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RESEARCH ARTICLE

Synthesis and biological evaluation of some novel 6-aryl-2-(p-sulfamylphenyl)-4,5-dihydropyridazin-3(2H)-ones as anti-cancer, antimicrobial, and anti-inflammatory agents

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Abstract

A series of 6-aryl-2-(*p*-sulfamylphenyl)-4,5-dihydropyridazin-3(*2H*)-ones (**2a–j**) were synthesized by condensation of the appropriate β-aroylpropionic acid and 4-hydrazinobenzenesulfonamide hydrochloride in ethanol and tested for anti-cancer, anti-inflammatory, and antimicrobial actions. According to the protocol of the National Cancer Institute (NCI) *in vitro* disease-oriented human cells screening panel assay, compound **2g** showed high activity against HL-60 (TB) (leukemia), SR (leukemia), NCI-H522 (non-small-cell lung cancer), and BT-549 (breast cancer) with a GI_{50} value of less than 2 μM. Two compounds (**2c** and **2f**) were found to have promising anti-inflammatory activity, while a fair number of compounds showed good antifungal activity.

Keywords: *Pyridazinones; benzenesulfonamides; anti-cancer; anti-inflammatory; antimicrobial*

Introduction

Though not commonly found in nature, pyridazinones have been used as scaffolds in the pharmaceutical industry for a wide range of structure–activity relationship (SAR) studies[1]. For example, azelastine is an antihistamine, while zardaverine exhibits phosphodiesterase (PDE) III and PDE IV inhibitory activity. The significant commercial interest in the pharmaceutical uses of pyridazinones is further illustrated by the large number of patents filed in this area that cover positive inotropic agents for the treatment of congestive heart failure, antidepressants, α1/α2 antagonists, potassium channel activators, anti-asthmatics, and others[2,3]. Recently, these derivatives have been reported as potent aldose reductase inhibitors[4], hepatoprotective agents[5], antibacterial and antifungal agents[6], and cyclo-oxygenase-2 (COX-2) inhibitors[7].

The benzenesulfonamide moiety has exhibited its importance by its presence in a large variety of pharmaceuticals covering a wide range of biological activities. These have been reported as carbonic anhydrase inhibitors[8]. They show affinities for endothelin receptors ET_A and ET_B in the low non-molar range and high functional antagonistic potency *in vitro*[9]. Antibacterial, antifungal, and cytotoxic activities have been similarly evaluated[10,11]. They have also been reported as COX-2 inhibitors[12]. The anti-inflammatory activity of celecoxib (Celebrex), a clinically marketed drug, is attributed to the presence of SO_2NH_2 pharmacophore. Structure–activity studies have shown that the SO_2NH_2 substituent at the *para* position of one aryl group usually confers optimal COX-2 inhibitor potency[13]. Therefore, it was thought worthwhile to synthesize novel pyridazinone derivatives bearing the benzenesulfonamide moiety and screen them for their anti-cancer, anti-inflammatory, and antimicrobial activity.

Materials and methods

Chemistry

Melting points were determined using open capillary tubes and are uncorrected. All Fourier transform infrared (FTIR) spectra were recorded on a Bio-rad FTS-135 spectrophotometer using KBr pellets; ν_{max} values are given in cm^{-1}. ^1H nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Spectrospin DPX 300-MHz spectrometer using deuterated dimethylsulfoxide (DMSO) as solvent and

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tetramethylsilane (TMS) as 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 using a fast atom bombardment (FAB) ionization Jeol JMS-DX 303 apparatus, equipped with direct inlet probe system. The *m/z* values of the more intense peaks are mentioned. Purity of the compounds was checked on thin layer chromatography (TLC) plates (silica gel G) which were visualized by exposing to iodine vapors. Elemental analysis was carried out on a CHNS Elementar (Vario EL III) system.

