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Short communication

Organoiodine (III)-mediated synthesis of 3-aryl/heteroaryl-5,7-dimethyl-1,2,4-triazolo[4,3-*c*]pyrimidines as antibacterial agents

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Abstract

Synthesis of 12 new 3-aryl/heteroaryl-5,7-dimethyl-1,2,4-triazolo[4,3-*c*]pyrimidines (**3a–l**) has been accomplished by the oxidation of pyrimidinylhydrazones (**2a–l**) of various aryl/heteroaryl aldehydes using 1.1 equiv. of iodobenzene diacetate (IBD) in dichloromethane. All the compounds **3a–l** tested in vitro for their antibacterial activity against two Gram-positive bacteria namely, *Bacillus subtilis*, *Bacillus stearothermophilus* and two Gram-negative bacteria *Pseudomonas putida*, *Escherichia coli*. Two compounds, namely 3-(2,4-dichlorophenyl)-5,7-dimethyl-1,2,4-triazolo [4,3-*c*]pyrimidine (**3j**) and 3-(4-hydroxy-2-methoxyphenyl)-5,7-dimethyl-1,2,4-triazolo[4,3-*c*]pyrimidine (**3l**) were found to be equipotent or more potent than the commercially available antibiotics (chloramphenicol and streptomycin).

Keywords: Iodobenzene diacetate; Triazolopyrimidines; Antibacterial activity

1. Introduction

Use of hypervalent iodine compounds as mild oxidizing agents has acquired much attention in organic synthesis [1–9]. In our previous papers [10–13], we reported the synthesis of fused 1,2,4-triazolopyridines, 1,2,4-triazolopyrimidines and 1,2,4-bistriazolopyrimidines. Some of the compounds were found to possess strong antibacterial activity. Encouraged by these results, we got interested in extending the scope of this approach for the synthesis of new 3-aryl/heteroaryl-5,7-dimethyl-1,2,4-triazolo[4,3-*c*]pyrimidines (**3a–l**) by using iodobenzene diacetate (IBD) as antibacterial agents.

2. Chemistry

The synthetic Scheme 1 used for the synthesis of 3-aryl/heteroaryl-5,7-dimethyl-1,2,4-triazolo[4,3-*c*] pyrimidines **3** is similar to the work reported from our laboratory [10–13]. Thus, treatment of substituted pyrimidinylhydrazones **2** with 1.1 equiv. of IBD in dichloromethane (DCM) for about 1 h at room temperature afforded desired products 3-aryl/heteroaryl-5,7-dimethyl-1,2,4-triazolo[4,3-*c*]pyrimidines **3** in high yields. The hydrazones (**2a–l**) were obtained by the condensation of 4-hydrazino-2,6-dimethyl pyrimidine (**1**) with different aromatic/heteroaromatic aldehydes.

The structures of all the compounds **3a–l** and **2a–l** were elaborated by their spectral data (IR, ^{1}H NMR and mass) and elemental analysis.

The I (III)-mediated oxidative cyclization of **2** to **3** is significant for the following reasons: (i) the method is eco-friendly, (ii) it involves mild conditions, and (iii) there is possibility of using this approach for the synthesis of a wide variety of heterocyclic compounds of potential biological interest.

Abbreviations: IBD, Iodobenzene diacetate; DCM, Dichloromethane; MIC, Minimum inhibitory concentration; *B. subtilis*, *Bacillus subtilis*; *E. coli*, *Escherichia coli*; *B. stearothermophilus*, *Bacillus stearothermophilus*; *P. putida*, *Pseudomonas putida*.

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3. Biological investigation and results

In earlier reported work from our laboratory [11], it was found that 1,2,4-triazolo[4,3-*a*]pyrimidines are associated with antibacterial activities. Keeping this in view, new isomeric compounds **3a–l** were synthesized and tested in vitro antibacterial activity against two Gram-positive bacteria namely *Bacillus subtilis* (MTCC 8509), *Bacillus stearothermophilus* (MTCC 8508) and against two Gram-negative bacteria namely *Escherichia coli* (MTCC 51), *Pseudomonas putida* (MTCC 121).

