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Synthesis, anti-inflammatory and analgesic activity evaluation of some amidine and hydrazone derivatives

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Abstract—A number of amidine derivatives (**3a**–**i**) were synthesized by condensation of cyanopyridine and cyanopyrazine with sulfonylhydrazides in the presence of sodium methoxide. 2-Acetylpyridine and 4-acetylpyridine were condensed with sulfonylhydrazides by microwave irradiation in solid phase to give corresponding hydrazones (**5a**–**d**). Indole-3-carboxaldehyde was condensed with sulfonylhydrazides by refluxing in acetic acid to give corresponding condensation product (**5e** and **f**). All the compounds, that is, **3a**–**i** and **5a**–**f** were purified by crystallization or by column chromatography. Structures of all the synthesized compounds are supported by correct IR, ^{1}H NMR, mass spectral and analytical data. Anti-inflammatory activity evaluation was carried out using carrageenin-induced paw oedema assay and compounds **3e**,**f** and **5e** exhibited good anti-inflammatory activity, that is 52%, 37% and 38% at 50 mg/kg po, respectively. Analgesic activity evaluation was carried out using acetic acid writhing assay and compounds **3a**,**c**,**e** and **5f** showed good analgesic activity, that is, 50%, 50%, 50% and 60% at 50 mg/kg po, respectively.

1. Introduction

Amidine and hydrazone derivatives form a class of compounds possessing a wide range of biological activities. Amidine derivatives possessing anti-degenerative,[1] anticancer,[2,3] anti-platelet[4] and antimicrobial[5] activities have been reported in the literature. Amidine derivatives also act as serine protease inhibitors[6] and nitric oxide synthase inhibitors.[7] Hydrazone derivatives possessing anti-inflammatory, analgesic,[8,9] antipyretic[10] and antibacterial[11] activities are also reported in the literature. In continuation[12–20] of our efforts in search of potent anti-inflammatory and analgesic compounds which can be developed as safer anti-inflammatory drugs, we have synthesized a number of amidine and hydrazone derivatives and screened them for anti-inflammatory and analgesic activities which we wish to report in this paper.

2. Results and discussion

2.1. Chemistry

2-Cyanopyridine, 4-cyanopyridine and 2-cyanopyrazine (**1a**–**c**, Scheme 1) on refluxing with sulfonylhydrazide (**2a**–**c**) in methanol do not give condensation product (**3**; Scheme 1). 2-Cyanopyridine, 4-cyanopyridine and 2-cyanopyrazine when first treated with catalytic amount of sodium methoxide in dry methanol at room temperature for 2–3 h and then adding sulfonylhydrazides and allowing the reaction contents to reflux for 4 h gave condensation products in good yields. Direct condensation of cyanopyridine or cyanopyrazine with sulfonylhydrazide is an addition reaction which does not take place, however, in the presence of sodium methoxide an intermediate **1′** (Scheme 1) will be formed in situ[21] which can easily undergo substitution reaction with sulfonylhydrazide to give condensation products **3a**–**i** (Scheme 1) in good yields. All these compounds were purified by crystallization. Spectral and analytical data of compounds **3a**–**i** reported in Section 4 of this paper fully support the structures assigned to them.

Condensation of 2-acetylpyridine and 4-acetylpyridine with sulfonylhydrazide (**2**, Scheme 2) was carried out

