Med Chem Res (2014) 23:1454–1464
DOI 10.1007/s00044-013-0751-9

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

p-Toluenesulfonic acid-catalyzed solvent-free synthesis and biological evaluation of new 1-(4′,6′-dimethylpyrimidin-2′-yl)-5-amino-4*H*-3-arylpyrazole derivatives

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Received: 29 April 2013 / Accepted: 19 August 2013 / Published online: 1 September 2013

Abstract A solvent-free quick synthesis of 1-(4′,6′-dimethylpyrimidin-2′-yl)-5-amino-4*H*-3-arylpyrazoles (**3**) was accomplished by grinding 2-hydrazino-4,6-dimethylpyrimidine (**1**) and β-ketonitriles (**2**) in the presence of *p*-Toluenesulfonic acid as a catalyst. Subsequently, 5-aminopyrazoles (**3**) were converted to their corresponding *N*-acetamide (**4**) and *N*-trifluoroacetamide (**5**) derivatives by treating (**3**) with acetic anhydride/acetic acid and trifluoroacetic anhydride/trifluoroacetic acid, respectively. The newly synthesized compounds were fully characterized using IR, NMR (^{1}H, ^{13}C, and ^{19}F), mass spectral studies, and elemental analyses. All of the fifteen compounds were screened for their in vitro antibacterial activity against two Gram-positive and two Gram-negative pathogenic bacteria such as *Bacillus pumilus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*, respectively. Additionally, nine of these compounds were screened for their cytotoxicity against the human Caucasian promyelocytic leukemia (HL-60) cell line using the alamarBlue® assay. Preliminary results reveal that compounds **3a**, **3b**, **3d**, **5a**, and **5d** are showing moderate-to-significant cytotoxic and antibacterial activity.

Keywords Solvent-free synthesis · 1-(4′,6′-Dimethylpyrimidin-2′-yl)-5-amino-4*H*-3-arylpyrazoles · *N*-acetamide/trifluoroacetamide derivatives · *p*-Toluenesulfonic acid · Antibacterial activity · Cytotoxicity

Introduction

Pyrazole is an important structural motif, which is found as an integral part of numerous pharmaceutically interesting compounds displaying antimicrobial (Liu *et al.*, 2008; Gilbert *et al.*, 2006), anti-inflammatory (Farag *et al.*, 2008), antileukemic (Manetti *et al.*, 2008), antidiabetic (Soliman and Darwish 1983), and antitumor (Benaamane *et al.* 2008) activities. Among pyrazole derivatives, 5-aminopyrazoles have attracted considerable attention because of their enormous applications as pharmaceuticals (Goldstein *et al.*, 2009) and agrochemicals (Lee *et al.*, 2003) as they have been found to exhibit antibacterial (Kane *et al.*, 2003; Kumar *et al.*, 2005; Kumar *et al.*, 2006), anticonvulsant (Michon *et al.*, 1995), antidepressant (Abdel-Aziz *et al.* 2009), or herbicidal (Jung *et al.*, 2002) activities. For example, several 5-aminopyrazoles have been reported to be potent and selective inhibitors of the p38α kinase (Das *et al.*, 2010). Moreover, the structurally simple 1-(4-methylphenyl)-5-aminopyrazole (Fig. 1a) has been described as an antagonist of the Neuropeptide Y receptor type 5 (NPY5) (Kordik *et al.*, 2001), whereas 5-amino-4-(3-methoxyphenyl)-3-thiomethyl-1-(2,6-dichloro-4-trifluoromethylphenyl)pyrazole (Fig. 1b), a potential insecticide, exerts potent GABA inhibition with selectivity toward the insect versus the

Electronic supplementary material The online version of this article (doi:10.1007/s00044-013-0751-9) contains supplementary material, which is available to authorized users.

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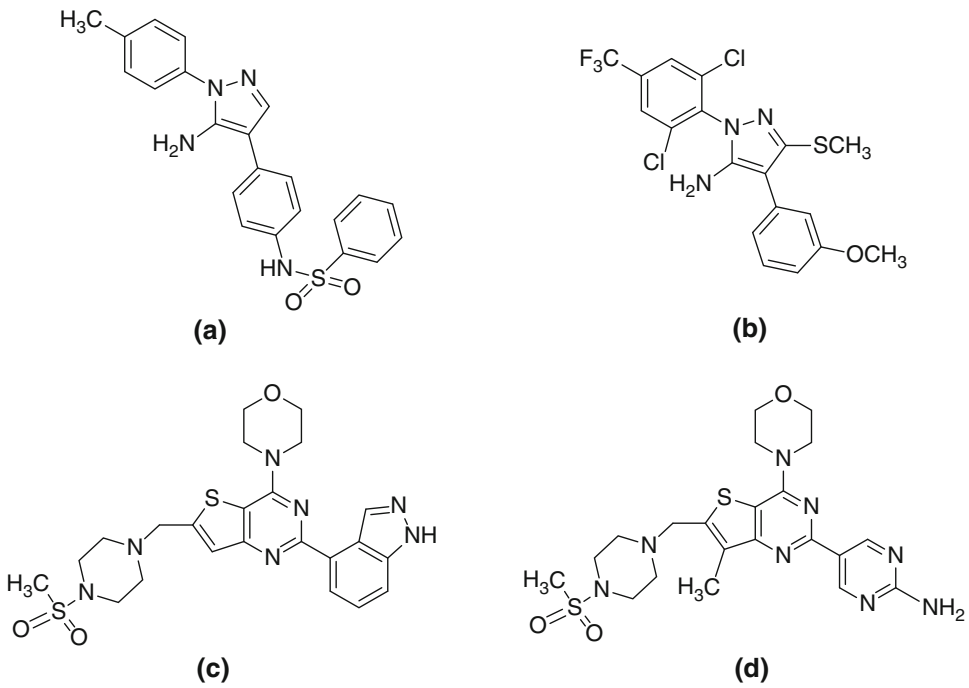


Fig. 1 Structures of 5-aminopyrazoles (**a** and **b**) and pyrimidines (**c** and **d**) of pharmaceutical relevance

mammalian receptor (Meegalla *et al.*, 2004). Besides their biological importance, they are also useful synthons for the preparation of many biologically important fused heterocycles such as pyrazolo[3,4-*d*]pyrimidines, pyrazolo[3,4-*b*]pyridines or imidazopyrazoles (An *et al.*, 2008; Abu Almaati and El-Taweel 2004; Sellery *et al.*, 2005; Gopalsamy *et al.* 2005). Furthermore, many pyrazole derivatives, especially those bearing aryl groups, are important for the creation of promising new antitumor agents (Barril *et al.*, 2006; Sherif 2006; Dymock *et al.*, 2005). For example, some *N*-arylpyrazoles have remarkable activities as tumor necrosis inhibitors (Mercep *et al.*, 2004), and also it has been reported that pyrazole derivatives showed significant activity toward Ehrlich ascites carcinoma tumor cells (Farag *et al.*, 2008). It has also been found that some 3-arylpyrazoles suppressed A549 lung cancer cell growth (Wei *et al.*, 2006).

Pyrimidine derivatives, the fundamental building blocks for DNA and RNA, also occupy a prominent place in the pharmaceutical area (Zoltewicz and Uray 1994). They have attracted a great deal of attention over many years due to their broad bioactivities, including antitumor (Lindvall *et al.*, 2011; Heffron *et al.*, 2010), antimicrobial (Shetty *et al.*, 2009), or anti-inflammatory (El-gazzar *et al.*, 2007). In particular, 4-morpholinothieno[3,2-*d*]pyrimidines such as GDC-0941 and GNE-477 (Fig. 1c and d, respectively) have shown marked antitumor activities (Heffron *et al.*, 2010; Hayakawa *et al.*, 2006). For example, GDC-0941, which is a potent, selective, and orally bioavailable inhibitor of PI3 K, exerts antiproliferative effects against an array of human tumor cell lines and is currently undergoing Phase I clinical trials (Junttila *et al.*, 2009).

Taking all this into account, we present the synthesis of a number of pyrazoles carrying both a pyrimidine and an amino group at positions 1 and 5 of the pyrazole ring, respectively. Our final aim is to find new and more potent antibacterial and cytotoxic agents.

