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EXPERIMENTAL PAPER

Development of 7-Aminopyrazolo[1,5-*a*]pyrimidines as Anti-Candida and Antibacterial Agents

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We have previously developed[1] a chemo and regioselective synthesis of pyrazol-1'-ylpyrazolo[1,5-*a*]pyrimidines **3a-h** (Scheme 1). This was achieved by treatment of 5-amino-3-hydrazinopyrazole dihydrochloride (**1**) with symmetrical and unsymmetrical *β*-diketones **2** in aqueous media. We have further found the pyrazol-1'-ylpyrazolo[1,5-*a*]pyrimidines to be excellent antibacterial and antifungal agents[2] for which structure-activity studies showed that the antimicrobial potency was mainly influenced by the substituents at position 7.

With these observations as background,[3] we now describe the formation of the title compounds by the reaction of **1** with 3(2)-aryl-3-oxoalkanenitriles. The novel compounds were screened for their anti-Candida and antibacterial potency. Given the advantages of green chemistry protocols,[4–6] it was envisaged to condense **1** with compounds **4** under solvent-free conditions. For example, one equivalent of **1** was thoroughly ground with two equiv of NaOAc and two equiv of **4a** at room temperature. Then the reaction mixture was heated on the water bath for 15 min to afford compound **5a** (70%) as the exclusive product (Scheme 2). The presence of NaOAc was found to be essential, as no product could be achieved without NaOAc. Following a similar protocol, other pyrazolo[1,5-*a*]pyrimidines **5b-g** were synthesized by condensing **1** with other 3(2)-aryl-3-oxoalkanenitriles **4b-g**. All of the products were fully characterized (see Experimental section).

The formation of compound **5a** is attributed to the *in situ* generation of hydrazine by C-N bond cleavage in **1** following the proposed mechanism depicted in Scheme 3.

To support the possible *in situ* generation of hydrazine, we undertook to synthesize **5a** directly from hydrazine hydrate itself (Scheme 4). In exploring this possibility, the reaction solvent was changed from toluene to a mixture of toluene and ethanol (9:1), with a small amount of *p*-toluenesulfonic acid (PTSA). This indeed led to **5a** (65%) in 10 hr, unambiguously determined from a comparison with an authentic sample.

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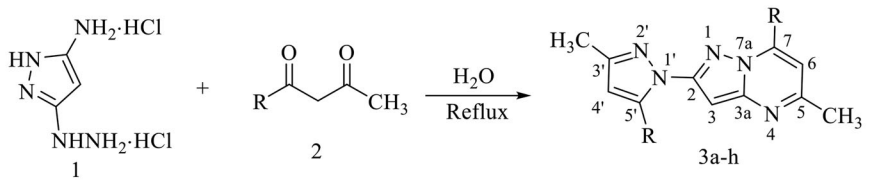
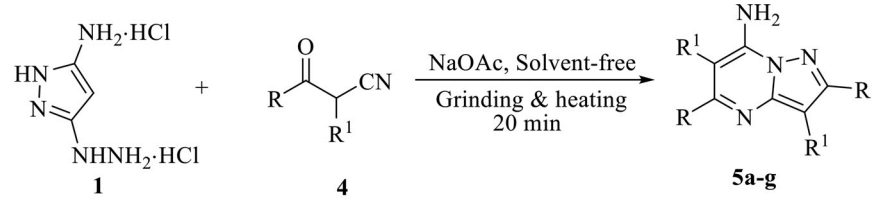


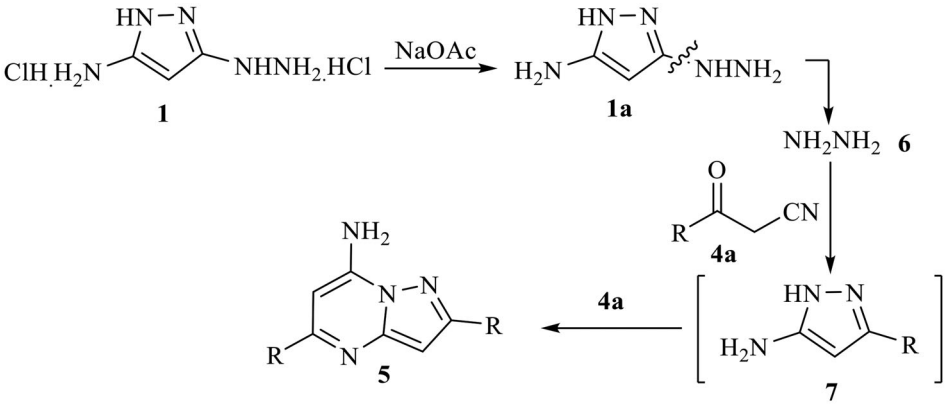
R = CH_3(a), C_6H_5(b) , p-$CH_3C_6H_4$(c), p-$OCH_3C_6H_4$(d), p-ClC_6H_4(e), p-BrC_6H_4(f), 2-thienyl (g), (-CH_2-)$_3$(h)

Scheme 1. General reaction of 5-amino-3-hydrazinopyrazole dihydrochloride (**1**) and β-diketones (**2**).