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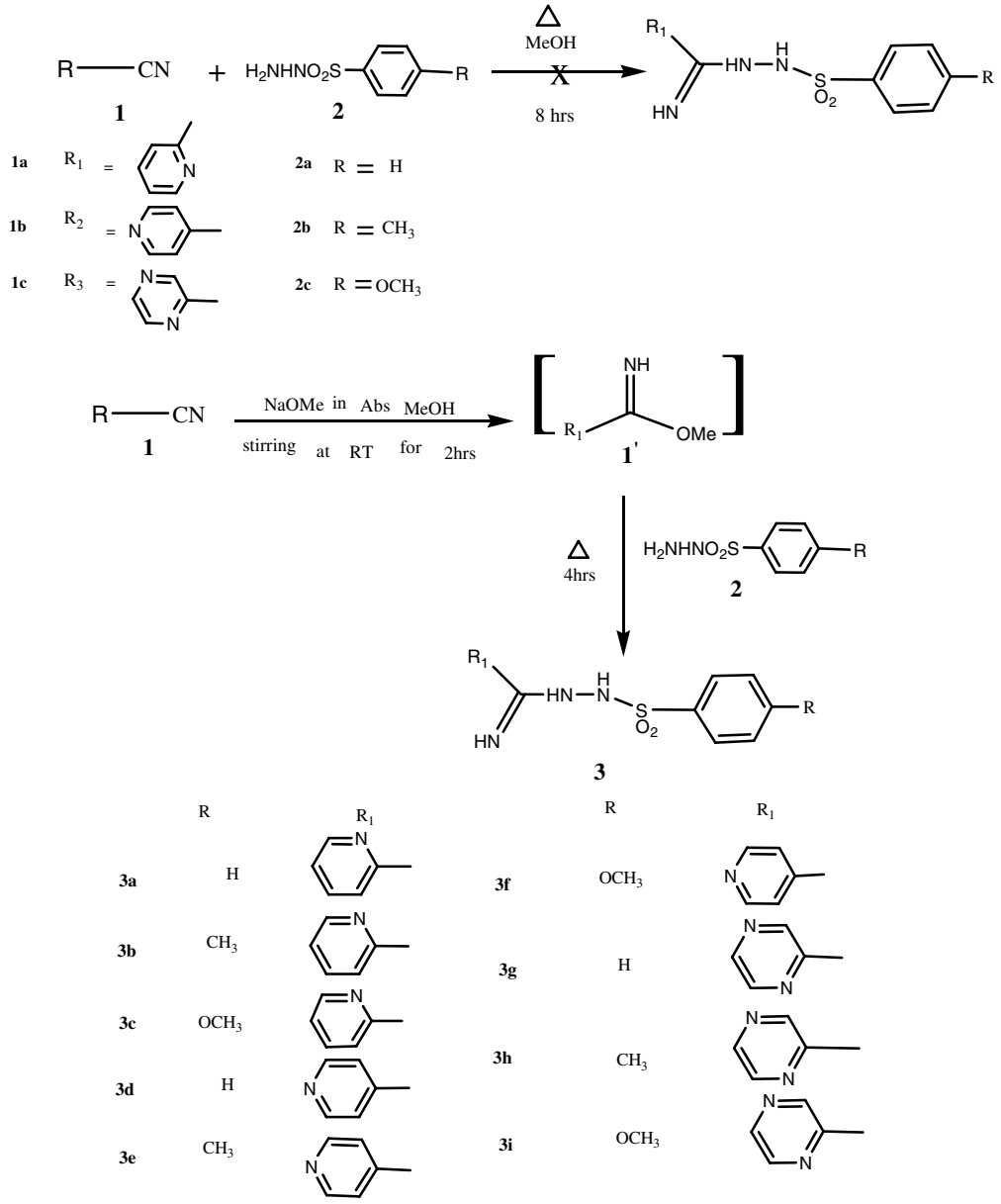
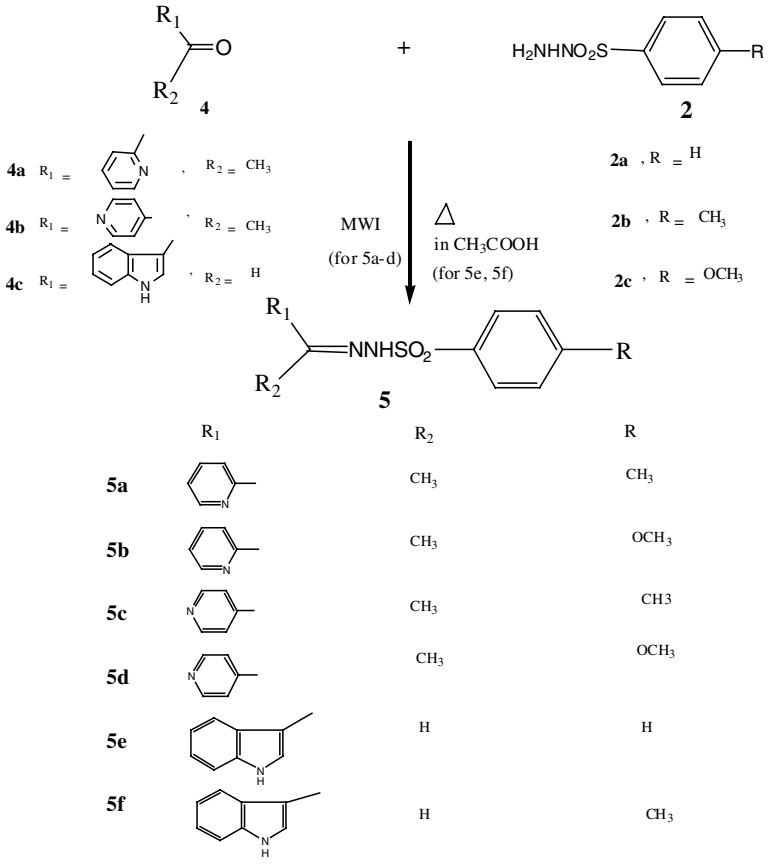