General procedure for the synthesis of aroylpropionic acids (1a–j)

To liquid aromatic hydrocarbon (30 mL), anhydrous aluminum chloride (16.6 g, 0.125 mol) was added. The mixture was stirred using a magnetic stirrer at room temperature for 30 min. To this, succinic anhydride (5 g, 0.05 mol) was added in five portions with continuous stirring. Vigorous reaction started with the evolution of HCl gas. Stirring was continued for another 6 h at room temperature. The mixture 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. The precipitated solid was treated with saturated sodium bicarbonate solution, filtered, washed with cold water, dried, and crystallized from the appropriate solvent to give **1a–j**[14,15].

General procedure for the synthesis of pyridazinones (2a–j)

A mixture of the appropriate aroylpropionic acid (**1a–j**) (0.001 mol) and 4-hydrazinobenzenesulfonamide hydrochloride (0.001 mol) in absolute ethanol (20–30 mL) was refluxed for 18–24 h. The reaction mixture was concentrated to one-third of its volume and left at room temperature, when a solid separated out. The crude product was filtered off, washed with a small volume of alcohol, and stirred with 5% sodium bicarbonate solution (25 mL). It was filtered, and washed with 2% acetic acid and then with water. The product was dried and crystallized from methanol (**2a–j**).

6-Phenyl-2-(p-sulfamylphenyl)-4,5-dihydropyridazine-3(2H)-one (2a)

Yield = 63%; m.p. 178°C; IR ν_{max} (KBr, in cm^{-1}): 3305 and 3191 (NH_2), 1662 (C=O), 1590 (C=N), 1327 and 1154 cm^{-1} (SO_2N); ^{1}H NMR: 2.77 and 3.17 (each t, 2 × -CH_2-), 7.39 (2H, s, SO_2NH_2), 7.49 (3H, m, Ar-H), 7.77–7.88 (6H, m, Ar-H); FAB-MS (*m/z*): 329 [M^+]; molecular formula $C_{16}H_{15}N_3O_3S$; Calculated: C = 58.53, H = 4.59, N = 12.76, S = 9.73; Found: C = 58.49, H = 4.37, N = 13.02, S = 9.61%.

6-(4-Methylphenyl)-2-(p-sulfamylphenyl)-4,5-dihydropyridazine-3(2H)-one (2b)

Yield = 66%; m.p. 192°C; IR ν_{max} (KBr, in cm^{-1}): 3303 and 3195 (NH_2), 1662 (C=O), 1591 (C=N), 1329 and 1155 cm^{-1} (SO_2N); ^{1}H NMR: 2.36 (3H, s, CH_3), 2.76 and 3.14 (each t, 2 × -CH_2-), 7.28 (2H, d, *J* = 7.5 Hz, Ar-H), 7.36 (2H, s, SO_2NH_2), 7.76–7.80 (4H, m, Ar-H), 7.88 (2H, d, *J* = 8.0 Hz, Ar-H); FAB-MS (*m/z*): 343 [M^+], 344 [M + 1]; molecular formula $C_{17}H_{17}N_3O_3S$; Calculated: C = 59.46, H = 4.99, N = 12.24, S = 9.34; Found: C = 58.98, H = 5.06, N = 12.09, S = 9.51%.

6-(4-Chlorophenyl)-2-(p-sulfamylphenyl)-4,5-dihydropyridazine-3(2H)-one (2c)

Yield = 70%; m.p. 220–222°C; IR ν_{max} (KBr, in cm^{-1}): 3303 and 3225 (NH_2), 1644 (C=O), 1591 (C=N), 1334 and 1154 cm^{-1} (SO_2N); ^{1}H NMR: 2.78 and 3.16 (each t, 2 × -CH_2-), 7.27 (2H, s, SO_2NH_2), 7.54 (2H, d, *J* = 8.5 Hz, Ar-H), 7.77 (2H, d, *J* = 8.6 Hz, Ar-H), 7.86 (2H, *J* = 6.2 Hz, Ar-H), and 7.89 (2H, d, *J* = 6.2 Hz, Ar-H); FAB-MS (*m/z*): 363 [M^+], 364 [M + 1]; molecular formula $C_{16}H_{14}ClN_3O_3S$; Calculated: C = 52.82, H = 3.88, N = 11.55, S = 8.81; Found: C = 52.59, H = 3.64, N = 11.52, S = 8.76%.

