Indian Journal of Chemistry
Vol. 59B, April 2020, pp. 445-453

Synthesis of novel benzo[4,5]imidazo[1,2-*a*]pyrimido- [4,5-*d*]pyrimidine derivatives as potent antimicrobial agents

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Received 23 September 2019; accepted (revised) 3 December 2019

Synthesis of novel benzo[4,5]imidazo[1,2-*a*]pyrimido[4,5-*d*] pyrimidines **5/6** has been achieved by reaction of 2-amino-4-aryl-4,10-dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimidine-3-carbonitriles **4** with formaldehyde/urea. The key intermediate **4,** is obtained by reaction of 2-aminobenzaldehyde **1** with aromatic aldehyde and malononitrile by a three-component one-pot process. The newly synthesized title compounds **5/6** have been evaluated for their *in vitro* antimicrobial activity. Compounds **5** and **6** exhibit potent antimicrobial activity compared to that of standard drugs.

Keywords: Multi-component reaction, benzo[4,5]imidazo[1,2-*a*]pyrimidine-3-carbonitriles, cyclization, benzo[4,5]imidazo[1,2-*a*]pyrimido[4,5-*d*]pyrimidines, antimicrobial activity

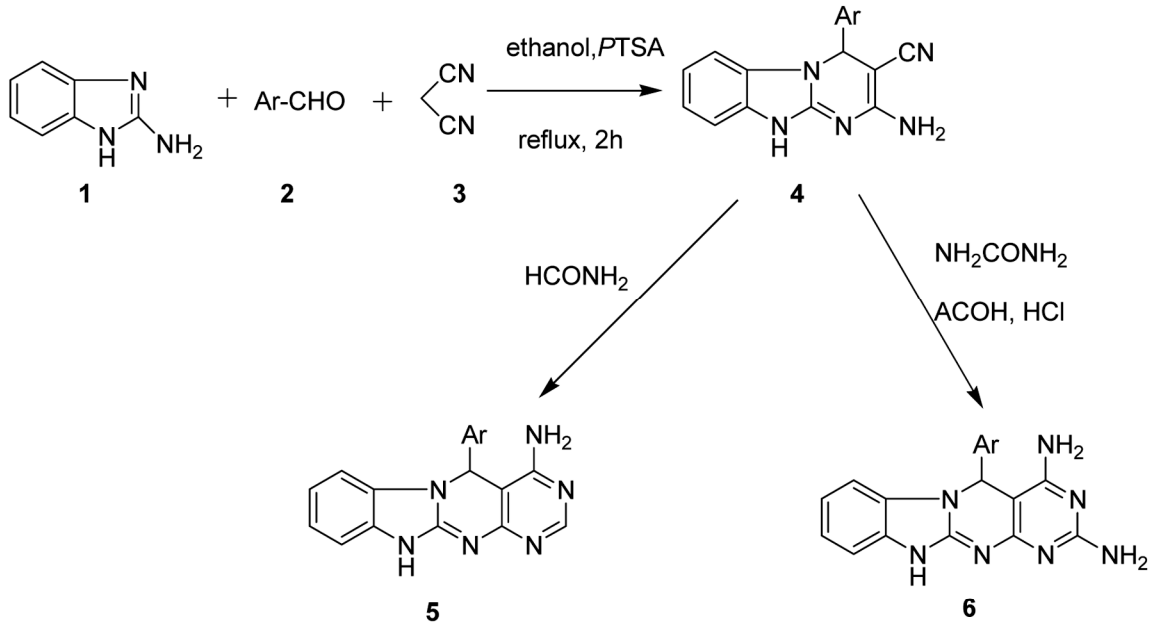
Multi-component reactions (MCRs) play an important role in organic and medicinal chemistry[1] as it furnishes products with a high degree of structural variability. MCRs are inexpensive, less time-consuming and eco-friendly in comparison to conventional multi-step synthesis[2,3]. The exploitation of a simple molecule with different functionalities for the synthesis of bio heterocycles is a useful contribution in the heterocyclic chemistry[4]. Heterocyclic moieties play a prominent role in the design and synthesis of bioactive molecules. Pyrimidines and fused pyrimidines have been found to possess diverse biological activity[5-7]. In particular pyrimido- [4,5-*d*]pyrimidines, a class of annulated uracils, have been found to possess a wide range of biological activity. They act as bronchodilators[8], antiallergic[9], cardiotonic[10], anti hypertensive[11] and anticancer[12] agents. Benzimidazole is an important nucleus that has been extensively used in medicinal chemistry, notable examples being the antihistaminic asterizole and the antiulcerative omeprazole[13]. Benzimidazoles are also known for their anti-inflammatory[14], antibiotic[15], anthelmintic[16], anticancer[17], and antiviral activities[18].

Molecular hybridization is a relatively new concept in the field of drug design, and development involving the fusion of two or more pharmocophoric groups which have an inhibitory effect against the target disease. The newly designed structure can lead to compounds having improved affinity and efficacies than the parent compounds with reduced side effects, while retaining the desired characteristics of original template[19-21]. Prompted by these reports, and as a sequel to our interest in the synthesis of benzimidazole derivatives with potent biological activity[22-25], we herein, report the synthesis and antimicrobial activity of benzo[4,5]imidazo[1,2-*a*] pyrimido[4,5-*d*]pyrimidine derivatives.

Results and Discussion

The synthesis of title compounds was accomplished by synthetic sequence shown in Scheme I**.** The three-component reaction of 2-amino benzimidazole **1**, substituted aromatic aldehyde **2,** and malononitrile **3** in presence of *p*-toluene sulphonic acid (*P*TSA), a Lewis acid catalyst, in ethanol furnished novel 2-amino-4-aryl-4, 10-dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimidine-3-carbonitriles **4** in good yields. This reaction is similar to the reaction reported earlier in the literature[26]. Compounds **4** on treatment with formamide and urea separately in glacial acetic acid gave the 5-aryl-5,11-dihydro benzo[4,5]imidazo[1,2-*a*]pyrimido[4,5-*d*] pyrimidine-4-amines **5**, and 5-aryl-5,11-dihydro benzo[4,5]imidazo[1,2-*a*]pyrimido[4,5-*d*] pyrimidine-2, 4-diamines **6**. This reaction is in accordance with earlier report on pyrimidine derivatives[27].

