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Synthesis and antimicrobial activity of novel benzo[4', 5']-imidazo[1',2':1,2]pyrrolo[3,4-*b*]isoxazolo[4,5-*e*]pyridines

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Synthesis of novel benzo [4',5'] imidazo [1',2':1,2] pyrrolo [3,4-*b*] isoxazolo [4,5-*e*] pyridines **8** has been achieved by reaction of 1-(prop-2-yn-1-yl)-1*H*-benzo [*d*] imidazo-2-carbaldehydes **5** with 5-amino -3-methylisoxazole **6** in presence of $InCl_3$ in CH_3CN media, followed by reaction of imine **7** with $BF_3.Et_2O$ in DCE at 80°C. The newly synthesized title compounds **8** have been evaluated for their *in vitro* antimicrobial activity. Compounds **8** exhibit potent antimicrobial activity compared to that of standard drugs.

Keywords: 1-(Prop-2-yn-1-yl)-1*H*-benzo[*d*]imidazo-2-carbaldehydes, condensation, intramolecular Povarov reaction, benzo[4',5']imidazo[1',2':1,2]pyrrolo[3,4-*b*]isoxazolo[4,5-*e*] pyridines, antimicrobial activity

Polycyclic nitrogen-containing heterocycles form the basic skeleton of numerous alkaloids and physiologically active compounds[1]. Benzimidazole is an important nucleus that has been extensively used in medicinal chemistry. They are found to possess antihistaminic and anti- ulcerative[2], anti-inflammatory[3], antibiotic[4], antihelmentic[5], anticancer[6] and antiviral[7] activities. Similarly, isoxazole derivatives have been reported with diverse structural features and versatile biological properties such as antitumor[8], CNS-active[9], analgesic[10], antimicrobial[11], muscular relaxant[12] activities, and are also used for the treatment of hypercholesterolemia and hyperlipidemia[13]. The present study is aimed at investigating the effect of polycyclic nitrogen-containing heterocycles on the anticipated antimicrobial activities. As a sequel to our work on the synthesis and biological activity of fused benzimidazole derivatives[14-20], we herein, report the synthesis and antimicrobial evaluation of novel benzo[4',5']imidazo [1',2':1,2]pyrrolo[3,4-*b*] isoxazolo[4,5-*e*] pyridines.

Results and Discussion

The synthesis of title compounds was accomplished by synthetic sequence shown in Scheme I. Condensation of *o*-diaminoarene **1** with ethyl diethyloxyacetate **2** in presence of sodium ethoxide afforded the 2-benzimidazole acetal **3**, which on further treatment with propargyl bromide in presence of sodium hydride in refluxing dry tetrahydrofuran furnished the *N*-propargyl derivative **4**. Acid-catalyzed acetal deprotection of **4** with HCl in THF yielded the carbaldehyde **5** according to literature procedure[21].

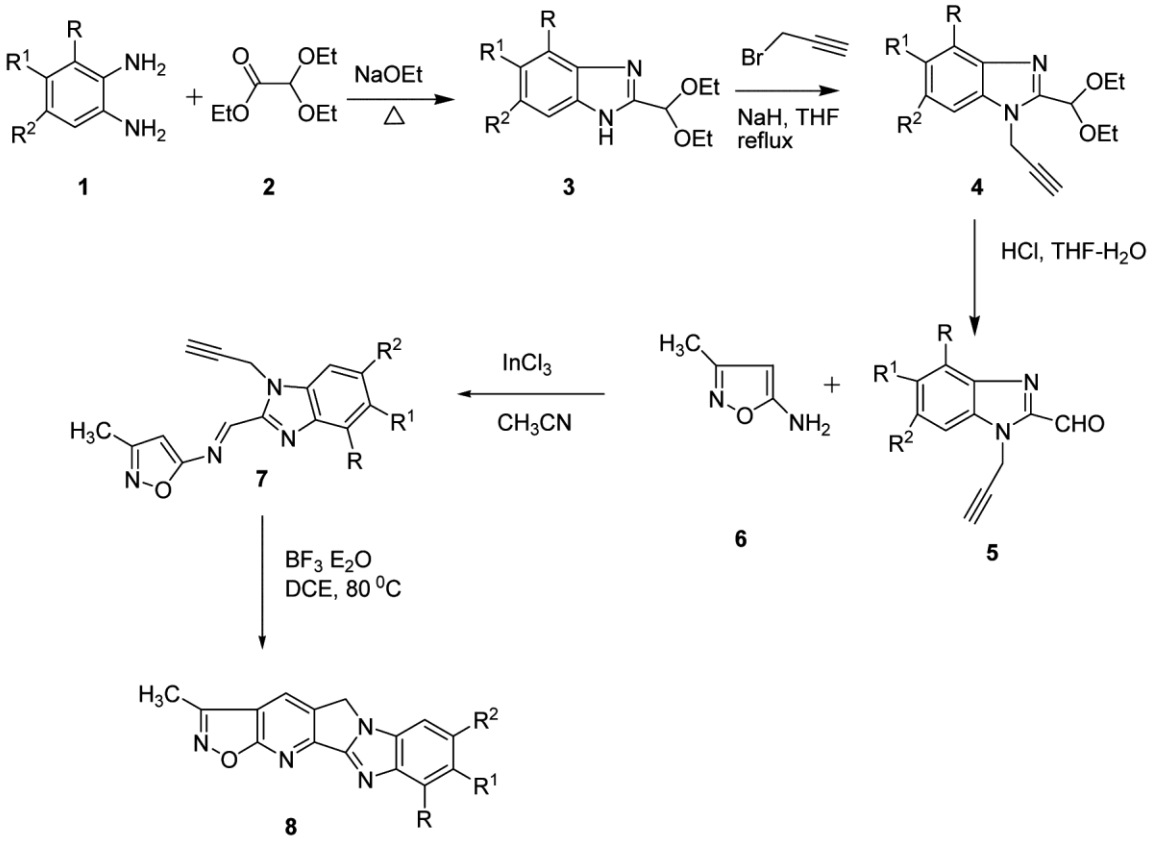
With *N*-propargyl benzimidazole carbaldehydes **5**, inhand, we studied the construction of the target compounds **8** by the reaction of carbaldehydes **5** with isoxazole amine **6** in presence of $InCl_3$ in CH_3CN solvent. The reaction was carried out at RT with stirring. The resulting imines **7** were then cyclized to benzo[4',5']imidazo[1',2':1,2]pyrrolo[3,4-*b*]isoxazolo-[4,5-*e*] pyridines **8** in good yields, in presence of boron triflouride etherate in dichloroethane solvent at 80°C, by intramolecular Povarov reaction, involving formal [4+2] cycloaddition. The structure of compounds **3, 4, 5, 7** and **8** were fully characterized by IR, ^{1}H and ^{13}C NMR and mass spectra and elemental analyses.

The ^{1}H NMR spectrum of compound **7a** exhibited propargyl proton as a singlet at δ 2.05, and methylene proton displayed as singlet at δ 5.30. The azomethine proton appeared as a singlet at δ 8.50. The ^{13}C NMR spectrum of **7a** displayed azomethine carbon at δ 163.7 confirming condensation. The mass spectrum of **7a** displayed the molecular ion $[M+H]^+$ peak at *m/z* 265.