6-(4-Methoxyphenyl)-2-(p-sulfamylphenyl)-4,5-dihydropyridazine-3(2H)-one (2d)

Yield = 63%; m.p. 228°C; IR ν_{max} (KBr, in cm^{-1}): 3304 and 3179 (NH_2), 1654 (C=O), 1599 (C=N), 1331 and 1156 (SO_2N), 1032 cm^{-1} (OCH_3); ^{1}H NMR: 2.75 and 3.13 (each t, 2 × -CH_2-), 3.82 (3H, s, OCH_3), 7.03 (2H, d, *J* = 7.8 Hz, Ar-H), 7.36 (2H, s, SO_2NH_2), 7.78–7.88 (6H, m, Ar-H); FAB-MS (*m/z*): 359 [M^+]; molecular formula $C_{17}H_{17}N_3O_4S$; Calculated: C = 56.81, H = 4.77, N = 11.69, S = 8.92; Found: C = 57.02, H = 4.73, N = 11.55, S = 8.96%.

6-(4-Ethylphenyl)-2-(p-sulfamylphenyl)-4,5-dihydropyridazine-3(2H)-one (2e)

Yield = 68%; m.p. 148°C; IR ν_{max} (KBr, in cm^{-1}): 3300 and 3186 (NH_2), 1660 (C=O), 1591 (C=N), 1331 and 1154 cm^{-1} (SO_2N); ^{1}H NMR: 1.20 (3H, t, -CH_2-CH_3), 2.65 (2H, q, -CH_2-CH_3), 2.76 and 3.15 (each t, 2 × -CH_2-), 7.32 (2H, d, *J* = 7.8 Hz, Ar-H), 7.37 (2H, s, SO_2NH_2), 7.77–7.89 (6H, m, Ar-H); FAB-MS (*m/z*): 357 [M^+]; molecular formula $C_{18}H_{19}N_3O_3S$; Calculated: C = 60.49, H = 5.36, N = 11.76, S = 8.97; Found: C = 60.27, H = 5.31, N = 11.57, S = 9.09%.

6-(2-Hydroxy-5-methylphenyl)-2-(p-sulfamylphenyl)-4,5-dihydropyridazine-3(2H)-one (2f)

Yield = 59%; m.p. 228°C; IR ν_{max} (KBr, in cm^{-1}): 3291, 1654 (C=O), 1592 (C=N), 1311 and 1152 cm^{-1} (SO_2N); ^{1}H NMR: 2.17 (3H, s, CH_3), 2.72 and 3.11 (each t, 2 × -CH_2-), 6.85 (1H, d, *J* = 8.3 Hz, Ar-H), 7.36 (2H, s, SO_2NH_2), 7.55 (1H, d, *J* = 8.3 Hz, Ar-H), 7.62 (1H, s, Ar-H), 7.77 (2H, d, *J* = 8.3 Hz, Ar-H), 7.86 (2H, d, *J* = 8.5 Hz, Ar-H), 9.82 (1H, s, OH); FAB-MS (*m/z*): 359 [M^+]; molecular formula $C_{17}H_{17}N_3O_4S$; Calculated: C = 56.81, H = 4.77, N = 11.69, S = 8.92; Found: C = 57.11, H = 4.59, N = 11.96, S = 9.15%.

