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Short communication

Synthesis and antibacterial activity of some new 1-heteroaryl-5-amino-4-phenyl-3-trifluoromethylpyrazoles

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Abstract

Treatment of 1,1,1-trifluoromethyl-3-cyano-3-phenylpropanone (**1**) with several heteroarylhydrazines (**2a**–**e**) in refluxing ethanol affords 1-heteroaryl-5-amino-4-phenyl-3-trifluoromethylpyrazoles (**4**) in a regioselective manner. The location of trifluoromethyl group at position-3 was established by a combined use of ^{13}C and ^{19}F NMR spectroscopy. The reaction proceeds through the intermediacy of the hydrazone which was isolated and characterized in one case (**3e**) by performing the reaction at room temperature. The compounds **3e** and **4** were tested for their antibacterial property against six Gram-positive and three Gram-negative bacteria. Two compounds, namely 1-(benzothiazol-2′-yl)-5-amino-4-phenyl-3-trifluoromethylpyrazole (**4a**) and 1-(6′-methylbenzothiazol-2′-yl)-5-amino-4-phenyl-3-trifluoromethylpyrazole (**4b**) have displayed antibacterial activity comparable to the commercial antibiotics.

Keywords: Trifluoromethylpyrazoles; NMR spectroscopy; Antibacterial activity

1. Introduction

Pyrazoles and their derivatives are widely used as agrochemicals [1] and pharmaceuticals [2], the earliest example being antipyrine dating back 1884. Since then the chemistry of pyrazoles has received much attention and many methods for their synthesis have been developed to obtain substituted pyrazole derivatives. There has been particular interest in the synthesis of 5-aminopyrazoles and 3-trifluoromethylpyrazoles with a wide array of groups at N-1 and C-4, as these were reported to be selective inhibitor of cyclooxygenase [3] and have antidiabetic [4], herbicidal [5] and antibacterial properties [6]. In view of these observations, it was envisaged in the present investigation to undertake the synthesis of a number of pyrazoles, having both the pharmacophores i.e. CF_3 and NH_2 at positions 3 and 5 of the pyrazole moiety, respectively, with an aim to find new and more potent antibacterial agents.

2. Chemistry

Synthesis of 1-heteroaryl-5-amino-4-phenyl-3-trifluoromethylpyrazoles (**4**) is summarized in Scheme 1. The starting compound 1,1,1-trifluoromethyl-3-cyano-3-phenylpropanone (α-phenyltrifluoroacetylacetonitrile, **1**), was readily prepared by the condensation of phenyl acetonitrile with ethyl trifluoroacetate under the influence of sodium ethoxide [7]. Reaction of **1** with heteroarylhydrazines (**2a**–**e**) in refluxing ethanol gave regioselectively, 1-heteroaryl-5-amino-4-phenyl-3-trifluoromethylpyrazoles **4a**–**e** in high yield. The compounds were characterized by a combined application of ^{13}C and ^{19}F NMR spectroscopy. The pyrazole 3 carbon resonated at δ 141–142 ppm in all these compounds, which is a characteristic signal for the location of trifluoromethyl group at that carbon. Had the trifluoromethyl group been located at position 5 of the pyrazole, there would have been signal at δ 133 ppm [8].

In conformity with the earlier reports, carbon 5 of the pyrazole ring in **4** resonated at 146–147 ppm, which is a charac-

Abbreviations: ATCC, American type culture collection; EtOH, ethanol; MHA, Muller Hinton agar; MIC, minimum inhibitory concentration; MTCC, microbial type culture collection and gene bank; SCDA, soybean casein digest agar.

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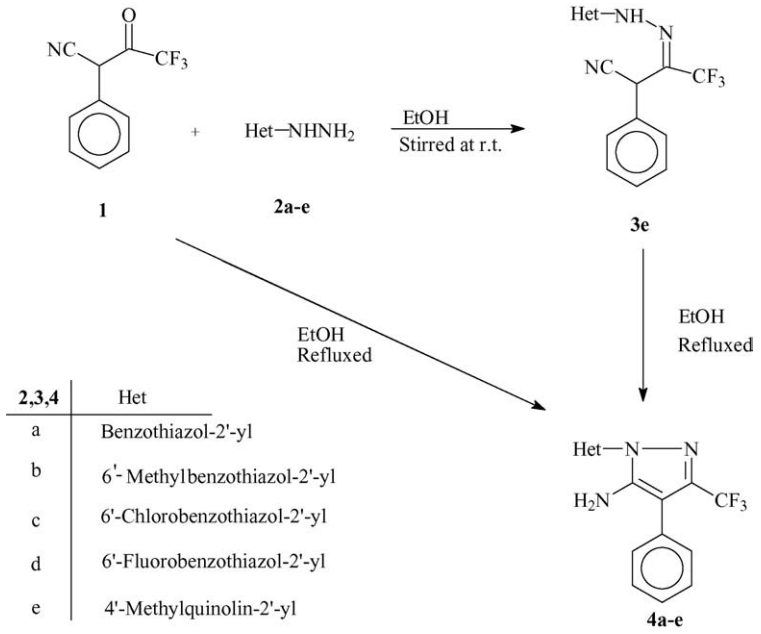