A recent overview of current methods for the synthesis of 5-aminopyrazoles (Aggarwal *et al.*, 2011) reveals that common routes involve the reactions of β-ketonitriles, malononitrile, alkylidenemalononitriles, and their derivatives with hydrazines in the presence of hydrochloric or acetic acid using ethanol as a solvent. Though these reactions afford 5-aminopyrazoles, they take 8–10 h for completion and the workup is cumbersome. Recently, solvent-free synthesis has gained much attention due to advantage over the conventional methods in terms of enhanced selectivity, efficiency, cleaner reaction profiles, ease of manipulation, and relatively benign conditions compared to their solution counterparts. In view of these observations and continuing with our work on solvent-free synthesis of heterocyclic compounds (Aggarwal *et al.*, 2007), we have developed a solvent-free protocol for the preparation of the mentioned 5-amino-1-pyrimidinopyrazole derivatives **3** using *p*-Toluenesulfonic acid (PTSA) as a catalyst. Further, the synthesis of their *N*-acetamide **4** and *N*-trifluoroacetamide **5** derivatives was considered as it has been observed that the acyl moiety, an integral part of many drugs, enhances the biological activity, e.g., acylation

converts the natural painkiller morphine into the far more potent heroin (Kaiko *et al.*, 1981) (diacetylmorphine).

Chemistry

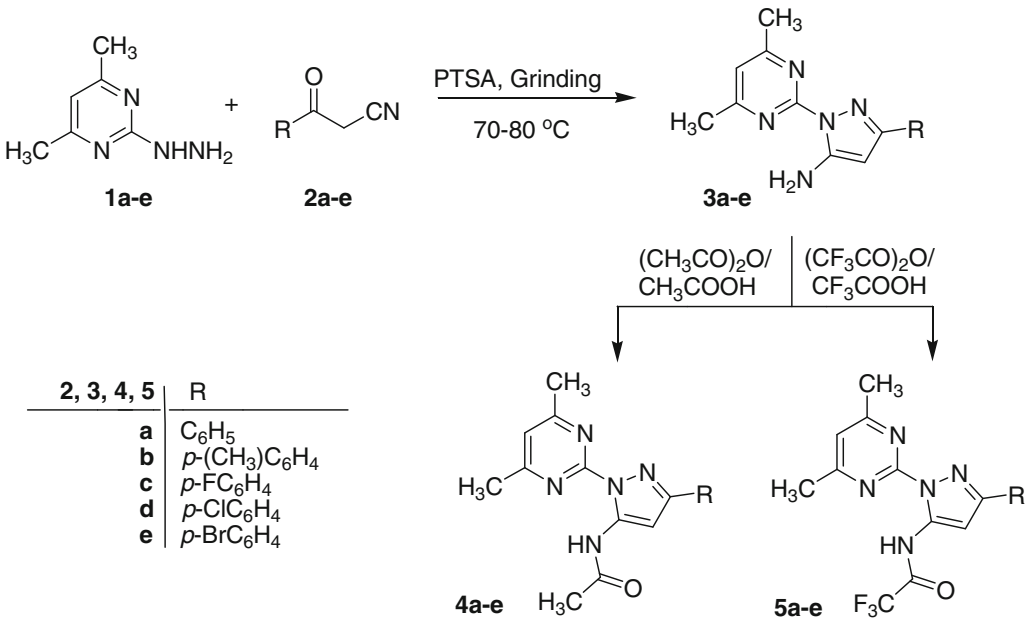
The synthesis of 1-(4′,6′-dimethylpyrimidin-2′-yl)-5-amino-3-arylpyrazoles **3a–e**, 1-(4′,6′-dimethylpyrimidin-2′-yl)-5-(*N*-acetamide)-3-arylpyrazoles **4a–e** and 1-(4′,6′-dimethylpyrimidin-2′-yl)-5-(2,2,2-trifluoro-*N*-acetamide)-3-arylpyrazoles **5a–e** is outlined in Scheme 1.

The corresponding starting compounds, the β-ketonitriles **2a–e**, were obtained by treating α-bromoacetophenones with potassium cyanide (Gakhar *et al.*, 1971). Then, grinding **2a–e** with 2-hydrazino-4,6-dimethylpyrimidine **1**, in the presence of PTSA as a catalyst, provided 1-(4′,6′-dimethylpyrimidin-2′-yl)-5-amino-3-arylpyrazoles **3a–e** as an exclusive product in excellent yields in just 2 h following a simple workup. Synthesis of the *N*-acetamide **4a–e** and *N*-trifluoroacetamide **5a–e** derivatives was accomplished by treating the corresponding 5-aminopyrazoles **3a–e** with acetic anhydride in the presence of acetic acid and trifluoroacetic anhydride in the presence of trifluoroacetic acid, respectively. The structure and purity of all the products **3a–e**, **4a–e,** and **5a–e** was confirmed by the corresponding spectral data (IR, MS, ^{1}H, ^{13}C, and ^{19}F NMR) and elemental microanalysis.

The presence of the amino group in compounds **3a–e** was confirmed with the help of IR, which showed two sharp absorption bands at 3,250 (symm.) and 3,400 cm^{-1} (asymm.) due to NH_2 stretching. The IR spectra of compounds **4a–e** confirmed the incorporation of acetamide by showing the strong absorption band at 1,690–1,700 cm^{-1} due to C=O stretch and only one band at about 3,160–3,170 cm^{-1} due to N–H stretch. Finally, the corresponding bands for the trifluoroacetamide derivatives appeared at 1,620–1,640 and 3,110 cm^{-1} in the IR spectra of **5a–e**.

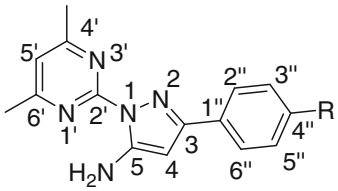
The ^{1}H NMR spectra of compounds **3a–e** displayed a characteristic sharp singlet integrating for one proton at δ 5.9 ppm due to the H-4 of the pyrazole ring, besides two singlets integrating for six and one protons at about δ 2.5 and 6.8 ppm corresponding to the two $–CH_3$ groups present on the pyrimidine ring and the H-5 of this ring, respectively. The corresponding signal for H-4 pyrazole for compounds **4a–e** and **5a–e** appeared at δ 7.30 and 6.10 ppm, respectively, as a sharp singlet. The significant downfield shift of δ 1.40 ppm in compounds **4a–e** as compared to **3a–e** may be attributed to the weak hydrogen bonding between the oxygen of the acetyl C=O group and the H-4 of the pyrazole ring. Formation of such weak hydrogen bonds has been previously reported (Burley and Petsko 1988; Banks *et al.*, 1999).

Further, the structure of compounds **3a–e** was supported by ^{13}C NMR spectroscopy that showed signals at δ 150, 87, and 152–153 ppm due to C-3, C-4, and C-5 of the pyrazole ring, respectively. These values are in accordance with the literature (Kumar *et al.*, 2006). The ^{13}C NMR spectra of **4a–e** displayed signals at δ 151, 95, and 152–154 ppm due to C-3, C-4, and C-5, respectively, whereas the corresponding signals appeared at δ 157, 93, and 160–162 ppm for compounds **5a–e**. The downfield shift in the value of



Scheme 1 Synthesis of 5-aminopyrazoles **3a–e** and their corresponding *N*-acetamide **4a–e** and *N*-trifluoroacetamide **5a–e** derivatives

Table 1 ^{13}C NMR δ (ppm) data for 1-(4′,6′-dimethylpyrimidin-2′-yl)-5-amino-3-arylpyrazoles **3a–e**



Compounds	3a	3b	3c	3d	3e
C-3	150.3	150.2	150.4	150.4	150.5
C-4	87.5	87.4	87.3	87.3	87.3
C-5	153.5	153.6	152.6	152.4	152.4
C-2′	168.4	168.4	168.5	168.5	168.5
C-4′, 6′	157.7	157.7	157.6	157.7	157.7
C-5′	116.4	116.3	115.3	116.6	116.5
C-1″	128.2	130.0	128.1	128.4	127.9
C-2″, 6″	127.4	126.3	128.0	127.6	122.3
C-3″, 5″	129.1	129.0	129.1	131.4	131.4
C-4″	132.9	130.2	141.4	134.1	131.9
Pyrimidine –CH_3	24.2	24.2	24.1	24.2	24.1
R (–CH_3)		21.3			

Table 3 ^{19}F NMR δ (ppm) data of compounds **5a–e**

Compound	5a	5b	5c	5d	5e
CF_3	−75.11	−75.76	−75.92	−75.43	−75.63

C-4 in **4a–e** as compared to **3a–e** supported the existence of the mentioned hydrogen bond between C–H and O atoms. The complete assignment of the signals in the ^{13}C NMR spectra of the compounds **3a–e**, **4a–e,** and **5a–e** is given in Tables 1 and 2, respectively.