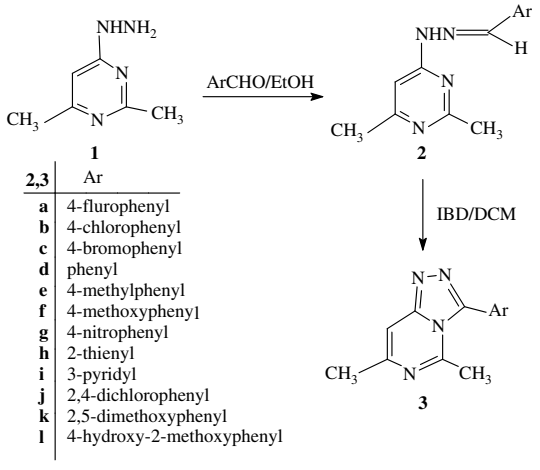
The primary screening of the compounds for antibacterial activity was determined by measuring the diameter of growth inhibition (Table 1) whereas the minimal inhibitory concentration (MIC) was given in Table 2. In general, the newly synthesized compounds **3a**, **c**, **e**, **f**, **i**, **3j** and **l** showed good antibacterial activity. Among the newly synthesized compounds, two **3j** and **l** are the strong inhibitors of bacterial growth.

The antibacterial activity of these compounds was also compared with two commercially available antibiotics namely chloramphenicol and streptomycin. It is clear from the results (Tables 1 and 2) that the compounds **3j** and **l** show maximum inhibition, which is even comparable to the commercially available antibiotics. It is to be mentioned that compound 3-(2,4-dichlorophenyl)-5,7-dimethyl-1,2,4-triazolo[4,3-*c*]pyrimidine (**3j**) was found to be more potent against Gram-negative bacteria, *P. putida*.

4. Experimental

4.1. Chemical synthesis

Melting points were determined in open capillaries in electrical melting point apparatus and are uncorrected. The IR (KBr) and ^{1}H NMR ($CDCl_3$) spectra were recorded on Buck Scientific IR M-500 and Bruker (300MHz) spectrophotometers, respectively. All the compounds **3** gave



2,3	Ar
a	4-flurophenyl
b	4-chlorophenyl
c	4-bromophenyl
d	phenyl
e	4-methylphenyl
f	4-methoxyphenyl
g	4-nitrophenyl
h	2-thienyl
i	3-pyridyl
j	2,4-dichlorophenyl
k	2,5-dimethoxyphenyl
l	4-hydroxy-2-methoxyphenyl

Scheme 1.

Table 1
In vitro antibacterial activity of newly synthesized compounds by using well diffusion method

Compound	Concentration (μg/mL)	Diameter of zone of growth inhibition[a]			
		B.S	*B.St*	*E.C*	*P.put*
3a	10	13.66	10.11	11.29	09.67
	50	22.26	23.43	14.28	15.34
	100	41.72	41.81	26.44	23.22
3b	10	–	–	–	–
	50	–	11.79	–	08.33
	100	11.33	18.67	10.67	14.55
3c	10	27.66	26.66	23.33	28.33
	50	40.66	41.66	31.66	48.66
	100	59.33	60.66	47.33	66.66
3d	10	–	–	–	–
	50	08.34	–	–	08.33
	100	13.33	10.66	11.33	12.66
3e	10	12.33	09.74	11.66	26.66
	50	21.29	19.66	22.18	40.33
	100	42.13	30.33	40.24	59.66
3f	10	27.33	12.33	12.33	11.66
	50	39.66	20.66	21.33	20.33
	100	59.66	41.33	40.66	40.66
3g	10	–	–	–	–
	50	09.66	10.33	08.33	12.33
	100	14.33	16.67	13.66	19.66
3h	10	–	09.74	10.33	–
	50	08.34	16.66	18.66	08.33
	100	13.66	25.66	26.33	12.33
3i	10	29.33	25.33	25.66	36.66
	50	46.33	39.66	39.33	59.33
	100	65.66	56.33	57.33	69.66
3j	10	30.66	29.66	37.33	29.33
	50	47.66	48.33	63.66	49.66
	100	67.33	66.33	75.33	67.66
3k	10	–	–	–	–
	50	–	08.33	10.66	09.66
	100	11.33	13.66	19.33	13.66
3l	10	27.33	30.66	29.33	32.66
	50	42.66	46.66	46.33	49.33
	100	61.66	66.33	66.66	72.66
Chloramphenicol	10	36.33	35.33	30.33	29.33
	50	57.20	53.20	50.40	53.40
	100	74.20	77.20	75.40	71.24
Streptomycin	10	36.40	33.20	26.40	38.20
	50	57.42	49.20	49.40	48.20
	100	73.24	67.20	69.40	72.24

