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

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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 chemistry¹ 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 chemistry⁴. 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 activity⁵⁻⁷. 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 bronchodilators⁸, antiallergic⁹, cardiogenic¹⁰, anti hypertensive¹¹ and anticancer¹² agents. Benzimidazole is an important nucleus that has been extensively used in medicinal chemistry, notable examples being the antihistaminic astemizole and the antiulcerative omeprazole¹³. Benzimidazoles are also known for their anti-inflammatory¹⁴, antibiotic¹⁵, anthelmintic¹⁶, anticancer¹⁷, and antiviral activities¹⁸.

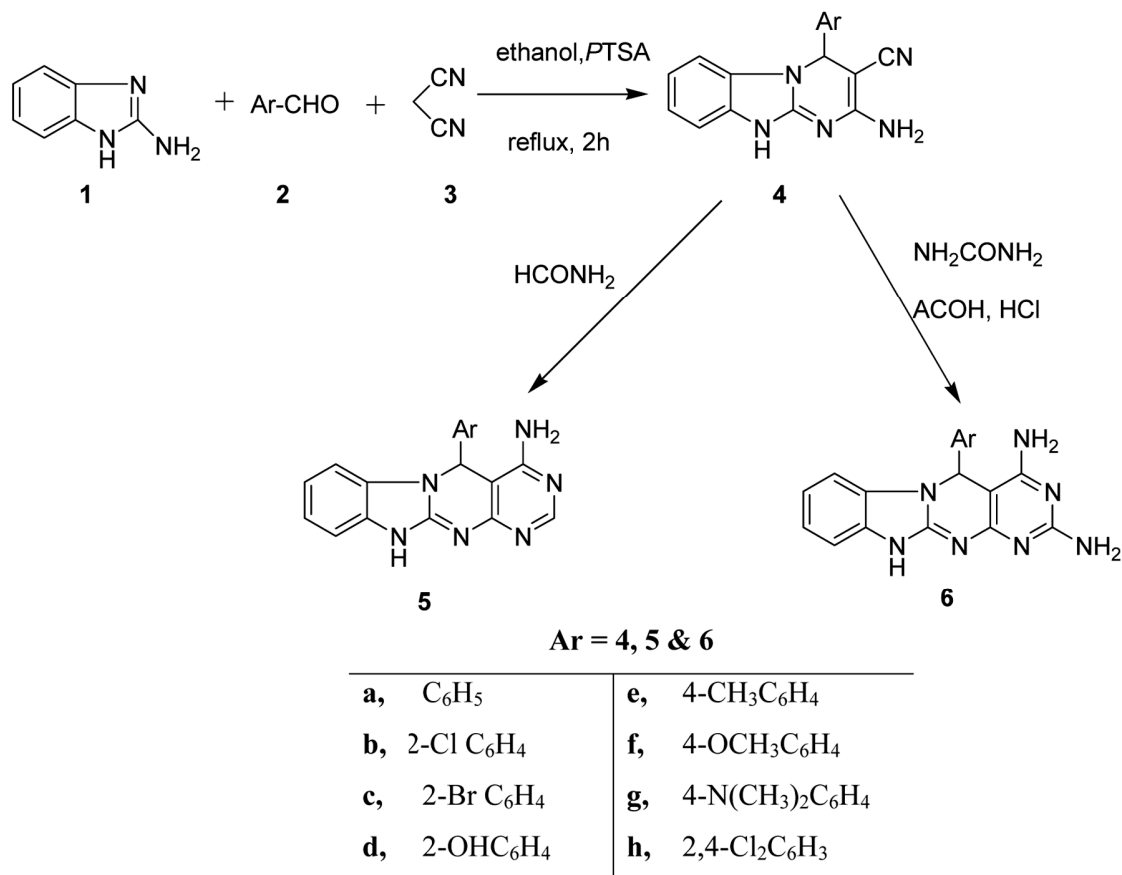
Molecular hybridization is a relatively new concept in the field of drug design, and development involving the fusion of two or more pharmacophoric groups which have an inhibitory effect against the target disease. The newly designed structure can lead to compounds having improved affinity and efficacies

than the parent compounds with reduced side effects, while retaining the desired characteristics of original template¹⁹⁻²¹. Prompted by these reports, and as a sequel to our interest in the synthesis of benzimidazole derivatives with potent biological activity²²⁻²⁵, 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 (PTSA), 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 literature²⁶. 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 derivatives²⁷.