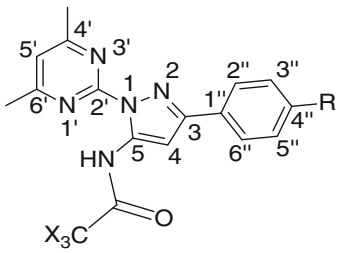
^{19}F NMR spectroscopy helped in establishing the presence of a trifluoroacetyl group ($COCF_3$) in compounds **5a–e**. A sharp singlet at about δ −75 ppm confirmed the presence of CF_3 group in these compounds (Table 3). (See supplementary information).

Biological results and discussion

Antibacterial activity

All the fifteen compounds were screened in vitro for their antibacterial activity against four pathogenic bacterial

Table 2 ^{13}C NMR δ (ppm) data for 1-(4′,6′-dimethylpyrimidin-2′-yl)-5-(*N*-acetamide)-3-arylpyrazoles **4a–e** and 1-(4′,6′-dimethylpyrimidin-2′-yl)-5-(2,2,2-trifluoro-*N*-acetamide)-3-arylpyrazoles **5a–e**



4 X= H
5 X= F

Cmpd.	4a	4b	4c	4d	4e	5a	5b	5c	5d	5e
C-3	151.3	151.2	151.3	151.4	151.4	157.3	157.2	157.3	157.4	157.4
C-4	95.5	95.4	95.4	95.4	95.4	93.3	93.3	93.6	93.1	93.2
C-5	154.0	154.1	153.1	152.9	152.9	160.8	161.8	162.0	161.6	161.4
C-2′	168.7	168.7	168.7	168.8	168.7	168.9	169.2	168.9	169.4	169.3
C-4′/6′	157.5	157.4	157.4	157.4	157.6	158.1	158.1	158.2	158.2	158.1
C-5′	117.0	116.9	115.2	117.1	117.2	117.8	117.8	115.7	118.4	118.4
C-1″	128.4	129.1	130.3	128.6	128.1	128.7	128.2	127.4	128.6	126.1
C-2″/6″	126.6	126.5	128.2	127.8	122.9	126.6	126.6	128.9	125.1	124.5
C-3″/5″	128.8	128.6	132.7	130.8	131.2	129.4	129.4	129.0	128.9	128.7
C-4″	132.3	130.7	137.4	134.6	131.6	131.3	130.7	136.5	132.6	131.9
$PyCH_3$	24.6	24.6	24.2	24.2	24.2	23.7	23.6	23.7	23.5	23.5
R(CH_3)	–	20.6	–	–		–	21.4	–	–	
CX_3	24.2	24.2	23.5	23.6	23.7	124.6	124.2	124.5	124.4	124.3
CO	166.2	166.3	166.4	166.4	166.4	170.2	169.8	170.4	170.6	170.0

Table 4 MIC values determined for compounds **3a–e**, **4a–e,** and **5a–e** using agar well diffusion method

Compound	MIC (μM)[a]			
	B. pumilus	*S. aureus*	*P. aeruginosa*	*E. coli*
3a	0.377	0.377	0.018	3.77
3b	1.79	NA	0.018	NA
3c	3.53	NA	3.53	NA
3d	3.34	NA	1.67	NA
3e	2.91	NA	1.16	2.91
4a	NA	NA	3.25	NA
4b	NA	NA	3.11	NA
4c	3.07	NA	3.07	NA
4d	2.93	NA	2.19	NA
4e	1.29	NA	1.29	2.59
5a	2.77	0.554	1.38	NA
5b	1.33	0.066	0.533	NA
5c	1.31	0.066	0.527	NA
5d	2.53	0.063	0.506	NA
5e	2.27	0.056	0.022	NA
Ampicillin	0.002	0.002	0.004	0.002
Chloramphenicol	0.003	0.003	0.003	0.003

NA no activity

[a] Mean of triplicates

Table 5 Percentage cell viability for compounds **3a**, **b**, **d**, **4a**, **b**, **d**, and **5a**, **b**, **d** at 50 and 100 μM

Compound	Conc. (μM)	% cell viability	±SEM
3a	100	2.3	0.3
	50	3.6	0.6
3b	100	0.5	0.1
	50	2.4	0.3
3d	100	1.1	0.5
	50	7.7	0.9
4a	100	35.5	2.7
	50	52.8	3.6
4b	100	72.5	9.1
	50	71.9	10.1
4d	100	51.2	1.4
	50	68.9	2.8
5a	100	6.3	1.2
	50	40.4	4.5
5b	100	33.9	9.2
	50	47.9	3.2
5d	100	1.3	0.6
	50	11.4	5.0

strains, two Gram-positive [*Bacillus pumilus* (MTCC 10414) and *Staphylococcus aureus* (MTCC 96)] and two Gram-negative [*Pseudomonas aeruginosa* (MTCC 741) and *Escherichia coli* (MTCC 119)]. Different parameters assessing the effect of compounds **3a–e**, **4a–e,** and **5a–e** against those four bacterial strains were evaluated: the diameter of zone of growth inhibition (see supplementary information) and the minimum inhibitory concentration (MIC), which is shown in Table 4. The antibacterial activity of these compounds was compared with two reference antibiotics, ampicillin and chloramphenicol.

A careful analysis of the data in Table 4 shows that all the compounds, except **4a** and **4b**, exhibited moderate-to-significant activity against *B. pumilus*, with MIC values ranging from 0.37 to 3.53 μM. Compound **3a** was found to be the most active, whereas the corresponding amide derivative **4a** was found to be completely inactive toward *B. pumilus*. However, the antibacterial activity was restored by incorporating the trifluoroacetamide group as in compound **5a** (MIC 2.77 μM). This trend is again visible, when comparing the MIC values against Gram-positive *S. aureus* of 5-amino-3-phenylpyrazole **3a** (0.37 μM), 5-(*N*-acetamide)-3-phenylpyrazole **4a** (no activity) and 5-(*N*-trifluoroacetamide)-3-phenylpyrazole **5a** (0.55 μM).

Compounds **5b–e** were found to be the most effective against *S. aureus* showing MIC values ranging from 0.056 to 0.066 μM (Table 4). This indicates that para substitution of CH_3, F, Cl, and Br on the phenyl ring of 5-(*N*-trifluoroacetamide)-3-phenylpyrazole **5a** increases the antibacterial activity to a significant extent.

All the fifteen compounds showed moderate-to-excellent activity against Gram-negative bacterium *P. aeruginosa* (Table 4). Compounds **3a** and **3b** showed the best results with very promising MIC values of 0.018 μM. Incorporation of acetyl or trifluoroacetyl groups on these compounds reduces their antibacterial activity; however, trifluoroacetyl derivatives **5a** and **5b** (1.38 and 0.533 μM) are still more active than the corresponding acetyl derivatives **4a** and **4b** (3.25 and 3.11 μM). Compound **5e** also showed promising antibacterial activity with a MIC value 0.022 μM. Another interesting feature that emerged out of the MIC data reveals that while the incorporation of substituents on phenyl rings in compounds **3a–e** decreases the activity, this activity increases significantly when the same substituents are present on the trifluoroacetamide derivatives **5a–e**.

Only compounds **3a**, **3e,** and **4e** were found to be active against *E. coli* (with the MIC values of 3.77, 2.91, and 2.59 μM, respectively, see Table 4).