Scheme 1. Synthesis of amidine derivatives.

using microwave irradiation. The condensation was done in solid phase. Both the reactants were dissolved in methanol and a small amount of silica gel was added to it. The solvent was removed under reduced pressure and the silica gel left behind (with both the reactants adsorbed on it) was irradiated in microwave oven at 450 W power for 4–6 min and then to this silica gel was added ethyl acetate. The reaction contents were stirred for 30 min and then filtered to remove silica gel. Filtrate on removal of solvent under reduced pressure gave crude product (**5a**–**d**; Scheme 2), which was purified by crystallization to give pure hydrazone derivatives (**5a**–**d**). The structures assigned to hydrazone derivatives **5a**–**d** are fully supported by correct IR, ^{1}H NMR, FAB-MS and elemental analysis reported in Section 4. Condensation of indole aldehyde (**4c**) with sulfonylhydrazide (**2**) was carried out by refluxing in glacial acetic acid. The crude product (**5e** and **f**) obtained was purified by column chromatography over silica gel to give pure hydrazone derivatives (**5e** and **f**; Scheme 2). The analytical and spectral data of **5e** and **f** reported in Section 4 fully support the structures assigned to them.

2.2. Biological results

Compounds **3a**–**c**,**e**–**i** and **5a**–**f** at 50 mg/kg po were tested for anti-inflammatory activity in the carrageenin-induced paw oedema model[22] and results are summarized in Table 1. Compounds **3a**–**c**,**e**–**i** and **5a**–**f** showed 36%, 32%, 28%, 52%, 37%, 16%, 18%, 29%, 14%, 2%, 3%, 11%, 38% and 36% anti-inflammatory activity, respectively, whereas standard drug aspirin exhibited 43% activity at 50 mg/kg po, compound **3e** exhibited 63% anti-inflammatory activity at 100 mg/kg po, whereas aspirin also exhibited 63% anti-inflammatory



Scheme 2. Synthesis of hydrazone derivatives.

Table 1. Anti-inflammatory, analgesic activity evaluation of compounds (**3a**–**i**) and (**5a**–**f**)

Compounds tested	Anti-inflammatory activity (%) 50 mg/kg po	Analgesic activity (%) 50 mg/kg po
3a	36	50
3b	32	30
3c	28	50
3d	—	0.0
3e	52	50
3f	37	30
3g	16	20
3h	18	10
3i	29	20
5a	14	25
5b	2	25
5c	3	—
5d	11	—
5e	38	40
5f	36	60
Aspirin	43	—
Ibuprofen	—	50

activity at 100 mg/kg po. Anti-inflammatory activity of **3e** was comparable to that of a standard drug aspirin. Compounds **3a**–**i** and **5a**,**b**,**e**,**f** on analgesic activity evaluation using acetic acid writhing assay[23] (Table 1) exhibited 50%, 30%, 50%, 0.0%, 50%, 30%, 20%, 10%, 20%, 25%, 25%, 40% and 60% analgesic activity, respectively. Compound **3e** exhibited 100% analgesic activity at 100 mg/kg po. Ibuprofen, a standard drug, exhibited 75% and 50% analgesic activity at 100 and 50 mg/kg po, respectively.

3. Conclusion

Various amidine (**3a**–**i**) and hydrazone (**5a**–**f**) derivatives have been synthesized and screened for anti-inflammatory and analgesic activities. Compound **3e** exhibited good anti-inflammatory and analgesic activities.

4. Experimental

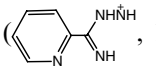
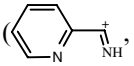
4.1. General

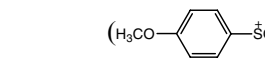
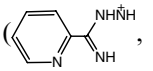
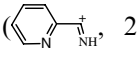
Melting points (mp) were determined on a JSGW apparatus and are uncorrected. Domestic microwave oven model M197 DL (SAMSUNG) was used for microwave irradiation. IR spectra were recorded using a Perkin Elmer 1600FT Spectrometer. ^{1}H NMR spectra were recorded on a Bruker WH-300 Spectrometer in a ca. 5–15% (w/v) solution in appropriate deuterated solvent. FAB-MS was recorded on a Jeol SX-120 (FAB) Spectrometer. Thin-layer chromatography (TLC) was performed on silica gel G for TLC (Merck) and spots were visualized by iodine vapours or by irradiation with UV light (254 nm). Column chromatography was performed by using Qualigens silica gel for column chromatography (60–120 mesh). Elemental analysis was performed using a Vario EL III elementar analyzer.

