Synthesis, Anti-Inflammatory, and Anticancer Activity Evaluation of Some Heterocyclic Amidine and Bis-Amidine Derivatives

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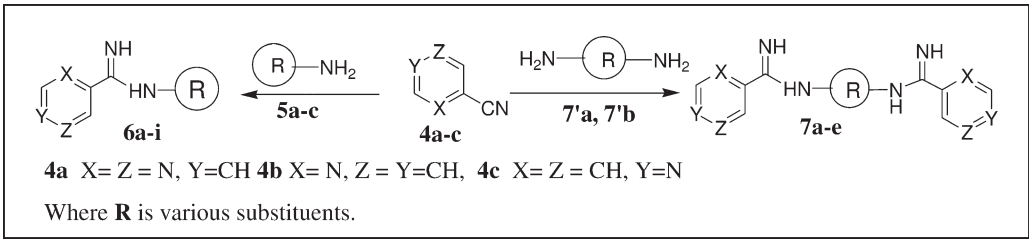

Heterocyclic amidine derivatives of benzothiazole (**6a–c**), benzimidazole (**6d–f**), benzoxazole (**6g–i**), and bis-amidine derivatives of pyrimidine, (**7a**, **7b**) & triazole (**7c–e**) ring system have been synthesized by nucleophilic addition reaction. All these compounds were screened for anti-inflammatory and anticancer activities. At a dose of 50 mg/kg p.o., compounds **6c** (39%), **6e** (39%), and **6f** (39%) exhibited anti-inflammatory activity comparable to standard drug ibuprofen, which showed 39% activity and compounds **6b**, **6e**, **7a**, and **7c** exhibited moderate anticancer activity against cervix (HELA); neuroblastoma (IMR-32); breast (MCF-7), leukemia (THP-1); and cervix (HELA) human cancer cell lines, respectively.

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INTRODUCTION

At present, inflammatory diseases and cancer are major health problems faced by human beings. Benzothiazole, benzimidazole, benzoxazole, pyrimidine, and triazole derivatives are the most promising class of heterocyclic molecules having many interesting activity profiles. These structural motifs possess wide variety of biological activities such as anti-inflammatory [1–4], anticancer [5–8], antimicrobial [9], antibacterial [10], and antifungal [11]. Riluzole drug, which is a derivative of benzothiazole, is useful in the treatment of brain diseases and prostate cancer [12]. Amidino-benzimidazole derivatives exhibit anticancer activity by targeting the minor groove of DNA [13]. During the last few decades, simple and small amidines and their derivatives have been widely tested as antitumor agents *in vitro* and *in vivo* treatment [14–17]. Amidine and bis-amidine derivatives itself possess anti-inflammatory [18–20], antimicrobial [21], antiparasitic [22], antibacterial [23], and antiprotozoal [24] activities.

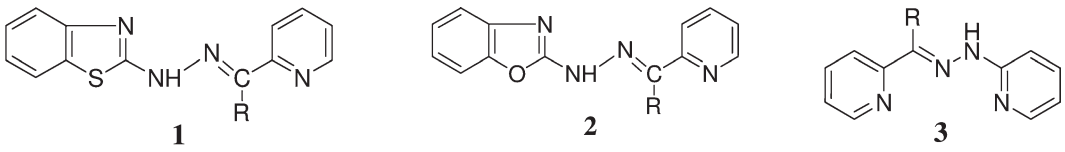
Benzothiazole derivatives [25] (**1**, Fig. 1), benzoxazole derivatives [25] (**2**, Fig. 1) and 2-acylpyridine-α-(*N*)-hetarylhydrazones [26] (**3**, Fig. 1) exhibiting good anticancer activity is reported in literature.

Based on this hypothesis and in continuation of our efforts [27,28] in search of potent molecules exhibiting anticancer and/or anti-inflammatory activity, we have synthesized a number of small heterocyclic molecules bearing benzothiazole, benzimidazole benzoxazole, pyrimidine & triazole moiety and screened them for anticancer and anti-inflammatory activities, which we wish to report in this article.