Cytotoxicity in HL-60 cancer cells

Nine compounds (**3a**, **b**, **d**, **4a**, **b**, **d**, and **5a**, **b**, **d**) were screened for their cytotoxicity in human Caucasian promyelocytic leukemia (HL-60) cell line using the well-established alamarBlue® assay (details can be found in the Experimental section). The HL-60 cell line is an efficient

in vitro model, which has been used in the literature for the evaluation of cytotoxic activity of different types of chemotherapeutic agents such as DNA minor groove binders (Hiraku *et al.*, 2002), protein kinase inhibitors (Zhao *et al.*, 2011), and caspases inhibitors (Studzinski *et al.*, 1986) among others. This assay was chosen as a suitable model for the assessment of the effect on cell viability of the compounds here tested. For first general screening, the viability of the compounds was evaluated at two concentrations, 100 μM and 50 μM. The results corresponding to the percentage cell viability are presented in Table 5.

The cell viability data revealed that the toxicity of all the compounds is directly related to the concentration. In particular, compounds **3a**, **3b**, **3d,** and **5d** are more potent (both at 100 and 50 μM) than the rest of the compounds in the different series. For instance, at 100 μM concentration, compounds **3a**, **3b**, **3d,** and **5d** show 2.3, 0.5, 1.1, and 1.3 % of viability, respectively. On the contrary, at the same concentration, the rest of the compounds show cell viability between 6.3 and 72.5 %, indicating that their ability to generate toxicity in cancer cells is scarce. For this reason, a more exhaustive study of the cytotoxicity of compounds **3a**, **3b**, **3d,** and **5d** was further carried out.

Thus, a broader range of concentrations (100, 50, 40, 35, 30, and 20 μM) was chosen to assess the activity of these compounds more in detail. In this way, the IC_{50} (half maximal inhibitory concentration) of the drugs in the HL-60 cell line was determined after 72-h treatment. The IC_{50} values represent the effectiveness of these compounds to inhibit cell viability by half. The results obtained together with those of furamidine (a DNA minor groove binder) and sorafenib (a protein kinases inhibitor), which were used as control, are shown in Fig. 2.

As can be seen in Fig. 2, the IC_{50} values measured for compounds **3a**, **3b**, **3d,** and **5d** are relatively similar and around 30 μM. Compare to known drugs such as furamidine or sorafenib, the cytotoxicity of these compounds, even though not outstanding, it is promising and future derivatives could improve it.

A careful analysis of the data, given in Fig. 2, shows some interesting patterns about the cytotoxicity of the compounds studied. Most of the compounds with the primary amine moiety (**3a**, **b**, **d**) were found to be very potent against cancer cell proliferation. However, incorporation of an amide group decreases their cytotoxicity even though the introduction of the CF_3 group in the amide produces some improvements. Considering that the target by which these molecules exert their activity is not known, some insights into the nature of the active site of this target could be hypothesized. It could be possible that the putative active site has a residue with a hydrogen bond (HB) acceptor group that can interact with the HB donor NH_2 group. Additionally, the active site of the target could have a lipophilic pocket, what could explain the best results obtained with those compounds carrying a CF_3 group.

These results are very encouraging, and thus, further biological studies need to be carried out to identify the specific target of these molecules cytotoxic activity; this will allow the identification of the corresponding pharmacophore that will help to synthesize more potent molecules.

Conclusion

A new series of biologically active 1-heteroaryl-5-amino-4*H*-3-arylpyrazoles **3a**–**e** and their corresponding *N*-acetamide **4a**–**e** and *N*-trifluoroacetamide **5a**–**e** derivatives were synthesized under solvent-free conditions using *p*-Toluenesulfonic acid as a catalyst.

The antibacterial activity of all the compounds prepared was tested against *B. pumilus*, *S. aureus*, *P. aeruginosa,* and *E. coli*. The potency observed for these compounds was mainly influenced by the substituents at position 5 (NH_2, $NHCOCH_3$, or $NHCOCF_3$) and 3 (various substituted phenyl groups) of the pyrazole ring. While the introduction of an *N*-acetyl group results in decrease or complete loss of activity, incorporation of the corresponding *N*-trifluoroacetyl group restores or increases the

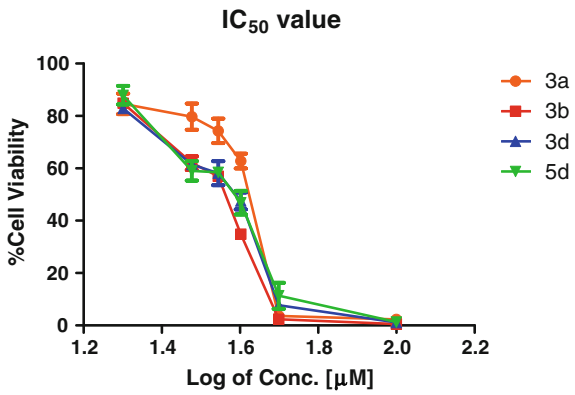


Fig. 2 Graph representing % cell viability versus logarithm of the concentration and IC_{50} values obtained for compounds **3a**, **3b**, **3d,** and **5d** as well as for furamidine and sorafenib

Compound	IC_{50} (μM) ± SEM
3a	42.6 ±1.3
3b	28.1 ±1.3
3d	31.3 ±1.2
5d	32.5 ±1.2
Furamidine	77.6 ±1.15
Sorafenib	1.68 ±1.27

activity. Additionally, the substitution on the phenyl ring decreases the antibacterial activity in 5-aminopyrazoles **3a–e**, whereas these substituents cause an increase in the activity of *N*-trifluoroacetamide pyrazoles **5a–e**.

The cytotoxicity of some of the compounds prepared (**3a**,**b**, **d**, **4a**, **b**, **d**, and **5a**, **b**, **d**) was evaluated against the human leukemia HL-60 cell line. First, a general screening of the effect of these compounds on the viability of the HL-60 cells was performed and then the IC_{50} values of the most interesting compounds (**3a**, **b**, **d**, and **5d**) were measured. A structure activity study showed that the amino derivatives **3a**, **b**, **d** are the most cytotoxic; the introduction of a *N*-acetyl group decreases cytotoxicity, while introducing *N*-trifluoroacetyl groups instead showed some improvements in the cancer cell toxicity.

Regarding anticancer activity, further derivatives of **3a**, **3b,** and **3d** and additional biochemical studies are required to improve the cytotoxic potential of these compounds. Regarding antibacterial activity, the nanomolar inhibition shown by compounds **5b**, **5c**, **5d,** and **5e** against *S. aureus* and **3a**, **3b,** and **5e** against *P. aeruginosa* is very promising and it will be investigated further.

Experimental protocols

Melting points were determined in open capillaries and are uncorrected. IR spectra were recorded on a Buck Scientific IR M-500 spectrophotometer in KBr pellets (ν max in cm^{-1}); ^1H and ^{13}C NMR spectra for analytical purpose were recorded in $CDCl_3$ on a Bruker instrument at 300 MHz and 75 MHz, respectively; chemical shifts are expressed in δ-scale downfield from TMS as an internal standard. ^{19}F NMR spectra were run on DRX 300 and DPX 400 at 282 and 376 MHz, respectively, using deuteriochloroform as a solvent. The internal standard for ^{19}F spectra was fluorotrichloromethane, setting the $CFCl_3$ signal at δ 0.0. Elemental analyses were performed at Sophisticated Analytical Instrument Facility, Central Drug Research Institute, Lucknow, India.

2-Hydrazino-4,6-dimethylpyrimidine **1** was synthesized according to the literature procedure (Danagulyan *et al.*, 1989).

General procedure for the preparation of 1-(4′,6′-dimethylpyrimidin-2′-yl)-5-amino-3-arylpyrazoles (3a–e)

Equimolar amounts of β-ketonitriles **2** (5 mmol) and 2-hydrazino-4,6-dimethylpyrimidine **1** (5 mmol) were grinded in the presence of *p*-Toluenesulfonic acid (PTSA) (1 mmol) as catalyst in mortar and pestle. Initially, the reactants melted on grinding and reaction mixture became hot and then the contents solidified. The reaction mixture was then heated on water bath for 1–2 h. The reaction was monitored by tlc. On completion of the reaction, the crude product was washed with water, filtered, and crystallized using ethanol. The tlc and ^1H NMR spectra showed the formation of a single product.