4, 5	a	b	c	d	e	f	g
R	C_6H_5	*p*-ClC_6H_4	*p*-$CH_3C_6H_4$	*p*-BrC_6H_4	*p*-FC_6H_4	H	CH_3
R^1	H	H	H	H	H	C_6H_5	C_6H_5

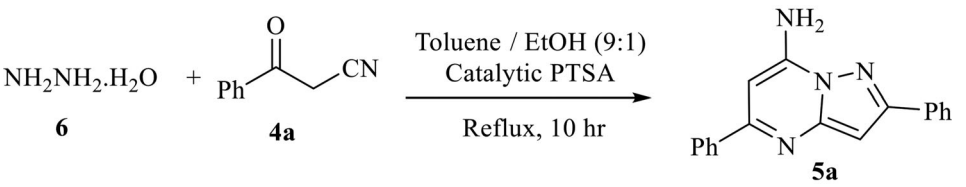
Scheme 2. Reaction of 5-amino-3-hydrazinopyrazole dihydrochloride **1** with 3(2)-aryl-3-oxoalkanenitriles **4**.



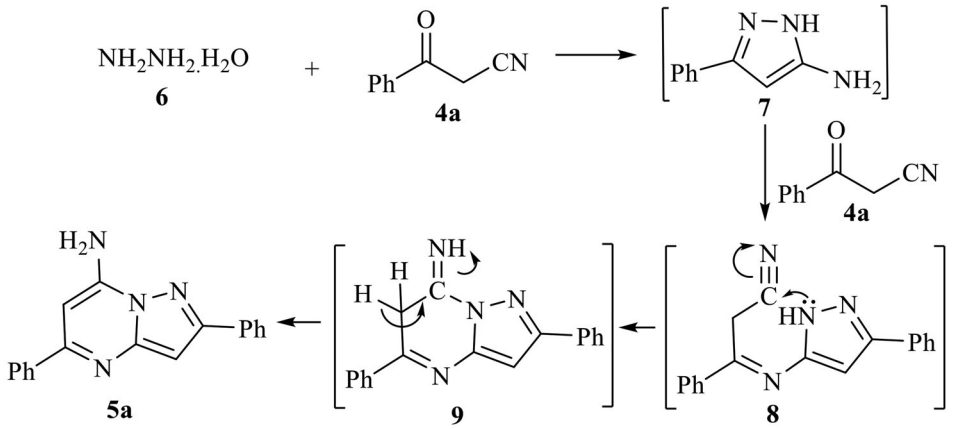
Scheme 3. Generation of hydrazine *in situ*.

We surmise that formation of **5a** directly from hydrazine hydrate follows the mechanistic pathway shown in Scheme 5. Thus hydrazine hydrate **6** condenses with one mole of **4a** to give the intermediate 5-amino-1*H*-pyrazole **7**. The exocyclic amino group of **7** reacts with the carbonyl group of a second molecule of **4a** to give the intermediate **8**. Finally, intramolecular nucleophilic addition gives **5a**.

The robust character of the protocol used in this mechanistic exploration encouraged us to use it to prepare more 7-aminopyrazolo[1,5-*a*]pyrimidines (**5h**-**5o**). These compounds had further structural variations at positions 2, 3, 5 and 6 of the pyrazolo[1,5-*a*]pyrimidine moiety (Scheme 6). We anticipated these would be useful for estimating



Scheme 4. Formation of **5a** directly from hydrazine hydrate.

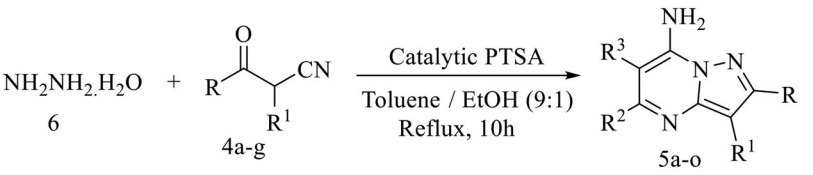


Scheme 5. Proposed mechanism for formation of **5a** from hydrazine.

structure-activity relationships in the microorganisms we wished to assay. We also used this method to again prepare compounds **5a**-**5g**. The results of our synthesis efforts are summarized in Table 3. Following our synthetic methods, no purification was needed.