Twenty four new derivatives were reported. The structures of newly synthesized compounds **4a-h, 5a-**



Ar = 4, 5 & 6

a,	C_6H_5	**e,**	4-$CH_3C_6H_4$
b,	2-Cl C_6H_4	**f,**	4-$OCH_3C_6H_4$
c,	2-Br C_6H_4	**g,**	4-$N(CH_3)_2C_6H_4$
d,	2-OHC_6H_4	**h,**	2,4-$Cl_2C_6H_3$

Scheme I

h, and 6a-h were confirmed by analytical and spectral data (IR, ^{1}H NMR, ^{13}C NMR and MS).

IR spectrum of **4a** exhibited absorption bands at 3435, 3415 cm^{-1} due to NH_2, where as CN absorption band was shown at 2215 cm^{-1}. In ^{1}H NMR spectrum of **4a** pyrimidine ring CH proton appeared as a sharp singlet at δ 5.13, NH_2 protons appeared at δ 8.25, which are D_2O exchangeable, whereas imidazole ring NH displayed as a singlet at δ 10.25, which is also D_2O exchangeable. Aromatic protons appeared as a complex multiplet between δ 7.00-7.83. ^{13}C NMR spectrum of **4a** exhibited CN and Ar-CH carbon signals at δ 83.25 and 52.54 confirming cyclization. The mass spectrum of **4a** displayed the molecular ion $[M+H]^+$ peak at *m/z* 288. In IR spectrum of **5a** NH_2 absorption bands appeared at 3388, 3305 cm^{-1}. ^{1}H NMR spectrum of **5a** exhibited a singlet at δ 8.50 due to the newly formed pyrimidine ring proton confirming cyclization. Rest of the signals are in agreement with the proposed structure. ^{13}C NMR spectrum of **5a** displayed –N=CH-N= carbon at δ 166.04. The mass spectrum of **5a** exhibited the molecular ion $[M+H]^+$ peak at *m/z* 315, which is in agreement with assigned structure. Similarly, The IR, ^{1}HNMR, and ^{13}C NMR spectra of **6** are very well in agreement with the proposed structure. The mass spectrum of **6a** displayed molecular ion $[M+H]^+$ peak at *m/z* 330. Data from the elemental analyses further confirmed the assigned structure of **4a-h, 5a-h, and 6a-h.**

Antimicrobial activity

Antibacterial activity

The newly synthesized 5-aryl-5,11-dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimido[4,5-*d*]pyrimidin-4-amines **5a-h,** 5-aryl-5,11-dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimido[4,5-*d*]pyrimidine-2,4-diamines **6a-h** were evaluated for their *in vitro* antibacterial activity against Gram-positive bacteria *viz*., *Bacillus subtilis* (MTCC 441), *Bacillus sphaericus* (MTCC 511) and *Staphylococcus aureus* (MTCC 96) and Gram-negative bacteria *viz*., *Pseudomonas aeruginosa* (MTCC 741), *Klobsinella*

aerogenes (MTCC 39) and *Chromobacterium violaceum* (MTCC 2656) at 100 µg/ml concentration. The *in vitro* antibacterial activity of the tested compounds was assessed by minimum inhibitory concentration (MIC) using broth dilution method[28]. *Ciprofloxacin* was used as standard drug for comparison.

The antibacterial activity results showed that compounds **5a-h and 6a-h** displayed a better activity and were more active than the standard drug *Ciprofloxacin* (Table I and Table II). The activity was expressed in terms of minimum inhibitory concentration (MIC). The compounds **5e, 5f** and **5g, 6e, 6f and 6g** are highly active, because the activity is considerably affected by the presence of methyl, methoxy and dimethyl amino as substituents on benzene ring. Compounds **5b**, **5c** and **5h, 6b, 6c and 6h** carrying chloro and bromo substitutions on benzene ring did not exhibit much activity. Compounds **5a and 6a** showed least activity, because they did not have any substituent on the benzene ring. However, the degree of inhibition varied both with the test compound as well as with the bacteria used in the present investigation.

In conclusion, the antibacterial activity of compounds **5f** and **5g, 6f and 6g** is promising compared to standard drug *Ciprofloxacin*, and they can be exploited for formulation of bacteriocides after further study.

Antifungal activity

The newly synthesized 5-aryl-5,11-dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimido[4,5-*d*]pyrimidin-4-amines **5a-h and** 5-aryl-5,11-dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimido[4,5-*d*]pyrimidine-2,4-diamines **6a-h** were also evaluated for

Table I — Antibacterial activity of 5-phenyl-5,11-dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimido[4,5-*d*]pyrimidin-4-amines. **5a-h**

		Minimum Inhibitory Concentration in µg/ml (MIC)					
		Gram + ve bacteria			Gram –ve bacteria		
Compd	Ar	B.subtilis	*B.sphaericus*	*S.aureus*	*P.aeruginosa*	*K.aerogenes*	*C.violaceum*
5a	C_6H_5	17	19	19	18	17	17
5b	2-ClC_6H_4	19	20	18	20	18	17
5c	2-BrC_6H_4	16	13	12	16	16	17
5d	2-OHC_6H_4	18	14	14	13	17	16
5e	4-$CH_3C_6H_4$	8	7	7	8	7	8
5f	4-$OCH_3C_6H_4$	7	8	8	9	9	8
5g	4-$N(CH_3)_2C_6H_4$	6	7	8	8	7	6
5h	2,4-$Cl_2C_6H_3$	15	17	16	14	16	15
Ciprofloxacin	---	20	22	26	25	20	22