*1-(4′,6′-dimethylpyrimidin-2′-yl)-5-amino-3-phenylpyrazole (**3a**)*

Reaction time 1 h 30 min; Yield 83 %; mp 192–196 °C; IR (KBr, cm^{-1}): 3,268 (symm.) and 3,391 (asymm.) due to NH_2; ^1H NMR (300 MHz, $CDCl_3$) δ: 2.57 (s, 6H, 4′, 6′–CH_3); 5.91 (s, 1H, 4-H); 6.02 (bs, 2H, –NH_2, exchangeable with D_2O); 6.88 (s, 1H, 5–H); 7.37–7.46 (m, 3H, 3″, 4″, 5″-H); 7.91–7.93 (m, 2H, 2″, 6″-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 24.2, 87.5, 116.4, 127.4, 128.2, 129.1, 132.9, 150.3, 153.5, 157.7, 168.4; MS: *m/z* $[M + 1]^+$ 266.1. Anal. Calcd. for $C_{15}H_{15}N_5$: C, 67.90; H, 5.70; N, 26.40. Found: C, 67.84; H, 5.39; N, 26.12.

*1-(4′,6′-dimethylpyrimidin-2′-yl)-5-amino-3-(4″-methylphenyl)pyrazole (**3b**)*

Reaction time 1 h 40 min; yield 85 %; mp 130–134; IR (KBr, cm^{-1}): 3,271 (symm.) and 3,373 (asymm.) due to NH_2; ^1H NMR (300 MHz, $CDCl_3$) δ: 2.38 (s, 3H, 4″–CH_3); 2.57 (s, 6H, 4′, 6′, –CH_3); 5.89 (s, 1H, 4-H); 6.02–6.06 (bs, 2H, –NH_2, exchangeable with D_2O); 6.87 (s, 1H, 5′–H); 7.19–7.22 (d, 2H, J = 8.1 Hz, 3″, 5″-H); 7.80–7.83 (d, 2H, J = 8.1 Hz, 2″, 6″-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 21.3, 24.2, 87.4, 116.3, 126.3, 129.0, 130.0, 130.2, 150.2, 153.6, 157.7, 168.4; MS: *m/z* $[M + 1]^+$ 280.1. Anal. Calcd. for $C_{16}H_{17}N_5$: C, 68.79; H, 6.13; N, 25.07. Found: C, 68.84; H, 6.39; N, 24.92.

*1-(4′,6′-dimethylpyrimidin-2′-yl)-5-amino-3-(4″-fluorophenyl)pyrazole (**3c**)*

Reaction time 1 h 40 min; yield 79 %; mp 160–164; IR (KBr, cm^{-1}): 3,298 (symm.) and 3,408 (asymm.) due to NH_2; ^1H NMR (300 MHz, $CDCl_3$) δ: 2.58 (s, 6H, 4′, 6′–CH_3); 5.88 (s, 1H, 4-H); 6.01–6.04 (bs, 2H, –NH_2, exchangeable with D_2O); 6.89 (s, 1H, 5′–H); 7.05–7.15 (m, 2H, 3″, 5″-H); 7.87–7.92 (m, 2H, 2″, 6″-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 24.1, 87.3, 115.3, 128.0, 128.1, 129.1, 141.4, 150.4, 152.6, 157.6, 168.5; MS: *m/z* $[M + 1]^+$ 284.1. Anal. Calcd. for $C_{15}H_{14}FN_5$: C, 63.59; H, 4.98; N, 24.72. Found: C, 63.84; H, 4.93; N, 24.46.

*1-(4′,6′-dimethylpyrimidin-2′-yl)-5-amino-3-(4″-chlorophenyl)pyrazole (**3d**)*

Reaction time 1 h 50 min; yield 81 %; mp 196–200; IR (KBr, cm^{-1}): 3,302 (symm.) and 3,418 (asymm.) due to NH_2; ^1H NMR (300 MHz, $CDCl_3$) δ: 2.58 (s, 6H, 4′,

6′–CH_3); 5.88 (s, 1H, 4-H); 6.04 (bs, 2H, –NH_2, exchangeable with D_2O); 6.90 (s, 1H, 5′–H); 7.36–7.39 (d, 2H, J = 8.7 Hz, 3″, 5″-H); 7.85–7.88 (d, 2H, J = 8.7 Hz, 2″, 6″-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 24.2, 87.3, 116.6, 127.6, 128.4, 131.4, 134.1, 150.4, 152.4, 157.7, 168.5; MS: *m/z* $[M + 1]^+$ 300.1/302.1 (3:1). Anal. Calcd. for $C_{15}H_{14}ClN_5$: C, 60.10; H, 4.71; N, 23.36. Found: C, 60.04; H, 4.63; N, 23.03.

*1-(4′,6′-dimethylpyrimidin-2′-yl)-5-amino-3-(4″-bromophenyl)pyrazole (**3e**)*

Reaction time 2 h; yield 82 %; mp 194–198; IR (KBr, cm^{-1}): 3,279 (symm.) and 3,418 (asymm.) due to NH_2; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.58 (s, 6H, 4′, 6′–CH_3); 5.88 (s, 1H, 4-H); 6.05 (bs, 2H, –NH_2, exchangeable with D_2O); 6.90 (s, 1H, 5′–H); 7.51–7.54 (d, 2H, J = 8.1 Hz, 3″, 5″-H); 7.78–7.81 (d, 2H, J = 8.1 Hz, 2″, 6″-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 24.1, 87.3, 116.5, 122.3, 127.9, 131.4, 131.9, 150.5, 152.4, 157.7, 168.5; MS: *m/z* $[M + 1]^+$ 344/346 (1:1). Anal. Calcd. for $C_{15}H_{14}BrN_5$: C, 52.34; H, 4.10; N, 20.35. Found: C, 52.44; H, 4.13; N, 20.18.

General procedure for the preparation of 1-(4′,6′-dimethylpyrimidin-2′-yl)-5-(*N*-acetamide)-3-arylpyrazoles **(4a–e)**

5-Aminopyrazoles (2 mmol) were treated with acetic anhydride (2 mmol) in the presence of acetic acid (20 ml) as a solvent. The reaction mixture was heated on water bath for about 15 min and then cooled. Solid separated on cooling was filtered, washed with water, and crystallized using ethanol. The tlc and ^{1}H NMR spectra showed the formation of a single product.

*1-(4′,6′-dimethylpyrimidin-2′-yl)-5-(N-acetamide)-3-phenylpyrazole (**4a**)*

Yield 75 %; mp 220–224; IR (KBr, cm^{-1}): 1,690 (C=O), 3,178 (NH); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.31 (s, 3H, –$COCH_3$), 2.63 (s, 6H, 4′, 6′–CH_3); 6.97 (s, 1H, 5′–H); 7.36 (s, 1H, 4-H); 7.39–7.46 (m, 3H, 3″, 4″, 5″-H); 8.00–8.02 (m, 2H, 2″, 6″-H); 12.18 (s, 1H, –NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 24.2, 24.6, 95.5, 117.0, 126.6, 128.4, 128.8, 132.3, 151.3, 154.0, 157.5, 166.2, 168.7; MS: *m/z* $[M + 1]^+$ 308.1. Anal. Calcd. for $C_{17}H_{17}N_5O$: C, 66.43; H, 5.58; N, 22.79. Found: C, 66.34; H, 5.43; N, 22.57.

*1-(4′,6′-dimethylpyrimidin-2′-yl)-5-(N-acetamide)-3-(4″-methylphenyl)pyrazole (**4b**)*

Yield 73 %; mp 252–256; IR (KBr, cm^{-1}): 1,703 (C=O), 3,170 (NH); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.30 (s, 3H, –$COCH_3$), 2.39 (s, 3H, 4″-H); 2.62 (s, 6H, 4′, 6′–CH_3); 6.95 (s, 1H, 5′–H); 7.23–7.25 (d, 2H, J = 7.8 Hz, 3″, 5″-H); 7.32 (s, 1H, 4-H); 7.89–7.92 (d, 2H, J = 7.8 Hz, 2″, 6″-H); 12.18 (s, 1H, –NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 20.6, 24.2, 24.6, 95.4, 116.9, 126.5, 128.6, 129.1, 130.7, 151.2, 154.1, 157.4, 166.3, 168.7; MS: *m/z* $[M + 1]^+$ 322.2. Anal. Calcd. for $C_{18}H_{19}N_5O$: C, 67.27; H, 5.96; N, 21.79. Found: C, 67.34; H, 5.93; N, 21.67.