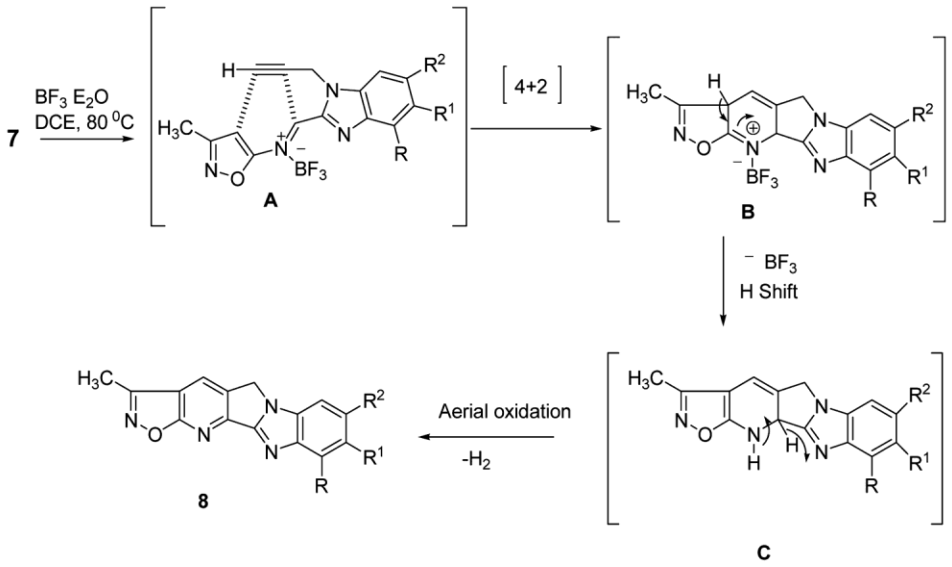
The ^{1}H NMR spectrum of compound **8a** exhibited a sharp singlet at δ 5.40 assignable to methylene protons, whereas isoxazole methyl protons resonated at δ 2.35 as a singlet. Isoxazolo- pyridine proton appeared as a singlet at δ 7.95, and rest of the aromatic protons resonated between δ 7.06 - 8.01 as a complex multiplet.

In the ^{13}C NMR spectrum of **8a**, methyl and methylene carbons appeared at δ 16.5 and δ 51.9 respectively. The isoxazolopyridine carbon appeared at δ 133.65. The mass spectrum of **8a** displayed the molecular ion $[M+H]^+$ peak at *m/z* 263. Data from the elemental analyses further confirmed the assigned structures of **7** and **8**.

A plausible mechanism for the formation of compounds **8** is depicted in Scheme II. The arylimine **7** arising from benzimidazole carbaldehyde **5** and



Scheme I



Scheme II — Plausible mechanism for the formation of 3-methyl-5*H*- benzo[4',5']imidazo[1',2':1,2]pyrrolo[3,4-*b*]isoxazolo[4,5-*e*]pyridines **8**

isoxazole amine **6**, underwent intramolecular formal [4+2] cycloaddition involving Povarov reaction to produce a dihydroquinoline derivative **C** *via* **B**, which on aerial oxidation furnished the title compounds **8**.

Antimicrobial activity

Antibacterial activity

The newly synthesized benzo[4',5']imidazo[1',2':1, 2]pyrrolo[3,4-*b*]isoxazolo[4,5-*e*]- pyridines **8a-h,** were evaluated for their *in vitro* antibacterial activity against three Gram-positive bacteria *viz*., *Bacillus subtilis* (MTCC 441), *Bacillus sphaericus* (MTCC 511) and *Staphylococcus aureus* (MTCC 96) and three Gram-negative bacteria *viz*., *Pseudomonas aeruginosa* (MTCC 741), *Klobsinella aerogenes* (MTCC 39) and *Chromobacterium violaceum* (MTCC 2656) at 100 µg/mL concentration. The activity was assessed by minimum inhibitory concentration (MIC) using broth dilution method[22]. *Ciprofloxacin* was used as standard drug for comparison.

The antibacterial activity results shown that compounds **8a-h** displayed a better activity and were more active than the standard drug *Ciprofloxacin* (Table I). The activity was expressed in minimum inhibitory concentration (MIC). The compounds **8d, 8e** and **8h,** are highly active, because the activity is considerably affected by the presence of methyl and methoxy groups as substituents on benzimidazole ring, besides the presence of basic skeleton. Compounds **8b**, **8c, 8f** and **8g** carrying nitro, chloro and bromo substitutions on benzimidazole ring have exhibited moderate activity. Compound **8a** has shown least activity, as it has not possessed any substitutents on benzimidazole ring.

In conclusion, the antibacterial activity of compounds **8d, 8e** and **8h** is promising when compared to standard drug *Ciprofloxacin*, and they can be selected as bactericides after structure-activity studies.

Antifungal activity

Compounds **8a-h** have been evaluated for their in-vitro antifungal activity against six fungal organisms *viz*., *Fusarium oxysporum*, *Verticillium dahliae*, *Alternaria solani*, *Rhizoctonia solani*, *Colletotrichum capsici* and *Pythium aphanidermatum* by agar cup bioassay method[23] at 100 µg/mL concentration.

Antifungal activity data (Table II) has revealed that compounds **8a-h** are highly toxic towards all the fungi under investigation. Compounds **8d, 8e** and **8h** have exhibited high antifungal activity by inhibiting the growth of fungi to a remarkable extent, when compared to the standard drug *Fluconazole*, it may be due to the presence of methoxy and methyl substituents on the benzimidazole ring, besides the presence of basic skeleton. Compounds **8b, 8c, 8f and 8g** are moderately active, may be due to the presence of nitro, chloro and bromo groups on benzimidazole ring. Compound **8a** has shown least activity.

In conclusion, the results indicated that compounds **8d, 8e** and **8h** are highly toxic towards the fungi under investigation and they are lethal even at 100 µg/mL concentrations in comparison with standard drug *Fluconazole* at the same concentration. They may be exploited for control of wilt diseases of different crops as fungicides after detailed study.

Experimental Section

Melting points have been determined on a Cintex melting point apparatus. TLC has been performed on Merck precoated 60 F_{254} silica gel plates. Visualization is done by exposing to iodine vapour.

Table I — Antibacterial activity of 3-methyl-5*H*-benzo[4',5']imidazo[1',2':1,2]pyrrolo[3,4-*b*]isoxazolo[4,5-*e*]pyridines **8a-h**

Compd				Minimum Inhibitory Concentration in µg/mL (MIC)					
				Gram + ve bacteria			Gram –ve bacteria		
	R	R^1	R^2	*B.subtilis*	*B.sphaericus*	*S.aureus*	*P.aeruginosa*	*K.aerogenes*	*C.violaceum*
8a	H	H	H	17	19	21	23	18	17
8b	NO_2	H	H	16	18	19	21	15	16
8c	H	NO_2	H	15	16	20	22	17	18
8d	OCH_3	H	H	7	8	9	11	7	6
8e	H	H	CH_3	8	9	11	13	6	7
8f	H	Br	H	16	15	17	20	13	14
8g	H	H	Cl	15	17	19	21	16	18
8h	H	H	OCH_3	6	8	10	12	7	9
Ciprofloxacin	–	–	–	20	22	26	25	20	22

Negative control (acetone) – No activity.