2,3,4	Het
a	Benzothiazol-2'-yl
b	6'-Methylbenzothiazol-2'-yl
c	6'-Chlorobenzothiazol-2'-yl
d	6'-Fluorobenzothiazol-2'-yl
e	4'-Methylquinolin-2'-yl

Scheme 1.

teristic signal for the carbon 5 bearing an amino group [9]. The complete assignment of the signals in ^{13}C NMR spectra of these compounds is given in Table 1.

Finally, the location of trifluoromethyl group at position-3 of the pyrazole ring was firmly established by ^{19}F NMR spectra of compounds **4**. It has already been reported by us that fluorine of trifluoromethyl group resonates at about δ –62 ppm when the trifluoromethyl group is located at position 3, and at about δ –58 ppm when the same group is located at position 5 [10]. ^{19}F NMR spectra of all these compounds (**4a–e**) display a signal between δ –61 and –62 ppm in conformity with our earlier observations. The exact values are given in Table 2.

The reaction apparently proceeds through the intermediacy of hydrazone, which was isolated in one case by performing the reaction in ethanol by stirring **1** and **2e** at room temperature. The formation of **3e** indicates that the unsubstituted nitrogen of heteroarylhydrazines preferentially, reacts with the carbonyl carbon of compound **1**. The hydrazone **3e** was characterized by IR and NMR spectroscopy. The IR spectrum of **3e** showed a characteristic band due to CN str. at 2179 cm^{-1}. The ^{19}F spectrum of compound **3e** showed fluorine signal at δ –65 ppm as expected. Refluxing of **3e** in ethanol afforded compound **4e** in quantitative yield.

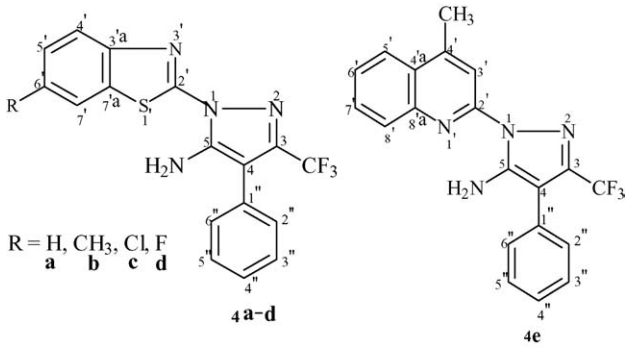
3. Biological results and discussion

Six chemically synthesized compounds were tested in vitro for their antibacterial activity against *Staphylococcus aureus* (MTCC 3160), *S. aureus* (ATCC 25923), methicillin-resistant *S. aureus* (MRSA) (ATCC 700698), *Bacillus pumilus* (MTCC 1456), *Bacillus megaterium* (MTCC 428) (Gram-positive) and *Staphylococcus epidermidis* (MTCC 2639) *Salmonella typhi* (MTCC 733), *Escherichia coli* (MTCC 51) and *Pseudomonas aeruginosa* (MTCC 3541) (Gram-negative) bacteria (Tables 3 and 4). Many of these compounds possess excellent antibacterial activity against both Gram-positive and Gram-negative bacteria. All of the compounds showed MIC 2–8 μg ml^{-1} against Gram-positive bacteria namely *S. aureus* (MTCC 3160) and *S. aureus* (ATCC 25923). All the compounds except **4a** showed MIC 16–32 μg ml^{-1} against MRSA (Gram-positive) whereas compound **4a**, showed MIC of 4 μg ml^{-1} against the same organism. Compound **4a–e** exhibited less antibacterial activity against *B. pumilus* and *B. megaterium* (Gram-positive) except compound **3e**, which showed noticeable activity i.e. MIC 8 μg ml^{-1} against these organisms (Table 4).