*1-(4′,6′-dimethylpyrimidin-2′-yl)-5-(N-acetamide)-3-(4″-fuorophenyl)pyrazole (**4c**)*

Yield 71 %; mp 226–230; IR (KBr, cm^{-1}): 1,694 (C=O), 3,145 (NH); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.31 (s, 3H, –$COCH_3$), 2.63 (s, 6H, 4′, 6′–CH_3); 6.98 (s, 1H, 5′–H); 7.09–7.15 (m, 2H, 3″, 5″-H); 7.31 (s, 1H, 4-H); 7.96–8.01 (m, 2H, 2″, 6″-H); 12.19 (s, 1H, –NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 23.5, 24.2, 95.4, 115.2, 128.2, 130.3, 132.7, 137.4, 151.3, 153.1, 157.4, 166.4, 168.7; MS: *m/z* $[M + 1]^+$ 326.1. Anal. Calcd. for $C_{17}H_{16}FN_5O$: C, 62.76; H, 4.96; N, 21.53. Found: C, 62.64; H, 4.93; N, 21.39.

*1-(4′,6′-dimethylpyrimidin-2′-yl)-5-(N-acetamide)-3-(4″-chlorophenyl)pyrazole (**4d**)*

Yield 72 %; mp 190–194; IR (KBr, cm^{-1}): 1,690 (C=O), 3,163 (NH); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.31 (s, 3H, –$COCH_3$), 2.63 (s, 6H, 4′, 6′–CH_3); 6.98 (s, 1H, 5′–H); 7.33 (s, 1H, 4-H); 7.39–7.42 (d, 2H, J = 7.8 Hz, 3″, 5″-H); 7.93–7.96 (d, 2H, J = 7.8 Hz, 2″, 6″-H); 12.18 (s, 1H, –NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 23.6, 24.2, 95.4, 117.1, 127.8, 128.6, 130.8, 134.6, 151.4, 152.9, 157.4, 166.4, 168.8; MS: *m/z* $[M + 1]^+$ 342.1/344.1 (3:1). Anal. Calcd. for $C_{17}H_{16}ClN_5O$: C, 59.74; H, 4.72; N, 20.49. Found: C, 59.64; H, 4.83; N, 21.37.

*1-(4′,6′-dimethylpyrimidin-2′-yl)-5-(N-acetamide)-3-(4″-bromophenyl)pyrazole (**4e**)*

Yield 71 %; mp 204–208; IR (KBr, cm^{-1}): 1,686 (C=O), 3,168 (NH); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.30 (s, 3H, –$COCH_3$), 2.62 (s, 6H, 4′, 6′–CH_3); 6.97 (s, 1H, 5′–H); 7.32 (s, 1H, 4-H); 7.53–7.56 (d, 2H, J = 7.8 Hz, 3″, 5″-H); 7.86–7.89 (d, 2H, J = 7.8 Hz, 2″,6″-H); 12.12–12.16 (s, 1H, –NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 23.7, 24.2, 95.4, 117.2, 122.9, 128.1, 131.2, 131.6, 151.4, 152.9, 157.6, 166.4, 168.7; MS: *m/z* $[M + 1]^+$ 386.1/388.1 (1:1). Anal. Calcd. for $C_{17}H_{16}BrN_5O$: C, 52.86; H, 4.18; N, 18.13. Found: C, 52.64; H, 4.26; N, 17.97.

General procedure for the preparation of 1-(4′,6′-dimethylpyrimidin-2′-yl)-5-(2,2,2-trifluoro-*N*-acetamide)-3-arylpyrazoles **(5a–e)**

5-Aminopyrazoles (2 mmol) were treated with trifluoroacetic anhydride (2 mmol) in the presence of trifluoroacetic

acid (20 mL) as solvent. The reaction mixture was heated on water bath for about 15 min and then cooled. Solid separated on cooling was filtered, washed with water, and crystallized using ethanol. The tlc and ^{1}H NMR spectra showed the formation of a single product.

*1-(4′,6′-dimethylpyrimidin-2′-yl)-5-(2,2,2-trifluoro-N-acetamide)-3-phenylpyrazole (**5a**)*

Yield 71 %; mp 138–142; IR (KBr, cm^{-1}): 1,609 (C=O), 3,112 (NH); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.59 (s, 6H, 4′, 6′–CH_3); 6.17 (s, 1H, 4-H); 7.03 (s, 1H, 5′–H); 7.44–7.49 (m, 3H, 3″, 4″, 5″-H); 7.95–7.98 (m, 2H, 2″, 6″-H); 12.18 (s, 1H, –NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 23.7, 93.3, 117.8, 124.6, 126.6, 128.7, 129.4, 131.3, 157.3, 158.1, 160.8, 168.9, 170.2; MS: *m/z* $[M + 1]^+$ 362.1. Anal. Calcd. for $C_{17}H_{14}F_3N_5O$: C, 56.51; H, 3.91; N, 19.38. Found: C, 56.64; H, 3.96; N, 19.47.

*1-(4′,6′-dimethylpyrimidin-2′-yl)-5-(2,2,2-trifluoro-N-acetamide)-3-(4″-methylphenyl)pyrazole (**5b**)*

Yield 69 %; mp 230–234; IR (KBr, cm^{-1}): 1,630 (C=O), 3,126 (NH); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.42 (s, 3H, 4″–CH_3); 2.61 (s, 6H, 4′, 6′–CH_3); 6.12 (s, 1H, 4-H); 7.02 (s, 1H, 5′–H); 7.35–7.38 (d, 2H, J = 7.8 Hz, 3″, 5″-H); 7.83–7.86 (d, 2H, J = 7.8 Hz, 2″, 6″-H); 12.18 (s, 1H, –NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 21.4, 23.6, 93.3, 117.8, 124.2, 126.6, 128.2, 129.4, 130.7, 157.2, 158.1, 161.8, 169.2, 169.8; MS: *m/z* $[M + 1]^+$ 376.1. Anal. Calcd. for $C_{18}H_{16}F_3N_5O$: C, 57.60; H, 4.30; N, 18.66. Found: C, 57.64; H, 4.36; N, 18.47.

*1-(4′,6′-dimethylpyrimidin-2′-yl)-5-(2,2,2-trifluoro-N-acetamide)-3-(4″-fluorophenyl)pyrazole (**5c**)*

Yield 67 %; mp 128–132; IR (KBr, cm^{-1}): 1,626 (C=O), 3,114 (NH); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.57 (s, 6H, 4′, 6′–CH_3); 6.05 (s, 1H, 4-H); 6.89 (s, 1H, 5′–H); 7.18–7.24 (m, 2H, 3″, 5″-H); 7.95–7.99 (m, 2H, 2″, 6″-H); 12.18 (s, 1H, –NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 23.7, 93.6, 115.7, 124.5, 127.4, 128.9, 129.0, 136.5, 157.3, 158.2, 162.0, 168.9, 170.4; MS: *m/z* $[M + 1]^+$ 380.1. Anal. Calcd. for $C_{17}H_{13}F_4N_5O$: C, 53.83; H, 3.45; N, 18.46. Found: C, 53.64; H, 3.36; N, 18.37.

*1-(4′,6′-dimethylpyrimidin-2′-yl)-5-(2,2,2-trifluoro-N-acetamide)-3-(4″-chlorophenyl)pyrazole (**5d**)*

Yield 72 %; mp 106–110; IR (KBr, cm^{-1}): 1,656 (C=O), 3,117 (NH); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.51 (s, 6H, 4′, 6′–CH_3); 6.15 (s, 1H, 4-H); 6.93 (s, 1H, 5′–H); 7.23–7.26 (d, 2H, J = 8.1 Hz, 3″, 5″-H); 7.70–7.73 (d, 2H, J = 8.1 Hz, 2″, 6″-H); 12.18 (s, 1H, –NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 23.5, 93.1, 118.4, 124.4, 125.1, 128.6, 128.9, 132.6, 157.4, 158.2, 161.6, 169.4, 170.6; MS: *m/z* $[M + 1]^+$ 396.1/398.1 (3:1). Anal. Calcd. for $C_{17}H_{13}ClF_3N_5O$: C, 51.59; H, 3.31; N, 17.70. Found: C, 51.64; H, 3.36; N, 17.54.