Negative control (acetone) – No activity
Values are indicated in µg/mL

Table II — Antibacterial activity of 5-phenyl-5,11-dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimido[4,5-*d*]pyrimidine-2,4-diamines **6a-h**

		Minimum Inhibitory Concentration in µg/ml (MIC)					
		Gram + ve bacteria			Gram –ve bacteria		
Compd	Ar	B.subtilis	*B.sphaericus*	*S.aureus*	*P.aeruginosa*	*K.aerogenes*	*C.violaceum*
6a	C_6H_5	17	17	19	20	19	19
6b	2-ClC_6H_4	17	20	19	20	18	17
6c	2-BrC_6H_4	16	13	13	16	15	16
6d	2-OHC_6H_4	18	16	14	13	16	17
6e	4-$CH_3C_6H_4$	8	7	6	7	8	6
6f	4-$OCH_3C_6H_4$	8	6	7	8	9	8
6g	4-$N(CH_3)_2C_6H_4$	8	7	6	8	8	9
6h	2,4-$Cl_2C_6H_3$	15	17	16	14	18	15
Ciprofloxacin	---	20	22	26	25	20	22

Negative control (acetone) – No activity
Values are indicated in µg/mL

their antifungal activity against *Fusarium oxysporum, Verticillium dahliae, Alternaria solani, Rhizoctonia solani, Colletotrichum capsici* and *Pythium aphanidermatum* in acetone by agar cup bioassay method[29], using *Fluconazole* as the standard drug.

Antifungal activity data (Table III and Table IV) revealed that compounds **5a-h** and **6a-h** are highly toxic towards all the fungi under investigation. Compounds **5e, 5f** and **5g, 6e, 6f** and **6g** exhibited high antifungal activity by inhibiting the growth of fungi to a remarkable extent, when compared to the standard drug *Fluconazole*, which may be due to the presence of methyl, methoxy and dimethylamino substituents on the benzene ring. Compounds **5a** and **6a** showed low activity. Compounds **5b**, **5c** and **5d** are moderately active. However, the degree of spore germination inhibition varied with the test compound as well as with the fungi under investigation. It is noteworthy that compounds **5f** and **5g and 6f, 6g** showed better activity, when compared with the standard drug *Fluconazole*, hence, they may be exploited for control of wilt diseases of different crops as fungicides after further studies.

In conclusion, the results revealed that compounds **5g** and **6g** are highly toxic towards the fungi under investigation and they are lethal even at 100 µg/mL concentration in comparison with standard *Fluconazole* at the same concentration, and may be exploited for control of wilt diseases of different crops as fungicides after detailed study.

Experimental Section

All the melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Analytical TLC was performed on Merck precoated 60 F_{254} silica gel plates. Visualization was carried out by exposure to iodine vapour. IR spectra (KBr pellet) were recorded on a Perkin-Elmer BX series FT-IR spectrometer. ^{1}H NMR spectra were recorded on a Varian Gemini 300 MHz spectrometer. ^{13}C NMR spectra were recorded on a Bruker 75 MHz spectrometer. Chemical shift values are given in δ ppm with tetramethyl silane as an internal standard. ESI

Table III — Antifungal activity of 5-phenyl-5,11-dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimido[4,5-*d*]pyrimidin-4-amines. **5a-h**

Compd		Minimum inhibitory Concentration in µg/mL(MIC)					
	Ar	*F.oxysporum*	*V. dahliae*	*A. solani*	*R. solani*	*C. capsici*	*P. aphanidermatum*
5a	C_6H_5	13	15	11	12	15	16
5b	2-ClC_6H_4	16	15	14	17	19	20
5c	2-BrC_6H_4	15	19	18	15	18	17
5d	2-OHC_6H_4	17	16	18	12	18	20
5e	4-$CH_3C_6H_4$	8	7	7	9	8	9
5f	4-$OCH_3C_6H_4$	8	7	8	9	8	9
5g	4-$N(CH_3)_2C_6H_4$	6	7	6	8	9	6
5h	2,4-$Cl_2C_6H_3$	11	12	13	14	13	14
Fluconazole	---	16	16	20	16	18	22

Negative control (acetone) – No activity.

Table IV — Antifungal activity of 5-phenyl-5,11-dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimido[4,5-*d*]pyrimidine-2,4-diamines. **6a-h**

Compd		Minimum inhibitory concentration in µg/mL(MIC)					
	Ar	*F.oxysporum*	*V. dahliae*	*A. solani*	*R. solani*	*C. capsici*	*P. aphanidermatum*
6a	C_6H_5	12	14	12	12	14	15
6b	2-ClC_6H_4	16	14	15	16	18	20
6c	2-BrC_6H_4	14	18	18	16	17	17
6d	2-OHC_6H_4	10	11	14	12	12	13
6e	4-$CH_3C_6H_4$	8	8	7	8	8	9
6f	4-$OCH_3C_6H_4$	8	6	9	8	8	8
6g	4-$N(CH_3)_2C_6H_4$	8	7	8	6	9	8
6h	2,4-$Cl_2C_6H_3$	10	11	14	12	12	13
Fluconazole	--	16	16	20	16	18	22

Negative control (acetone) – No activity.

Mass spectra were recorded on a Agilent LC-MSD mass spectrometer. Elemental analyses were performed on a Carlo Erba 106 and Perkin-Elmer model 240 analyzers.

General procedure for the synthesis of 2-amino-4-aryl-4,10-dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimidine-3-carbonitriles, 4a-h

To a vigorously stirred aromatic aldehyde 2 (1mmol), and malononitrile 3 (1mmol), 2-amino benzimidazole in ethanol was added and contents were refluxed while stirring for 4 h. The termination of the reaction was monitored by TLC. After completion of the reaction, the reaction was poured on to crushed ice, The separated solid was filtered, and purified by recrystallization from benzene-ethyl acetate to obtain pure compounds (**4a-h**).