6-(4-Hydroxy-2-Methylphenyl)-2-(p-sulfamylphenyl)-4,5-dihydropyridazine-3(2H)-one (2g)

Yield = 57%; m.p. 290°C; IR ν_{max} (KBr, in cm^{-1}): 3307 and 3204 (NH_2), 1665 (C=O), 1600 (C=N), 1334 and 1157 cm^{-1} (SO_2N); ^{1}H NMR: 2.29 (3H, s, CH_3), 2.56 and 3.01 (each t, 2 × -CH_2-), 6.56 (2H, m, Ar-H), 7.18 (2H, s, SO_2NH_2), 7.31 (1H, d, *J* = 7.6 Hz, Ar-H), 7.50 (2H, d, *J* = 8.3 Hz, Ar-H), 7.69 (2H, d, *J* = 8.3 Hz, Ar-H), 10.93 (1H, s, OH); FAB-MS (*m/z*): 359 [M^+]; molecular

formula $C_{17}H_{17}N_3O_4S$; Calculated: C = 56.81, H = 4.77, N = 11.69, S = 8.92; Found: C = 56.26, H = 4.48, N = 11.38, S = 8.80%.

6-(4-Hydroxy-3-Methylphenyl)-2-(p-sulfamylphenyl)-4,5-dihydropyridazine-3(2H)-one (2h)

Yield = 54%; m.p. 240°C; IR ν_{max} (KBr, in cm^{-1}): 3395 and 3292 (NH_2), 1654 (C=O), 1592 (C=N), 1314 and 1152 cm^{-1} (SO_2N); ^1H NMR: 2.17 (3H, s, CH_3), 2.72 and 3.09 (each t, 2 × -CH_2-), 6.85 (1H, d, *J* = 8.4 Hz, Ar-H), 7.36 (2H, s, SO_2NH_2), 7.55 (1H, d, *J* = 7.8 Hz, Ar-H), 7.62 (1H, s, Ar-H), 7.78 (2H, d, *J* = 8.5 Hz, Ar-H), 7.87 (2H, d, *J* = 8.5 Hz, Ar-H), 9.82 (1H, s, OH); FAB-MS (*m/z*): 359 [M^+]; molecular formula $C_{17}H_{17}N_3O_4S$; Calculated: C = 56.81, H = 4.77, N = 11.69, S = 8.92; Found: C = 56.62, H = 4.61, N = 11.65, S = 8.98%.

6-(3-Chloro-4-hydroxyphenyl)-2-(p-sulfamylphenyl)-4,5-dihydropyridazine-3(2H)-one (2i)

Yield = 53%; m.p. 248°C; IR ν_{max} (KBr, in cm^{-1}): 3279, 1641 (C=O), 1590 (C=N), 1335 and 1147 cm^{-1} (SO_2N); ^1H NMR: 2.74 and 3.10 (each t, 2 × -CH_2-), 7.05 (1H, d, *J* = 8.5 Hz, Ar-H), 7.37 (2H, s, SO_2NH_2), 7.69 (1H, d, *J* = 8.4 Hz, Ar-H), 7.76–7.88 (5H, m, Ar-H), 10.71 (1H, s, OH); FAB-MS (*m/z*): 379 [M^+]; molecular formula $C_{16}H_{14}ClN_3O_4S$; Calculated: C = 50.60, H = 3.72, N = 11.06, S = 8.44; Found: C = 50.31, H = 4.02, N = 10.92, S = 8.22%.

6-(2,5-Dimethylphenyl)-2-(p-sulfamylphenyl)-4,5-dihydropyridazine-3(2H)-one (2j)

Yield = 61%; m.p. 156–158°C; IR ν_{max} (KBr, in cm^{-1}): 3314, 1654 (C=O), 1591 (C=N), 1327 and 1154 cm^{-1} (SO_2N); ^1H NMR: 2.31 (3H, s, CH_3), 2.38 (3H, s, CH_3), 2.78 and 3.04 (each t, 2 × -CH_2-), 7.15 (2H, m, Ar-H), 7.29 (1H, s, Ar-H), 7.36 (2H, s, SO_2NH_2), 7.73 (1H, d, *J* = 8.5 Hz, Ar-H), 7.85 (2H, d, *J* = 8.5 Hz, Ar-H); FAB-MS (*m/z*): 379 [M^+]; molecular formula $C_{18}H_{19}N_3O_3S$; Calculated: C = 60.49, H = 5.36, N = 11.76, S = 8.97; Found: C = 60.87, H = 4.98, N = 12.02, S = 9.13%.