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



Scheme I

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

IR spectrum of **4a** exhibited absorption bands at 3435, 3415 cm⁻¹ due to NH₂, where as CN absorption band was shown at 2215 cm⁻¹. In ¹H NMR spectrum of **4a** pyrimidine ring CH proton appeared as a sharp singlet at δ 5.13, NH₂ protons appeared at δ 8.25, which are D₂O exchangeable, whereas imidazole ring NH displayed as a singlet at δ 10.25, which is also D₂O exchangeable. Aromatic protons appeared as a complex multiplet between δ 7.00-7.83. ¹³C 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** NH₂ absorption bands appeared at 3388, 3305 cm⁻¹. ¹H 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. ¹³C 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, ¹H NMR, and ¹³C 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), *Klebsiella*

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.

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

Minimum Inhibitory Concentration in µg/ml (MIC)	
Gram + ve bacteria	Gram -ve bacteria
100	100
50	50
25	25
12.5	12.5
6.25	6.25
3.125	3.125
1.5625	1.5625
0.78125	0.78125
0.390625	0.390625
0.1953125	0.1953125
0.09765625	0.09765625
0.048828125	0.048828125
0.0244140625	0.0244140625
0.01220703125	0.01220703125
0.006103515625	0.006103515625
0.0030517578125	0.0030517578125
0.00152587890625	0.00152587890625
0.000762939453125	0.000762939453125
0.0003814697265625	0.0003814697265625
0.00019073486328125	0.00019073486328125
0.000095367431640625	0.000095367431640625
0.0000476837158203125	0.0000476837158203125
0.00002384185791015625	0.00002384185791015625
0.000011920928955078125	0.000011920928955078125
0.0000059604644775390625	0.0000059604644775390625
0.00000298023223876953125	0.00000298023223876953125
0.000001490116119384765625	0.000001490116119384765625
0.0000007450580596923828125	0.0000007450580596923828125
0.00000037252902984619140625	0.00000037252902984619140625
0.000000186264514923095703125	0.000000186264514923095703125
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0.0000000116415321826934814453125	0.0000000116415321826934814453125
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0.0000000000000055511151231257827021181583340541015625	0.0000000000000055511151231257827021181583340541015625

Negative control (acetone) – No activity
Values are indicated in $\mu\text{g/mL}$

Gram + ve bacteria	Gram -ve bacteria
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Negative control (acetone) – No activity
Values are indicated in $\mu\text{g/mL}$