Compounds **5a**-**o** were screened for their *in vitro* anti-Candida activity against four pathogenic *Candida* strains, namely *Candida albicans* (MTCC 3017), *Candida glabrata* (MTCC 3019), *Candida tropicalis* (MTCC 184) and *Candida parapsilosis* (MTCC 1965) obtained from Microbial Type Culture Collection, Institute of Microbial Technology (IMTECH), Chandigarh. Compounds showing zones of inhibition of 12 mm or more were further screened for their minimum inhibitory concentration (MIC) through the agar-well diffusion assay technique. The results are compiled in Table 1. For evaluating anti-Candida activity the known antifungal fluconazole was used as standard drug. It is worth mentioning that most of these compounds showed anti-Candida activity against *C. albicans*. Interestingly, compounds **5i**, **5l**, and **5o** showed activity against *C. glabrata*, an organism which was resistant to fluconazole. The anti-Candida activity against *C. tropicalis* increases to a significant extent when the hydrogen of the phenyl residue of compound **5a** is replaced with a chlorine in **5b**, bromine in **5d** and fluorine in **5c**.

Compounds **5a**-**o** were assayed *in vitro* for their antibacterial activity against two Gram-positive bacteria {*Staphylococcus aureus* (MTCC 890), *Streptococcus mutans* (MTCC 3160)} and two Gram-negative bacteria {*Pseudomonas aeruginosa* (MTCC 2295), *Escherichia coli* (MTCC 43)} obtained from MTCC, IMTECH, Chandigarh. For evaluating antibacterial activity, ciprofloxacin was used as the standard drug. The results of antibacterial activity are presented in Table 2. In general, the results reveal that many of the tested compounds showed antibacterial potency towards *S. mutans* and *P.*



4	R	R^1
a	C_6H_5	H
b	*p*-ClC_6H_4	H
c	*p*-$CH_3C_6H_4$	H
d	*p*-BrC_6H_4	H
e	*p*-FC_6H_4	H
f	H	C_6H_5
g	CH_3	C_6H_5

5	R	R^1	R^2	R^3
a	C_6H_5	H	C_6H_5	H
b	*p*-ClC_6H_4	H	*p*-ClC_6H_4	H
c	*p*-$CH_3C_6H_4$	H	*p*-$CH_3C_6H_4$	H
d	*p*-BrC_6H_4	H	*p*-BrC_6H_4	H
e	*p*-FC_6H_4	H	*p*-FC_6H_4	H
f	H	C_6H_5	H	C_6H_5
g	CH_3	C_6H_5	CH_3	C_6H_5
h	H	C_6H_5	*p*-$CH_3C_6H_4$	H
i	H	C_6H_5	C_6H_5	H
j	H	C_6H_5	*p*-ClC_6H_4	H
k	H	C_6H_5	*p*-BrC_6H_4	H
l	CH_3	C_6H_5	*p*-$CH_3C_6H_4$	H
m	CH_3	C_6H_5	C_6H_5	H
n	CH_3	C_6H_5	*p*-ClC_6H_4	H
o	CH_3	C_6H_5	*p*-BrC_6H_4	H

Scheme 6. Preparation of compounds **5a-o**.

aeruginosa. The data in Table 2 suggest that the movement of an aryl residue from position-2 to position-3 increases the antibacterial potency of the compounds. Replacement of hydrogen (**5a**) and chlorine (**5b**) with a methyl group (**5c**) causes a huge loss of antibacterial activity. The presence of a methyl group on positions 2 and 5 decreases the potency drastically (**5f**).

In summary, we have disclosed convenient methods for the preparation of novel 7-aminopyrazolo[1,5*a*]pyrimidines in good to very good yields and high purity. We have examined the activities of our compounds in eight micro-organisms and discovered useful activities. Some of the activities appear to be related to substitution patterns on the pyrimidines, suggesting that further work will be warranted in forming wider structure-activity relationships. Finally, our work demonstrates the productive interaction of mechanistic organic chemistry with organic synthesis.

Experimental section

Melting points were determined in open capillaries and are uncorrected. The IR spectra of the compounds were recorded on a Buck Scientific IR M-500 spectrophotometer

Table 1. *In vitro* anti-Candida activity and minimum inhibitory concentration (MIC) of **5a-o**.