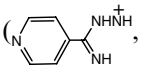
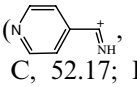
4.2. General procedure for the synthesis of amidine derivatives (3a–i)

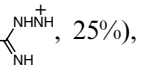
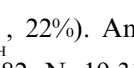
4.2.1. Synthesis of *N*-(phenyl sulfonamido)picolinamidine (3a). Sodium metal 23 mg was dissolved in 20 ml absolute methanol and was labelled as sodium methoxide solution in methanol. 2-Cyanopyridine (0.104 g; 1 mmol) was dissolved in absolute methanol (10 ml) and to it was added sodium methoxide solution (2 ml) prepared above, the reaction contents were stirred at room temperature for 4 h and then benzenesulfonylhydrazide (0.172 g; 1 mmol) was added to it. The reaction contents were heated under reflux for 4 h. Solvent was removed under reduced pressure and to the residue left behind was added distilled water (15 ml) and the solid separated out was filtered, washed with water and air-dried to give crude product, which was purified by crystallization from methanol to give pure product **3a**. Yield 0.180 g, (65%); mp 130 °C; IR (KBr) ν_{max}: 3369 and 3211 (NH), 1635 (C=N), 1577 and 1474 (Ar) cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6 + D_2O) δ: 7.32 (q, J = 5.4 Hz, 1H, Py), 7.49–7.69 (m, J = 8.1 Hz, 7.5 Hz, 5.7 Hz, 4H, 2H (Ar) + 2H (Py)), 7.94–8.01 (dd, J = 8.1 Hz, 7.5 Hz, 3H, Ar), 8.51 (d, J = 4.0 Hz, 1H, Py). FAB-MS m/z 277 (MH^+, 100%), 276 (M^+, 12%). Anal. Calcd for $C_{12}H_{12}N_4O_2S$: C, 52.17; H, 4.34; N, 20.28; S, 11.59. Found: C, 52.00; H, 4.21; N, 20.51; S, 11.31.

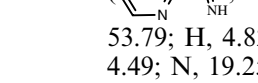
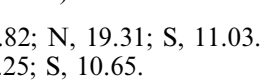
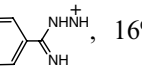
Similarly were synthesized compounds **3b**–**i**.

4.2.2. *N*-(4-Methyl phenyl sulfonamido)picolinamidine (3b). Solvent of crystallization: MeOH; 0.141 g (48%); mp 175 °C; IR (KBr) ν_{max}: 3454 and 3347 (NH), 1645 (C=N), 1592 and 1470 (Ar) cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6 + $CDCl_3$) δ: 2.40 (s, 3H, CH_3), 6.12 (br s, 2H, NH, NH, exch), 6.37 (br s, 1H, NH, exch), 7.29 (t, J = 8.1 Hz, 7.9 Hz, 6.9 Hz, 3H, 2H (Ar) + 1H (Py)), 7.62 (m, J = 6.9 Hz, 1H, (Py)), 7.87 (d, J = 8.1 Hz, 2H, Ar), 7.95 (d, J = 7.9 Hz, 1H, Py), 8.48 (d, J = 4.5 Hz, 1H, Py). FAB-MS m/z 291 (MH^+, 100%), m/z 290 (M^+, 13%), 135 ( , 15%), 105 ( , 20%). Anal. Calcd for $C_{13}H_{14}N_4O_2S$: C, 53.79; H, 4.82; N, 19.31; S, 11.03. Found: C, 53.74; H, 4.49; N, 19.25; S, 10.65.

4.2.3. *N*-(4-Methoxy phenyl sulfonamido)picolinamidine (3c). Solvent of crystallization: MeOH; 0.180 g (59%); mp 140 °C; IR (KBr) ν_{max}: 3362 and 3190 (NH), 1650 (C=N), 1592 and 1497 (Ar) cm^{-1}. ^1H NMR (300 MHz; DMSO-d_6 + $CDCl_3$) δ: 3.87 (s, 3H, OCH_3), 6.12 (br s, 2H, NH, NH, exch), 7.00 (q, J = 8.7 Hz, 2H, Ar), 7.32 (t, J = 6.9 Hz, 1H (Py)), 7.47 (br s, 1H, NH, exch), 7.67 (m, J = 6.9 Hz, 1H, Py), 7.97 (m, J = 8.7 Hz, 3H, Ar), 8.52 (d, J = 4.5 Hz, 1H, Ar). FAB-MS m/z 307 (MH^+, 100%), 306 (M^+, 7%), 171 ( , 10%), 135 ( , 22%), 105 ( , 21%). Anal. Calcd for $C_{13}H_{14}N_4O_3S$: C, 50.98; H, 4.57; N, 18.30; S, 10.45. Found: C, 50.59; H, 4.40; N, 18.15; S, 10.63.