Biological evaluation

Evaluation of in vitro anti-cancer activity

An *in vitro* anti-cancer assay was performed using a full panel of about 60 human tumor cell lines derived from nine different cancer types: leukemia, melanoma, lung, colon, central nervous system (CNS), ovarian, renal, prostate, and breast cancers, in accordance with the protocol of the Drug Evaluation Branch, National Cancer Institute (NCI), Bethesda, and described elsewhere[16–18]. Two standard drugs, meaning that their activities against the cell lines are well documented, were tested against each cell line: NSC 19893 (5-fluorouracil, 5-FU) and NSC 123127 (Adriamycin).

Anti-inflammatory activity

The carrageenan-induced hind paw edema method was used for evaluating anti-inflammatory activity[19]. Wistar rats (either sex) weighing 150–175 g were procured from the 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, Government of India, January 2000. Overnight-fasted rats (16 h) were divided into groups of six animals each. One group of rats, which served as control, was given vehicle (1% carboxymethyl cellulose (CMC) in water in a volume of 10 mL/kg) only. Test compounds (20 mg/kg body weight (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 the left hind paw to induce inflammation, and the volume of the injected paw was measured immediately (at 0 h) using a plethysmometer. The paw volume was measured again after 3 h and 5 h. The average paw volume in a group of treated rats was compared with vehicle (control group) and the percentage inhibition of edema was calculated using the formula:

$$\text{Percent inhibition} = (1 - Vt/Vc) \times 100$$

where Vt is the mean paw volume of the test drug-treated rats and Vc is the mean paw volume of the controls.

The results were analyzed for statistical significance using one-way analysis of variance (ANOVA) followed by Dunnett's test. A value $p < 0.05$ was considered significant.

The acute gastric ulcerogenic effect of compounds **2c** and **2f** was evaluated in Wistar rats[20]. Albino rats of the Wistar strain (160–220g) 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. Compounds **2c** (60 mg/kg) and **2f** (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 anesthesia after 6 h of treatment at a dose of 60 mg/kg (three times). Their stomachs were removed and opened through the greater curvature for examining lesions or bleeding.

Antimicrobial activity

Antifungal and antibacterial activities were evaluated using the cup and plate method[21]. Sabouraud dextrose agar (Hi Media, Mumbai, India) and nutrient agar medium (peptone, beef extract, NaCl, and agar-agar) were used for antifungal and antibacterial screening, respectively. Inocula of different fungi and bacteria were spread over the agar medium using the garden culture method. Test drug (100 μL; 500 μg/mL in DMSO) was poured in each cavity of 6 mm diameter. Standard drug, ciprofloxacin (25 μg/disk) or fluconazole (10 μg/disk), was placed aseptically in a separate Petri dish. The plates were kept at room temperature for 1 h to diffuse the drug in the surrounding medium and then incubated at 37°C for 24 h for bacterial organisms and 32°C for 4 days for fungal organisms. The diameter of the zone of inhibition was measured in mm and compared with that of the standard drug (ciprofloxacin or fluconazole).