*1-(4′,6′-dimethylpyrimidin-2′-yl)-5-(2,2,2-trifluoro-N-acetamide)-3-(4″-bromophenyl)pyrazole (**5e**)*

Yield 69 %; mp 76–80; IR (KBr, cm^{-1}): 1,643 (C=O), 3,108 (NH); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.51 (s, 6H, 4′, 6′–CH_3); 6.16 (s, 1H, 4-H); 6.93 (s, 1H, 5′–H); 7.38–7.41 (d, 2H, J = 8.1 Hz, 3″, 5″-H); 7.63–7.65 (d, 2H, J = 8.1 Hz, 2″, 6″-H); 12.18 (s, 1H, –NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 23.5, 93.2, 118.4, 124.3, 124.5, 126.1, 128.7, 131.9, 157.4, 158.1, 161.4, 169.3, 170.0; MS: *m/z* $[M + 1]^+$ 440/442 (1:1). Anal. Calcd. for $C_{17}H_{13}BrF_3N_5O$: C, 46.38; H, 2.98; N, 15.91. Found: C, 46.54; H, 2.86; N, 15.84.

Evaluation of antibacterial assay: preliminary screening

The agar well diffusion method (Perez *et al.*, 1990) was used for the determination of antimicrobial activity. Overnight broth culture of the respective bacterial strains was adjusted to approximately 10^8 colony-forming units (CFU/mL) with sterile distilled water, and 1 mL of diluted inoculum was spread over the Petri plates containing 25 mL of nutrient agar media. Six to eight wells (7 mm in diameter) were made equidistance in each of the plates using a sterile cork borer. The test compounds were dissolved in dimethyl sulfoxide (DMSO) and then antimicrobial effect of the test compounds was tested. The wells were filled with 1 mL of test compound having concentration 1 mg/mL. The plates were incubated at 37 °C for 48 h. The antimicrobial activity was evaluated by measuring the zone of growth inhibition of bacteria surrounding the wells after 24 h and 48 h. Ampicillin and Chloramphenicol (10 μg/mL) served as positive antibacterial controls. DMSO was taken as negative control.

Determination of minimum inhibitory concentration

The minimum inhibitory concentration (MIC) of each compound giving an inhibitory zone at the concentration of 1 mg/mL was also tested by a modified agar well diffusion method (Okunji *et al.*, 1990; Rios *et al.*, 1988). Different concentrations (1,000–1 μg/mL) of a single compound were applied to number of wells in the agar plates. About 1 mL of each dilution was poured into wells. All test plates were incubated at 37 °C for 48 h. The minimum concentration of each compound showing a clear zone of inhibition was considered to be MIC. The determinations were performed in triplicates and the results averaged.

Evaluation of cytotoxic activity

AlamarBlue™ assays were carried out in HL-60 by seeding cells at 2×10^5 per mL in RPMI solution and transferring 200 μL into each well on a 96-well plate. Ethanol solutions of 100 μM and 50 μM concentration of each drug were prepared and tested. Once treated, the plate of cells was incubated over a period of 72 h. After this, into each well, 20 μL of alamarBlue® dye was added and left in darkness in the incubator for 5 h at 37 °C. The alamarBlue® assay incorporates a fluorometric/colorimetric growth indicator based on the detection of metabolic activity. The system comprises an oxidation–reduction redox reaction that is reflected in fluorescence and color change. These changes occur as a result of the cellular metabolic reduction of the indicator. Only leaving cells metabolise the indicator, reducing it, and emitting fluorescence. After incubation, the fluorescence of the dye was read using a plate reader. The reader measures absorption of the alamarBlue® at two wavelengths and produces figures that are used to calculate the percentage viability of the cells. The mean values and the standard error of the mean (SEM) of all the experiments for each compound were then used to plot graphs using GraphPad Prism and Excel. The IC_{50} was calculated using GraphPad Prism.

Acknowledgments We (RA and IR) thank the Department of Science and Technology, New Delhi, India, and Trinity College, Dublin, Ireland, for providing financial assistance under India–Ireland Cooperative Science Programme. Anshul Bansal is thankful to the Council of Scientific and Industrial Research, New Delhi, India, for Senior Research Fellowship. Elena Diez-Cecilia thanks IRCSET (Ireland) for financial support.

References

Abdel-Aziz M, Abuo-Rahma G, El-Din A, Hassan AA (2009) Synthesis of novel pyrazole derivatives and evaluation of their antidepressant and anticonvulsant activities. Eur J Med Chem 44:3480–3487

Abu Almaati TM, El-Taweel FM (2004) New trends in the chemistry of 5-aminopyrazoles. J Heterocycl Chem 41:109–134

Aggarwal R, Kumar V, Kumar R (2007) A facile and rapid one-pot synthesis of 1,4-diaryl-2-mercaptoimidazoles under solvent-free conditions. J Sulfur Chem 28:617–623

Aggarwal R, Kumar V, Kumar R, Singh SP (2011) Approaches towards the synthesis of 5-aminopyrazoles. Beilstein J Org Chem 7:179–197

An H, Eum S, Koh M, Lee SK, Park SB (2008) Diversity-oriented synthesis of privileged benzopyranyl heterocycles from *s-cis*-Enones. J Org Chem 73:1752–1761

Banks JW, Batsanov AS, Howard JAK, O'Hagan D, Rzepa HS, Santamaria SM (1999) The preferred conformation of α-fluoroamides. J Chem Soc Perkin Trans 2:2409–2411

Barril X, Beswick MC, Collier A, Drysdale MJ, Dymock BW, Fink A, Grant K, Howes R, Jordan AM, Massey A, Wayne AS, Workman JP, Wright L (2006) 4-Amino derivatives of the Hsp90 inhibitor CCT018159. Bioorg Med Chem Lett 16:2543–2548

Benaamane N, Nedjar-Kolli B, Bentarzi Y, Hammal L, Geronikaki A, Eleftheriou P, Langunin A (2008) Synthesis and in silico biological activity evaluation of new *N*-substituted pyrazolo-oxazin-2-one systems. Bioorg Med Chem 6:3059–3066

Burley SK, Petsko GA (1988) Weakly polar interactions in proteins: advances in protein chemistry. Academic Press, New York, pp 125–189

Danagulyan GG, Balasanyan NG, Terent'ev PB, Zalinyan MG (1989) Chemistry of heterocyclic compounds. Kluwer, New York, pp 1369–1373

Das J, Moquin RV, Dyckman AJ, Li T, Pitt S, Zhang R, Shen DR, McIntyre KW, Gillooly K, Doweyko AM, Newitt JA, Sack JS, Zhang H, Kiefer SE, Kish K, McKinnon M, Barrish JC, Dodd JH, Schieven GL, Leftheris K (2010) 5-Amino-pyrazoles as potent and selective p38a inhibitors. Bioorg Med Chem Lett 20:6886–6889

Dymock BW, Barril X, Brough PA, Cansfield JE, Massey A, McDonald E, Hubbard RE, Surgenor A, Roughley SD, Webb P, Workman P, Wright L, Drysdale MJ (2005) Novel, potent small-molecule inhibitors of the molecular chaperone Hsp90 discovered through structure-based design. J Med Chem 48:4212–4215

El-gazzar ABA, Hussein HAR, Hafez HN (2007) Synthesis and biological evaluation of thieno[2,3-d]pyrimidine derivatives for anti-inflammatory, analgesic and ulcerogenic activity. Acta Pharm 57:395–411

Farag AM, Mayhoub AS, Barakat SE, Bayomi AH (2008) Regioselective synthesis and antitumor screening of some novel *N*-phenylpyrazole derivatives. Bioorg Med Chem 16:881–889

Gakhar HK, Gill GS, Multani JS (1971) Thiopegan derivatives part XLIX. J Indian Chem Soc 48:953–956