RESULTS AND DISCUSSION

Chemistry. The 2-aminobenzimidazole **5b** (Scheme 1) was synthesized according to reported procedure [29]. 2-Cyanopyrazine (**4a**), was first allowed to react with sodium methoxide by stirring at room temperature for 2 h, using absolute methanol as solvent of reaction to give *in situ* intermediate [30] **4′** (Scheme 1). Intermediate **4′** undergoes substitution reaction with 2-aminobenzothiazole **5a** (Scheme 1) to give product **6a** in 8 h. Compound **6a** was purified by recrystallization from methanol and was obtained in good yield. IR, ^{1}H Nuclear Magnetic Resonance (NMR) and gas chromatography mass spectroscopy (GC-MS) spectral data reported in experimental section fully support the structure assigned to compound **6a**.

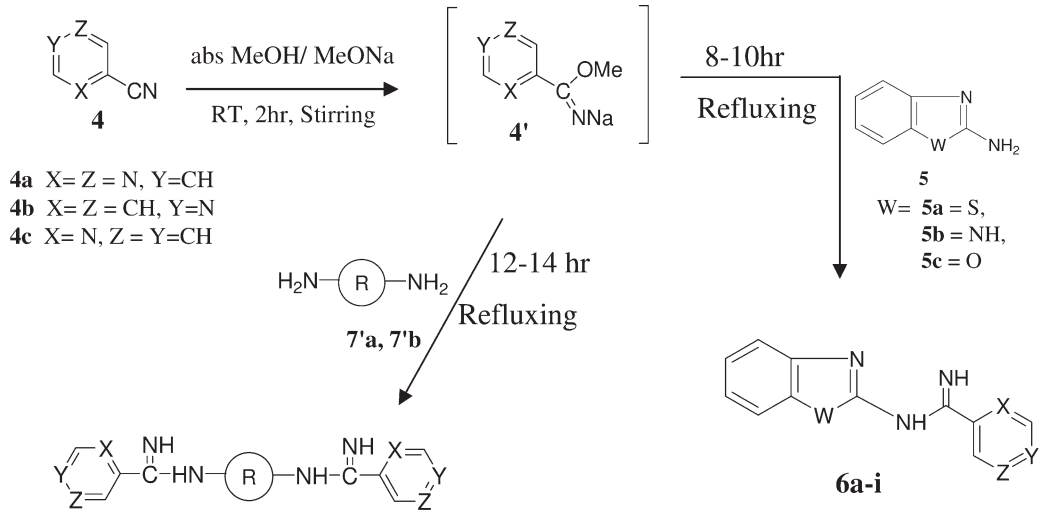
Condensation of 2-cyanopyrazine (**4a**), 2-cyanopyridine (**4b**), and 4-cyanopyridine (**4c**) with 2-aminobenzothiazole (**5a**), 2-aminobenzimidazole (**5b**), and 2-

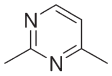
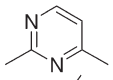
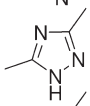
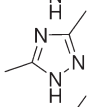
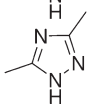


1 R=H, CH_3 **2** R=H, CH_3 **3** R=H, CH_3

Figure 1. Anticancer agents.

Scheme 1. Synthesis of amidine and bis-amidine derivatives.



	R	X	Y	Z
7a		N	CH	CH
7b		CH	N	CH
7c		N	CH	CH
7d		CH	N	CH
7e		N	CH	N

	W	X	Y	Z
6a	S	N	CH	N
6b	S	N	CH	CH
6c	S	CH	N	CH
6d	NH	N	CH	N
6e	NH	N	CH	CH
6f	NH	CH	N	CH
6g	O	N	CH	N
6h	O	N	CH	CH
6i	O	CH	N	CH

Table I

Anti-inflammatory and anticancer activity evaluation of compounds **6a-i** and **7a-e.**