– No activity; *B.S* – *Bacillus subtilis*; *B.st* – *Bacillus stearothermophilus*; *E.C*– *E. coli*; *P.put* – *Pseudomonas putida*.
[a] Mean of three replicates.

satisfactory analytical results (with ±0.4 of the theoretical values).

4-Hydrazinopyrimidine **1** was synthesized according to the literature procedure commencing with acetamidine hydrochloride and ethylacetoacetate [14].

4.1.1. Hydrazones *2a–l*

4.1.1.1. General procedure. 4-Hydrazinopyrimidine **1** was dissolved in ethanol and aryl/heteroaryl aldehydes were added to it. The contents were refluxed on a water bath for 1 h and

Table 2
MIC (μg/mL) of test compounds

Compound	*BS*	*B.st*	*P.put*	*EC*
3a	16	16	32	32
3b	>250	125	>250	>250
3c	08	08	16	04
3d	>250	>250	>250	>250
3e	16	32	16	08
3f	08	16	16	16
3g	250	>250	250	125
3h	>250	64	64	>250
3i	04	08	08	02
3j	04	04	01	04
3k	>250	>250	125	>250
3l	08	04	04	02
Chloramphenicol	02	02	04	02
Streptomycin	02	02	04	04

BS – *Bacillus subtilis*; *B.st* – *Bacillus stearothermophilus*; *EC* – *E. coli*; *P.put* – *Pseudomonas putida*.

allowed to stand at room temperature. The crystalline solid, thus obtained, was filtered, washed with ethanol and dried to afford hydrazones **2**.

*4.1.1.2. Characterization data of hydrazones **2a–l***

*4.1.1.2.1. Compound **2a***. M.p. 110 °C, yield 78%, ^{1}H NMR ($CDCl_3$) δ 2.48 (s, 3H, CH_3); 2.56 (s, 3H, CH_3); 6.97 (s, 1H); 7.10–7.12 (d, 2H, $J = 8.7$ Hz); 7.69–7.71 (d, 2H, $J = 8.7$ Hz); 7.81 (s, 1H, N=CH).

*4.1.1.2.2. Compound **2b***. M.p. 199–200 °C, yield 80%, ^{1}H NMR ($CDCl_3$) δ 2.46 (s, 3H, CH_3); 2.54 (s, 3H, CH_3); 6.96 (s, 1H); 7.40–7.38 (d, 2H, $J = 6.9$ Hz); 7.64–7.62 (d, 2H, $J = 6.9$ Hz); 7.73 (s, 1H, N=CH).

*4.1.1.2.3. Compound **2c***. M.p. 208–210 °C, yield 79%, ^{1}H NMR ($CDCl_3$) δ 2.48 (s, 3H, CH_3); 2.56 (s, 3H, CH_3); 6.97 (s, 1H); 7.49–7.55 (m, 4H); 7.79 (s, 1H, N=CH).