Table II — Antifungal activity of 3-methyl-5*H*-benzo[4',5']imidazo[1',2':1,2]pyrrolo [3,4-*b*]isoxazolo[4,5-*e*]pyridines 8a-h

Compd	R	R^1	R^2	Minimum Inhibitory Concentration in µg/mL (MIC)					
				F.oxysporum	*V. dahliae*	*A. solani*	*R. solani*	*C. capsici*	*P. aphanidermatum*
8a	H	H	H	15	14	18	15	17	21
8b	NO_2	H	H	13	12	16	13	16	20
8c	H	NO_2	H	14	13	15	14	15	19
8d	OCH_3	H	H	7	6	10	7	9	11
8e	H	H	CH_3	6	7	11	8	10	12
8f	H	Br	H	14	14	18	15	16	21
8g	H	H	Cl	15	13	14	13	17	18
8h	H	H	OCH_3	7	8	9	11	13	15
Fluconazole	–	–	–	16	16	20	16	18	22

Negative control (acetone) – No activity.

IR spectra (KBr pellet) have been recorded on a Perkin- Elmer BX series FT-IR spectrometer. ^{1}H NMR spectra are recorded on a Bruker 300 MHz spectrometer. ^{13}C NMR spectra are recorded on a Bruker 75 MHz spectrometer. Chemical shift values are given in δ (ppm) with tetramethyl silane as an internal standard. ESI -MS spectra are recorded on a Agilent LC-MSD mass spectrometer. Elemental analyses are performed on a Carlo Erba 106 and Perkin-Elmer model 240 analyzers.

General procedure for the synthesis of 2-(diethoxymethyl)-1*H*-benzo[*d*]imidazole, 3a-h

To a solution of sodium (5 mmol) in dry ethanol (10 mL) were added 1, 2-diaminobenzene (1 mmol) and ethyl diethoxyacetate (1 mmol). The mixture was refluxed for 24 h. and, allowed to cool to RT, and then the solvent was removed under reduced pressure. The residue obtained was dissolved in water, neutralized with acetic acid, and the contents are extracted with ethyl acetate. The separated organic layer was dried over anhy. sodium sulphate, and evaporated to dryness. The crude product obtained was recrystallized from ethylacetate-n-hexane.

2-(Diethoxymethyl)-1*H*-benzo[*d*]imidazole, 3a: Orange solid. Yield 78%. m.p. 120-122°C; IR (KBr): 3345 cm^{-1} (NH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.35 (t, 6H,CH_3), 3.85 (q, 4H, CH_2), 5.58 (s, 1H, CH), 7.03-7.86 (m, 4H, Ar-H), 10.15 (bs, 1H, benzimidazole-NH, D_2O exchangeable); ESI-MS: *m/z* 221 $[M+H]^+$. Anal. Calcd for $C_{12}H_{16}N_2O_2$: C, 65.45; H, 7.27; N, 12.72. Found: C, 65.42; H, 7.24; N, 12.75%.

2-(Diethoxymethyl)-4-nitro-1*H*-benzo[*d*]imidazole, 3b: Orange solid. Yield 68%. m.p. 148-150°C; IR (KBr): 3342 cm^{-1} (NH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.37 (t, 6H,CH_3), 3.88 (q, 4H, CH_2), 5.68 (s, 1H, CH), 7.08-7.89 (m, 3H, Ar-H), 10.17 (bs, 1H, benzimidazole-NH, D_2O exchangeable); ^{13}C NMR (75MHz, $CDCl_3$): δ 16.27, 17.20, 64.14, 65.56, 97.90, 116.81, 123.14, 125.01, 132.25, 135.10, 139.21, 143.16; ESI-MS: *m/z* 266 $[M+H]^+$. Anal. Calcd for $C_{12}H_{15}N_3O_4$: C, 54.33; H, 5.66; N, 15.84. Found: C, 54.36; H, 5.64; N, 15.81%.

2-(Diethoxymethyl)-5-nitro-1*H*-benzo[*d*]imidazole, 3c: Orange solid. Yield 66%. m.p. 156-158°C; IR (KBr): 3348 cm^{-1} (NH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.39 (t, 6H,CH_3), 3.79 (q, 4H, CH_2), 5.66 (s, 1H, CH), 7.10-7.91 (m, 3H, Ar-H), 10.19 (bs, 1H, benzimidazole-NH, D_2O exchangeable); ESI-MS: *m/z* 266 $[M+H]^+$. Anal. Calcd $C_{12}H_{15}N_3O_4$: C, 54.33; H, 5.66; N, 15.84. Found: C, 54.30; H, 5.69; N, 15.87%.

2-(Diethoxymethyl)-4-methoxy-1*H*-benzo[*d*]imidazole, 3d: Orange solid. Yield 80%. m.p. 136-138°C; IR (KBr): 3350 cm^{-1} (NH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.40 (t, 6H, CH_3), 3.68 (s, 3H, OCH_3), 3.81 (q, 4H, CH_2) 5.61 (s, 1H, CH), 7.12-7.90 (m, 3H, Ar-H), 10.18 (bs, 1H, benzimidazole-NH, D_2O exchangeable); ESI-MS: *m/z* 251 $[M+H]^+$. Anal. Calcd for $C_{13}H_{18}N_2O_3$: C, 62.40; H, 7.20; N, 11.20. Found: 62.44; H, 7.23; N, 11.23%.

2-(Diethoxymethyl)-6-methyl-1*H*-benzo[*d*]-imidazole, 3e: Orange solid. Yield 82%. m.p. 130-132°C; IR (KBr): 3343 cm^{-1} (NH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.38 (t, 6H, CH_3), 2,53 (s, 3H, CH_3), 3.86 (q, 4H, CH_2), 5.67 (s, 1H, CH), 7.07-7.81 (m, 3H, Ar-H), 10.15 (bs, 1H, benzimidazole-NH, D_2O exchangeable); ESI-MS: *m/z* 235 $[M+H]^+$. Anal. Calcd for $C_{13}H_{18}N_2O_2$: C, 66.66; H, 7.69; N, 11.96. Found: 66.63; H, 7.67; N, 11.99%.

5-Bromo-2-(diethoxymethyl)-1*H*-benzo[*d*]imidazole, 3f: Brown solid. Yield 65%. m.p. 162-164°C; IR (KBr): 3352 cm^{-1} (NH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.41 (t, 6H, CH_3), 3.80 (q, 4H, CH_2), 5.63 (s, 1H,

CH), 7.10-7.91 (m, 3H, Ar-H), 10.17 (bs, 1H, benzimidazole-NH, D_2O exchangeable); ESI-MS: *m/z* 300 $[M+H]^+$. Anal. Calcd for $C_{12}H_{15}BrN_2O_2$: C, 48.16; H, 5.01; N, 9.36. Found: 48.13; H, 5.04; N, 9.33%.