their antifungal activity against *Fusarium oxysporum*, *Verticillium dahliae*, *Alternaria solani*, *Rhizoctonia solani*, *Colletotrichum capsici* and *Pythium aphanidermatum* in acetone by agar cup bioassay method²⁹, 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 F₂₅₄ 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. ¹H NMR spectra were recorded on a Varian Gemini 300 MHz spectrometer. ¹³C 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	Ar	Minimum inhibitory Concentration in µg/mL(MIC)					
		<i>F.oxysporum</i>	<i>V. dahliae</i>	<i>A. solani</i>	<i>R. solani</i>	<i>C. capsici</i>	<i>P. aphanidermatum</i>
5a	C ₆ H ₅	13	15	11	12	15	16
5b	2-ClC ₆ H ₄	16	15	14	17	19	20
5c	2-BrC ₆ H ₄	15	19	18	15	18	17
5d	2-OHC ₆ H ₄	17	16	18	12	18	20
5e	4-CH ₃ C ₆ H ₄	8	7	7	9	8	9
5f	4-OCH ₃ C ₆ H ₄	8	7	8	9	8	9
5g	4-N(CH ₃) ₂ C ₆ H ₄	6	7	6	8	9	6
5h	2,4-Cl ₂ C ₆ H ₃	11	12	13	14	13	14
<i>Fluconazole</i>	---	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	Ar	Minimum inhibitory concentration in µg/mL(MIC)					
		<i>F.oxysporum</i>	<i>V. dahliae</i>	<i>A. solani</i>	<i>R. solani</i>	<i>C. capsici</i>	<i>P. aphanidermatum</i>
6a	C ₆ H ₅	12	14	12	12	14	15
6b	2-ClC ₆ H ₄	16	14	15	16	18	20
6c	2-BrC ₆ H ₄	14	18	18	16	17	17
6d	2-OHC ₆ H ₄	10	11	14	12	12	13
6e	4-CH ₃ C ₆ H ₄	8	8	7	8	8	9
6f	4-OCH ₃ C ₆ H ₄	8	6	9	8	8	8
6g	4-N(CH ₃) ₂ C ₆ H ₄	8	7	8	6	9	8
6h	2,4-Cl ₂ C ₆ H ₃	10	11	14	12	12	13
<i>Fluconazole</i>	--	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 (NH₂), 2215 (CN) cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 5.13 (s, 1H, CH), 7.00-7.83 (m, 9H), 8.25 (s, 2H, NH₂, D₂O exchangeable), 10.25 (s, 1NH, D₂O exchangeable); ¹³C NMR (75MHz, CDCl₃): 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.calcd for C₁₇H₁₃N₅: 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 (NH₂), 2225 (CN) cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 5.16 (s, 1H, CH), 7.05-7.89 (m, 8H), 8.28 (s, 2H, NH₂, D₂O exchangeable), 10.28 (s, 1NH, D₂O exchangeable); ¹³C NMR (75MHz, CDCl₃): 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.calcd for C₁₇H₁₂N₅Cl: 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 (NH₂), 2222 (CN) cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 5.15 (s, 1H, CH), 7.03-7.85 (m, 8H), 8.26 (s, 2H, NH₂, D₂O exchangeable), 10.24 (s, 1NH, D₂O exchangeable); ¹³C NMR (75MHz, CDCl₃): 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.calcd for C₁₇H₁₂N₅Br: 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 (NH₂), 2227 (CN) cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 5.17 (s, 1H, CH), 7.10-7.99 (m, 8H), 8.30 (s, 2H, NH₂, D₂O exchangeable), 8.56 (s, 1H, OH, D₂O exchangeable), 10.29 (s, 1NH, D₂O exchangeable); ¹³C NMR (75MHz, CDCl₃): 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.calcd for C₁₇H₁₃N₅O: 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 (NH₂), 2223 (CN) cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 2.35 (s, 3H, CH₃), 5.17 (s, 1H, CH), 7.06-7.92 (m, 8H), 8.25 (s, 2H, NH₂, D₂O exchangeable), 10.26 (s, 1NH, D₂O exchangeable); ¹³C NMR (75MHz, CDCl₃): 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.calcd for C₁₈H₁₅N₅: 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 (NH₂), 2228 (CN) cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 3.78 (s, 3H, OCH₃), 5.19 (s, 1H, CH), 7.09-7.95 (m, 8H), 8.28 (s, 2H, NH₂, D₂O exchangeable), 10.28 (s, 1NH, D₂O exchangeable); ¹³C NMR (75MHz, CDCl₃): 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.calcd for C₁₈H₁₅N₅O: 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 (NH₂), 2230 (CN) cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 2.80 (s, 6H, N(CH₃)₂), 5.21 (s, 1H, CH), 7.10-7.90 (m, 8H), 8.30 (s, 2H, NH₂, D₂O exchangeable), 10.25 (s, 1NH, D₂O