*4.1.1.2.4. Compound **2d***. M.p. 160 °C, (Lit. −161 to 164 °C) [15], yield 75%, ^{1}H NMR ($CDCl_3$) δ 2.48 (s, 3H, CH_3); 2.56 (s, 3H, CH_3); 6.99 (s, 1H); 7.43–7.46 (m, 3H); 7.70–7.72 (m, 2H); 7.81 (s, 1H, N=CH).

*4.1.1.2.5. Compound **2e***. M.p. 173–175 °C, yield 75%, ^{1}H NMR ($CDCl_3$) δ 2.42 (s, 3H, CH_3); 2.64 (s, 3H, CH_3); 2.70 (s, 3H, CH_3); 7.05 (s, 1H); 7.24–7.26 (d, 2H, $J = 7.5$ Hz); 7.63–7.65 (d, 2H, $J = 7.5$ Hz); 8.28 (s, 1H, N=CH).

*4.1.1.2.6. Compound **2f***. M.p. 154–155 °C, yield 77%, ^{1}H NMR ($CDCl_3$) δ 2.49 (s, 3H, CH_3); 2.53 (s, 3H, CH_3); 3.86 (s, 3H, OMe); 6.93 (s, 1H); 6.93–6.96 (d, 2H, $J = 9$ Hz); 7.64–7.67 (d, 2H, $J = 9$ Hz); 7.83 (s, 1H, N=CH).

*4.1.1.2.7. Compound **2g***. M.p. 225 °C, yield 90%, ^{1}H NMR ($CDCl_3$) δ 2.55 (s, 3H, CH_3); 2.57 (s, 3H, CH_3); 7.01 (s, 1H); 7.82–7.86 (d, 2H, $J = 8.5$ Hz); 7.87 (s, 1H, N=CH); 8.27–8.33 (d, 2H, $J = 8.5$ Hz).

*4.1.1.2.8. Compound **2h***. M.p. 150 °C, yield 89%, ^{1}H NMR ($CDCl_3$) δ 2.49 (s, 3H, CH_3); 2.56 (s, 3H, CH_3); 6.94 (s, 1H); 7.06–7.09 (dd, 1H, $J = 3.9$ Hz, $J' = 3.3$ Hz); 7.38–7.39 (d, 1H, $J = 3.9$ Hz); 7.24–7.25 (d, 1H, $J = 3.3$ Hz); 8.02 (s, 1H, N=CH).

*4.1.1.2.9. Compound **2i***. M.p. 197–198 °C, yield 68%, ^{1}H NMR ($CDCl_3$) δ 2.46 (s, 3H, CH_3); 2.55 (s, 3H, CH_3); 6.98 (s, 1H); 7.33–7.37 (m, 1H); 7.78 (s, 1H); 8.06–8.08 (m, 1H); 8.61–8.63 (m, 1H); 8.84 (s, 1H, N=CH).

*4.1.1.2.10. Compound **2j***. M.p. 183–185 °C, yield 72%, ^{1}H NMR ($CDCl_3$) δ 2.56 (s, 3H, CH_3); 2.62 (s, 3H, CH_3); 6.99 (s, 1H); 7.29–7.32 (m, 1H); 7.42–7.43 (m, 1H); 7.99–8.02 (m, 1H); 8.21 (s, 1H, N=CH).

*4.1.1.2.11. Compound **2k***. M.p. 104–105 °C, yield 72%, ^{1}H NMR ($CDCl_3$) δ 2.45 (s, 3H, CH_3); 2.52 (s, 3H, CH_3); 3.63 (s, 3H, OCH_3); 3.66 (s, 3H, OCH_3); 6.84 (s, 1H); 6.94–7.10 (m, 3H); 8.24 (s, 1H, N=CH).

*4.1.1.2.12. Compound **2l***. M.p. 82 °C, yield 72%, ^{1}H NMR ($CDCl_3$) δ 2.59 (s, 3H, CH_3); 2.60 (s, 3H, CH_3); 3.76 (s, 3H, OCH_3); 6.98 (s, 1H); 7.42–7.62 (m, 3H); 7.94 (s, 1H).