4.2.4. *N*-(Phenyl sulfonamido)isonicotiamidine (3d). Solvent of crystallization: MeOH; 0.110 g (40%); mp 180 °C; IR (KBr) ν_{max}: 3469 (NH), 1650 (C=N), 1546 (Ar) cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6 + $CDCl_3$), 6.00 (br s, 2H, NH, NH, exch), 7.49–7.59 (m, J = 7.2 Hz, 5.7 Hz, 5H, 3H (Ar) + 2H (Py)), 7.92–7.99 (t, J = 7.2 Hz, 2H, Ar), 8.54–8.56 (t, J = 5.7 Hz, 2H, Py), 9.30 (br s, 1H, NH, exch). FAB-MS m/z 277 (MH^+, 100%), m/z 276 (M^+, 15%), 135 ( , 17%), 105 ( , 5%). Anal. Calcd for $C_{12}H_{12}N_4O_2S$: C, 52.17; H, 4.34; N, 20.28; S, 11.59. Found: C, 51.72; H, 4.03; N, 19.81; S, 11.14.

4.2.5. *N*-(4-Methyl phenyl sulfonamido)isonicotiamidine (3e). Solvent of crystallization: MeOH; 0.143 g (49%); mp 197–200 °C; IR (KBr) ν_{max}: 3458 and 3352 (NH), 1644 (C=N), 1602 (Ar) cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6 + $CDCl_3$) δ: 2.42 (s, 3H, CH_3), 6.14 (br s, 2H, NH, NH, exch), 7.31–7.34 (d, J = 8.1 Hz, 2H (Ar)), 7.58–7.60 (dd, J = 6.0 Hz, 2H, (Py)), 7.82–7.85 (d, J = 8.1 Hz, 2H, Ar), 8.54–8.56 (t, J = 6 Hz, 2H, Py), 9.30 (br s, 1H, NH, exch). FAB-MS m/z 291 (MH^+, 100%), 290 (M^+, 12%), 135 ( , 25%), 105 ( , 22%). Anal. Calcd for $C_{13}H_{14}N_4O_2S$: C, 53.79; H, 4.82; N, 19.31; S, 11.03. Found: C, 53.54; H, 5.03; N, 19.52; S, 10.84.

4.2.6. *N*-(4-Methoxy phenyl sulfonamido)isonicotiamidine (3f). Solvent of crystallization: MeOH; 0.138 g (45%); mp 210 °C; IR (KBr) ν_{max}: 3433 and 3347 (NH), 1648 (C=N), 1598 and 1495 (Ar) cm^{-1}. ^1H NMR (300 MHz; DMSO-d_6 + $CDCl_3$) δ: 3.86 (s, 3H, OCH_3), 6.05 (br s, 2H, NH, NH, exch), 6.97–7.02 (m, J = 6.9 Hz, 2H, Ar), 7.57–7.61 (q, J = 4.6 Hz, 2H (Py)), 7.87–7.9 1 (m, J = 6.9 Hz, 2H, Ar), 8.55–8.57 (q, J = 4.8 Hz, 2H, Py), 9.13 (s, 1H, NH, exch). FAB-MS m/z 307 (MH^+, 100%), m/z 306 (M^+, 7%), 228 ( , 8%), 226 ( , 50%) 135 ( , 16%), 105 ( , 18%). Anal. Calcd for $C_{13}H_{14}N_4O_3S$: C, 50.98; H, 4.57; N, 18.30; S, 10.45. Found: C, 50.49; H, 4.29; N, 18.17; S, 9.95.

4.2.7. *N*-(Phenyl sulfonamido)pyrazine-2-carboxamidine (3g). Solvent of crystallization: MeOH; 0.145 g (52%) mp 205 °C; IR (KBr) ν_{max}: 3324 (NH), 1615 (C=N), 1515 (Ar) cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6 + $CDCl_3$) δ: 6.25 (s, 2H, NH, NH, exch), 7.50–7.61 (m, J = 8.1 Hz, 3H, Ar), 7.97–8.00 (d, J = 8.1 Hz, 2H, Ar), 8.45 (d, 1H, Pyr), 8.54 (d, 1H, Pyr), 9.20 (s, 1H, Pyr), 9.53 (br s, 1H, NH, exch). FAB-MS m/z 278 (MH^+, 55%), 277 (M^+, 10%). Anal. Calcd for

$C_{11}H_{11}N_5O_2S$: C, 47.65; H, 3.97; N, 25.27; S, 11.55. Found: C, 47.44; H, 3.92; N, 25.29; S, 11.66.