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 $[\text{M}+\text{H}]^+$. Anal. calcd for $\text{C}_{19}\text{H}_{18}\text{N}_6$: C, 69.09; H, 5.45; N, 25.45 % Found: C, 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} ; ^1H 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 $[\text{M}+\text{H}]^+$. Anal. calcd 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} ; ^1H 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, $\text{N}=\text{CH}$), 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 $[\text{M}+\text{H}]^+$. Anal. calcd for $\text{C}_{18}\text{H}_{14}\text{N}_6$: 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} ; ^1H 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, $\text{N}=\text{CH}$), 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 $[\text{M}+\text{H}]^+$. Anal. calcd for $\text{C}_{18}\text{H}_{13}\text{N}_6\text{Cl}$: 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} ; ^1H 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, $\text{N}=\text{CH}$), 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 $[\text{M}+\text{H}]^+$. Anal. calcd for $\text{C}_{18}\text{H}_{13}\text{N}_6\text{Br}$: 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} ; ^1H 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, $\text{N}=\text{CH}$), 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 $[\text{M}+\text{H}]^+$. Anal. calcd for $\text{C}_{18}\text{H}_{14}\text{N}_6\text{O}$: 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} ; ^1H 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, $\text{N}=\text{CH}$), 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 $[\text{M}+\text{H}]^+$. Anal. calcd for $\text{C}_{19}\text{H}_{16}\text{N}_6$: 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 (NH₂), cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 3.68 (s, 3H, OCH₃), 5.29 (s, 1H, CH), 7.09-8.05 (m, 8H), 7.98 (s, 2H, NH₂, D₂O exchangeable), 8.52 (s, 1H, N=CH), 10.24 (s, 1NH, D₂O exchangeable); ¹³C NMR (75MHz, CDCl₃): 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.caclcd for C₁₉H₁₆N₆O: 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 (NH₂), cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 2.45 (s, 6H, (NH₃)₂), 5.28 (s, 1H, CH), 7.05-8.01 (m, 8H), 7.98 (s, 2H, NH₂, D₂O exchangeable), 8.50 (s, 1H, N=CH), 10.22 (s, 1NH, D₂O exchangeable); ¹³C NMR (75MHz, CDCl₃): 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.caclcd for C₂₀H₁₉N₇: 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 (NH₂), cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 5.27 (s, 1H, CH), 7.06-8.10 (m, 7H), 7.99 (s, 2H, NH₂, D₂O exchangeable), 8.59 (s, 1H, N=CH), 10.29 (s, 1NH, D₂O exchangeable); ¹³C NMR (75MHz, CDCl₃): 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.caclcd for C₁₈H₁₂N₆Cl₂: 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 (2NH₂), cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 5.25 (s, 1H, CH), 7.01-7.95 (m, 9H), 7.43 (s, 2H, NH₂, D₂O exchangeable), 7.91 (s, 2H, NH₂, D₂O exchangeable), 10.21 (s, 1NH, D₂O exchangeable); ¹³C NMR (75MHz, CDCl₃): 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.caclcd for C₁₈H₁₅N₇: 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 (2NH₂), cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 5.28 (s, 1H, CH), 7.01-7.99 (m, 8H), 7.46 (s, 2H, NH₂, D₂O exchangeable), 7.95 (s, 2H, NH₂, D₂O exchangeable), 10.26 (s, 1NH, D₂O exchangeable); ¹³C NMR (75MHz, CDCl₃): 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.caclcd for C₁₈H₁₄N₇ 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 (2NH₂), cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 5.22 (s, 1H, CH), 6.98-7.90 (m, 8H), 7.42 (s, 2H, NH₂, D₂O exchangeable), 7.90 (s, 2H, NH₂, D₂O exchangeable), 10.20 (s, 1NH, D₂O exchangeable); ¹³C NMR (75MHz, CDCl₃): 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.caclcd for C₁₈H₁₄N₇ 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 (2NH₂), 3436 (OH) cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 5.29 (s, 1H, CH), 7.01-7.99

(m, 8H), 7.43 (s, 2H, NH₂, D₂O exchangeable), 7.91 (s, 2H, NH₂, D₂O exchangeable), 8.55 (s, 1H, OH, D₂O exchangeable), 10.22 (s, 1NH, D₂O exchangeable); ¹³C NMR (75MHz, CDCl₃): 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. calcd for C₁₈H₁₅N₇O: 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 (2NH₂), cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 2.35 (s, 3H, CH₃), 5.27 (s, 1H, CH), 6.99-7.97 (m, 8H), 7.46 (s, 2H, NH₂, D₂O exchangeable), 7.99 (s, 2H, NH₂, D₂O exchangeable), 10.26 (s, 1NH, D₂O exchangeable); ¹³C NMR (75MHz, CDCl₃): 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. calcd for C₁₉H₁₇N₇: 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 (2NH₂), cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 3.68 (s, 3H, OCH₃), 5.26 (s, 1H, CH), 6.98-7.90 (m, 8H), 7.45 (s, 2H, NH₂, D₂O exchangeable), 7.94 (s, 2H, NH₂, D₂O exchangeable), 10.24 (s, 1NH, D₂O exchangeable); ¹³C NMR (75MHz, CDCl₃): 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. calcd for C₁₉H₁₇N₇O: 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 (2NH₂), cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 2.36 (s, 6H, N(CH₃)₂), 5.27 (s, 1H, CH), 7.01-7.93 (m, 8H), 7.45 (s, 2H, NH₂, D₂O exchangeable), 7.96 (s, 2H, NH₂, D₂O exchangeable), 10.27 (s, 1NH, D₂O exchangeable); ¹³C NMR (75MHz, CDCl₃): 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. calcd for C₂₀H₂₀N₈: 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 (2NH₂), cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 5.29 (s, 1H, CH), 7.03-7.99 (m, 7H), 7.49 (s, 2H, NH₂, D₂O exchangeable), 7.98 (s, 2H, NH₂, D₂O exchangeable), 10.29 (s, 1NH, D₂O exchangeable); ¹³C NMR (75MHz, CDCl₃): 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. calcd for C₁₈H₁₃N₇Cl₂: 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 method²⁸ 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/ inc² 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 method²⁹. 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/ inc² 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 analogue can be generated with desired activity and good efficacy.

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