*4.1.2. 3-Aryl/heteroaryl-5,7-dimethyl-1,2,4-triazolo [4,3-c]pyrimidines (**3a–l**)*

4.1.2.1. General procedure. To a stirred solution of pyrimidinylhydrazones **2** (0.01 mol) in dichloromethane (25 mL) at room temperature, IBD (0.011 mol) was added in four to five portions during 5 min. The solvent was evaporated on a steam bath and the residual mass containing product and iodobenzene was triturated with petroleum ether to give solid product, which was recrystallised from methanol to yield pure triazolopyrimidines **3**.

*4.1.2.2. Characterization data of triazolopyrimidines **3a–l***. In IR spectral data of compounds **3a–l**, the characteristic peak for NH stretch present in pyrimidinylhydrazones **2** was disappeared.

*4.1.2.2.1. Compound **3a***. M.p. 180 °C, yield 68%, ^{1}H NMR ($CDCl_3$) δ 2.60 (s, 3H, CH_3); 3.02 (s, 3H, CH_3); 7.17–7.23 (d, 2H, $J = 8.6$ Hz); 7.36 (s, 1H); 8.32–8.35 (d, 2H, $J = 8.6$ Hz). Elemental analysis: $C_{13}H_{11}FN_4$ Found (C, 64.01; H, 4.43; N, 22.94%). Requires (C, 64.46; H, 4.54; N, 23.14%). *m/z* M^+ 242.

*4.1.2.2.2. Compound **3b***. M.p. 202–203 °C, yield 72%, ^{1}H NMR ($CDCl_3$) δ 2.51 (s, 3H, CH_3); 2.93 (s, 3H, CH_3); 7.39 (s, 1H); 7.41–7.42 (d, 2H, $J = 6.9$ Hz); 8.17–8.18 (d, 2H, $J = 6.9$ Hz). Elemental analysis: $C_{13}H_{11}ClN_4$ Found (C, 60.13; H, 4.12; N, 21.54%). Requires (C, 60.23; H, 4.25; N, 21.66%). *m/z* M^+ 259, 261.

*4.1.2.2.3. Compound **3c***. M.p. 197 °C, yield 75%, ^{1}H NMR ($CDCl_3$) δ 2.51 (s, 3H, CH_3); 2.93 (s, 3H, CH_3); 7.27 (s, 1H); 7.55–7.57 (d, 2H, $J = 7.5$ Hz); 8.10–8.12 (d, 2H, $J = 7.5$ Hz). Elemental analysis: $C_{13}H_{11}BrN_4$ Found (C, 51.24; H, 3.43; N, 18.36%). Requires (C, 51.48; H, 3.60; N, 18.48%). *m/z* M^+ 303, 305.

*4.1.2.2.4. Compound **3d***. M.p. 118–120 °C, yield 62%, ^{1}H NMR ($CDCl_3$) δ 2.61 (s, 3H, CH_3); 3.04 (s, 3H, CH_3); 7.41 (s, 1H); 7.54–7.52 (m, 3H); 8.32–8.35 (m, 2H). Elemental analysis: $C_{13}H_{12}N_4$ Found (C, 69.55; H, 5.27; N, 24.94%). Requires (C, 69.65; H, 5.35; N, 25.00%). *m/z* M^+ 224.

*4.1.2.2.5. Compound **3e***. M.p. 159–160 °C, yield 72%, ^{1}H NMR ($CDCl_3$) δ 2.36 (s, 3H, CH_3); 2.51 (s, 3H, CH_3); 2.93 (s, 3H); 7.22–7.25 (d, 2H, $J = 9$ Hz); 7.27 (s, 1H); 8.14–8.11 (d, 2H, $J = 9$ Hz). Elemental analysis: $C_{14}H_{14}N_4$ Found (C, 70.41;

H, 5.66; N, 23.44%). Requires (C, 70.58; H, 5.88; N, 23.52%). *m/z* M^+ 238.