2-Amino-4-phenyl-4,10-dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimidine-3-carbonitrile, 4a: Orange solid, yield 67%, m.p. 175-77°C, IR (KBr): 3435, 3415 (NH_2), 2215 (CN) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 5.13 (s, 1H, CH), 7.00-7.83 (m, 9H), 8.25 (s, 2H, NH_2, D_2O exchangeable), 10.25 (s,1NH, D_2O exchangeable); ^{13}C NMR (75MHz, $CDCl_3$): δ 52.54, 83.25, 117.21, 120.23, 123.56, 125.37, 128.31, 131.28, 133.56, 135.21, 137.23, 138.00, 140.01, 142.20, 146.21, 168.21, 169.31; ESI-MS: *m/z* 288 $[M+H]^+$. Anal.cacld for $C_{17}H_{13}N_5$: C, 71.08; H, 4.52; N, 24.39 % Found: C, 71.05; H, 4.55; N, 24.43%.

2-Amino-4-(2-chlorophenyl)-4,10-dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimidine-3-carbonitrile, 4b: Pale yellow solid, yield 70%, m.p. 180-82°C, IR (KBr): 3445, 3425 (NH_2), 2225 (CN) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 5.16 (s, 1H, CH), 7.05-7.89 (m, 8H), 8.28 (s, 2H, NH_2, D_2O exchangeable), 10.28 (s,1NH, D_2O exchangeable); ^{13}C NMR (75MHz, $CDCl_3$): δ 53.14, 84.27, 118.25, 121.23, 124.66, 126.39, 128.31, 132.23, 134.59, 136.21, 138.50, 139.09, 141.11, 142.29, 147.28, 169.21, 170.30; ESI-MS: *m/z* 322 $[M+H]^+$. Anal.cacld for $C_{17}H_{12}N_5Cl$: C, 63.55; H, 3.73; N, 21.80 % Found: C, 63.51; H, 3.76; N, 21.84 %.

2-Amino-4-(2-bromophenyl)-4,10-dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimidine-3-carbonitrile, 4c: Brown solid, yield 73%, m.p.220-22°C. IR (KBr): 3443, 3423 (NH_2), 2222 (CN) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 5.15 (s, 1H, CH), 7.03-7.85 (m, 8H), 8.26 (s, 2H, NH_2, D_2O exchangeable), 10.24 (s,1NH, D_2O exchangeable); ^{13}C NMR (75MHz, $CDCl_3$): δ 52.89, 85.29, 117.79, 122.29, 123.79, 127.41, 127.36, 133.29, 134.568, 135.20, 139.76, 139.99, 140.51, 143.39, 146.08, 168.11, 169.20; ESI-MS: *m/z* 366 $[M+H]^+$. Anal.cacld for $C_{17}H_{12}N_5Br$: C, 55.89; H, 3.28; N, 19.17 % Found: C, 55.92; H, 3.24; N, 19.21 %.

2-Amino-4-(2-hydroxyphenyl)-4,10-dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimidine-3-carbonitrile, 4d: yellow solid, yield 75%, m.p. 200-02°C, IR (KBr): 3454 (OH), 3447, 3426 (NH_2), 2227 (CN) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 5.17 (s, 1H, CH), 7.10-7.99 (m, 8H), 8.30 (s, 2H, NH_2, D_2O exchangeable), 8.56 (s, 1H, OH, D_2O exchangeable), 10.29 (s,1NH, D_2O exchangeable); ^{13}C NMR (75MHz, $CDCl_3$): δ 55.34, 86.27, 120.15, 121.23, 124.69, 127.39, 127.99, 133.53, 135.39, 136.29, 139.30, 139.89, 142.01, 143.19, 147.55, 168.11, 171.00; ESI-MS: *m/z* 304 $[M+H]^+$. Anal.cacld for $C_{17}H_{13}N_5O$: C, 63.32; H, 4.29; N, 23.10 % Found: C, 63.35; H, 4.26; N, 23.14 %.

2-Amino-4-(p-tolyl)-4,10-dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimidine-3-carbonitrile, 4e: Pale yellow solid, yield 78%, m.p. 195-97°C, IR (KBr): 3443, 3423 (NH_2), 2223 (CN) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.35 (s, 3H, CH_3), 5.17 (s, 1H, CH), 7.06-7.92 (m, 8H), 8.25 (s, 2H, NH_2, D_2O exchangeable), 10.26 (s,1NH, D_2O exchangeable); ^{13}C NMR (75MHz, $CDCl_3$): δ 23.45, 54.44, 85.37, 121.25, 122.25, 123.69, 128.59, 126.90, 134.53, 136.33, 137.19, 139.50, 140.09, 143.01, 143.59, 147.75, 168.19, 170.05; ESI-MS: *m/z* 302 $[M+H]^+$. Anal.cacld for $C_{18}H_{15}N_5$: C, 71.76; H, 4.98; N, 23.25 % Found: 71.72; H, 4.95; N, 23.28 %.

2-Amino-4-(4-methoxyphenyl)-4,10-dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimidine-3-carbonitrile, 4f: yellow solid, yield 85%, m.p. 207-09°C, IR (KBr): 3446, 3427 (NH_2), 2228 (CN) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 3.78 (s, 3H, OCH_3), 5.19 (s, 1H, CH), 7.09-7.95 (m, 8H), 8.28 (s, 2H, NH_2, D_2O exchangeable), 10.28 (s,1NH, D_2O exchangeable); ^{13}C NMR (75MHz, $CDCl_3$): δ 54.44, 64.48, 85.39, 120.29, 121.35, 122.69, 124.79, 127.94, 135.59, 138.33, 138.89, 139.59, 141.19, 144.21, 144.89, 147.79, 169.19, 171.03; ESI-MS: *m/z* 319 $[M+H]^+$. Anal.cacld for $C_{18}H_{15}N_5O$: C, 67.92; H, 4.71; N, 22.01 % Found: 67.95; H, 4.75; N, 22.02 %.