	Diameter of zone of inhibition (mm)[a] (MIC (μg/ml))			
Compound	*C. glabrata*	*C. albicans*	*C. tropicalis*	*C. parapsilosis*
5a	–	28 (125)	12 (210)	–
5b	–	12 (210)	24 (250)	–
5c	–	14 (300)	14 (320)	–
5d	–	12 (260)	26 (500)	–
5e	–	26 (250)	18 (400)	12 (250)
5f	–	–	–	–
5g	10	12	11	–
5h	–	10	13 (625)	–
5i	–	–	12 (1250)	–
5j	–	–	10	–
5k	–	11	12	–
5l	11	12 (1250)	13 (1250)	–
5m	–	10	–	–
5n	–	–	–	–
5o	10	10	10	–
Fluconazole	–	–	34 (50)	–

[a]Mean of three replicates.

using KBr pellets (ν_{max} in cm^{-1}); ^{1}H and ^{13}C NMR spectra were determined on a Bruker instrument at 300 and 75 MHz, respectively. Chemical shifts are expressed on the δ-scale downfield from TMS as an internal standard. Elemental analyses were performed at NIPER (Mohali), Chandigarh, India. All the compounds gave C, H and N analysis within ±0.40% of the theoretical values. Mass spectra were measured in EI mode on a Shimadzu QP-5000 spectrometer at NIPER (Mohali), Chandigarh, India. Thin layer chromatography (TLC) was performed on silica gel (100-200), using ethyl acetate/petroleum ether (20% v/v) as the eluting solvent. 3(2)-Aryl-3-oxoalkanenitriles **4a-e** were prepared from α-bromoacetophenones and potassium cyanide following the literature procedure.[7] Similarly, 2-aryl-3-oxoalkanenitriles **4f-g** were also prepared following the literature protocol.[8,9] 5-Amino-3-hydrazinopyrazole dihydrochloride **1** was prepared by condensing hydrazine hydrate with malononitrile.[10]

Representative solvent-free preparation. 7-Amino-2,5-diphenylpyrazolo[1,5-a]pyrimidine (5a)

5-Amino-3-hydrazinopyrazole dihydrochloride (0.378 g, 2 mmol) **1** was ground with sodium acetate (0.328 g, 4 mmol) with a dry pestle and mortar at room temperature under solvent-free conditions. To the resulting mixture was added 3-phenyl-3-oxopropanenitrile (0.580 g, 4 mmol) **4a** and the mixture was ground thoroughly for 5 minutes at room temperature, then heated on a water bath for 15 minutes until TLC showed complete disappearance of starting materials. The addition of aqueous ethanol (50%) to the crude mixture led to the separation of the solid product which was filtered, dried and recrystallized from aqueous ethanol (50%) to give pure **5a** (70%). Following the same synthetic protocol, compounds **5b-g** were synthesized, with mean yields of 73%.

Direct Reactions with Hydrazine Hydrate. 7-Aminopyrazolo[1,5-a]pyrimidines 5a-5g, 5h-5o

To hydrazine hydrate **6** (0.05 g, 1 mmol) was added the appropriate 3(2)-aryl-3-oxoalkanenitrile (2 mmol) **4a-g** in toluene/EtOH (9:1) followed by a catalytic amount of *p*-

Table 2. *In vitro* antibacterial activity and minimum inhibitory concentrations (MIC) of **5a-o**.

	Diameter of zone of inhibition (mm)[a] (MIC (μg/ml))			
Compound	*E. Coli*	*S. aureus*	*S. mutans*	*P. aeruginosa*
5a	13 (125)	–	12 (125)	13 (125)
5b	14 (250)	12 (250)	13 (125)	12 (250)
5c	Not determined	–	12 (500)	–
5d	11 (500)	–	13 (500)	–
5e	11 (250)	–	14 (250)	–
5f	–	–	–	10
5g	–	–	–	10
5h	19 (313)	13 (78)	13 (78)	12
5i	15 (1250)	–	17 (380)	12 (78)
5j	12	12	12	11
5k	–	–	12	10
5l	21 (313)	–	19 (20)	11
5m	12	–	16 (1250)	14 (1250)
5n	–	–	15 (313)	13 (78)
5o	–	10	11	12
Ciprofloxacin	37 (5)	40 (4)	31	32

[a]Mean of three replicates.

toluenesulfonic acid. The mixture was refluxed until the completion of reaction (4 hr), as determined by TLC. On completion, the excess solvent was distilled off, and the solid thus obtained was filtered off. The solid was dissolved in water and neutralized with aqueous sodium bicarbonate solution. Finally, the product was filtered and dried in air to give **5a-g**. No further purification was needed.