*4.1.2.2.6. Compound **3f***. M.p. 142 °C, yield 72%, ^{1}H NMR ($CDCl_3$) δ 2.63 (s, 3H, CH_3); 3.05 (s, 3H, CH_3); 3.91 (s, 3H, OMe); 7.04–7.07 (d, 2H, $J = 9$ Hz); 7.48 (s, 1H); 8.30–8.33 (d, 2H, $J = 9$ Hz). Elemental analysis: $C_{14}H_{14}N_4O$ Found (C, 65.91; H, 5.43; N, 21.94%); Requires (C, 66.14; H, 5.51; N, 22.04%). *m/z* M^+ 254.

*4.1.2.2.7. Compound **3g***. M.p. 229–230 °C, yield 75%, ^{1}H NMR ($CDCl_3$) δ 2.64 (s, 3H, CH_3); 3.07 (s, 3H, CH_3); 7.42 (s, 1H); 8.36–8.39 (d, 2H, $J = 9$ Hz); 8.52–8.57 (d, 2H, $J = 9$ Hz). Elemental analysis: $C_{13}H_{11}N_5O_2$ Found (C, 57.88; H, 3.93; N, 25.94%). Requires (C, 57.99; H, 4.08; N, 26.02%). *m/z* M^+ 269.

*4.1.2.2.8. Compound **3h***. M.p. 145 °C, yield 65%, ^{1}H NMR ($CDCl_3$) δ 2.50 (s, 3H, CH_3); 2.92 (s, 3H, CH_3); 7.25 (s, 1H); 7.89 (m, 1H); 7.39–7.42 (m, 1H); 7.08–7.10 (m, 1H). Elemental analysis: $C_{11}H_{10}N_4S$ Found (C, 57.21; H, 4.23; N, 24.14%). Requires (C, 57.39; H, 4.34; N, 24.34%). *m/z* M^+ 230.

*4.1.2.2.9. Compound **3i***. M.p. 149–150 °C, yield 72%, ^{1}H NMR ($CDCl_3$) δ 2.70 (s, 3H, CH_3); 3.04 (s, 3H, CH_3); 7.39 (s, 1H); 7.58–7.60 (m, 1H); 8.74–8.76 (m, 2H); 9.58 (s, 1H). Elemental analysis: $C_{12}H_{11}N_5$ Found (C, 63.88; H, 4.77; N, 31.01%); Requires (C, 64.00; H, 4.88; N, 31.11%). *m/z* M^+ 225.

*4.1.2.2.10. Compound **3j***. M.p. 135 °C, yield 75%, ^{1}H NMR ($CDCl_3$) δ 2.06 (s, 3H, CH_3); 2.60 (s, 3H, CH_3); 7.40–7.43 (d, 1H, $J = 8.2$ Hz); 7.47 (s, 1H); 7.60 (m, 1H); 8.01–8.04 (d, 1H, $J = 8.2$ Hz). Elemental analysis: $C_{13}H_{10}Cl_2N_4$ Found (C, 53.34; H, 3.30; N, 18.94%). Requires (C, 53.42; H, 3.42; N, 19.17%). *m/z* M^+ 292.

*4.1.2.2.11. Compound **3k***. M.p. 157–158 °C, yield 72%, ^{1}H NMR ($CDCl_3$) δ 2.37 (s, 3H, CH_3); 2.44 (s, 3H, CH_3); 3.61 (s, 3H, OCH_3); 3.73 (s, 3H, OCH_3); 6.86–7.05 (m, 3H); 7.35 (s, 1H). Elemental analysis: $C_{15}H_{16}N_4O_2$ Found (C, 63.11; H, 5.52; N, 19.62%). Requires (C, 63.34; H, 5.63; N, 19.71%). *m/z* M^+ 284.

*4.1.2.2.12. Compound **3l***. M.p. 182–183 °C, yield 73%, ^{1}H NMR ($CDCl_3$) δ 2.55 (s, 3H, CH_3); 2.57 (s, 3H, CH_3); 4.03 (s, 3H, OCH_3); 7.03 (s, 1H); 7.91–7.23 (m, 3H). Elemental analysis: $C_{14}H_{14}N_4O_2$ Found (C, 62.11; H, 5.03; N, 20.54%); Requires (C, 62.22; H, 5.18; N, 20.74%). *m/z* M^+ 270.