4.2.8. *N*-(4-Methyl phenyl sulfonamido)pyrazine-2-carboxamidine (3h). Solvent of crystallization: MeOH; 0.252 g (86%); mp 182–185 °C; IR (KBr) ν_{max}: 3426 and 3330 (NH), 1665 (C=N), 1604 and 1478 (Ar) cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6 + $CDCl_3$) δ: 2.44 (s, 3H, CH_3), 5.97 (br s, 2H, NH, NH, exch), 6.73 (br s, 1H, NH, exch), 7.33–7.36 (d, J = 8.1 Hz, 2H, Ar), 7.89–7.92 (d, J = 8.1 Hz, 2H, Ar), 8.47–8.48 (t, J = 2.7 Hz, 1.2 Hz, 1H, Pyr), 8.60–8.61 (d, J = 2.7 Hz, 1H, Pyr), 9.220–9.224 (d, J = 1.2 Hz, 1H, Pyr). ES-MS *m/z* 314 (MNa^+, 100%), *m/z* 292 (MH^+, 45%). Anal. Calcd for $C_{12}H_{13}N_5O_2S$: C, 49.48; H, 4.46; N, 24.05; S, 10.99. Found: C, 49.55; H, 4.11; N, 23.99; S, 10.49.

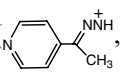
4.2.9. *N*-(4-Methoxy phenyl sulfonamido)pyrazine-2-carboxamidine (3i). Solvent of crystallization: MeOH; 0.178 g (58%); mp 175 °C; IR (KBr) ν_{max}: 3420 and 3308 (NH), 1603 (C=N), 1515 and 1471 (Ar) cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6 + $CDCl_3$) δ: 3.83 (s, 3H, OCH_3), 6.27 (s, 2H, NH, NH, exch), 6.98–7.02 (t, J = 8.7 Hz, 2H, Ar), 7.90–7.93 (t, J = 8.7 Hz, 2H, Ar), 8.44–8.45 (t, J = 2.7 Hz, 1H, Pyr), 8.54–8.55 (d, J = 2.7 Hz, 1H, Pyr), 9.20 (d, J = 1.1 Hz, 1H, Pyr), 9.51 (br s, 1H, NH, exch). FAB-MS *m/z* 308 (MH^+, 100%), 307 (M^+, 32%). Anal. Calcd for $C_{12}H_{13}N_5O_3S$: C, 46.90; H, 4.23; N, 22.80; S, 10.42. Found: C, 47.01; H, 4.34; N, 23.01; S, 10.03.

4.3. General procedure for synthesis of hydrazide derivatives (5a–d)

4.3.1. 4-Methyl-*N*′-(1-pyridin-2-yl)ethylidine benzenesulfonohydrazide (5a)[24]. 2-Acetyl pyridine (0.121 g; 1 mmol) and *p*-tolylsulfonylhydrazide (0.186 g; 1 mmol) were dissolved in methanol (10 ml) and to this solution was added silica gel (5 g; 60–120 mesh). Solvent from above solution was removed under reduced pressure and the silica gel (adsorbed with acetyl pyridine and *p*-tolylsulfonylhydrazide) left behind was irradiated in microwave oven at 450 W for 4 min (with 1 min interval after 1 min irradiation). Microwave irradiated silica gel was stirred in ethyl acetate (30 ml) for 30 min and then filtered. The silica gel left behind on the filter paper was washed with ethyl acetate (2× 5 ml). Solvent from ethyl acetate solution was removed under reduced pressure and the solid residue left behind was crystallized from $CHCl_3$:pet. ether (50:50) to give pure product **5a**. Yield 0.188 g (65%); mp 130 °C; IR (KBr) ν_{max}: 3226 (NH), 1634 (C=N), 1595 (Ar) cm^{-1}. ^1H NMR (300 MHz; $CDCl_3$) δ: 2.27 (s, 3H, $CH_3-\underset{}{\overset{}{C}}=N-$), 2.41 (s, 3H, CH_3), 7.2 (m, J = 1.8 Hz, 1H; Py), 7.32 (d, J = 8.1 Hz, 2H, Ar), 7.68 (m, J = 7.8 Hz; 1.8 Hz, 1H, Py), 7.90 (d, J = 8.1 Hz, 2H, Ar), 7.99 (d, 1H, J = 7.8 Hz., Py), 8.50 (d, 1H, Py), NH is expected downfield. ES-MS *m/z* 312 (MNa^+, 100%), 290 (MH^+, 44%). Anal. Calcd for $C_{14}H_{15}N_3O_2S$: C, 58.13; H, 5.19; N, 14.53; S, 11.07. Found: C, 58.00; H, 5.00; N, 14.71; S, 11.38.