2-Amino-4-(4-(dimethylamino)phenyl)-4,10-dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimidine-3-carbonitrile, 4g: yellow solid, yield 87%, m.p. 213-15°C, IR (KBr): 3446, 3428 (NH_2), 2230 (CN) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.80 (s, 6H, $N(CH_3)_2$), 5.21 (s, 1H, CH), 7.10-7.90 (m, 8H), 8.30 (s, 2H, NH_2, D_2O exchangeable), 10.25 (s,1NH, D_2O

exchangeable); ^{13}C NMR (75MHz, $CDCl_3$): δ 48.32, 51.79, 54.49, 86.37, 121.39, 122.45, 123.60, 125.77, 128.94, 136.60, 137.03, 138.39, 138.89, 140.19, 145.11, 146.39, 147.89, 168.12, 170.53; ESI-MS: *m/z* 331 $[M+H]^+$. Anal.cacld for $C_{19}H_{18}N_6$: C, 69.09; H, 5.45; N, 25.45 % Found: 69.05; H, 5.48; N, 25.43 %.

2-Amino-4-(2,4-dichlorophenyl)-4,10-dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimidine-3-carbonitrile, 4h: Pale yellow solid, yield 68%, m.p. 188-90°C, IR (KBr): 3448, 3429 (NH_2), 2230 (CN) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.21 (s, 1H, CH), 7.05-7.95 (m, 7H), 8.35 (s, 2H, NH_2, D_2O exchangeable), 10.39 (s,1NH, D_2O exchangeable); ^{13}C NMR (75MHz, $CDCl_3$): δ 54.24, 87.27, 119.35, 122.33, 124.69, 127.69, 129.30, 131.93, 135.09, 137.11, 138.70, 139.89, 142.11, 143.34, 147.29, 168.61, 171.10; ESI-MS: *m/z* 356 $[M+H]^+$. Anal.cacld for: C, 57.46; H, 3.09; N, 19.71 % Found: C, 57.43; H, 3.13; N, 19.73 %.

General procedure for the synthesis of 5-phenyl-5,11-dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimido[4,5-*d*]pyrimidin-4-amines, 5a-h

A mixture of compound **4** (1.06 g, 5 mmol), and formamide (10 mL) was refluxed for 5 h. After the completion of the reaction (monitored by TLC) the reaction mixture was cooled, separated solid is filtered off, washed with methanol, and recrystallized from ethyl acetate to give pure compounds (**5a-h).**

5-Phenyl-5,11-dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimido[4,5-*d*]pyrimidin-4-amine, 5a: Orange solid, yield 69%, m.p. 225-27°C, IR (KBr): 3388, 3305 (NH_2), cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.19 (s, 1H, CH), 7.00-7.98 (m, 9H), 7.95 (s, 2H, NH_2, D_2O exchangeable), 8.50 (s, 1H, N=CH), 10.20 (s,1NH, D_2O exchangeable); ^{13}C NMR (75MHz, $CDCl_3$): δ 44.52, 109.34, 115.43, 117.54, 118.64, 120.02, 127.86, 128.99, 129.03, 130.02, 130.95, 131.23, 134.84, 143.94, 156.84, 159.94, 164.93, 166. 04; ESI-MS: *m/z* 315 $[M+H]^+$. Anal.cacld for $C_{18}H_{14}N_6$: C, 68.78; H, 4.45; N, 26.75 % Found: C, 68.75; H, 4.43; N, 26.78 %.

5-(2-Chlorophenyl)-5,11-dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimido[4,5-*d*]pyrimidin-4-amine, 5b: Pale Yellow solid, yield 65%, m.p. 234-36°C, IR (KBr): 3396, 3315 (NH_2), cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.25 (s, 1H, CH), 7.06-8.02 (m, 8H), 7.99 (s, 2H, NH_2, D_2O exchangeable), 8.55 (s, 1H, N=CH), 10.25 (s, 1NH, D_2O exchangeable); ^{13}C NMR (75MHz, $CDCl_3$): δ 45.58, 110.24, 116.73, 118.44, 118.94, 121.02, 128.02, 129.09, 129.95, 131.09, 131.95, 132.03, 134.84, 144.34, 157.80, 160.94, 165.91, 167. 01; ESI-MS: *m/z* 349 $[M+H]^+$. Anal.cacld for $C_{18}H_{13}N_6Cl$: C, 62.06; H, 3.73; N, 24.13 % Found: C, 62.03; H, 3.76; N, 24.10 %.

5-(2-Bromophenyl)-5,11-dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimido[4,5-*d*]pyrimidin-4-amine, 5c: Brown solid, yield 60%, m.p. 244-46°C, IR (KBr): 3394, 3313 (NH_2), cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.22 (s, 1H, CH), 7.06-8.00 (m, 8H), 7.96 (s, 2H, NH_2, D_2O exchangeable), 8.53 (s, 1H, N=CH), 10.22 (s, 1NH, D_2O exchangeable); ^{13}C NMR (75MHz, $CDCl_3$): δ 44.59, 109.24, 115.79, 117.40, 117.90, 120.02, 127.76, 128.34, 128.95, 130.09, 131.05, 132.03, 133.80, 143.24, 156.82, 160.35, 164.31, 166.67; ESI-MS: *m/z* 393 $[M+H]^+$. Anal.cacld for $C_{18}H_{13}N_6Br$: C, 55.10; H, 3.31; N, 21.42 % Found: C, 55.14; H, 3.35; N, 21.46%.