4.2. Biological assay

4.2.1. Medium

Media used for the biological testing was nutrient agar media (NAM) of the following composition: peptone 10 g; yeast extract 3 g; sodium chloride 5 g; nutrient agar 2% and final volume of media was adjusted to 1000 mL with sterile distilled water having pH 7.

4.2.2. In vitro antibacterial assay

The 12 newly synthesized compounds **3a–l** were screened for their antibacterial activity against bacterial culture using agar well diffusion assay technique and minimum inhibitory concentration (MIC) method.

4.2.3. Primary screening

The antibacterial activities of the newly synthesized compounds were evaluated by agar well diffusion assay technique against two Gram-positive bacteria i.e. *B. subtilis* (MTCC 8509) and *B. stearothermophilus* (MTCC 8508) and two Gram-negative bacteria i.e. *E. coli* (MTCC 51) and *P. putida* (MTCC 121). The bacterial cultures were maintained on the nutrient agar media by sub-culturing them on the fresh slants after every 4–6 weeks and incubating them at the appropriate temperature for 24 h. All stock cultures were stored at 4 °C. For the evaluation of antimicrobial activity of the synthetic compounds, suspension of each test microorganism was prepared. Turbidity of each suspension was adjusted to 0.5 McFarland units by suspending the cultures in sterile distilled water. The size of final inoculum was adjusted to 5×10^7 CFU/mL.

A volume of 20 mL of agar media was poured into each petri plate and plates were swabbed with broth cultures of the respective microorganisms and kept for 15 min for adsorption to take place. Using a punch, ≈8 mm diameter well was bored in the seeded agar plates and a 100 μL volume of each test compound reconstituted in DMSO was added into the wells. DMSO was used as control for all the test compounds. After holding the plates at room temperature for 2 h to allow diffusion of the compounds in to the agar, the palates were incubated at 37 °C for 24 h. Antibacterial activity was determined by measuring the inhibition zone diameter. The entire tests were made in triplicates and mean of the diameter of inhibition was calculated. The antimicrobial activities of the compounds were compared against the standard drugs, chloroamphenicol and streptomycin.

4.2.4. Minimum inhibitory concentration (MIC)

MIC is the lowest concentration of the antimicrobial agents that prevents the development of visible growth of microorganism after overnight incubation. MIC of chemically synthesized compounds against two Gram-positive and two Gram-negative bacteria namely *B. subtilis* (MTCC 121), *B. stearothermophilus*, *E. coli* (MTCC 51), and *P. putida* (MTCC 121) was determined by reported methods [16].

Nutrient broth was adjusted to pH 7.0 used for the determination of MIC. The inoculum of the test microorganisms was prepared by using 16 h old cultures adjusted by reference to the 0.5 McFarland standards (10^8 cells/mL) [17]. These cultures were further diluted upto 10-folds with nutrient broth to get the inoculum size of 1.2×10^7 CFU/mL. A positive control (containing inoculum but no compound) and a negative control (containing compound but no inoculum) were also prepared. A stock solution of 4 mg/mL of each compound was prepared in DMSO and further appropriately diluted to get final concentration ranging from 250 to 0.03 μg/mL [18]. Requisite quantity of antifungal compound (cycloheximide) was added to the broth to get its desirable final concentration of 100 μg/mL. Separate flasks were taken for each test dilution. To each flask was added the 100 μL of inoculum.

Then appropriately diluted test sample was added to each flask having broth and microbial inocula. The contents of the flask were mixed and incubated for 24–48 h at 37 °C. The test bacterial culture was spotted in a predefined pattern by aseptically transferring 5 μL of each bacterial culture on the surface of solidified agar–agar plates and incubated at 37 °C for 24 h for determining the MIC value.

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