Similarly were prepared compounds **5b–d**.

4.3.2. 4-Methoxy-*N*′-(1-pyridin-2-yl)ethylidine benzenesulfonohydrazide (5b). Solvent of crystallization: $CHCl_3$:pet. ether (50:50), 0.302 g (99%); mp 135 °C; IR (KBr) ν_{max}: 3168 (NH), 1634 (C=N), 1596 and 1499 (Ar) cm^{-1}. ^1H NMR (300 MHz; $CDCl_3$) δ: 2.27 (s, 3H, $CH_3-\underset{}{\overset{}{C}}=N-$), 3.85 (s, 3H, OCH_3), 6.90 (d, J = 8.7 Hz, 2H, Ar), 7.26 (dd, J = 6.3 Hz, 1H, Py), 7.69 (m, J = 6.3 Hz, 1H, Py), 7.96 (d + m, J = 8.7 Hz, 4.9 Hz; 3H, 2H (Ar) + IH (Py)), 8.66 (dd, J = 4.8 Hz, 1H, Py), NH is expected downfield. ES-MS *m/z* 328 (MNa^+, 100%), 306 (MH^+, 10%). Anal. Calcd for $C_{14}H_{15}N_3O_3S$: C, 55.08; H, 4.91; N, 13.77; S, 10.49. Found: C, 54.74; H, 4.71; N, 13.40; S, 10.73.

4.3.3. 4-Methyl-*N*′-(1-pyridin-4-yl)ethylidine benzenesulfonohydrazide (5c). Solvent of crystallization: $CHCl_3$:pet. ether (50:50), 0.145 g (50%) mp 158 °C; IR (KBr) ν_{max}: 3009 (NH), 1596 (C=N) and 1495 (Ar) cm^{-1}. ^1H NMR (300 MHz; $CDCl_3$) δ: 2.16 (s, 3H, $CH_3-\underset{}{\overset{}{C}}=N-$) 2.42 (s, 3H, CH_3), 7.32 (d, J = 8.4 Hz, 2H, Ar), 7.59 (d, J = 6 Hz, 2H, Py), 7.89 (d, J = 8.4 Hz, 2H, Ar), 8.64 (d, J = 6 Hz, 2H, Py), NH is expected downfield. FAB-MS *m/z* 290 (MH^+, 100%), 289 (M^+, 24%), 155 ($H_3CO-C_6H_4-\overset{+}{S}O_2$, 24%), 134 ( , 15%). Anal. Calcd for $C_{14}H_{15}N_3O_2S$: C, 58.13; H, 5.19; N, 14.53; S, 11.07. Found: C, 58.31; H, 5.02; N, 14.71; S, 11.35.

4.3.4. 4-Methoxy-*N*′-(1-pyridin-4-yl)ethylidine benzenesulfonohydrazide (5d). Solvent of crystallization: $CHCl_3$:pet. ether (50:50), 0.153 g (50%); mp 137 °C; IR (KBr) ν_{max}: 3069 (NH), 1589 (C=N) and 1494 (Ar) cm^{-1}. ^1H NMR (300 MHz; $CDCl_3$) δ: 2.16 (s, 3H, $CH_3-\underset{}{\overset{}{C}}=N-$), 3.86 (s, 3H, OCH_3), 7.0 (d, J = 7.2 Hz, 2H, Ar), 7.60 (d, J = 4.8 Hz, 2H, Py), 7.96 (d, J = 7.2 Hz, 2H, Ar), 8.73 (d, J = 4.8 Hz, 2H, Py), NH is expected downfield, ESMS *m/z* 328 (MNa^+, 65%), 306 (MH^+, 30%). Anal. Calcd for $C_{14}H_{15}N_3O_3S$: C, 55.08; H, 4.91; N, 13.77; S, 10.49. Found: C, 55.41; H, 4.81; N, 13.51; S, 9.97.