A mixture of hydrazine hydrate **6** (0.05 g, 1 mmol) and 3(2)-aryl-3-oxoalkanenitrile (1 mmol) **4a-g** in toluene/EtOH (9:1) was refluxed for 30 min. Then, to this reaction mixture another 1 mmol of 3(2)-aryl-3-oxoalkanenitrile **4a-e** was added along with a catalytic amount of *p*-toluenesulfonic acid. The mixture was again refluxed until completion of reaction (4 hr), as determined by TLC. On completion of reaction as monitored on TLC, the excess solvent was removed by distillation and the solid obtained was filtered off. The solid was dissolved in water and treated with aqueous sodium bicarbonate solution. The product was filtered off and dried in air to give **5h-o**. No further purification was needed.

Evaluation of anti-Candida and antibacterial activity: preliminary screening

The approximate number of microorganisms in a liquid suspension is determined by comparing the turbidity of the test organism suspension with a McFarland standard. It is a mixture of two chemical constituents {9.95 ml barium chloride (1.175%) + 0.05 mL sulfuric acid (1.0%)}. The reaction between the two chemicals results in production of a fine precipitate of barium sulfate. When shaken well, the turbidity of 0.5 McFarland standard is visually comparable to a bacterial suspension of approximately $15x10^8$ cells/mL. The inocula of different test pathogens were adjusted according to the above McFarland standard. Anti-Candida and antibacterial activities of the compounds were evaluated by the agar-well diffusion method. Using a sterilized cork borer, well plates of 6 mm diameter were punched in solidified malt extract agar plates containing the test pathogens adjusted as above at 0.5 McFarland standard. Then 250 μL of the chemical compound (1 mg/mL) was added into the well with the help of a sterilized micropipette.

Table 3. Analytical and spectral data of compounds **5a-o**.

Product	Yield %	mp (°C) Lit mp (°C)	IR (KBr, cm^{-1})	^{1}H-NMR (δ)	^{13}C-NMR (δ)	MS (m/z)	Elemental Analysis
5a	70[a]	262 (lit[28] mp 265)	3212, 3454	6.61 (s, 1H, 6-H), 6.92 (s, 1H, 3-H), 7.40-8.07 (m, 12H, Ph-H, 7-NH_2)	84.45, 91.45, 126.13, 126.68, 128.67, 128.73, 129.61, 132.98, 138.06, 148.07, 150.14, 154.45, 155.57	287 $[M+1]^+$	Found: C, 75.41; H, 4.89; N, 19.46; $C_{18}H_{14}N_4$ requires C, 75.50; H, 4.93; N, 19.57
5b	74[a]	292	3302, 3441	6.66 (s, 1H, 6-H), 7.04 (s, 1H, 3-H), 7.59-7.68 (d, 2H, $J=8.1$ Hz, Ph-H), 7.65-7.68 (d, 2H, $J=8.4$ Hz, Ph-H), 7.99-8.01 (d, 2H, $J=8.1$ Hz, Ph-H), 8.12-8.14 (d, 2H, $J=8.4$ Hz, Ph-H), 9.05 (bs, 2H, 7-NH_2 exchangeable with D_2O)	87.32, 90.44, 128.69, 129.04, 129.39, 129.74, 131.05, 132.85, 134.70, 136.59, 145.20, 150.30, 152.18, 155.13	355/357/359 $[M+1]^+$/ $[M+1+2]^+$ $[M+1+4]^+$ (9:1:6)	C, 60.81; H, 3.38; N, 15.68; $C_{18}H_{12}Cl_2N_4$ requires C, 60.86; H, 3.41; N, 15.77
5c	71[a]	256	3240, 3418, (-NH_2)	2.36 (s, 3H, 4′-CH_3), 2.40 (s, 3H, 4"- CH_3), 6.69 (s, 1H, 6-H), 6.98 (s, 1H, 3-H), 7.33-7.98 (m, 8H, Ph-H), 9.55 (bs, 7-NH_2 exchangeable with D_2O)	20.95, 20.97, 86.82, 88.75, 126.53, 127.53, 128.48, 128.99, 129.46, 129.78, 139.53, 142.11, 142.57, 150.37, 151.53, 156.22	315 $[M+1]^+$	Found: C, 76.39; H, 5.69; N, 17.64; $C_{20}H_{18}N_4$ requires C, 76.41; H, 5.77; N, 17.82
5d	68[a]	194	3302, 3456 (-NH_2)	6.63 (s, 1H, 6-H), 7.03 (s, 1H, 3-H), 7.69-7.72 (d, 2H, $J=8.4$ Hz, Ph-H), 7.78-7.80 (d, 2H, $J=8.1$ Hz, Ph-H), 7.91-7.94 (d, 2H, $J=8.4$ Hz, Ph-H), 8.04-8.07 (d, 2H, $J=8.1$ Hz, Ph-H), 8.89 (bs, 2H, 7-NH_2 exchangeable with D_2O)	87.05, 90.63, 123.34, 125.27, 127.96, 128.92, 129.85, 131.55, 131.96, 132.31, 132.51, 150.13, 152.65, 155.07	442/444/446 $[M+1]^+$/ $[M+1+2]^+$ $[M+1+4]^+$ (1:2:1)	Found: C, 48.61; H, 2.64; N, 12.53; $C_{18}H_{12}Br_2N_4$ requires C, 48.68; H, 2.72; N, 12.62
5e	82[a]	272	3232, 3310 (-NH_2)	6.68 (s, 1H, 6-H), 7.02 (s, 1H, 3-H), 7.37-8.15 (m, 8H, Ph-H), 9.5 (bs, 7-NH_2, exchangeable with D_2O)	87.1, 89.3, 116.9, 116.2, 128.0, 128.8, 129.2, 130.2, 143.6, 150.1, 151.3, 155.1, 163.0, 164.0	323 $[M+1]^+$	Found: C, 66.97; H, 3.69; N, 17.24; $C_{18}H_{12}F_2N_4$ requires C, 67.08; H, 3.75; N, 17.38
5f	65[b]	208-210	3495, 3317 (-NH_2)	5.92 (bs, 2H, 7-NH_2 exchangeable with D_2O), 7.23-7.27 (m, 2H, 3", 5"-H), 7.41-7.47 (m, 4H, 3′, 4′, 5′, 4"-H), 7.50-7.56 (m, 2H, 2′,6′-H), 8.05-8.07 (d, $J=8.4$ Hz, 2H, 2",6"-H), 8.36 (s, 1H, 5-H), 8.40 (s, 1H, 2-H)	102.36, 107.43, 125.10, 125.16, 127.23, 128.36, 128.95, 129.20, 132.77, 133.93, 141.67, 144.57, 145.32,150.24	287.15 $[M+1]^+$;	Found: C, 75.42; H, 4.85; N, 19.48; $C_{18}H_{14}BN_4$ requires: C, 75.50; H, 4.93; N, 19.57