Compds tested	Anti-inflammatory activity at 50 mg/kg p.o.	Anticancer activity at a concentration of $1x10^{-5}M$ % growth inhibition					
		Lung A-549	Cervix HELA	Prostate DU-145	Breast MCF-7	Leukaemia THP-1	Neuroblastoma IMR-32
6a	37	3	22	35	1	13	46
6b	29	0	53	13	15	10	28
6c	39	0	34	30	0	11	22
6d	25	0	34	21	0	13	43
6e	39	3	18	31	5	0	53
6f	39	0	16	33	0	15	30
6g	25	4	15	20	0	0	07
6h	36	2	0	28	0	36	0
6i	30	5	19	8	18	3	37
7a	26	2	22	4	52	54	23
7b	22	0	8	5	15	10	28
7c	26	0	55	8	12	18	35
7d	0	0	37	10	14	17	34
7e	11	0	20	16	7	18	39
Ibuprofen	39	–	–	–	–	–	–
Mitomycin-C	–	–	60	–	77	–	–
Adriamycin	–	82	44	65	75	76	85
Paclitaxel	–	52	–	–	72	–	66
5-FU	–	42	–	–	–	–	–

aminobenzoxazole (**5c**) was carried out as mentioned above, using absolute methanol as solvent of reaction to give amidine derivatives, *i.e*., **6a–i**. All these compounds were purified by crystallization from methanol and structures assigned to **6a–i** are fully supported by spectral data, *i.e*., IR, ^{1}H-NMR, GC-MS and elemental analysis.

A solution of 2-cyanopyridine **4b** (Scheme 1) (0.208 g; 2 m mol) and sodium methoxide (0.4 mL) in absolute methanol was stirred for 2 h at room temperature to get the intermediate **4′**, after that pyrimidine-2,4-diamine **7′a** (0.110 g; 1 m mol) was added to the reaction mixture. The reaction contents were heated under reflux for 12 h to give **7a**. Crude product **7a** was purified by crystallization from methanol to give pure product in 75% yield. Spectral and analytical data of **7a** reported in experimental section of this article fully support the structure assigned to it.

Condensation of 2-cyanopyridine (**4b**) and 4-cyanopyridine (**4c**) with pyrimidine-2,4-diamine **7′a**, and 2-cyanopyrazine (**4a**), 2-cyanopyridine (**4b**) and 4-cyanopyridine (**4c**) with 1*H*-1,2,4-triazole-3,5-diamine **7′b** by following reaction procedure mentioned above gave **7a–e** in good yields after purification by crystallization from methanol. Structures assigned to 7**a–e** are fully supported by spectral and analytical data reported in experimental section of this article.

PHARMACOLOGICAL EVALUATION

Compounds **6a–i** and **7a–e** were screened for anti-inflammatory activity [31] using carrageenan-induced paw oedema assay, and results are summarized in Table 1. Compounds **6a–i** and **7a–e** at 50 mg/kg *p.o*. exhibited 37, 29, 39, 25, 39, 39, 25, 37, 30, 26, 22, 26, 0, 11% activity, respectively, whereas ibuprofen exhibited 39% anti-inflammatory activity at 50 mg/kg p.o. A look at Table 1 indicates that compounds **6c**, **6e**, and **6f** exhibited anti-inflammatory activity comparable with standard drug ibuprofen.

In vitro anticancer activity [32,33] evaluation of compounds **6a–i** and **7a–f** was carried out against six human cancer cell lines consisting of lung (A-549), cervix (HELA), prostate (DU-145), breast (MCF-7), leukaemia (THP-1), and neuroblastoma (IMR-32). Percentage (%) growth inhibition of cancer cell lines was determined at a concentration of 1×10^{-5} *M*, and results are summarized in Table 1. A look at Table 1 indicates that compounds **6b** and **7c** possess moderate anticancer activity against cervix (HELA) cancer cell line. Compound **6e** exhibited moderate anticancer activity against neuroblastoma (IMR-32), and **7a** showed moderate anticancer activity against two cancer cell lines, *i.e*., breast (MCF-7) and leukaemia (THP-1). These activities may be due to the fact that these molecules meet stereochemical and electronic requirements of the target in a better way as compared with other molecules.

STRUCTURE ACTIVITY RELATIONSHIP

Mono amidine derivatives **6a–i** exhibited good anti-inflammatory activity where as bis-amidine derivatives

exhibited moderate to weak anti-inflammatory activity. From this observation, it may be concluded that small molecules can interact with the target in a better way as compared with big molecules. As derivatives of both the systems, *i.e.*, **6b**, **6e**, and **7a**, **7c** exhibited moderate anticancer activity, it can be concluded that these types of amidines and bis-amidines do not interact very well with targets responsible for causing cancer.