Results and discussion

Synthesis of compounds

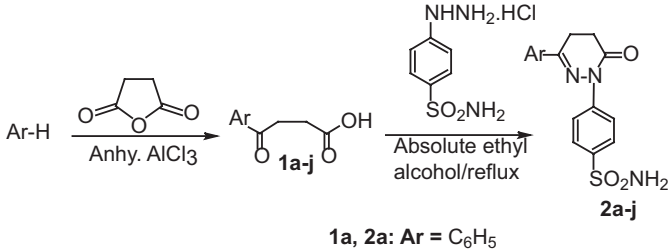
The synthetic route used to synthesize title compounds (**2a–j**) is outlined in Scheme 1. The aroylpropionic acids (**1a–j**) required for the synthesis of pyridazinones were obtained by Friedel–Crafts acylation through reported methods[14,15]. The cyclization to pyridazinone derivatives bearing a benzene sulfonamide moiety was afforded by condensation of the appropriate aroylpropionic acid and 4-hydrazinobenzenesulfonamide hydrochloride in ethanol in 53–70% yield. A literature survey revealed that **2a** was registered in SciFinder Scholar with number 930847-74-8, but no reference was available for work on its method of synthesis and biological study. The structures of the pyridazinone derivatives (**2a–j**) were determined on the basis of elemental analysis and various spectroscopic methods such as IR, ^{1}H-NMR, and MS. Elemental analyses (C, H, N, and S) data were within ±0.4% of the theoretical values. Support for the structure was evidenced by the presence of prominent bands in the IR spectra for NH_2 (3395–3294 cm^{-1} and 3292–3158 cm^{-1}), cyclic carbonyl (1665–1641 cm^{-1}), C=N (1600–1579 cm^{-1}), and SO_2N (1343–1314 cm^{-1} and 1166–1147 cm^{-1}). The structures were further established by ^{1}H-NMR spectral data. The singlet for SO_2NH_2 was observed at δ 7.18–7.53. Two triplets at δ 2.56–2.80 and δ 3.01–3.17, each integrating two protons, can be ascribed to -CH_2-CH_2- of the dihydropyridazinone ring.

Biological studies

Evaluation of anti-cancer activity in vitro

A primary *in vitro* one-dose (10^{-5} M) anticancer assay was performed using a 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[16–18]. The human tumor cell lines were derived from nine different cancer types: leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate, and breast cancers. Two standard drugs, meaning that their activities against the cell lines are well documented, were tested against each cell line: NSC 19893 (5-FU) and NSC 123127 (Adriamycin). From the synthesized compounds (**2a–j**), four compounds, namely **2a**, **2c**, **2d**, and **2g**, were selected by the NCI. The compounds **2a**, **2c**, and **2d** displayed mild sensitivity toward some leukemia cell lines (Table 1). Compound **2g** possessed considerable activity, and was selected for an advanced assay against a full panel (approximately 60 cell lines) at five concentrations (100, 10, 1, 0.1, and 0.001 μM). Based on the cytotoxicity assays, three antitumor activity dose–response parameters were calculated for each experimental agent against each cell line: GI_{50}, molar concentration of the compound that inhibits 50% net cell growth; TGI, molar concentration of the compound leading to total inhibition; and LC_{50}, molar concentration of the compound leading to 50% net cell death. 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 calculation of the MG_MID, insensitive cell lines were included with the highest concentration tested. In the present study, **2g** exhibited remarkable antitumor activities against most of the tested subpanel tumor cell lines (GI_{50}, TGI, and LC_{50} values less than 100 μM) (Table 2). It showed high activity against HL-60 (TB) (leukemia), SR (leukemia), NCI-H522 (non-small-cell lung), and BT-549 (breast cancer) with a GI_{50} value of less than 2 μM. It also displayed good activity against leukemia (K-562, MOLT-4, RPMI-8226), colon (HCT-15, HT29, KMI2, SW-620), CNS (SF-295, SNB-75), melanoma (MALME-3M, M14, SK-MEL-5, UACC-62), ovarian (OVCAR-3, NCI/ADR-RES), and breast (MCF7) cancer cell lines with a GI_{50} range of 2.01–3.00 μM (Table 2).

With regard to the SAR, we observed that the introduction of an electron withdrawing group at the *para* position of the 6-phenyl unit of **2a** (**2a** was sensitive to a certain leukemia cell line only) made the molecules sensitive to more leukemia cell lines (**2a** vs. **2c**, **2a** vs. **2d**). When two electron donating groups were introduced, one at the *ortho* and the other at the *para* position of the 6-phenyl unit of **2a**, these not only enhanced the remarkable activity against leukemia cell lines but also made the molecule sensitive to the other cancer cell lines, viz. colon, melanoma, ovarian, and breast (**2a** vs. **2g**).