6-Chloro-2-(diethoxymethyl)-1*H*-benzo[*d*]imidazole, 3g: Orange solid. Yield 69%. m.p. 140-142°C; IR (KBr): 3356 cm^{-1} (NH); ^1H NMR (300 MHz, $CDCl_3$): δ 1.43 (t, 6H, CH_3), 3.72 (q, 4H, CH_2), 5.64 (s, 1H, CH), 7.10-7.95 (m, 3H, Ar-H), 10.19 (bs, 1H, benzimidazole-NH, D_2O exchangeable); ESI-MS: *m/z* 255 $[M+H]^+$. Anal. Calcd for $C_{12}H_{15}ClN_2O_2$: C, 56.69; H, 5.90; N, 11.02. Found: 56.66; H, 5.93; N, 11.05%.

2-(Diethoxymethyl)-6-methoxy-1*H*-benzo[*d*]-imidazole, 3h: Orange solid. Yield 84%. m.p. 125-127°C; IR (KBr): 3351 cm^{-1} (NH); ^1H NMR (300 MHz, $CDCl_3$): δ 1.41 (t, 6H,CH_3), 3.69 (s, 3H, OCH_3), 3.80 (q, 4H, CH_2), 5.61 (s, 1H, CH), 7.12-7.95 (m, 3H, Ar-H), 10.19 (bs, 1H, benzimidazole-NH, D_2O exchangeable); ESI-MS: *m/z* 251 $[M+H]^+$. Anal. Calcd for $C_{13}H_{18}N_2O_3$: C, 62.40; H, 7.20; N, 11.20. Found: 62.38; H, 7.19; N, 11.22%.

General procedure for the synthesis of 2-(diethoxymethyl)-1-(prop-2-ynyl)-1*H*-benzo[*d*]imidazoles, 4a-h

2-(Diethoxymethyl)-1*H*-benzo[*d*]imidazole **3** (1.0 mmol) was added to NaH (2 mmol) in dry THF (20 mL) and the contents are refluxed for 30 min. To this propargyl bromide (1 mmol) was added and the resulting solution was refluxed for 6h. The reaction was monitored by TLC. After completion of the reaction, the reaction mixture was cooled to RT and water (50 mL) was added. The solution was extracted with diethyl ether (2×50 mL) and the organic phase was dried with $MgSO_4$, filtered, and evaporated to dryness to yield the desired product. The product was recrystallized from ethanol to yield the *N*-alkylated benzimidazole **4**.

2-(Diethoxymethyl)-1-(prop-2-ynyl)-1*H*-benzo-[*d*]imidazole, 4a: Orange solid. Yield 76%. m.p. 144-146°C; IR (KBr): 2185 cm^{-1} (C≡C); ^1H NMR (300 MHz, $CDCl_3$): δ 1.48 (t, 6H, CH_3), 2.12 (s, 1H, ≡CH), 3.67 (q, 4H, CH_2), 4.97 (s, 2H, CH_2) 5.65 (s, 1H, CH), 7.09-7.86 (m, 4H, Ar-H); ESI-MS: *m/z* 259 $[M+H]^+$. Anal. Calcd for $C_{15}H_{18}N_2O_2$: C, 69.76; H, 6.97; N, 10.85. Found: C, 69.73; H, 6.95; N, 10.81%.

2-(Diethoxymethyl)-4-nitro-1-(prop-2-ynyl)-1*H*-benzo[*d*]imidazole, 4b: Orange solid. Yield 69%. m.p. 178-180°C; IR (KBr): 2188 cm^{-1} (C≡C); ^1H NMR (300 MHz, $CDCl_3$): δ 1.49 (t, 6H, CH_3), 2.15 (s, 1H, ≡CH), 3.69 (q, 4H, CH_2), 5.02 (s, 2H, CH_2), 5.68 (s, 1H, CH), 7.11-7.90 (m, 3H, Ar-H); ^{13}C NMR (75MHz, $CDCl_3$): δ 15.81, 16.86, 33.02, 61.81, 62.76, 71.05, 78.95, 90.12, 116.91, 124.02, 125.85, 133.56, 135.05, 139.18, 141.58; ESI-MS: *m/z* 304 $[M+H]^+$. Anal. Calcd for $C_{15}H_{17}N_3O_4$: C, 59.40; H, 5.61; N, 13.86. Found: C, 59.44; H, 5.63; N, 13.84%.

2-(Diethoxymethyl)-5-nitro-1-(prop-2-ynyl)-1*H*-benzo[*d*]imidazole, 4c: Orange solid. Yield 64%. m.p. 185-187°C; IR (KBr): 2190 cm^{-1} (C≡C); ^1H NMR (300 MHz, $CDCl_3$): δ 1.50 (t, 6H, CH_3), 2.17 (s, 1H, ≡CH), 3.60 (q, 4H, CH_2), 5.00 (s, 2H, CH_2), 5.60 (s, 1H, CH), 7.15-7.92 (m, 3H, Ar-H); ESI-MS: *m/z* 304 $[M+H]^+$. Anal. Calcd for $C_{15}H_{17}N_3O_4$: C, 59.40; H, 5.61; N, 13.86. Found: C, 59.38; H, 5.60; N, 13.89%.

2-(Diethoxymethyl)-4-methoxy-1-(prop-2-ynyl)-1*H*-benzo[*d*]imidazole, 4d: Orange solid. Yield 77%. m.p. 142-144°C; IR (KBr): 1187 (C-O-C), 1626 (C=N), 2189 cm^{-1} (C≡C); ^1H NMR (300 MHz, $CDCl_3$): δ 1.51 (t, 6H, CH_3), 2.19 (s, 1H, ≡CH), 3.68 (s, 3H, OCH_3), 3.89 (q, 4H, CH_2), 5.02 (s, 2H, CH_2), 5.68 (s, 1H, CH), 7.11-7.91 (m, 3H, Ar-H); ESI-MS: *m/z* 289 $[M+H]^+$. Anal. Calcd for $C_{16}H_{20}N_2O_3$: C, 66.66; H, 6.94; N, 9.72. Found: C, 66.63; H, 6.91; N, 9.75%.

2-(Diethoxymethyl)-6-methyl-1-(prop-2-ynyl)-1*H*-benzo[*d*]imidazole, 4e: Orange solid. Yield 78%. m.p. 155-157°C; IR (KBr): 2184 cm^{-1} (C≡C); ^1H NMR (300 MHz, $CDCl_3$): δ 1.49 (t, 6H, CH_3), 2.30 (s, 1H, ≡CH), 2.54 (s, 3H, CH_3), 3.66 (q, 4H, CH_2), 5.10 (s, 2H, CH_2), 5.65 (s, 1H, CH), 7.08-7.85 (m, 3H, Ar-H); ESI-MS: *m/z* 273 $[M+H]^+$. Anal. Calcd for $C_{16}H_{20}N_2O_2$: C, 70.58; H, 7.35; N, 10.29. Found: C, 70.55; H, 7.38; N, 10.26%.