2-(4-Amino-5,11-dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimido[4,5-*d*]pyrimidin-5-yl)phenol, 5d: Pale Yellow solid, yield 75%, m.p. 239-41°C, IR (KBr): 3456 (OH), 3395, 3316 (NH_2),cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.26 (s, 1H, CH), 7.10-8.02 (m, 8H), 7.98 (s, 2H, NH_2, D_2O exchangeable), 8.53 (s, 1H, N=CH), 8.59 (s, 1H, OH, D_2O exchangeable), 10.25 (s, 1NH, D_2O exchangeable); ^{13}C NMR (75MHz, $CDCl_3$): δ 45.29, 110.14, 116.39, 118.20, 118.98, 121.02, 128.72, 129.34, 130.00, 130.89, 131.65, 132.03, 134.88, 144.24, 157.42, 161.55, 165.31, 167.17; ESI-MS: *m/z* 331 $[M+H]^+$. Anal.cacld for $C_{18}H_{14}N_6O$: C, 65.45; H, 4.24; N, 25.45 % Found: C, 65.49; H, 4.22; N, 25.47%.

5-(p-Tolyl)-5,11-dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimido[4,5-*d*]pyrimidin-4-amine, 5e: Pale Yellow solid, yield 78%, m.p. 232-34°C, IR (KBr): 3393, 3315 (NH_2), cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.45 (s, 3H, CH_3), 5.24 (s, 1H, CH), 7.04-8.00 (m, 8H), 7.96 (s, 2H, NH_2, D_2O exchangeable), 8.50 (s, 1H, N=CH), 10.22 (s, 1NH, D_2O exchangeable); ^{13}C NMR (75MHz, $CDCl_3$): δ 24.12, 44.09, 109.14, 115.39, 117.10, 117.98, 120.92, 127.72, 128.34, 129.12,130.89, 131.65, 131.98, 133.48, 143.24, 156.40, 160.65, 164.21, 166.27; ESI-MS: *m/z* 329 $[M+H]^+$. Anal.cacld for $C_{19}H_{16}N_6$: C, 69.51; H, 4.87; N, 25.60 % Found: C, 69.54; H, 4.86; N, 25.63%.

5-(4-Methoxyphenyl)-5,11-dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimido[4,5-

***d*]pyrimidin-4-amine, 5f**: Pale Yellow solid, yield 80%, m.p. 250-52°C, IR (KBr): 3397, 3319 (NH_2), cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 3.68 (s, 3H, OCH_3), 5.29 (s, 1H, CH), 7.09-8.05 (m, 8H), 7.98 (s, 2H, NH_2, D_2O exchangeable), 8.52 (s, 1H, N=CH), 10.24 (s, 1NH, D_2O exchangeable); ^{13}C NMR (75MHz, $CDCl_3$): δ 45.19, 64.47, 110.24, 116.45, 118.10, 118.98, 121.42, 128.32, 129.04, 129.96, 131.29, 132.25, 132.98, 134.48, 144.14, 157.40, 161.75, 165.31, 167.17; ESI-MS: *m/z* 345 $[M+H]^+$. Anal.cacld for $C_{19}H_{16}N_6O$: C, 66.27; H, 4.65; N, 24.41 % Found: C, 66.24; H, 4.68; N, 24.43 %.

5-(4-(Dimethylamino)phenyl)-5,11-dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimido[4,5-*d*]pyrimidin-4-amine, 5g: Pale Yellow solid, yield 81%, m.p. 256-58°C, IR (KBr): 3396, 3318 (NH_2), cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.45 (s, 6H, $(NH_3)_2$), 5.28 (s, 1H, CH), 7.05-8.01 (m, 8H), 7.98 (s, 2H, NH_2, D_2O exchangeable), 8.50 (s, 1H, N=CH), 10.22 (s, 1NH, D_2O exchangeable); ^{13}C NMR (75MHz, $CDCl_3$): δ 44.29, 48.51, 51.23, 63.47, 109.24, 115.25, 117.11, 117.98, 120.62, 127.32, 128.01, 128.96, 130.24, 131.15, 132.35, 133.28, 143.24, 156.30, 160.25, 164.21, 166.17; ESI-MS: *m/z* 358 $[M+H]^+$. Anal.cacld for $C_{20}H_{19}N_7$: C, 67.22; H, 5.32; N, 27.45 % Found: C, 67.20; H, 5.35; N, 27.43 %.

5-(2,4-Dichlorophenyl)-5,11-dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimido[4,5-*d*]pyrimidin-4-amine, 5h: Pale Yellow solid, yield 64%, m.p. 249-51°C, IR (KBr): 3398, 3320 (NH_2), cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 5.27 (s, 1H, CH), 7.06-8.10 (m, 7H), 7.99 (s, 2H, NH_2, D_2O exchangeable), 8.59 (s, 1H, N=CH), 10.29 (s, 1NH, D_2O exchangeable); ^{13}C NMR (75MHz, $CDCl_3$): δ 46.38, 111.14, 117.13, 119.24, 119.99, 122.02, 129.12, 129.89, 130.25, 132.09, 132.95, 133.03, 134.84, 145.34, 156.86, 161.96, 164.91, 168.06; ESI-MS: *m/z* 383 $[M+H]^+$. Anal.cacld for $C_{18}H_{12}N_6Cl_2$: C, 56.54; H, 3.14; N, 21.98 % Found: C, 56.56; H, 3.12; N, 21.95 %

General procedure for the Synthesis of phenyl-5,11-dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimido[4,5-*d*]pyrimidine-2,4-diamines, 6a-h

A mixture of compound **4** (1.06 g, 5 mmol), and urea (0.3 g, 5 mmol) was refluxed in glacial acetic acid and hydrochloric acid (10 mL; 3:1) for 10 h. After being cooled, the precipitated solid was filtered off, and recrystallized from ethanol to give **6a-h** in good yield**.**

5-Phenyl-5,11-dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimido[4,5-*d*]pyrimidine-2,4-diamine 6a: Pale Yellow solid, yield 68%, m.p. 258-60°C, IR (KBr): 3410 ($2NH_2$), cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 5.25 (s, 1H, CH), 7.01-7.95 (m, 9H), 7.43 (s, 2H, NH_2, D_2O exchangeable), 7.91 (s, 2H, NH_2, D_2O exchangeable), 10.21 (s, 1NH, D_2O exchangeable); ^{13}C NMR (75MHz, $CDCl_3$): δ 45.59, 110.34, 116.53, 118.24, 119.34, 121.12, 128.86, 129.32, 130.03, 131.09, 131.25, 132.35, 135.84, 144.25, 159.96, 162.34, 164.96, 166.04; ESI-MS: *m/z* 330 $[M+H]^+$. Anal.cacld for $C_{18}H_{15}N_7$: C, 65.65; H, 4.55; N, 29.78 % Found: C, 65.63; H, 4.52; N, 29.75 %.