1a, 2a: Ar = C_6H_5
1b, 2b: Ar = 4-$CH_3C_6H_4$
1c, 2c: Ar = 4-ClC_6H_4
1d, 2d: Ar = 4-OCH_3C_6H
1e, 2e: Ar = 4-$C_2H_5C_6H$
1f, 2f: Ar = 2-OH-5-$CH_3C_6H_3$
1g, 2g: Ar = 4-OH-2-$CH_3C_6H_3$
1h, 2h: Ar = 4-OH-3-$CH_3C_6H_3$
1i, 2i: Ar = 3-Cl-4-OHC_6H_3
1j, 2j: Ar = 2,5-(CH3)$2C_6H_3$

Scheme 1. Synthetic route for the preparation of pyridazinones.

Table 1. Anticancer screening data for **2a**, **2c**, and **2d** at concentration of 10^{-5} M.

Compound	NCI code	Mean growth (%)	Most sensitive cell line	Growth of most sensitive cell line (%)
2a	NSC: 747554	108.96	HL-60 (TB) (leukemia)	64.19
2c	NSC: 747553	93.33	RPMI-8226 (leukemia)	38.71
2d	NSC: 747555	88.83	RPMI-8226 (leukemia)	24.18

Table 2. Full panel (60) human tumor cell line anticancer screening data for **2g** (NSC: 747556).

Cancer type	Cell	GI_{50}
Leukemia	CCRF-CEM	9.88
	HL-60 (TB)	1.85
	K-562	3.00
	MOLT-4	2.67
	RPMI-8226	2.35
	SR	1.05
Non-small-cell lung cancer	A549/ATCC	14.0
	EKVX	3.87
	HOP-62	12.8
	HOP-92	11.1
	NCI-H226	10.9
	NCI-H23	14.5
	NCI-H322M	>50
	NCI-H460	2.19
	NCI-H522	1.12
Colon cancer	COLO 205	8.8
	HCC-2998	>50
	HCT-116	3.5
	HCT-15	2.67
	HT29	2.52
	KM12	2.89
	SW-620	2.34
CNS cancer	SF-268	10.1
	SF-295	2.21
	SF-539	6.66
	SNB-19	27.4
	SNB-75	2.95
	U251	6.97
Melanoma	LOX IMVI	10.8
	MALME-3M	2.01
	M14	2.55
	MDA-MB-435	0.99
	SK-MEL-2	16.8
	SK-MEL-28	25.7
	SK-MEL-5	2.10
	UACC-257	31.3
	UACC-62	2.74
Ovarian cancer	IGROV1	6.75
	OVCAR-3	2.69
	OVCAR-4	>50
	OVCAR-5	>50
	OVCAR-8	15.4
	NCI/ADR-RES	2.36
	SK-OV-3	10.8
Renal cancer	786-0	18.9
	A498	9.08
	ACHN	19.3
	CAKI-1	4.08
	RXF 393	6.31
	SN12C	6.68
	TK-10	>50
	UO-31	18.1
Prostate cancer	PC-3	23.5
	DU-145	15.1
Breast cancer	MCF7	2.26
	MDA-MB-231/ATCC	10.9
	HS 578T	6.64
	BT-549	1.49
	T-47D	3.23

Note. GI_{50} represents the concentration of test compound in μM providing 50% inhibition of tumor cell growth.

Anti-inflammatory activity

In the present study, all compounds (**2a–j**) were tested for anti-inflammatory activity by using the carrageenan-induced rat hind paw edema method[19]. Two compounds, namely **2c** and **2f**, exhibited maximum activity.

Structure–activity relationship studies showed that the introduction of lipophilic groups such as methyl and ethyl at the *para* position of the phenyl group attached at C-6 of dihydropyridazinone led to a significant decrease in the activity (**2a** vs. **2b**, **2a** vs. **2e**), while the introduction of a chlorine atom at the same position enhanced the anti-inflammatory activity (**2a** vs. **2c**; Table 3).