5g	54[b]		3478, 3315 (-NH_2)	2.14 (s, 3H, 2-CH_3), 2.18 (s, 3H, 5-CH_3), 7.21-7.24 (m, 1H, 4′-H), 7.34-7.38 (m, 6H, 2′, 3′, 5′, 6′, 7-NH_2), 7.42-7.46 (m, 3H, 3", 4", 5"-H), 7.79-7.81 (d, $J = 7.2$ Hz, 2H, 2",6"-H)	11.15, 14.50, 101.86, 105.23, 125.05, 125.15, 127.80, 127.85, 128.48, 129.11, 130.92, 133.28, 134.12, 144.71, 144.84, 150.45	315.21 $[M+1]^+$	Found: C, 76.52; H, 5.89; N, 17.48; $C_{20}H_{18}N_4$ requires: C, 76.41; H, 5.77; N, 17.82
5h	65[b]	162-163	3479, 3371 (-NH_2)	2.29 (s, 3H, 4"-CH_3), 6.72 (s, 1H, 6-H), 7.15 (d, $J = 7.8$ Hz, 2H, 3", 5"-H), 7.24-7.31 (m, 1H, 4′-H), 7.41-7.45 (m, 2H, 3′,5′-H), 7.52 (bs, 2H, 7-NH_2 exchangeable with D_2O), 8.04 (d, $J = 7.8$ Hz, 2H, 2",6"-H), 8.11 (d, $J = 7.4$ Hz, 2H, 2′,6′-H), 8.66 (s, 1H, 2-H)	20.76, 86.01, 107.26, 127.36, 128.21, 128.57, 128.89, 129.06, 129.24, 129.41, 129.96, 130.39, 131.92, 149.39, 155.00	301.14 $[M+1]^+$	Found: C, 75.88; H, 5.42; N, 18.70; $C_{19}H_{16}N_4$ requires: C, 75.98; H, 5.37; N, 18.65
5i	68[b]	160-162	3441, 3387 (-NH_2)	6.71 (s, 1H, 6-H), 7.13-8.29 (m, 12H, 2′,3′,4′,5′,6′, 2",3",4",5",6"-H, 7-NH_2), 8.66 (s, 1H, 2-H)	84.51, 107.36, 126.20, 126.42, 126.67, 127.50, 128.15, 128.79, 129.68, 129.34, 132.09, 135.30, 148.46, 155.49	287.12 $[M+1]^+$	Found: C, 75.58; H, 4.99; N, 19.43. $C_{18}H_{14}N_4$ requires: C, 75.50; H, 4.93; N, 19.57
5j	69[b]	173-175	3441, 3297(-NH_2)	6.68 (s, 1H, 6-H), 7.17-7.20 (m, 1H, 4′-H), 7.40-7.44 (m, 2H, 3′,5′-H), 7.53 (d, $J = 8.5$ Hz, 2H, 3",5"-H), 8.08 (d, $J = 8.5$ Hz, 2H, 2",6"-H), 8.12 (bs, 2H, 7-NH_2 exchangeable with D_2O), 8.15 (d, $J = 7.5$ Hz, 2H, 2′,6′-H), 8.52 (s, 1H, 2-H)	85.12, 107.45, 127.74, 127.95, 128.06, 128.34, 128.52, 128.57, 128.68, 132.43, 134.95, 135.88, 148.82, 154.16	321.08/323.08 (3:1) $[M+1]^+$/ $[M+1+2]^+$	Found: C, 67.58; H, 4.12; N, 17.53; $C_{18}H_{13}ClN_4$ requires : C, 67.40; H, 4.08; N, 17.47
5k	71[b]	240-244	3441, 3378 (-NH_2)	6.66 (s, 1H, 6-H), 7.16-7.19 (m, 1H, 4′-H), 7.39-7.43 (m, 2H, 3′,5′-H), 7.64 (d, $J = 8.5$ Hz, 2H, 3",5"-H), 7.68 (bs, 2H, 7-NH_2 exchangeable with D_2O), 8.04 (d, $J = 8.5$ Hz, 2H, 2",6"-H), 8.19 (d, $J = 7.5$ Hz, 2H, 2′,6′-H), 8.45 (s, 1H, 2-H)	84.43, 107.55, 123.26, 124.48, 124.88, 125.05, 128.25, 131.33, 132.87, 137.25, 148.32, 154.65	365.03/367.03 (1:1) $[M+1]^+$/ $[M+1+2]^+$	Found: C, 59.60; H, 3.44; N, 15.38; $C_{18}H_{13}BrN_4$ requires: C, 59.19; H, 3.59; N, 15.34
5l	75[b]	115-116	3217, 3148 (-NH_2)	2.23 (s, 3H, 4"- CH_3), 2.59 (s, 3H, 2-CH_3), 6.64 (s, 1H, 6-H), 7.23-7.27 (m, 1H, 4′-H), 7.31-7.40 (m, 4H, 2′,3′,5′,6′-H), 7.82 (bs, 2H, 7-NH_2 exchangeable with D_2O), 7.89 (d, $J = 8.2$ Hz, 2H, 3", 5"-H), 8.06 (d, $J = 8.4$ Hz, 2H, 2",6"-H)	14.65, 84.26, 106.26, 126.56, 127.96, 128.22, 128.66, 128.94, 129.22, 129.34, 136.98, 139.16, 133.54, 147.90, 155.48	315.15 $[M+1]^+$	Found: C, 76.48; H, 5.89; N, 17.63; $C_{20}H_{18}N_4$ requires : C, 76.41; H, 5.77; N, 17.82