5-(2-Chlorophenyl)-5,11-dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimido[4,5-*d*]pyrimidine-2,4-diamine 6b: Pale Yellow solid, yield 65%, m.p. 267-69°C, IR (KBr): 3415 ($2NH_2$), cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 5.28 (s, 1H, CH), 7.01-7.99 (m, 8H), 7.46 (s, 2H, NH_2, D_2O exchangeable), 7.95 (s, 2H, NH_2, D_2O exchangeable), 10.26 (s, 1NH, D_2O exchangeable); ^{13}C NMR (75MHz, $CDCl_3$): δ 46.79, 111.44, 117.53, 118.84, 120.04, 122.22, 129.02, 129.89, 131.03, 131.99, 132.15, 133.45, 136.84, 145.45, 158.96, 163.34, 164.99, 167.14; ESI-MS: *m/z* 364 $[M+H]^+$. Anal.cacld for $C_{18}H_{14}N_7$ Cl: C, 59.50; H, 3.85; N, 26.99 % Found: C, 59.54; H, 3.82; N, 26.97%.

5-(2-Bromophenyl)-5,11-dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimido[4,5-*d*]pyrimidine-2,4-diamine

6c: Brown solid, yield 60%, m.p. 289-91°C, IR (KBr): 3409 ($2NH_2$), cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 5.22 (s, 1H, CH), 6.98-7.90 (m, 8H), 7.42 (s, 2H, NH_2, D_2O exchangeable), 7.90 (s, 2H, NH_2, D_2O exchangeable), 10.20 (s, 1NH, D_2O exchangeable); ^{13}C NMR (75MHz, $CDCl_3$): δ 45.19, 110.44, 116.43, 117.84, 120.04, 121.42, 128.12, 128.84, 130.13, 130.99, 131.25, 132.85, 135.84, 144.45, 157.96, 162.64, 163.95, 166.24; ESI-MS: *m/z* 408 $[M+H]^+$. Anal.cacld for $C_{18}H_{14}N_7$ Br: C, 53.07; H, 3.43; N, 24.07 % Found: C, 53.05; H, 3.40; N, 24.09 %.

2-(2,4-Diamino-5,11-dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimido[4,5-*d*]pyrimidin-5-yl)phenol

6d: Pale Yellow solid, yield 73%, m.p. 255-57°C, IR (KBr): 3417 ($2NH_2$), 3436 (OH) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 5.29 (s, 1H, CH), 7.01-7.99

(m, 8H), 7.43 (s, 2H, NH_2, D_2O exchangeable), 7.91 (s, 2H, NH_2, D_2O exchangeable), 8.55 (s, 1H, OH, D_2O exchangeable), 10.22 (s, 1NH, D_2O exchangeable); ^{13}C NMR (75MHz, $CDCl_3$): δ 46.49, 111.24, 117.63, 119.64, 120.34, 121.78, 129.36, 130.02, 130.93, 132.09, 132.85, 133.01, 136.84, 145.05, 159.96, 163.14, 165.36, 166.04; ESI-MS: *m/z* 364 $[M+H]^+$. Anal.cacld for $C_{18}H_{15}N_7$ O: C, 59.50; H, 3.85; N, 26.99 % Found: C, 59.54; H, 3.82; N, 26.97%.

5-(p-Tolyl)-5,11-dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimido[4,5-*d*]pyrimidine-2,4-diamine 6e: Pale Yellow solid, yield 78%, m.p. 284-86°C, IR (KBr): 3416 ($2NH_2$), cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ2.35 (s.3H, CH_3), 5.27 (s, 1H, CH), 6.99-7.97 (m, 8H), 7.46 (s, 2H, NH_2, D_2O exchangeable), 7.99 (s, 2H, NH_2, D_2O exchangeable), 10.26 (s, 1NH, D_2O exchangeable); ^{13}C NMR (75MHz, $CDCl_3$): δ 24.65, 46.49, 111.34, 117.83, 118.56, 120.24, 122.17, 128.97, 130.02, 131.13, 131.89, 132.45, 133.35, 136.44, 145.35, 158.95, 163.44, 165.66, 166.54; ESI-MS: *m/z* 344 $[M+H]^+$. Anal.cacld for $C_{19}H_{17}N_7$: C, 66.47; H, 4.95; N, 28.57 % Found: C, 66.43; H, 4.97; N, 28.53 %.

5-(4-Methoxyphenyl)-5,11-dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimido[4,5-*d*]pyrimidine-2,4-diamine 6f: Pale Yellow solid, yield 73%, m.p. 272-74°C, IR (KBr): 3412 ($2NH_2$), cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 3.68 (s.3H, OCH_3), 5.26 (s, 1H, CH), 6.98-7.90 (m, 8H), 7.45 (s, 2H, NH_2, D_2O exchangeable), 7.94 (s, 2H, NH_2, D_2O exchangeable), 10.24 (s, 1NH, D_2O exchangeable); ^{13}C NMR (75MHz, $CDCl_3$): δ 46.49, 63.64, 112.24, 118.63, 119.26, 120.74, 123.07, 129.37, 130.72, 132.18, 132.89, 133.25, 133.95, 137.54, 146.25, 159.95, 164.34, 165.16, 167.24; ESI-MS: *m/z* 360 $[M+H]^+$. Anal.cacld for $C_{19}H_{17}N_7O$: C, 63.50; H, 4.73; N, 27.29 % Found: C, 63.54; H, 4.70; N, 27.26 %.