Among the Gram-negative bacteria, namely *S. typhi*, *E. coli*, *P. aeruginosa* and *S. epidermidis*, which were used for antibacterial activity, all the compounds of the series showed MIC ranging from 2 to 32 μg ml^{-1}, except against *S. epidermidis* having MIC 64 μg ml^{-1}. Compound **4a–d** were more effective in inhibiting *E. coli* and *P. aeruginosa* as compared to compound **4e** and **3e**. The antibacterial activity of these compounds was also compared with three commercial antibiotics namely Linezolid, Cefaclor and Cefuroxime axetial, as Linezolid is an active drug against resistant staphylococci as well as MRSA and other two against Gram-positive and Gram-negative bacteria, respectively. Many of these compounds showed comparable activity as displayed in Table 4 and Fig. 1.

Kane et al. [6] reported seventeen ureas of 5-aminopyrazoles only having antibacterial activity against MRSA. The MIC of many of these compounds was ranging from 0.7 to 22.9 μg ml^{-1}. Of these four were found to be inactive and some of them having MIC 12–22.9 μg ml^{-1}. In comparison to these findings, the results obtained of the synthesized

Table 1
^{13}C NMR data for compounds **4a–e**



Carbon atoms	**4a**	**4b**	**4c**	**4d**	**4e**
C-2′	160.86	161.58	161.12	162.73	152.80
C-3′					113.63
C-4′	121.64	122.59	122.29	123.90	145.20
C-5′	128.79	128.45	126.22	128.33	123.99
C-6′	123.89	136.17	130.24	160.11	123.70
C-7′	120.76	122.19	120.55	122.00	126.55
C-8′					129.52
C-3′a	151.28	149.33	148.41	150.02	–
C-4′a					126.09
C-7′a	132.68	132.89	132.51	134.11	–
C-8′a					148.15
C-3	142.15	142.15	142.52	142.26	140.9
	(q, J^2_{C-F} = 36.6 Hz)	(q, J^2_{C-F} = 36.6 Hz)	(q, J^2_{C-F} = 36.75 Hz)	(q, J^2_{C-F} = 36.5 Hz)	(q, J^2_{C-F} = 36.2 Hz)
C-4	102.56	103.00	102.56	103.37	102.56
C-5	146.40	147.10	146.40	147.26	147.35
C-1″	130.05	130.35	128.66	130.26	130.34
C-2″, 6″	128.39	128.59	128.58	128.81	128.45
C-3″, 5″	129.85	130.13	127.21	130.17	130.13
C-4″	128.75	128.89	128.39	129.98	128.95
CH_3	–	22.22	–	–	19.04
CF_3	119.81	119.85	119.80	119.85	119.71
	(q, J^1_{C-F} = 269.25 Hz)	(q, J^1_{C-F} = 270.93 Hz)	(q, J^1_{C-F} = 269.25 Hz)	(q, J^1_{C-F} = 269.39 Hz)	(q, J^1_{C-F} = 265.25 Hz)

Table 2
^{19}F chemical shifts (ppm) of **4a–e** and **3e**

Compounds	CF_3 position	CF_3	6′-F
4a	3	–61.6 (s)	–
4b	3	–61.6 (s)	–
4c	3	–61.7 (s)	–
4d	3	–62.0 (s)	–116.0 (s)
4e	3	–61.3 (s)	–
3e	–	–65.0 (s)	–

compounds in the present investigation exhibited antibacterial activity not only against Gram-positive bacteria including MRSA but also against Gram-negative bacteria.

Keeping in view the emerging resistance of the important immunity acquired pathogens such as MRSA, *Staphylococcus pneumoniae*, *S. epidermidis* and *Mycobacterium tuberculosis* to many standard antibiotics, there is an urgent need

Table 3
In vitro antibacterial spectrum of **4a–e** and **3e** by using agar diffusion assay

Compounds	Diameter of zone of growth inhibition (mm)[a]								
	Sa	Sa*	MRSA	St	Ec	Pa	Se	Bp	Bm
4a	21.22	20.58	16.64	14.79	20.36	18.16	–	–	–
4b	25.98	24.16	1815	18.01	19.18	17.26	–	–	–
4c	22.67	20.15	17.96	14.25	18.67	16.79	10.11	16.71	15.93
4d	20.56	18.25	14.67	22.36	17.77	18.45	12.64	–	–
4e	20.45	20.62	15.77	22.16	16.32	18.76	12.11	–	16.51
3e	22.99	20.35	16.35	18.16	14.67	16.52	10.26	20.37	22.16
Ethanol	6.83	6.83	7.00	7.16	7.00	7.16	6.83	6.83	7.16