CONCLUSION

A number of amidine (**6a–i**) and bis-amidine (**7a–e**) derivatives have been synthesized in good yield. These heterocyclic compounds were screened for anti-inflammatory and anticancer activities. Out of 14 compounds screened, three compounds, *i.e.*, **6c**, **6e**, and **6f** exhibited good anti-inflammatory activity.

EXPERIMENTAL

General. Melting points (mp) were determined on a JSGW apparatus and are uncorrected. Infrared (IR) spectra were recorded using a Perkin Elmer 1600 FT spectrometer. ^{1}H-NMR spectra were recorded on a Bruker WH-500 spectrometer at a ca 5–15% (w/v) solution in DMSO-d_6 (tetramethylsilane (TMS) as internal standard). GC-MS was recorded on Perkin Elmer Clarus 500 gas chromatograph with built-in mass spectrometer detector. Elemental analysis was carried out on a Vario EL III elementor. Thin layer chromatography (TLC) was performed on silica gel G for TLC (Merck), and spots were visualized by iodine vapor or by irradiation with ultraviolet light (254 nm).

Reaction procedure for synthesis of amidine derivatives. Sodium metal (23 mg) was dissolved in absolute methanol (20 mL) and was labeled as sodium methoxide solution in methanol. 2-Cyanopyrazine (0.105 g; 1 mmol) was dissolved in absolute methanol (10 mL), and to it was added sodium methoxide solution (0.5 mL) prepared above, and the reaction contents were stirred at room temperature for 2 h. 2-Aminobenzothiazole (0.150 g; 1 mmol) was added to the reaction mixture. The reaction contents were heated under reflux for 8 h. Solvent was removed under reduced pressure, and to the residue left behind was added diethyl ether, and the solid separated out was filtered, washed with ether and air-dried to give crude product, which was purified by crystallization from methanol to give pure product **6a**, *i.e., N-(Benzo[d]thiazol-2-yl)pyrazine-2-carboxamidine*. Yield: (0.235 g) 92%; m.p. 240–242°C; IR(KBr) ν_{max}: 3352 (NH), 1623 (C=N), 1566, 1513, and 1466 (Ar) cm^{-1}. ^{1}H-NMR (500MHz; DMSO-d_6) δ: 7.32–7.35 (t, 1H, Ar), 7.44–7.47 (t, 1H, Ar), 7.84–7.85 (d, 1H, Ar), 7.94–7.96 (d, 1H, Ar), 8.899–8.903 (d, 1H, Ar), 9.04–9.05 (d, 1H, Ar), 9.31 (bs, 1H, NH exch.), 9.77 (s, 1H, Ar), 10.05 (bs, 1H, NH exch). GC-MS *m/z*: 255 (M^+, 35%). Anal. Calcd. for $C_{12}H_9N_5S$: C, 56.47; H, 3.53; N, 27.45; S,12.55%. Found: C, 56.43; H, 3.53; N, 27.42; S, 12.53%.

Similarly compounds **6b–i** were synthesized and purified by crystallization from MeOH.

N-(Benzo[d]thiazol-2-yl)picolinamidine (6b). Solvent of crystallization: MeOH; Yield: 85%; m.p. 188–189°C; IR(KBr) ν_{max}: 3401 (NH), 1614 (C=N), 1570, 1465, and 1426 (Ar) cm^{-1}. ^{1}H-NMR (500MHz; DMSO-d_6) δ: 7.30–7.33 (t, 1H, Ar), 7.43–7.46 (t, 1H, Ar), 7.64–7.67 (m, 1H, Ar), 7.81–7.82 (d, 1H, Ar), 7.91–7.93 (d, 1H, Ar), 8.02–8.04 (m, 1H, Ar), 8.38–8.40 (d, 1H, Ar), 8.745–8.754 (d, 1H, Ar), 9.26 (bs, 1H, NH exch.), 10.025 (bs, 1H, NH exch). GC-MS *m/z*: 254 ($M^{+\cdot}$, 43%). Anal. Calcd. for $C_{13}H_{10}N_4S$: C, 61.42; H, 3.94; N, 22.05; S,12.59%. Found: C, 61.40; H, 3.90; N, 22.01; S, 12.58%.