5-Bromo-2-(diethoxymethyl)-1-(prop-2-ynyl)-1*H*-benzo[*d*]imidazole, 4f: Brown solid. Yield 64%. m.p. 185-187°C; IR (KBr): 2190 cm^{-1} (C≡C); ^1H NMR (300 MHz, $CDCl_3$): δ 1.52 (t, 6H, CH_3), 2.25 (s, 1H, ≡CH), 3.60 (q, 4H, CH_2), 5.12 (s, 2H, CH_2), 5.60 (s, 1H, CH), 7.12-7.98 (m, 3H, Ar-H); ESI-MS: *m/z* 338 $[M+H]^+$. Anal. Calcd for $C_{15}H_{17}BrN_2O_2$: C, 53.41; H, 5.04; N, 8.30. Found: C, 53.44; H, 5.01; N, 8.33%.

6-Chloro-2-(diethoxymethyl)-1-(prop-2-ynyl)-1*H*-benzo[*d*]imidazole, 4g: Orange solid. Yield 66%. m.p. 164-166°C; IR (KBr): 2190 cm^{-1} (C≡C); ^1H NMR (300 MHz, $CDCl_3$): δ 1.52 (t, 6H, CH_3), 2.15 (s, 1H, ≡CH), 3.60 (q, 4H, CH_2), 5.08 (s, 2H, CH_2), 5.62 (s, 1H, CH), 7.10-7.96 (m, 3H, Ar-H); ESI-MS: *m/z* 293 $[M+H]^+$. Anal. Calcd for $C_{15}H_{17}ClN_2O_2$: C,

61.64; H, 5.82; N, 9.58. Found: 61.67; H, 5.85; N, 9.55%.

2-(Diethoxymethyl)-6-methoxy-1-(prop-2-ynyl)-1*H*-benzo[*d*]imidazole, 4h: Orange solid. Yield 82%. m.p. 137-139°C; IR (KBr): 2190 cm^{-1} (C≡C); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.50 (t, 6H, CH_3), 2.11 (s, 1H, ≡CH), 3.65 (s, 3H, OCH_3), 3.76 (q, 4H, CH_2), 5.03 (s, 2H, CH_2), 5.67 (s, 1H, CH), 7.11-7.94 (m, 3H, Ar-H); ESI-MS: *m/z* 289 $[M+H]^+$. Anal. Calcd for $C_{16}H_{20}N_2O_3$: C, 66.66; H, 6.94; N, 9.72. Found: C, 66.68; H, 6.97; N, 9.70%.

General procedure for the synthesis of 1-(prop-2-ynyl)-1*H*-benzo[*d*]imidazole-2-carbaldehyde, 5a

Water (10 mL) and 37% HCl (5 mL) were added to a THF (20 mL) solution of *N*-alkylated benzimidazole **4** (1 mmol) and the contents are refluxed for 12 h. The reaction mixture was then neutralized with saturated aq. $NaHCO_3$ and extracted with EtoAC (3×50 mL). The extract was washed with brine solution (2×25 mL), dried, filtered and evaporated. The resulting solid was purified by recrystallization from EtoAC to get benzimidazole carbaldehyde **5**.

1-(Prop-2-ynyl)-1*H*-benzo[*d*]imidazole-2-carbaldehyde, 5a: Orange solid. Yield 74%. m.p. 160-162°C; IR (KBr): 1718 (CO), 2190 cm^{-1} (C≡C); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.05 (s, 1H, ≡CH), 5.00 (s, 2H, CH_2), 7.06-7.86 (m, 4H, Ar-H), 9.80 (s, 1H, CHO); ESI-MS: *m/z* 185 $[M+H]^+$. Anal. Calcd for $C_{11}H_8N_2O$: C, 71.73; H, 4.34; N, 15.21. Found: C, 71.70; H, 4.37; N, 15.24%.

4-Nitro-1-(prop-2-ynyl)-1*H*-benzo[*d*]imidazole-2-carbaldehyde, 5b: Orange solid. Yield 66%. m.p. 188-190°C; IR (KBr): 1715 (CO), 2192 cm^{-1} (C≡C); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.20 (s, 1H, ≡CH), 5.10 (s, 2H, CH_2), 7.07-7.90 (m, 3H, Ar-H), 9.82 (s, 1H, CHO); ^{13}C NMR (75MHz, $CDCl_3$): δ 33.11, 72.56, 78.32, 119.81, 123.25, 125.11, 134.61, 138.78, 139.01, 143.25, 192.25; ESI-MS: *m/z* 230 $[M+H]^+$. Anal. Calcd for $C_{11}H_7N_3O_3$: C, 57.64; H, 3.05; N, 18.34. Found: C, 57.61; H, 3.08; N, 18.31%.

5-Nitro-1-(prop-2-ynyl)-1*H*-benzo[*d*]imidazole-2-carbaldehyde, 5c: Orange solid. Yield 63%. m.p. 200-202°C; IR (KBr): 1714 (CO), 2190 cm^{-1} (C≡C); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.25 (s, 1H, ≡CH), 5.11 (s, 2H, CH_2), 7.07-7.98 (m, 3H, Ar-H), 9.83 (s, 1H, CHO); ESI-MS: *m/z* 230 $[M+H]^+$. Anal. Calcd for $C_{11}H_7N_3O_3$: C, 57.64; H, 3.05; N, 18.34. Found: C, 57.67; H, 3.03; N, 18.37%.

4-Methoxy-1-(prop-2-ynyl)-1*H*-benzo[*d*]imidazole-2-carbaldehyde, 5d: Orange solid. Yield 76%. m.p. 155-157°C; IR (KBr): 1714 (CO), 2192 cm^{-1} (C≡C); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.05 (s, 1H, ≡CH), 3.89 (s, 3H, OCH_3), 5.18 (s, 2H, CH_2), 7.08-7.88 (m, 3H, Ar-H), 9.81 (s, 1H, CHO); ESI-MS: *m/z* 215 $[M+H]^+$. Anal. Calcd for $C_{12}H_{10}N_2O_2$: C, 67.28; H, 4.67; N, 13.08. Found: C, 67.24; H, 4.69; N, 13.05%.

6-Methyl-1-(prop-2-ynyl)-1*H*-benzo[*d*]imidazole-2-carbaldehyde, 5e: Orange solid. Yield 77%. m.p. 168-170°C; IR (KBr): 1719 (CO), 2187 cm^{-1} (C≡C); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.11 (s, 1H, ≡CH), 2.38 (s, 3H, CH_3), 5.20 (s, 2H, CH_2), 7.05-7.89 (m, 3H, Ar-H), 10.00 (s, 1H, CHO); ESI-MS: *m/z* 199 $[M+H]^+$. Anal. Calcd for $C_{12}H_{10}N_2O$: C, 72.72; H, 5.05; N, 14.14. Found: C, 72.75; H, 5.03; N, 14.17%.

