility of Jamia Hamdard, New Delhi (registration no. 173/CPCSEA) were used. 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. CMC 1%, w/v in distilled water was used as vehicle for dosing (10 mL/kg). All the treatments suspended in vehicle were given orally. Overnight fasted rats (150–200 g) were taken and divided into 15 groups of six animals each. Group I was administered orally with vehicle (1% Cween-10, 10 mL/kg) and

served as control. Group II was treated with etoricoxib (0.05 mmol/kg b.w.) served as standards. Test groups were administered orally with respective test drugs (**2a–p**) 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 volume of paw was measured by using plethysmometer at 0, 3, and 5 h postcarageenan 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 following formula:

$$\% \text{ Inhibition} = (1 - V_t/V_c) \times 100$$

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.

Ulcerogenic activity

Acute gastric ulcerogenic effect of the compounds **2b** and **2i** were evaluated in Wistar rats [39]. Albino rats of Wistar strain (150–200 g) fasted over 24 h were randomly allotted into four groups of six animals each. The animals of first group were given vehicle 10 mL/kg (CMC 1% w/v in distilled water) orally. Group II served as standard and was administered orally etoricoxib (0.15 mmol/kg) suspended in vehicle. Groups III and IV were administered orally compounds **2b** and **2i** at a dose of 0.25 mmol/kg po 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.

Cyclooxygenase inhibition assay

The ability of the compounds **2b**, **2j**, **2i**, and **2k** to inhibit ovine COX-1 and human recombinant COX-2 were determined using an enzyme immune-assay (EIA) kit (catalog no. 560131, Cayman Chemical, Ann Arbor, MI, USA) according to the instructions given in the kit manual.

5-Lipoxygenase inhibition assay

The ability of compounds **2b**, **2j**, **2i**, and **2k** to inhibit potato 5-LOX (catalog no. 60401, Cayman Chemical, Ann Arbor, MI, USA) (IC_{50} values, l μM) were determined using an enzyme immune assay (EIA) kit (catalog no. 760700, Cayman Chemical, Ann Arbor, MI, USA) according to the instructions given in the kit manual.

In vitro anti-proliferative activity

A total of 60 human tumor cell lines, derived from nine cancer types (leukemia, lung, colon, brain, melanoma, ovarian, renal, prostate, and breast) formed the basis of this test. The tumor cells were cultured in PMI1640 medium supplemented with 5% fetal calf serum and 2 mM L-glutamine. The tumor cells are inoculated over a series of standard 96-well microtiter plates in 100 mL of medium [40, 41]. Density of inoculum depends on the type of tumor cell and from its growth characteristics. These cells are then pre-incubated on the microtiter plate for 24 h before adding the compounds. These were tested in DMSO solution at 10^{-5} M concentrations. After an incubation of the chemical agent for 48 h with the tumor cell lines, a sulforhodamine B (SRB) protein assay was used to estimate cell viability or growth. The cytotoxic effects are evaluated and the assay results and dose–response parameters were calculated as previously described [42].

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