N-(Benzo[d]thiazol-2-yl)isonicotinamidine (6c). Solvent of crystallization: MeOH; Yield: 88%; m.p. 102–103°C; IR(KBr) ν_{max}: 3309 (NH), 1641 (C=N), 1530, 1447, and 1409 (Ar) cm^{-1}. ^{1}H-NMR (500MHz; DMSO-d_6) δ: 7.29–7.33 (t, 1H, Ar), 7.43–7.46 (t, 1H, Ar), 7.64–7.67 (m, 1H, Ar), 7.81–7.82 (d, 1H, Ar), 7.91–7.93 (d, 1H, Ar), 8.02–8.05 (m, 1H, Ar), 8.38–8.40 (d, 1H, Ar), 8.74–8.75 (d, 1H, Ar), 9.25 (bs, 1H, NH exch.), 10.02 (bs, 1H, NH exch.). GC-MS *m/z*: 254 (M^+, 35%). Anal. Calcd. for $C_{13}H_{10}N_4S$: C, 61.42; H, 3.93; N, 22.05; S, 12.59%. Found: C, 61.40; H, 3.91; N, 22.05; S, 12.56%.

N-(1H-benzo[d]imidazol-2-yl)pyrazine-2-carboxamidine (6d). Solvent of crystallization: MeOH; Yield: 60%; m.p. 210–215°C; IR(KBr) ν_{max}: 3396 (NH), 1615 (C=N), 1564, 1534, and 1467 (Ar) cm^{-1}. ^{1}H-NMR (500MHz; DMSO-d_6) δ: 6.970–6.972 (d, 2H, Ar), 7.44 (bs, 1H, NH exch.), 7.59 (bs, 1H, NH exch.), 8.62–8.73 (d+s, 3H, Ar), 9.34–9.36 (d, 1H, Ar), 10.28 (bs, 1H, Ar), 12.11 (bs, 1H, NH exch). GC-MS *m/z*: 238 (M^+, 2.5%). Anal. Calcd. for $C_{12}H_{10}N_6$: C, 60.50; H, 4.20; N, 35.29%. Found: C, 60.48; H, 4.17; N, 35.26%.

N-(1H-benzo[d]imidazol-2-yl)picolinamidine (6e). Solvent of crystallization: MeOH; Yield: 65%; m.p. 183–186°C; IR(KBr) ν_{max}: 3403 (NH), 1613 (C=N), 1576 1464, and 1425 (Ar) cm^{-1}. ^{1}H-NMR (500MHz; DMSO-d_6) δ: 7.29–7.32 (m, 1H, Ar),7.42–7.45 (m, 1H, Ar), 7.64–7.66 (m, 1H, Ar), 7.80–7.82 (d, 1H, Ar), 7.91–7.92 (d, 1H, Ar), 8.01–8.04 (m, 1H, Ar), 8.38–8.40 (d, 1H, Ar), 8.740–8.75 (d, 1H, Ar), 9.14 (bs, 1H, NH exch), 9.98 (bs, 1H, NH exch). GC-MS *m/z*: 237 (M^+, 1%). Anal. Calcd. for $C_{13}H_{11}N_5$: C, 65.82; H, 4.64; N, 29.53%. Found: C, 65.80; H, 4.60; N, 29.51%.

N-(1H-benzo[d]imidazol-2-yl)isonicotinamidine (6f). Solvent of crystallization: MeOH; Yield: 61%; m.p. 212–215°C; IR(KBr) ν_{max}: 3383 (NH), 1661 (C=N), 1564, 1453, and 1425 (Ar) cm^{-1}. ^{1}H-NMR (500MHz; DMSO-d_6) δ: 7.08–7.11 (t, 1H, Ar), 7.21–7.24 (t, 1H, Ar), 7.43–7.45 (m, 1H, Ar), 7.59–7.61(d, 1H, Ar), 7.70–7.72 (d, 1H, Ar), 7.81–7.84 (m, 1H, Ar), 8.17–8.19 (d, 1H, Ar), 8.53–8.54 (d, 1H, Ar), 9.04 (bs, 1H, NH exch), 9.81 (bs, 1H, NH exch). 10.53 (bs, 1H, NH exch). GC-MS *m/z*: 237 (M^+, 1%). Anal. Calcd. for $C_{13}H_{11}N_5$: C, 65.82; H, 4.64; N, 29.53%. Found: C, 65.78; H, 4.61; N, 29.50%.