5-Bromo-1-(prop-2-ynyl)-1*H*-benzo[*d*]imidazole-2-carbaldehyde, 5f: Brown solid. Yield 63%. m.p. 220-222°C; IR (KBr): 1715 (CO), 2195 cm^{-1} (C≡C); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.15 (s, 1H, ≡CH), 5.14 (s, 2H, CH_2), 7.08-7.90 (m, 3H, Ar-H), 9.82 (s, 1H, CHO); ESI-MS: *m/z* 264 $[M+H]^+$. Anal. Calcd for $C_{11}H_7BrN_2O$: C, 50.19; H, 2.66; N, 10.64. Found: C, 50.16; H, 2.64; N, 10.61%.

6-Chloro-1-(prop-2-ynyl)-1*H*-benzo[*d*]imidazole-2-carbaldehyde, 5g: Orange solid. Yield 67%. m.p. 175-177°C; IR (KBr): 1715 (CO), 2197 cm^{-1} (C≡C); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.12 (s, 1H, ≡CH), 5.10 (s, 2H, CH_2), 7.10-7.93 (m, 3H, Ar-H), 9.81 (s, 1H, CHO); ESI-MS: *m/z* 219 $[M+H]^+$. Anal. Calcd for $C_{11}H_7ClN_2O$: C, 60.55; H, 3.21; N, 12.84. Found: C, 60.53; H, 3.24; N, 12.87%.

6-Methoxy-1-(prop-2-ynyl)-1*H*-benzo[*d*]imidazole-2-carbaldehyde, 5h: Orange solid. Yield 80%. m.p. 148-150°C; IR (KBr): 1709 (CO), 2190 cm^{-1} (C≡C); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.20 (s, 1H, ≡CH), 3.68 (s, 3H, OCH_3), 5.15 (s, 2H, CH_2), 7.08-7.90 (m, 3H, Ar-H), 9.90 (s, 1H, CHO); ESI-MS: *m/z* 215 $[M+H]^+$. Anal. Calcd for $C_{12}H_{10}N_2O_2$: C, 67.28; H, 4.67; N, 13.08. Found: C, 67.25; H, 4.64; N, 13.10%.

General procedure for the synthesis of (*E*)-3-methyl-*N*-((1-(prop-2-ynyl)-1*H*-benzo[*d*]imidazol-2-yl)methylene)isoxazol-5-amine, 7a

Benzimidazole carbaldehyde **5** (1 mmol) and 5-amino-3-methylisoxazole **6** (1 mmol) were dissolved in dry acetonotrile (10 mL) and 10% of $InCl_3$ was added. The reaction mixture was stirred for 15 min, and the

resulting solid was filtered, and dried. Recrystallization was effected from benzene-ethylacetate.

(*E*)-3-methyl-*N*-((1-(prop-2-ynyl)-1*H*-benzo-[*d*]imidazol-2-yl)methylene)isoxazol-5-amine, 7a: Orange solid. Yield 77%. m.p. 170-172°C; IR (KBr): 1638 (C=N), 2198 cm^{-1} (C≡C); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.05 (s, 1H, ≡CH), 2.28 (s, 3H, CH_3), 5.30 (s, 2H, CH_2), 6.12 (s, 1H, isoxazole-CH), 7.11-7.92 (m, 4H, Ar-H), 8.50 (s, 1H, =CH); ^{13}C NMR (75MHz, $CDCl_3$): δ 18.26, 34.51, 74.29, 80.08, 95.76, 115.11, 116.23, 123.10, 124.01, 134.21, 138.21, 141.32, 159.32, 160.87, 163.51; ESI-MS: *m/z* 265 $[M+H]^+$. Anal. Calcd for $C_{15}H_{12}N_4O$: C, 68.18; H, 4.54; N, 21.21. Found: C, 68.14; H, 4.51; N, 21.24%.

(*E*)-3-methyl-*N*-((4-nitro-1-(prop-2-ynyl)-1*H*-benzo[*d*]imidazol-2-yl)methylene)isoxazol-5-amine, 7b: Orange solid. Yield 68%. m.p. 210-212°C; IR (KBr): 1640 (C=N), 2199 cm^{-1} (C≡C); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.15 (s, 1H, ≡CH), 2.30 (s, 3H, CH_3), 5.26 (s, 2H, CH_2), 6.16 (s,1H, isoxazole-CH), 7.15-7.95 (m, 3H, Ar-H), 8.62 (s, 1H, =CH); ^{13}C NMR (75MHz, $CDCl_3$): δ 18.31, 34.98, 74.88, 80.35, 95.16, 115.81, 116.68, 124.10, 124.95, 134.81, 138.65, 142.02, 159.65, 160.96, 163.69; ESI-MS: *m/z* 310 $[M+H]^+$. Anal. Calcd for $C_{15}H_{11}N_5O_3$: C, 58.25; H, 3.55; N, 22.87. Found: C, 58.21; H, 3.53; N, 22.84%.

(*E*)-3-methyl-*N*-((5-nitro-1-(prop-2-ynyl)-1*H*-benzo-[*d*]imidazol-2-yl)methylene)isoxazol-5-amine, 7c: Orange solid. Yield 69%. m.p. 220-222°C; IR (KBr): 1645 (C=N), 2200 cm^{-1} (C≡C); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.18 (s, 1H, ≡CH), 2.30 (s, 1H, CH_3), 5.27 (s, 2H, CH_2), 6.14 (s,1H, isoxazole-CH), 7.17-7.97 (m, 3H, Ar-H), 8.70 (s, 1H, =CH); ^{13}C NMR (75MHz, $CDCl_3$): δ 18.59, 35.08, 75.28, 80.85, 95.69, 116.11, 116.98, 124.69, 125.15, 135.21, 139.65, 143.02, 160.15, 161.66, 164.09 ESI-MS: *m/z* 310 $[M+H]^+$. Anal. Calcd for $C_{15}H_{11}N_5O_3$: C, 58.25; H, 3.55; N, 22.87. Found: C, 57.96; H, 3.51; N, 22.89%.

(E)-*N*-((4-methoxy-1-(prop-2-ynyl)-1*H*-benzo-[*d*]imidazol-2-yl)methylene)-3-methylisoxazol-5-amine, 7d: Orange solid. Yield 80%. m.p. 163-165°C; IR (KBr): 1639 (C=N), 2201 cm^{-1} (C≡C); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.25 (s, 1H, ≡CH), 2.35 (s, 3H, CH_3), 3.78 (s, 3H, OCH_3), 5.15 (s, 2H, CH_2), 6.17 (s, 1H, isoxazole-CH), 7.15-7.96 (m, 3H, Ar-H), 8.62 (s, 1H, =CH); ^{13}C NMR (75MHz, $CDCl_3$): δ 18.61, 35.68, 65.31, 76.28, 81.15, 96.39, 116.01, 117.08, 124.99, 126.25, 136.01, 139.69, 144.02, 161.05, 161.85, 165.09; ESI-MS: *m/z* 295 $[M+H]^+$. Anal. Calcd for $C_{16}H_{14}N_4O_2$: C, 65.30; H, 4.76; N, 19.04. Found: C, 65.34; H, 4.72; N, 19.07%.