(*continued*)

Table 3. Continued.

Product	Yield %	mp (°C) Lit mp (°C)	IR (KBr, cm^{-1})	^{1}H-NMR (δ)	^{13}C-NMR (δ)	MS (m/z)	Elemental Analysis
5m	72[b]	155-158	3441, 3297 (-NH_2)	2.51 (s, 3H, 2-CH_3), 6.55 (s, 1H, 6-H), 7.70 (bs, 2H, 7-NH_2 exchangeable with D_2O), 7.38-8.00 (m, 10H, 2′,3′,4′,5′,6′, 2",3",4",5",6"-H);	14.65, 20.75, 84.40. 106.64, 125.15, 127.99, 128.08, 128.52, 128.67, 128.76, 129.54, 130.91, 138.15, 147.87, 155.47	346.07 $[M+1]^+$	Found: C, 75.86; H, 5.23; N, 18.91; $C_{19}H_{16}N_4$ requires: C, 75.98; H, 5.37; N, 18.65
5n	75[b]	104-106	3109, 3024 (-NH_2)	2.54 (s, 3H, 2- CH_3), 6.58 (s, 1H, 6-H), 7.25-7.29 (m, 1H, 4′-H), 7.44-7.47 (m, 2H, 3′,5′-H), 7.52 (d, $J=8.2$ Hz, 2H, 3", 5"-H), 7.77 (d, $J=7.6$ Hz, 2H, 2′,6′-H), 8.00 (d, $J=8.2$ Hz, 2H, 2",6"-H), 8.20 (bs, 2H, 7-NH_2 exchangeable with D_2O)	14.27, 99.49, 106.22, 125.47, 125.55, 127.96, 128.16, 128.35, 128.51, 128.65, 137.66, 148.35, 153.59	335.10 (3:1) $[M+1]^+$/ $[M+1+2]^+$	Found: C, 68.38; H, 4.65; N, 16.56; $C_{19}H_{15}ClN_4$ requires: C, 68.16; H, 4.52; N, 16.73
5o	67[b]	161-164	3240, 3055 (-NH_2)	2.54 (s, 3H, 2- CH_3), 6.60 (s, 1H, 6-H), 7.26-7.30 (m, 1H, 4′-H), 7.44-7.48 (m, 2H, 3′,5′-H), 7.70 (d, $J=8.5$ Hz, 2H, 3", 5"-H), 7.78 (d, $J=7.8$ Hz, 2H, 2′,6′-H), 7.92 (d, $J=8.5$ Hz, 2H, 2",6"-H), 8.28 (bs, 2H, 7-NH_2 exchangeable with D_2O)	14.31, 85.18, 106.18, 125.69, 128.12, 128.33, 128.40, 129.02, 130.27, 132.42, 135.94, 137.81, 148.41, 153.66	379.05/381.05 (1:1) $[M+1]^+$/$[M+1+2]^+$	Found: C, 59.92; H, 4.20; N, 14.58; $C_{19}H_{15}BrN_4$ requires: C, 60.17; H, 3.99; N, 14.77.