N-(Benzo[d]oxazol-2-yl)pyrazine-2-carboxamidine (6g). Solvent of crystallization: MeOH; Yield: 75%; m.p. 218–219°C; IR(KBr) ν_{max}: 3351 (NH), 1623 (C=N), 1517, 1465, and 1422 (Ar) cm^{-1}. ^{1}H-NMR (500MHz; DMSO-d_6) δ: 7.33–7.35 (m, 1H, Ar), 7.44–7.47 (m, 1H, Ar), 7.83–7.85 (d, 1H, Ar), 7.94–7.96 (d, 1H, Ar), 8.82–8.84 (d, 1H, Ar), 8.899–8.903 (d, 1H, Ar), 9.31 (bs, 1H, NH exch.), 9.77 (s, 1H, Ar), 10.05 (s, 1H, NH exch). GC-MS *m/z*: 239 (M^+, 2.4%). Anal. Calcd. for $C_{12}H_9N_5O$: C, 60.25; H, 3.76; N, 29.29%. Found: C, 60.41; H, 3.75; N, 29.22%.

N-(Benzo[d]oxazol-2-yl)picolinamidine (6h). Solvent of crystallization: MeOH; Yield: 80%; m.p. 185–186°C; IR(KBr) ν_{max}: 3353 (NH), 1623 (C=N), 1513, 1466, and 1425 (Ar)

cm^{-1}. ^{1}H-NMR (500MHz; DMSO-d_6) δ: 7.58–7.61 (t, 1H, Ar), 7.17–7.42 (t, 1H, Ar), 7.93–7.95 (q, 1H, Ar), 8.09–8.11 (d, 1H, Ar), 8.20–8.22 (d, 1H, Ar), 8.30–8.34 (m, 1H, Ar), 8.67–8.69 (d, 1H, Ar), 9.03–9.04 (d, 1H, Ar), 9.54 (bs, 1H, NH exch.), 10.31 (bs, 1H, NH exch). GC-MS *m/z*: 238 (M^+, 28%). Anal. Calcd. for $C_{13}H_{10}N_4O$: C, 65.55; H, 4.20; N, 23.53%. Found: C, 65.52; H, 4.18; N, 23.50%.

N-(Benzo[d]oxazol-2-yl)isonicotinamidine (6i). Solvent of crystallization: MeOH; Yield: 75%; m.p. 165–168°C; IR(KBr) ν_{max}: 3386 (NH), 1646 (C=N), 1589, 1552, and 1487 (Ar) cm^{-1}. ^{1}H-NMR (500 MHz; DMSO-d_6) δ: 7.29–7.33 (t, 1H, Ar), 7.43–7.46 (t, 1H, Ar), 7.64–7.67 (q, 1H, Ar), 7.80–7.82 (d, 1H, Ar), 7.91–7.93 (d, 1H, Ar), 8.02–8.05 (d, 1H, Ar), 8.38–8.40 (d, 1H, Ar), 8.74–8.75 (d, 1H, Ar), 9.25 (bs, 1H, NH exch.), 10.02 (bs, 1H, NH exch). GC-MS *m/z*: 238 (M^+, 28%). Anal. Calcd. for $C_{13}H_{10}N_4O$: C, 65.55; H, 4.20; N, 23.53%. Found: C, 65.50; H, 4.19; N, 23.51%.