(*E*)-3-methyl-*N*-((6-methyl-1-(prop-2-ynyl)-1*H*-benzo[*d*]imidazol-2-yl)methylene)isoxazol-5-amine, 7e: Orange solid. Yield 80%. m.p. 176-178°C; IR (KBr): 1635 (C=N), 2194 cm^{-1} (C≡C); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.20 (s, 1H, ≡CH), 2.27 (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 5.40 (s, 2H, CH_2), 6.15 (s, 1H, isoxazole-CH), 7.05-7.85 (m, 3H, Ar-H), 8.60 (s, 1H, =CH); ^{13}C NMR (75MHz, $CDCl_3$): δ 18.38, 24.31, 35.11, 75.09, 80.68, 95.98, 116.21, 117.13, 123.80, 125.01, 134.65, 139.11, 142.12, 159.82, 161.87, 164.21; ESI-MS: *m/z* 279 $[M+H]^+$. Anal. Calcd for $C_{16}H_{14}N_4O$: C, 69.06; H, 5.03; N, 20.14. Found: C, 69.03; H, 5.06; N, 20.16%.

(*E*)-*N*-((5-bromo-1-(prop-2-ynyl)-1*H*-benzo[*d*]-imidazol-2-yl)methylene)-3-methylisoxazol-5-amine, 7f: Brown solid. Yield 66%. m.p. 233-235°C; IR (KBr): 1640 (C=N), 2196 cm^{-1} (C≡C); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.25 (s, 1H, ≡CH), 2.28 (s, 3H, CH_3), 5.33 (s, 2H, CH_2), 6.18 (s,1H, isoxazole-CH), 7.01-7.96 (m, 3H, Ar-H), 8.72 (s, 1H, =CH); ^{13}C NMR (75MHz, $CDCl_3$): δ 18.41, 34.92, 74.65, 81.18, 95.86, 115.41, 116.83, 123.21, 124.11, 134.69, 139.11, 141.68, 159.98, 161.17, 164.01; ESI-MS: *m/z* 344 $[M+H]^+$. Anal. Calcd for $C_{15}H_{11}BrN_4O$: C, 52.47; H, 3.20; N, 16.32. Found: C, 52.44; H, 3.23; N, 16.35%.

(E)-*N*-((6-chloro-1-(prop-2-ynyl)-1*H*-benzo-[*d*]imidazol-2-yl)methylene)-3-methylisoxazol-5-amine, 7g: Orange solid. Yield 64%. m.p. 188-190°C; IR (KBr): 1647 (C=N), 2190 cm^{-1} (C≡C); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.20 (s, 1H, ≡CH), 2.31 (s, 3H, CH_3), 5.53 (s, 2H, CH_2), 6.19 (s,1H, isoxazole-CH), 7.01-7.81 (m, 3H, Ar-H), 8.70 (s, 1H, =CH); ^{13}C NMR (75MHz, $CDCl_3$): δ 18.29, 35.21, 74.95, 81.71, 95.96, 116.11, 117.13, 123.55, 124.61, 135.19, 139.96, 141.93, 160.18, 162.07, 165.11; ESI-MS: *m/z* 299 $[M+H]^+$. Anal. Calcd for $C_{15}H_{11}ClN_4O$: C, 60.40; H, 3.69; N, 18.79. Found: C, 60.43; H, 3.66; N, 18.75%.

(E)-*N*-((6-methoxy-1-(prop-2-ynyl)-1*H*-benzo-[*d*]imidazol-2-yl)methylene)-3-methylisoxazol-5-amine, 7h: Orange solid. Yield 79%. m.p. 166-168°C; IR (KBr): 1640 (C=N), 2203 cm^{-1} (C≡C); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.18 (s, 1H, ≡CH), 2.36 (s, 3H, CH_3), 3.69 (s, 3H, OCH_3), 5.55 (s, 2H, CH_2), 6.15 (s,1H, isoxazole-CH), 7.15-7.86 (m, 3H, Ar-H), 8.70 (s, 1H, =CH); ^{13}C NMR (75MHz, $CDCl_3$): δ 18.51, 35.81, 66.21, 76.65, 82.05, 97.19, 117.01, 117.55, 124.69, 126.68, 137.11, 139.69, 145.02, 162.05, 163.25, 165.69; ESI-MS: *m/z* 295 $[M+H]^+$. Anal. Calcd for $C_{16}H_{14}N_4O_2$: C, 65.30; H, 4.76; N, 19.04. Found: C, 65.34; H, 4.79; N, 19.01%.

General procedure for the synthesis of 3-methyl-5*H*-benzo [4',5'] imidazo [1',2':1,2] pyrrolo [3,4-*b*] isoxazolo [4,5-*e*] pyridine, 8a-h

Compound **7** (1 mmol) was dissolved in dry dichloroethane (10 mL) and 20 mol% of BF_3. E_2O (10 mL) was added to it, and the contents were heated at 80°C for 12 h. under nitrogen atmosphere. The reaction was monitored by TLC. After completion of the reaction, the reaction mixture was cooled, and the precipitate obtained was filtered and washed with ethylacetate. The crude product was recrystallized from benzene-ethylacetate.

3-Methyl-5*H*-benzo[4',5']imidazo[1',2':1,2] pyrrolo[3,4-*b*]isoxazolo[4,5-*e*]pyridine, 8a: Orange solid. Yield 75%. m.p. 185-187°C; IR (KBr): 1638 cm^{-1} (C=N); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.35 (s, 3H, CH_3), 5.40 (s, 2H, CH_2), 7.06-8.01 (m, 4H, Ar-H),8.55 (s, 1H, pyridine ring -H); ^{13}C NMR (75MHz, $CDCl_3$): δ 18.51, 52.31, 119.21, 120.01, 121.98, 123.68, 132.25, 133.65, 134.01, 135.21, 136.21, 142.51, 156.34, 160.21, 169.65; ESI-MS: *m/z* 263 $[M+H]^+$. Anal. Calcd for $C_{15}H_{10}N_4O$: C, 68.70; H, 3.81; N, 21.37. Found: C, 68.73; H, 3.84; N, 21.34%.

3-Methyl-10-nitro-5*H*-benzo[4',5']imidazo [1',2':1,2]pyrrolo[3,4-*b*]isoxazolo [4,5-*e*]pyridine, 8b: Orange solid. Yield 67%. m.p. 250-252°C; IR (KBr): 1640 cm^{-1} (C=N); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.36 (s, 3H, CH_3), 5.53 (s, 2H, CH_2), 7.06-8.11 (m, 3H, Ar-H), 8.65 (s, 1H, pyridine ring -H); ^{13}C NMR (75MHz, $CDCl_3$): δ 18.71, 53.21, 120.11, 121.21, 122.38, 123.61, 132.79, 133.88, 135.01, 135.91, 137.21, 143.21, 157.24, 161.11, 170.55; ESI-MS: *m/z* 308 $[M+H]^+$. Anal. Calcd for $C_{15}H_9N_5O_3$: C, 58.63; H, 2.93; N, 22.80. Found: C, 58.60; H, 2.95; N, 22.83%.