[a]Solvent-free.
[b]Hydrazine hydrate, Toluene/ETOH reflux, PTSA catalyst.

The inoculated plates were kept in an upright position in an incubator at 37 °C for 24 hrs. The plates were observed for the diameter of the zone of growth inhibition around the well which was measured in mm on a Himedia zone scale.[11]

Determination of minimum inhibitory concentration (MIC)

A twofold serial dilution of compound (1 mg/mL) in 20% dimethylsulfoxide (DMSO) was made. Dilution was done in sterile distilled water (1:1) to achieve a decreasing concentration range. A 250 μL volume of each dilution was introduced into each well (in triplicate) in the specific media agar plates, already seeded with 100 μL of standardized microbial inocula of the test microbial strain. All test plates were incubated aerobically at 37 °C for 24 hrs and observed for the inhibition zones. The lowest concentration of each extract showing a clear zone of inhibition (>8 mm) was considered as the MIC.[12]

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References

1. R. Aggarwal, G. Sumran, R. M. Claramunt, D. Sanz and J. Elguero, *J. Mol. Struct.*, **934**, 96 (2009) .
2. R. Aggarwal, G. Sumran, N. Garg and A. Aggarwal, *Eur. J. Med. Chem.*, **46**, 3038, (2011). doi:10.1016/j.ejmech.2011.04.041
3. R. Aggarwal, V. Kumar, A. Bansal, D. Sanz and R. M. Claramunt, *J. Fluorine Chem.*, **140**, 31 (2012).
4. S. Samai, G. Chandra Nandi, R. Kumar and M. S. Singh, *Tetrahedron Lett.*, **50**, 7096 (2009).
5. R. Aggarwal, S. Kumar and S. P. Singh, *J. Sulfur Chem.*, **33**, 521 (2012).
6. R. Aggarwal, A. Bansal, I. Rozas, E. Diez-Cecilia, A. Kaur, R. Mahajan and J. Sharma, *Med. Chem. Res.*, **23**, 1454 (2014).
7. H. K. Gakhar, G. S. Gill and J. S. Multani, *J. Indian Chem. Soc.*, **48**, 953 (1971).
8. C. L. Bumgardner and J. C. Sloop, *J. Fluorine Chem.*, **56**, 141 (1992).
9. H. Tye, S. G. Mueller, J. Prestle, S. Scheuerer, M. Schindler, B. Nosse, N. Prevost, C. J. 1. Brown, A. Heifetz, C. Moeller, A. Pedret-Dunn and M. Whittaker, *Bioorg. Med. Chem. Lett.*, **21**, 34 (2011).
10. E. J. Gray, H. N. E. Stevens and M. F. G. Stevens, *J. Chem. Soc., Perkin Trans. I*, **8**, 885 (1978).
11. R. S. Kumar, N. Ayyadurai, P. Pandiaraja, A. V. Reddy, Y. Venkateshwarlu, O. Prakashand N. Sakthivel, *J. Appl. Microbiol.*, **98**, 145 (2005). doi:10.1111/j.1365-2672.2004.02435.x
12. J. M. Andrews, *J. Antimicrob. Chemother.*, **48**, 5 (2001).