4.3.5. Synthesis of *N*′-((1*H*-indol-3-yl)methylene)-benzenesulfonohydrazide (5e)[25]. Indole-3-carboxaldehyde (0.145 g; 1 mmol) and benzenesulfonohydrazide (0.172 g; 1 mmol) were dissolved in glacial acetic acid (10 ml) and reaction contents were heated under reflux for 9 h and the solvent was removed under reduced pressure. To the residue left behind was added 10% sodium carbonate solution (10 ml) and then extracted with ethyl acetate (2× 20 ml). The ethyl acetate extract was washed with water and then dried over anhydrous sodium sulfate. Ethyl acetate was removed under reduced pressure and the residue left behind was purified by column chromatography over silica gel. Elution with $CHCl_3$:ethyl acetate (8:2) removed side products and further elution with ethyl acetate gave pure product **5e**.

Yield 0.111 g (37%); mp 150 °C; IR (KBr) ν_{max}: 3206 (NH), 1639 (C=N), 1521 (Ar) cm^{-1}. ^1H NMR (300 MHz; $CDCl_3$) δ: 7.23 (t, 3H, Ar), 7.49 (m, 3H, Ar), 7.57 (q, 1H, Ar), 7.88 (q, 2H, Ar), 8.23 (t, 1H, Ar), 9.99 (s, 1H, H—C=N—), 10.10 (s, 1H, NH, exch), 11.46 (s, 1H, NH, exch). FAB-MS *m/z* 300 (MH^+, 5%), 299 (M^+, 10%). Anal. Calcd for $C_{15}H_{13}N_3O_2S$: C, 60.20; H, 4.34; N, 14.04; S, 10.70. Found: C, 60.41; H, 4.21; N, 14.00; S, 10.91.

Similarly was synthesized compound **5f**.

4.3.6. *N*′-((1*H*-Indol-3-yl)methylene)-4-methylbenzenesulfonohydrazide (5f). Elution solvent: ethyl acetate; 0.163 g (52%); mp 165 °C; IR (KBr) ν_{max}: 3384 (NH), 1597 (C=N), 1527 and 1493 (Ar) cm^{-1}. ^1H NMR (300 MHz; $CDCl_3$) δ: 2.42 (s, 3H, CH_3) 7.30 (m, 6H, 5H (Ar) + 1H (NH) exch), 7.41 (q, 1H, Ar), 7.79 (t, 2H, Ar), 7.86 (d, 1H, Ar), 8.31 (t, 1H, Ar), 8.95 (br s, 1H, NH exch), 10.06 (s, 1H, H—C=N—). FAB-MS *m/z* 314 (MH^+, 100%), 313 (M^+, 40%). Anal. Calcd for $C_{16}H_{15}N_3O_2S$: C, 61.34; H, 4.79; N, 13.41; S, 10.22. Found: C, 61.08; H, 4.38; N, 13.12; S, 9.91.

4.4. Anti-inflammatory activity

Paw oedema inhibition test was used on albino rats of Charles Foster strain by adopting the method of Winter.[22] Groups of five animals of both sexes (body weight 120–160 g), pregnant females excluded, were given a dose of a test compound. Thirty minutes later, 0.20 ml of 1% freshly prepared carrageenan suspension in 0.9% NaCl solution was injected subcutaneously into the plantar aponeurosis of the hind paw and the volume was measured by a water plethysmometer apparatus and then measured again 1–3 h later. The mean increase of paw volume at each time interval was compared with that of control group (five rats treated with carrageenan, but not with test compounds) at the same time intervals and percent inhibition values were calculated by the formula given below.

$$\% \text{ anti-inflammatory activity} = [1 - D_t/D_c] \times 100$$

D_t and D_c are paw volumes of oedema in tested and control groups, respectively. Compounds **3a**–**c**,**e**–**i** and **5a**–**f** were screened for anti-inflammatory activity at 50 mg/kg po. All these compounds exhibited 36%, 32%, 28%, 52%, 37%, 16%, 18%, 29%, 14%, 2%, 3%, 11%, 38% and 36% anti-inflammatory activity as compared to standard drug aspirin which showed 43% activity at 50 mg/kg po.

4.5. Analgesic activity

Acetic acid writhing test was performed on mice by following the method of Davis et al.[23] Groups of five mice (body weight 20–30 g) of both sexes, pregnant female excluded, were given a dose of a test compound. Thirty minutes later, the animals were injected intraperitoneally with 0.25 ml/mouse of 0.5% acetic acid solution and writhes were counted during the following 60 min. The mean number of writhes for each experimental group and percent decrease compared with control group (five mice not treated with test compounds) were calculated. Compounds **3a**–**i** and **5a**,**b**,**e**,**f** at 50 mg/kg po exhibited 50%, 30%, 50%, 0.0%, 50%, 30%, 20%, 10%, 20%, 25%, 25%, 40% and 60%, respectively, analgesic activity compared to Ibuprofen which showed 50% analgesic activity at 50 mg/kg po.

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