3-Methyl-9-nitro-5*H*-benzo[4',5']imidazo[1',2':1,2] pyrrolo[3,4-*b*]isoxazolo [4,5-*e*]pyridine, 8c: Orange solid. Yield 68%. m.p. 256-258°C; IR (KBr): 1640 cm^{-1} (C=N); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.34 (s, 3H, CH_3), 5.52 (s, 2H, CH_2), 7.06-8.21 (m, 3H, Ar-H), 8.62 (s, 1H, pyridine ring -H); ^{13}C NMR (75MHz, $CDCl_3$): δ 18.61, 53.61, 120.88, 122.11, 123.28, 124.11, 132.89, 134.68, 135.55, 136.31, 138.11, 144.21, 158.14, 162.21, 170.63; ESI-MS: *m/z* 308 $[M+H]^+$. Anal. Calcd for $C_{15}H_9N_5O_3$: C, 58.63; H, 2.93; N, 22.80. Found: C, 58.66; H, 2.91; N, 22.78%.

10-Methoxy-3-methyl-5*H*-benzo[4',5']imidazo [1',2':1,2]pyrrolo[3,4-*b*]isoxazolo [4,5-*e*]pyridine, 8d: Orange solid. Yield 78%. m.p. 175-177°C; IR (KBr): 1640 cm^{-1} (C=N); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.28 (s, 3H, CH_3), 3.68 (s, 3H, OCH_3), 5.44 (s, 2H, CH_2), 7.06-8.11 (m, 3H, Ar-H), 8.55 (s, 1H, pyridine ring -H),; ^{13}C NMR (75MHz, $CDCl_3$): δ 18.31, 52.62, 64.32, 119.66, 121.11, 122.08, 123.55, 133.05, 134.55, 135.01, 136.11, 136.81, 142.65, 156.62, 160.62, 169.75; ESI-MS: *m/z* 293 $[M+H]^+$. Anal. Calcd for $C_{16}H_{12}N_4O_2$: C, 65.75; H, 4.10; N, 19.17. Found: C, 65.72; H, 4.13; N, 19.15%.

3,8-Dimethyl-5*H*-benzo[4',5']imidazo[1',2':1,2] pyrrolo[3,4-*b*]isoxazolo[4,5-*e*]pyridine, 8e: Orange solid. Yield 82%. m.p. 211-213°C; IR (KBr): 1642 cm^{-1} (C=N); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.28 (s, 3H, CH_3), 2.52 (s, 3H, CH_3), 5.35 (s, 2H, CH_2), 7.04-7.96 (m, 3H, Ar-H), 8.61 (s, 1H, pyridine ring -H); ^{13}C NMR (75MHz, $CDCl_3$): δ 18.25, 24.21, 51.92, 118.96, 120.11, 122.68, 123.77, 133.41, 134.79, 135.65, 136.10, 137.11, 143.15, 156.32, 161.02, 168.85; ESI-MS: *m/z* 277 $[M+H]^+$. Anal. Calcd for $C_{16}H_{12}N_4O$: C, 69.56; H, 4.34; N, 20.28. Found: C, 69.53; H, 4.32; N, 20.25%.

9-Bromo-3-methyl-5*H*-benzo[4',5']imidazo [1',2':1,2]pyrrolo[3,4-*b*]isoxazolo [4,5-*e*]pyridine, 8f: Brown solid. Yield 65%. m.p. 270-272°C; IR (KBr): 1641 cm^{-1} (C=N); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.28 (s, 3H, CH_3), 5.22 (s, 2H, CH_2), 7.06-8.00 (m, 3H, Ar-H), 8.61 (s, 1H, pyridine ring -H); ^{13}C NMR (75MHz, $CDCl_3$): δ 18.21, 52.65, 119.17, 121.01, 122.28, 123.87, 132.55, 134.15, 134.81, 135.11, 136.26, 143.21, 157.44, 161.11, 169.95; ESI-MS: *m/z* 342 $[M+H]^+$. Anal. Calcd for $C_{15}H_9BrN_4O$: C, 52.78; H, 2.63; N, 16.42. Found: C, 52.75; H, 2.60; N, 16.45%.

8-Chloro-3-methyl-5*H*-benzo[4',5']imidazo [1',2':1,2]pyrrolo[3,4-*b*]isoxazolo [4,5-*e*]pyridine, 8g: Orange solid. Yield 68%. m.p. 262-264°C; IR (KBr): 1643 cm^{-1} (C=N); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.30 (s,3H, CH_3), 5.23 (s, 2H, CH_2), 7.09-8.03 (m, 3H, Ar-H), 8.60 (s, 1H, pyridine ring -H); ^{13}C NMR (75MHz, $CDCl_3$): δ 18.85, 53.25, 119.89, 121.61, 123.21, 124.17, 132.68, 135.05, 135.81, 136.31, 137.06, 143.41, 158.34, 162.31, 169.86;; ESI-MS: *m/z* 297 $[M+H]^+$. Anal. Calcd for $C_{15}H_9ClN_4O$: C, 60.81; H, 3.04; N, 18.91. Found: C, 60.84; H, 3.07; N, 18.94%.

8-Methoxy-3-methyl-5*H*-benzo[4',5']imidazo [1',2':1,2]pyrrolo[3,4-*b*]isoxazolo [4,5-*e*]pyridine, 8h: Orange solid. Yield 84%. m.p. 190-192°C; IR (KBr): 1642 cm^{-1} (C=N); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.25 (s, 3H, CH_3), 3.76 (s, 3H, OCH_3), 5.22 (s, 2H, CH_2), 7.06-7.98 (m, 3H, Ar-H), 8.60 (s, 1H, pyridine ring -H); ^{13}C NMR (75MHz, $CDCl_3$): δ 18.51, 53.02,

64.57, 120.16, 121.11, 122.16, 123.65, 133.88, 134.66, 135.85, 137.21, 137.89, 142.60, 157.12, 161.32, 169.89; ESI-MS: *m/z* 293 $[M+H]^+$. Anal. Calcd for $C_{16}H_{12}N_4O_2$: C, 65.75; H, 4.10; N, 19.17. Found: C, 65.78; H, 4.08; N, 19.19%.

Antibacterial activity

The antibacterial activity was done by broth dilution method[22], 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 **8** dissolved in suitable solvent (acetone) and concentration of 100 µg/mL of test compound **8** 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[23]. 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 **8** 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 *Flucanozole*. The treated and the controls were kept at RT 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

The synthesis of novel benzo[4',5']imidazo [1',2':1,2]pyrrolo[3,4-*b*]isoxazolo[4,5-*e*]pyridines has been achieved in good yields through boron triflouride etherate catalyzed intramolecular Povarov reaction. The newly synthesized title compounds **8** have been evaluated for their *in vitro* antimicrobial activity. Compounds **8d**, **8e** and **8h** exhibited significant antimicrobial activities, herein they may be considered as drug candidates after the detailed study by structure-activity relationship.

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