Reaction procedure for synthesis of bis-amidine derivatives. Sodium metal (23 mg) was dissolved in absolute methanol (20 mL) and was labelled as sodium methoxide solution in methanol. 2-Cyanopyridine (0.208 g; 2 mmol) was dissolved in absolute methanol (10 mL) and to it was added sodium methoxide solution (0.4 mL) prepared above, the reaction contents were stirred at room temperature for 2 h. Pyrimidine-2,4-diamine (0.110 g; 1 mmol) was added to the reaction mixture. The reaction contents were heated under reflux for 12 h. Solvent was removed under reduced pressure and to the residue left behind was added diethyl ether, and the solid separated out was filtered, washed with ether and air dried to give crude product, which was purified by crystallization from methanol to give pure product **7a.,** *i.e., N-(2-(picolinamidino)pyrimidin-4-yl)picolinamidine (**7a**)* Yield: (0.240 g) 75%; m.p. 198–199°C; IR(KBr) ν_{max}: 3423 (NH), 1634 (C=N), 1609, and 1455 (Ar) cm^{-1}. ^{1}H-NMR (500 MHz; DMSO-d_6) δ: 5.97–5.98 (m, 1H, Ar), 6.09 (bs, 2H, NH exch), 6.54 (bs, 2H, NH exch.), 7.63 (s, 2H, Ar), 7.90–7.91 (d, 1H, Ar), 8.06–8.08 (t, 2H, Ar), 8.25–8.26 (d, 2H, Ar), 8.77–8.81 (d, 2H, Ar). GC-MS *m/z*: 318 (M^+, 18%). Anal. Calcd. for $C_{16}H_{14}N_8$: C, 60.38; H, 4.40; N, 35.22%. Found: C, 60.35; H, 4.35; N, 35.20%.

Similarly were synthesized compounds **7b–e** and purified by crystallization from MeOH.

N-(2-(isonicotinamidino)pyrimidin-4-yl)isonicotinamidine (7b). Solvent of crystallization: MeOH; Yield: 68%; m.p. 210–212°C; IR(KBr) ν_{max}: 3425 (NH), 1702 (C=N), 1597, and 1520 (Ar) cm^{-1}. ^{1}H-NMR (500MHz; DMSO-d_6) δ: 5.58–5.60 (d, 1H, Ar), 5.71 (bs, 2H, NH exch), 6.15 (bs, 2H, NH exch.), 7.24–7.26 (t, 2H, Ar), 7.52–7.53 (d, 1H, Ar), 7.68–7.71 (t, 2H, Ar), 7.87–7.88 (d, 2H, Ar), 8.55–8.56(d, 2H, Ar)). GC-MS *m/z*: 318 (M^+, 0.2%), 317 (M^+-1, 12%), 316 ($M^{+\cdot}-2$, 100%), 302 ($M^{+\cdot}$-NH_2, 100%). Anal. Calcd. for $C_{16}H_{14}N_8$: C, 60.37; H, 4.40; N, 35.22%. Found: C, 60.35; H, 4.35; N, 35.20%.

N-(5-(Pyrazine-2-carboxamidino)-1H-1,2,4-triazol-3-yl)pyrazine-2-carboxamidine (7c). Solvent of crystallization: MeOH; Yield: 56%; m.p. 179–180°C; IR(KBr) ν_{max}: 3396 (NH), 1629 (C=N), 1580, 1535, and 1483 (Ar) cm^{-1}. ^{1}H-NMR (500MHz; DMSO-d_6) δ: 8.803–8.806 (d, 2H, Ar), 8.84–8.89 (d, 2H, Ar), 8.98–8.99 (d, 2H, Ar), 9.35–9.37 (d, 4H, NH, exch.), 10.68 (bs, 1H, NH, exch.). GC-MS M^+ ion peak not observed but fragmentation ion peaks were obtained, *m/z*: 230 ($C_8H_8N_9^+$, 6.5%), 106 ($C_5H_4N_3^+$, 74.3%), Anal. Calcd. for $C_{12}H_{11}N_{11}$: C, 46.60; H, 3.56; N, 49.84%. Found: C, 46.54; H, 3.51; N, 49.80%.

N-(5-(Picolinamidino)-1H-1,2,4-triazol-3-yl)picolinamidine (7d). Solvent of crystallization: MeOH; Yield: 65%; m.p. 218–219°C; IR(KBr) ν_{max}: 3310 (NH), 1628 (C=N), 1574, and 1486 (Ar) cm^{-1}. ^{1}H-NMR (500MHz; DMSO-d_6) δ: 5.86 (bs, 2H, NH exch.), 7.11–7.14 (t, 2H, Ar), 7.29–7.32 (t, 1H, Ar), 7.53–7.59 (m, 4H, Ar), 7.83 (bs, 2H, NH, exch.), 8.72–8.74 (d, 2H, Ar). GC-MS M^+ ion peak not observed but fragmentation ion peaks were obtained, *m/z*: 229 ($C_9H_9N_8^+$, 1.5%), 105 ($C_6H_5N_2^+$, 85%). Anal. Calcd. for $C_{14}H_{13}N_9$: C, 54.72; H, 4.23; N, 41.04%. Found: C, 54.70; H, 4.21; N, 41.00%.