5-(4-(Dimethylamino)phenyl)-5,11-dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimido[4,5-*d*]pyrimidine-2,4-diamine 6g: Pale Yellow solid, yield 76%, m.p. 275-77°C, IR (KBr): 3418 ($2NH_2$), cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.36 (s, 6H, $N(CH_3)_2$), 5.27 (s, 1H, CH), 7.01-7.93 (m, 8H), 7.45 (s, 2H, NH_2, D_2O exchangeable), 7.96 (s, 2H, NH_2, D_2O exchangeable), 10.27 (s, 1NH, D_2O exchangeable); ^{13}C NMR (75MHz, $CDCl_3$): δ 45.79, 49.35, 52.23, 110.24, 116.53, 117.64, 121.04, 123.32, 129.22, 130.49, 132.03, 132.99, 133.05, 134.45, 137.80, 146.25, 159.96, 162.64, 163.90, 168.04; ESI-MS: *m/z* 373 $[M+H]^+$. Anal.cacld for $C_{20}H_{20}N_8$: C, 64.51; H, 5.37; N, 30.10 % Found: C, 64.53; H, 5.34; N, 30.12%.

5-(2,4-Dichlorophenyl)-5,11-dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimido[4,5-*d*]pyrimidine-2,4-diamine 6h: Pale Yellow solid, yield 63%, m.p. 288-90°C, IR (KBr): 3418 ($2NH_2$), cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 5.29 (s, 1H, CH), 7.03-7.99 (m, 7H), 7.49 (s, 2H, NH_2, D_2O exchangeable), 7.98 (s, 2H, NH_2, D_2O exchangeable), 10.29 (s, 1NH, D_2O exchangeable); ^{13}C NMR (75MHz, $CDCl_3$): δ 45.89, 112.44, 118.63, 119.84, 121.14, 122.62, 129.32, 130.19, 131.23, 131.99, 133.35, 134.25, 137.84, 146.45, 159.96, 162.44, 165.99, 166.74; ESI-MS: *m/z* 398 $[M+H]^+$. Anal.cacld for $C_{18}H_{13}N_7Cl_2$: C, 54.40; H, 3.27; N, 24.68 % Found: C, 54.42; H, 3.25; N, 24.65 %

Antibacterial activity

The antibacterial activity was done by broth dilution method[28] and expressed as minimum inhibitory concentration. The readymade nutrient broth medium (Himedia, 24 g) was suspended in distilled water (100 mL) and heated to boiling until it dissolved completely. The medium and test tubes were autoclaved at pressure of 15 lb/ inc^2 for 20 min. A set of sterilized test tubes with nutrient broth medium was capped with cotton plugs. The test compounds **5 and 6** were dissolved in suitable solvent (acetone) and concentration of 100 µg/ mL of test compound is added in the first test tube, which is serially diluted. A fixed volume of 0.5 mL overnight culture is added in all test tubes, and are incubated at 37°C for 24 h. After 24 h, these tubes were measured for turbidity. Bacterial strains used for the present investigation, *Bacillus subtilis* (MTCC 441), *Bacillus sphaericus* (MTCC 511), *Staphylococcus aureus* (MTCC 96), *Pseudomonas aeruginosa* (MTCC 741), *Klobsiella aerogenes* (MTCC 39) and *Chromobacterium violaceum* (MTCC 2656), were obtained from the Institute of Microbial Technology, Chandigarh.

Antifungal activity

The antifungal activity was done by using agar cup bioassay method[29]. The readymade potato dextrose agar (PDA) medium (Himedia, 39g) was suspended in distilled water (100 mL) and heated to boiling until it dissolved completely. The medium and petri-dishes were autoclaved at pressure of 15 lb/ inc^2 for 20 min.

The medium was poured in to sterile petri-dishes under aseptic conditions in a laminar flow chamber. When the medium in the plates solidified, 0.5 mL of (week old) culture of test organism was inoculated and uniformly spread over the agar surface with a sterile L-shaped rod. Solutions were prepared by dissolving test compounds **5/6** in acetone and different concentrations were made. Agar inoculated cups were scooped out with 6 mm sterile cork borer and the lids of the dishes were replaced. To each cup different concentrations of test solutions were added. Controls were maintained with acetone and *Fluconazole*. The treated and the controls were kept at room temperature for 72-96 h. The minimum inhibitory concentration (MIC) was recorded in µg/ml. Three to four replicates were maintained for each treatment. *Fusarium oxysporum*, *Verticillium dahliae*, *Alternaria solani*, *Rhizoctonia solani*, *Colletotrichum capsici* and *Pythium aphanidermatum* were used as fungal strains and procured from the Institute of Microbial Technology, Chandigarh.

Conclusion

In Conclusion, the synthesis of novel benzo[4,5]imidazo[1,2-*a*]pyrimido[4,5-*d*] pyrimidines has been achieved from readily accessible starting materials in good yields. The newly synthesized title compounds **5/6** have been evaluated for their *in vitro* antimicrobial activity. Compounds **5e, 5f, 5g and 6e, 6f, 6g** exhibited significant antimicrobial activity. Thus, they may be considered as future drug candidates and by doing a simple modification in the structure, a new potent analouge can be generated with desired activity and good efficacy.

Acknowledgments

The authors are thankful to the Head, Department of Chemistry, Kakatiya University, Warangal for providing necessary facilities, the Director, CSIR-Indian Institute of Chemical Technology, Hyderabad for recording spectra. The authors are grateful to Prof. M. V. Rajam, Department of Genetics, University of Delhi, South Campus, New Delhi for screening the Compounds for antimicrobial activity.

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