The acute gastric ulcerogenic effect of compounds **2c** and **2f** was evaluated in Wistar rats[20]. Six hours after treatment at a dose of 60 mg/kg (three times) they were scarified under deep ether anesthesia and their stomachs removed and opened through the greater curvature for the examination of lesions or bleeding. These compounds did not cause any gastric ulceration.

Antimicrobial activity

All of the compounds at a concentration of 500 μg/mL were screened for their *in vitro* antifungal activity against *Candida albicans*, *Aspergillus fumigatus*, *Aspergillus versicolor*, and *Aspergillus flavus*, and for *in vitro* antibacterial activity against gram-negative *Escherichia coli* and gram-positive *Staphylococcus aureus*, using the cup–plate agar diffusion method by measuring the zone of inhibition in mm[21]. Ciprofloxacin (25 μg/disk) was used as standard drug for antibacterial activity while fluconazole (10 μg/disk) was used for antifungal activity.

Although it seems impossible to extract an obvious structure–activity relationship from the data shown in Table 4, it may be concluded these derivatives exhibited moderate to strong activity against the fungus and weak activity against bacteria.

Table 3. Effect of pyridazinones (**2a–j**, 20 mg/kg) and celecoxib (20 mg/kg) on carrageenan-induced hind paw edema in rats.

	Increase in paw volume after carrageenan administration (mL ± SEM)	
Treatment	3 h	5 h
Vehicle, 10 mL/kg	0.58 ± 0.0026	0.71 ± 0.0045
Celecoxib	0.11 ± 0.0031 (81.03%)	0.30 ± 0.0031 (57.74%)
2a	0.36 ± 0.0022* (37.93%)	0.53 ± 0.0021* (25.35%)
2b	0.45 ± 0.0023* (22.41%)	0.63 ± 0.0031 (11.26%)
2c	0.28 ± 0.0033* (51.72%)	0.41 ± 0.0036* (42.25%)
2d	0.43 ± 0.0023* (25.86%)	0.62 ± 0.0023 (12.67%)
2e	0.47 ± 0.0036 (18.96%)	0.63 ± 0.0040 (11.26%)
2f	0.28 ± 0.0021* (51.72%)	0.49 ± 0.0034* (30.98%)
2g	0.36 ± 0.0021* (37.93%)	0.53 ± 0.0040* (25.35%)
2h	0.33 ± 0.0021* (43.10%)	0.53 ± 0.0026* (25.35%)
2i	0.47 ± 0.0031 (18.96%)	0.64 ± 0.0026 (9.86%)
2j	0.44 ± 0.0021* (24.14%)	0.61 ± 0.0022 (14.08%)

Note. $n = 6$; $^*p < 0.05$ compared to control (one-way ANOVA followed by Dunnett's test). Values in parentheses represent percent inhibition of edema.

Table 4. Antimicrobial activity of pyridazinone derivatives (500 μg/mL).

Compound	*C. albicans*	*A. fumigatus*	*A. versicolor*	*A. flavus*	*S. aureus*	*E. coli*
2a	++	+++	++	++	++	+
2b	++	++	+++	++	+	+
2c	+++	++	++	++	++	+
2d	++	+++	++	++	+	+
2e	++	+++	++	++	+	++
2f	++	++	++	++	–	++
2g	++	+++	++	++	++	+
2h	++	+++	++	++	–	++
2i	+++	++	++	++	+	++
2j	+++	++	++	++	++	++
Fluconazole, 10 μg	++++	++++	+++	+++		
Ciprofloxacin, 25 μg					++++	++++

Note. Zone of inhibition: –, <8 mm (no activity); +, 8–9 mm (weak activity); ++, 10–15 mm (moderate activity); +++, 16–22 mm (strong activity); ++++, >22 mm (standard).

Conclusion

The structures proposed for the synthesized compounds (**2a–j**) are well supported by spectroscopic data and elemental analysis. One compound, **2g**, showed promising broad-spectrum antitumor activity. Two compounds (**2c** and **2f**) were found to have promising anti-inflammatory activity, while a fair number of compounds showed good antifungal activity.

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Declaration of interest

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