N-(5-(isonicotinamidino)-1H-1,2,4-triazol-3-yl) isonicotinamidino (7e). Solvent of crystallization: MeOH; Yield: 60%; m.p. 210–213°C; IR(KBr) ν_{max}: 3396 (NH), 1629 (C=N), 1567, 1486, and 1415(Ar) cm^{-1}. ^{1}H-NMR (500MHz; DMSO-d_6) δ: 8.54 (bs, 2H, NH exch.), 8.91–8.92 (d, 2H, Ar), 8.98–8.99 (d, 2H, Ar), 9.07–9.08 (d, 2H, Ar), 9.38–9.39 (d, 2H, Ar), 10.23 (bs, 2H, NH, exch.), 10.85 (d, 1H, NH, exch.), GC-MS *m/z*: 307 (M^+, 38%). Anal. Calcd. for $C_{14}H_{13}N_9$: C, 54.72; H, 4.23; N, 41.04%. Found: C, 54.68; H, 4.20; N, 41.01%.

Anti-inflammatory activity [31]. Paw oedema inhibition test was used on albino rats of Charles Foster by adopting the method of Winter *et al*. [31]. Groups of five animals of both sexes (body weight 120–160 g), excluding pregnant females, were given a dose of test compound. Thirty minutes later, 0.20 mL of 1% freshly prepared carrageenan suspension in 0.9% NaCl solution was injected subcutaneously into the planter 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 interval was compared with that of control group (five rats treated with carrageenan but not with test compound) at the same intervals and percent inhibition value calculated by the formula given below.

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

D_t and D_c are paw volumes of oedema in tested and control groups, respectively. Compounds **6a–i** and **7a–e** were screened for anti-inflammatory activity, and results are summarized in Table 1.

In vitro cytotoxicity against human cancer cell lines [32,33]. The human cancer cell lines procured from National Cancer Institute, Frederick, MD were used in this study. Cells were grown in tissue culture flasks in complete growth medium (RPMI-1640 medium with 2 m*M* glutamine, pH 7.4 supplemented with 10% fetal bovine serum, 100 μg/mL streptomycin, and 100 units/mL penicillin) in a carbon dioxide incubator (37°C, 5% CO_2, 90% RH). The cells at subconfluent stage were harvested from the flask by treatment with trypsin (0.05% in PBS (pH 7.4) containing 0.02% ethylenediaminetetraacetic acid (EDTA)). Cells with viability of more than 98%, as determined by trypan blue exclusion, were used for determination of cytotoxicity. The cell suspension of 1×10^5 cells/mL was prepared in complete growth medium.

Stock 4×10^{-2} *M* compound solutions were prepared in DMSO. The stock solutions were serially diluted with complete growth medium containing 50 μg/mL of gentamycin to obtain working test solution of required concentrations.

In vitro cytotoxicity against various human cancer cell lines was determined (Monks *et al*.) [32] using 96-well tissue culture plates. The 100 μL of cell suspension was added to each well of the 96-well tissue culture plates. The cells were allowed to grow in CO_2 incubator (37°C, 5% CO_2, 90% RH) for 24 h. The test materials in complete growth medium (100 μL) were added after 24 h incubation to the wells containing cell suspension. The plates were further incubated for 48 h (37°C in an atmosphere of 5% CO_2 and 90% relative humidity) in a carbon dioxide incubator after addition of test material, and then the cell growth was stopped by gently layering trichloroacetic acid (50% TCA, 50 μL) on top of the medium in all the wells. The plates were incubated at 4°C for 1 h to fix the cells attached to the bottom of the wells. The liquid of all the wells was gently pipetted out and discarded. The plates were washed five times with distilled water to remove TCA, growth medium low molecular weight metabolites, serum proteins, etc., and air-dried. Cell growth was measured by staining with sulforhodamine B dye (Skehan *et al*.) [33]. The adsorbed dye was dissolved in Tris-HCl Buffer (100 μL, 0.01 *M*, pH 10.4), and plates were gently stirred for 10 min on a mechanical stirrer. The optical density was recorded on ELISA reader at 540 nm.

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