No activity; [a] mean of three replicates.
Sa-*S. aureus* (MTCC 3160), Sa*- *S. aureus* (ATCC 25923), MRSA- methicillin-resistant *S. aureus* (ATCC 700698), St- *S. typhi* (MTCC 733), Ec- *E. coli* (MTCC 51), Pa- *P. aeruginosa* (MTCC 3541), Se-*S. epidermidis* (MTCC 2639), Bp- *B. pumilus* (MTCC 1456) and Bm- *B. megaterium* (MTCC 428).

Table 4
MIC of **4a–e** and **3e** against test bacteria by using agar dilution assay

Compounds	MIC (μg ml^{-1}) [a]								
	Sa	Sa*	MRSA	St	Ec	Pa	Se	Bp	Bm
4a	4	4	4	8	4	8	> 64	> 64	> 64
4b	2	2	16	8	4	8	> 64	> 64	> 64
4c	4	4	16	8	8	16	64	> 64	> 64
4d	8	8	32	2	8	8	64	> 64	> 64
4e	4	4	32	4	16	8	64	> 64	16
3e	4	4	32	8	32	16	64	8	8
Linezolid	4	2	2	8	< 16	< 16	< 16	4	4
Cefaclor	2	2	4	8	2	< 16	2	8	8
Cefuroxime axetial	8	8	16	8	8	> 16	8	> 16	> 16

[a] Mean of three replicates.

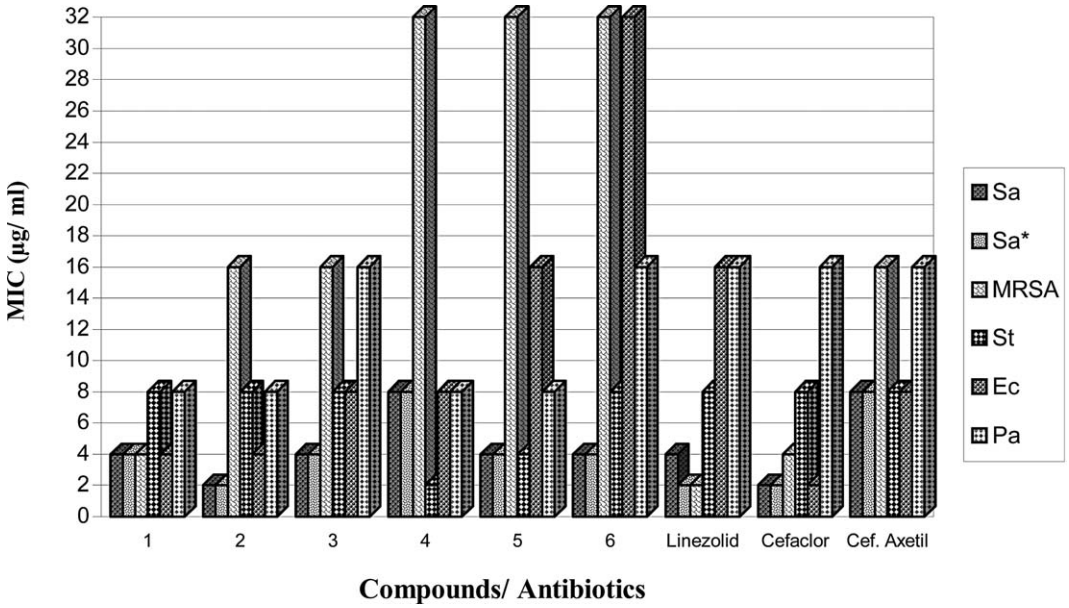


Fig. 1. Comparison of MIC of test compounds with commercial anitbiotics up to MIC 32 (μg ml^{-1}).

to discover new agents to treat patients infected with drug-resistant bacteria. The compounds, **4a–e** showed excellent antibacterial activity against test bacteria namely *S. aureus* (MTCC 3160) and *S. aureus* (ATCC 25923) that were Gram-positive and *S. typhi, E. coli* and *P. aeruginosa* (Gram-negative). Thus, these compounds represent promising new leads for combating the emerging drug-resistant pathogens. Efforts will be made to test these compounds against drug-resistance pathogens and their evaluation in human system for their toxicity.