Gilbert AM, Failli A, Shumsky J, Yang Y, Severin A, Singh G, Hu W, Keeney D, Petersen PJ, Katz AH (2006) Pyrazolidine-3,5-diones and 5-hydroxy-1*H*-pyrazol-3(2*H*)-ones, inhibitors of UDP-*N*-acetylenolpyruvyl glucosamine reductase. J Med Chem 49:6027–6036

Goldstein DM, Kuglstatter YL, Soth MJ (2009) Selective p38r inhibitors clinically evaluated for the treatment of chronic inflammatory disorders. J Med Chem 53:2345–2353

Gopalsamy A, Yang H, Ellingboe JW, Tsou HR, Zhang N, Honores E, Powell D, Miranda M, McGinnis JP, Rabindran SK (2005) Pyrazolo[1,5-*a*]pyrimidin-7-yl phenyl amides as novel anti-proliferative agents: parallel synthesis for lead optimization of amide region. Bioorg Med Chem Lett 15:1591–1594

Hayakawa M, Kaizawa H, Moritomo H, Koizumi T, Ohishi T, Okada M, Ohta M, Tsukamoto S, Parker P, Workman P, Waterfield M (2006) Synthesis and biological evaluation of 4-morpholino-2-phenylquinazolines and related derivatives as novel PI3 kinase p110α inhibitors. Bioorg Med Chem 14:6847–6858

Heffron TP, Berry M, Castanedo G et al (2010) Identification of GNE-477, a potent and efficacious dual PI3 K/mTOR inhibitor. Bioorg Med Chem Lett 20:2408–2411

Hiraku Y, Oikawa S, Kawanishi S (2002) Distamycin A, a minor groove binder, changes enediyne-induced DNA cleavage sites and enhances apoptosis. Nucl Acids Res Suppl 2:95–96

Jung JC, Walkins EB, Avery MA (2002) Synthesis of 3-substituted and 3,4-disubstituted pyrazolin-5-ones. Tetrahedron 58:3639–3646

Junttila TT, Akita RW, Parsons K, Fields C, Lewis Phillips GD, Friedman LS, Sampath D, Sliwkowski MX (2009) Ligand-independent HER2/HER3/PI3 K complex is disrupted by trastuzumab and is effectively inhibited by the PI3 K inhibitor GDC-0941. Cancer Cell 15:429–440

Kaiko RF, Wallenstein SL, Rogers AG, Grabinski PY, Houde RW (1981) Analgesic and mood effects of heroin and morphine in

cancer patients with postoperative pain. N Engl J Med 304:1501–1505

Kane JL, Hirth BH, Laing O, Gourlie BB, Nahill S, Barsomiam G (2003) Ureas of 5-aminopyrazole and 2-aminothiazole inhibit growth of gram-positive bacteria. Bioorg Med Chem Lett 13:4463–4466

Kordik CP, Luo C, Zanoni BC, Dax SL, McNally JJ, Lovenberg TW, Wilson SJ, Reitz AB (2001) Aminopyrazoles with high affinity for the human neuropeptide Y5 receptor. Bioorg Med Chem Lett 11:2283–2286

Kumar V, Aggarwal R, Tyagi P, Singh SP (2005) Synthesis and antibacterial activity of some new 1-heteroaryl-5-amino-4-phenyl-3-trifluoromethylpyrazoles. Eur J Med Chem 40:922–927

Kumar V, Aggarwal R, Tyagi P, Singh SP (2006) Synthesis and antibacterial activity of some new 1-heteroaryl-5-amino-3H/methyl-4-phenylpyrazoles. Bioorg Med Chem 14:1785–1791

Lee KY, Kim JM, Kim JN (2003) Regioselective synthesis of 1,3,4,5-tetrasubstituted pyrazoles from Baylis–Hillman adducts. Tetrahedron Lett 44:6737–6740

Lindvall M, McBride C, McKenna M, Gesner TG, Yabannavar A, Wong K, Lin S, Walter A, Shafer CM (2011) 3D pharmacophore model-assisted discovery of novel CDC7 inhibitors. Med Chem Lett 2:720–723

Liu XH, Cui P, Song BA, Bhadury PS, Zhu HL, Wang SF (2008) Synthesis, structure and antibacterial activity of novel 1-(5-substituted-3-substituted-4,5-dihydropyrazol-1-yl)ethanone oxime ester derivatives. Bioorg Med Chem 16:4075–4082

Manetti F, Brullo C, Magnani M, Mosci F, Chelli B, Crespan E, Schenone S, Naldini A, Bruno O, Trincavelli ML, Maga G, Carraro F, Martini C, Bondavalli F, Botta M (2008) Structure-based optimization of pyrazolo[3,4-*d*]pyrimidines as Abl inhibitors and antiproliferative agents toward human leukemia cell lines. J Med Chem 51:1252–1259

Meegalla SK, Doller D, Sha DY, Soll R, Wisnewski N, Silverb GM, Dhanoaa D (2004) Synthesis and GABA receptor potency of 3-thiomethyl-4-(hetero)aryl-5-amino-1-phenylpyrazoles. Bioorg Med Chem Lett 14:4949–4953

Mercep M, Mesic M, Pesic D (2004) PCT Int Appl WO 03 99, 822. Chem Abstr 140:16724j

Michon V, Hervé du Penhoat C, Tombret F, Gillardin JM, Lepage F, Berthon L (1995) Preparation, structural analysis and anticonvulsant activity of 3- and 5-aminopyrazole *N*-benzoyl derivatives. Eur J Med Chem 30:147–155

Okunji CO, Okeke CN, Gugnani HC, Iwu MM (1990) An antifungal Spirostanol Saponin from Fruit Pulp of Dracaena mannii. Int J Crude Drug Res 28:193–199

Perez C, Pauli M, Bezerque P (1990) An antibiotic assay by the agar-well diffusion method. J Aotabiol 15:113–115

Rios JL, Recio MC, Vilar (1988) A. Screening methods for natural products with antimicrobial activity: a review of the literature. J Ethnopharmacol 23:127–149

Sellery S, Gratteri P, Costagli C, Bonaccini C, Coztanzo A, Melani F, Guerrini G, Ciciani G, Costa B, Spinetti F, Martini C, Bruni F (2005) Insight into 2-phenylpyrazolo[1,5-a]pyrimidin-3-yl acetamides as peripheral benzodiazepine receptor ligands: synthesis, biological evaluation and 3D-QSAR investigation. Bioorg Med Chem 13:4821–4834

Sherif AFR (2006) Synthesis and in vitro antitumor evaluation of some indeno[1,2-*c*]pyrazol(in)es substituted with sulfonamide, sulfonylurea(-thiourea) pharmacophores, and some derived thiazole ring systems. Bioorg Med Chem 14:6475–6485

Shetty NS, Lamani RS, Khazi IM (2009) Synthesis and antimicrobial activity of some novel thienopyrimidines and triazolothinopyrimidines. J Chem Sci 121:301–307

Soliman R, Darwish SAS (1983) Antidiabetic activity of some 1-substituted 3,5-dimethylpyrazoles. J Med Chem 26:1659–1663

Studzinski GP, Bhandal AK, Brelvi ZS (1986) Potentiation by 1-α,25-dihydroxyvitamin D_3 of cytotoxicity to HL-60 cells produced by cytarabine and hydroxyurea. J Natl Cancer Inst 76(4):641–648

Wei F, Zhao B, Huang B, Zhang L, Sun C, Dong W, Shin D, Miao J (2006) Design, synthesis, and preliminary biological evaluation of novel ethyl 1-(2′-hydroxy-3′-aroxypropyl)-3-aryl-1H-pyrazole-5-carboxylate. Bioorg Med Chem Lett 16:6342–6347

Zhao W, Zhang T, Qu B, Wu X, Zhu X, Meng F, Gu Y, Shu Y, Shen Y, Sun Y, Xu Q (2011) Sorafenib induces apoptosis in HL60 cells by inhibiting Src kinase-mediated STAT3 phosphorylation. Anticancer Drugs 22(1):79–88

Zoltewicz JA, Uray G (1994) Thiamin: a critical evaluation of recent chemistry of the pyrimidine ring. Bioorg Chem 22:1–28