4. Experimental

4.1. Chemical synthesis

Melting points were determined in open capillaries in electrical apparatus and are uncorrected. IR spectra were recorded on a Buck Scientific IR M500 instrument and ^{1}H and ^{13}C NMR spectra on a Bruker instrument at 300 and 75 MHz, respectively. ^{19}F 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. ^{13}C and ^{19}F NMR data for the compounds are presented in Tables 1 and 2, respectively. High resolution mass spectra were measured in EI mode on a Kratos MS-50 spectrometer. Elemental analysis was performed at RSIC, Lucknow, India. The compounds gave satisfactory analytical results (within ± 0.4 of the calculated values).

Heteroarylhydrazines (**2a–e**) were synthesized according to the literature procedure [11].

*4.1.1. 1-Heteroaryl-5-amino-4-phenyl-3-trifluoromethyl-pyrazoles (**4a–e**)*

General procedure: 1,1,1-Trifluoromethyl-3-cyano-3-phenylpropanone (**1**, 2.13 g, 0.01 mol) was dissolved in 25 ml of ethanol and equimolar amount of an appropriate heteroarylhydrazine (**2a–e**, 0.01 mol) was added to it. The reaction mixture was refluxed for 1 h. Excess of the solvent was removed by distillation. The crude product so obtained was recrystallized from ethanol.

4a: Yield 74%, m.p. 118–119 °C, IR cm^{-1} 3326, 3450 NH_2 str., ^1H NMR δ 6.05 (bs, 2H, NH_2), 7.30–7.53 (m, 7H, Bz-5, 6H and 5 Ph-H), 7.85–7.9 (m, 2H, Bz-4, 7H), MS: M^+, *m/z* 360.

4b: Yield 76%, m.p. 120 °C, IR cm^{-1} 3318, 3435 NH_2 str., ^1H NMR δ 2.49 (s, 3H, CH_3), 6.04 (bs, 2H, NH_2), 7.29 (dd, 1H, Bz-5H, J = 8.4 Hz, J = 1.5 Hz), 7.34–7.49 (m, 5H, Ph-H), 7.65 (s, 1H, Bz-7H), 7.76 (d, 1H, Bz-4H, J = 8.4 Hz), MS: M^+, *m/z* 374.

4c: Yield 72%, m.p. 154–155 °C, IR cm^{-1} 3289, 3398 NH_2 str., ^1H NMR δ 6.01 (bs, 2H, NH_2), 7.38–7.47 (m, 6H, Bz-5H and Ph-H), 7.79 (d, 1H, Bz-4H, J = 8.7 Hz), 7.84 (d, 1H, Bz-7H, J = 2.1 Hz); MS: M^+, *m/z* 394 (M), 396 (M + 2).

4d: Yield 79%, m.p. 165–166 °C, IR cm^{-1} 3341, 3468 NH_2 str., ^1H NMR δ 5.94 (bs, 2H, NH_2), 7.29–7.43 (m, 6H, Bz-5H and Ph-H), 7.71–7.74 (d, 1H, Bz-4H, J = 8.7 Hz), 7.77–7.78 (d, 1H, Bz-7H, J = 2.1 Hz), MS: M^+, *m/z* 378.0568 ($C_{17}H_{10}F_4N_4S$ requires: 378.05623).

4e: Yield 71%, m.p. 98–100 °C, IR cm^{-1} 3330, 3452 NH_2 str., ^1H NMR δ 2.79 (s, 3H, CH_3), 6.43 (bs, 2H, NH_2), 7.33–7.50 (m, 5H, Ph-H), 7.53–7.58 (m, 1H, Qu-6H), 7.68–7.74 (m, 1H, Qu-7H), 7.91–7.94 (dd, 1H, Qu-5H, J = 8.4 Hz, J = 0.6 Hz), 7.99–8.02 (dd, 1H, Qu 8H, J = 8.4 Hz, J = 0.6 Hz), 8.13 (s, 1H, Qu 3H), MS: M^+, *m/z* 368.

*4.1.2. 11,1,1-Trifluoromethyl-3-cyano-3-phenylpropanone-4′-methyl quinolin-2′-ylhydrazone (**3e**)*

Equimolar amount of 1,1,1-trifluoromethyl-3-cyano-3-phenylpropanone (**1**, 2.13 g, 0.01 mol) and 2-hydrazino-4-methylquinoline (**2e**, 1.73 g, 0.01 mol) in ethanol (20 ml) were stirred at room temperature for 15 min. Excess of solvent was evaporated in vacuo to give the title compound **3e**.

Yield 81%, m.p. 115–116 °C, IR cm^{-1} 2179 CN str., 3209 C=N–H str., 3347 N–H str.; ^1H NMR δ 2.65 (s, 3H, CH_3), 7.08–7.53 (m, 9H, N-H, CH, Ph-H, Qu-6, 3H), 7.63–7.68 (m, 1H, Qu-7H), 7.80–7.83 (d, 1H, Qu-5H, J = 8.4 Hz), 7.90–7.93 (d, 1H, Qu-8H, J = 7.5 Hz), MS: M^+, *m/z* 368.1233 ($C_{20}H_{15}F_3N_4$ requires: 368.1234).

*4.1.2.1. **4e** from **3e**.* The hydrazone **3e** was refluxed in ethanol (20 ml) for 1 h. After refluxing, volume of the reaction mixture was reduced to half and cooled. On cooling the solid separated was identified as **4e**.

5. Biological assays

5.1. Medium

Solid medium used for the study were Muller Hinton agar (MHA) and soybean casein digest agar (SCDA) of the following composition; beef infusion 300 g l^{-1}, casein acid hydrolysate 17.5 g l^{-1}, starch 1.5 g l^{-1}, agar–agar 17 g l^{-1} and sterile distilled water 1000 ml, adjusted to pH 7.4 and casein enzymatic hydrolysate 17.0 g l^{-1}, papain digest of soybean 3.0 g l^{-1}, NaCl 5.0 g l^{-1}, dipotassium phosphate 2.5 g l^{-1}, dextrose 2.5 g l^{-1}, sterile distilled water 1000 ml, adjusted to pH 7.3, respectively.

5.2. Primary screening

Primary screening of six chemically synthesized compounds (**3e** and **4a**–**e**) was done by well diffusion assay technique. The 24 h old bacterial cultures of all above-mentioned Gram-positive and Gram-negative bacteria were used for the assay. The bacterial cultures used in the assay were adjusted to 0.5 McFarland Standard, i.e. 1.5×10^8 CFU ml^{-1} [12]. The test bacterial cultures were set at 0.5 McFarland Standard using Wickerham paper. The stock solution (1 mg ml^{-1}) of all the test chemicals was prepared by dissolving 1 mg of the test chemical in 1 ml of ethanol. Ethanol was used as control for all the test compounds.

Twenty milliliters MHA and 500 μl of each test bacterial culture of 24 h incubation adjusted at 0.5 McFarland was mixed. After mixing, it was poured in sterilized and labeled petri plates. The wells of 6 mm were punched in the solidified petri plates. With the help of micropipette, 100 μl of test chemicals were added to individual wells. The loaded plates were incubated at 35 °C for 24 h. The diameter of zone of growth inhibition around each well was measured after incubation using a Vernier Caliper (Table 3).

5.3. Determination of minimum inhibitory concentration (MIC)

The MIC is the lowest concentration of the antimicrobial agent that prevents the development of viable growth after overnight incubation [13]. MIC of compounds against Gram-positive and Gram-negative test bacteria was determined by method of NCCLS [14]. MHA was used for MIC determination. All the test cultures were streaked on the SCDA and incubated overnight at 37 °C. Turbidity of all the bacterial cultures were adjusted to 0.5 McFarland Standard by preparing bacterial suspension of four to six well isolated colonies. The cultures were further diluted 10-fold to get inoculum size of 1.2×10^7 CFU ml^{-1}. Stock solution of 4 mg ml^{-1} was prepared in ethanol and was appropriately diluted to get final concentration of 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25 and 0.12 μg ml^{-1}. Standard antibiotics (Linezolid, Manufacturer-Alembic, Batch no. 6893002; Cefaclor, Manufacturer- Glaxo, Batch no. 1305911; Cefuroxime axetial, Manufacturer- Galaxo, Batch no. HD-313) were also diluted in same manner to make comparison. Three hundred and twenty microliters of each dilution was added to 20 ml cooled 45 °C molten MHA (separate flask was taken for each dilution). After thorough mixing, the medium was poured in sterilized petri plates. The test bacterial cultures were spotted in a predefined pattern by ascetically transferring 10 μl of each culture on the surface of presolidified agar plates. The spotted plates were incubated at 35 °C for 24 h.

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