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Synthesis, spectral characterization and antimicrobial studies of novel imidazole derivatives

C. M. Mahalakshmi, M. Karthick, M. Shanmugam and V. Chidambaranathan*

Chemistry Section, FEAT, Annamalai University, Annamalainagar

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ABSTRACT

A series of six novel Imidazole derivatives, with natural nucleobases by mono, di and tri substitution in 2, 4, 5-Tribromo Imidazole at the 2, 4 – and 5 – position was synthesized. Target molecules were synthesized by stoichiometric addition of various nucleophiles to 2,4,5 – Tribromo Imidazole in the presence of suitable base. The newly synthesized Imidazole derivatives have been characterized by IR, ^{1}H NMR, ^{13}C NMR (1D, 2DNMR), mass spectral and elemental analysis. All the synthesized compounds were screened for in vitro and microbial activity against a panel of selected bacterial and fungal strains using streptomycin and Amphotericin B as standards.

Keywords: 2,4,5-Tribromo Imidazole, Nucleobases, Antimicrobial acitivity.

INTRODUCTION

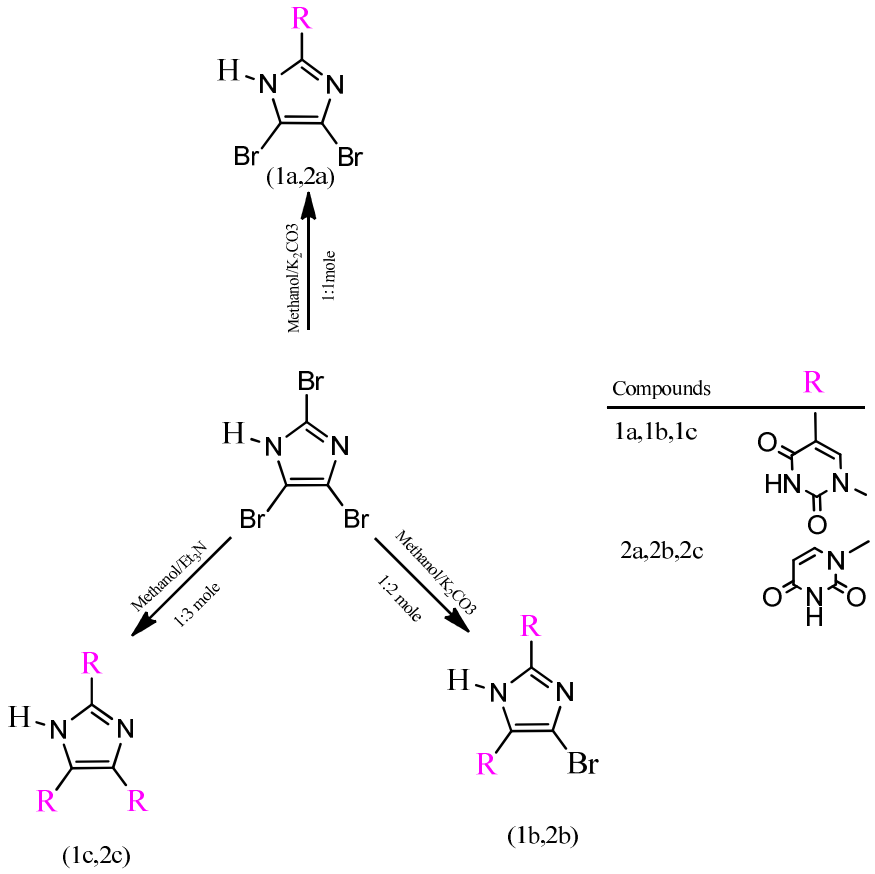
The chemistry of nitrogen heterocyclic compounds especially Imidazole has attracted more attention during recent years due to their wide range of biological and pharmacological activities. Imidazole is a well known common heterocyclic compound which is present in many natural products and medicinal drugs. Imidazole ring system is present in histidine, as an important biological building blocks and related hormone histamine. Many drugs include ketoconazole, miconazole, clotimazole. Imidazole is reported to possess varied activities like antimicrobial[1], analgesic[2], CNS depressants[3], antitubercular[4], anticancer[5], anthelmistic[6],etc. 2,4,5-Tribromo Imidazole has been found in nature[7] and is an effective fire redardant agent[8]. Halogenated Imidazoles exhibit insecticidal [9], parasiticidal[10], acaricidal[11] and herbicidal[12]acitivity. Based on the above observations, we have planned to synthesized a novel series of nucleobase derivatives derived from 2,4,5 – Tribromo Imidazole followed by their In-Vitro antibacterial, antifungal activities[13]. There is no report in the literature regarding the synthesis of Imidazole derivatives with thymine and uracil. As an inception, various Imidazole based nucleobases were synthesized and characterized by FT-IR, 1DNMR, 2D NMR (^{1}H, ^{13C}), mass (HRMS), CHN analysis and the antimicrobial activities were screened.

MATERIALS AND METHODS

Characterization Techniques

Melting point (mps) were determined by open capillary method and are uncorrected. IR spectra were recorded by Jasco FTS 3000 HX(KBr pellets). 1HNMR spectra were recorded on Bruker ADVANCE III NMR spectrometer (500 MHZ) using TMS as internal standard (Chemical shifts in ppm). ^{13}CNMR spectra were recorded on the same instrument at 125.76 MHZ and are referenced using the central line of the solvent signal (DMSO –d6 septet at S

=39.5 ppm). Mass spectra were recorded with JOEL ac MATE II instrument. Elemental anaylsis (C,H and N) were performed with a Perkin Elmer 2400 series II CHN Analyzer.



Scheme-I

Table 1

Product code	Yield (%)	Reaction Time (h)	Elemental Analysis (%) Calculated			Found			Molecular Weight	Molecular Formula
			C	H	N	C	H	N		
1a	85	5	27.6	1.73	16.01	27.71	1.71	16.13	349.97	349.97
1b	79	12	36.60	3.07	21.34	36.67	3.07	21.45	395.17	395.17
1c	85	16	49.09	3.66	25.45	49.08	3.70	25.55	440.37	440.37
2a	74	7	25.03	1.20	16.68	25.09	1.25	16.80	335.94	335.94
2b	71	16	35.99	1.92	22.89	34.01	2.01	23.01	367.12	367.12
2c	61	21	45.23	2.53	28.13	45.24	2.50	28.21	398.29	398.29

General procedure for synthesis of compounds 1a-2a

1- (4,5-dibromo-1H-imidazol-2-yl)-5-methylpyrimidine-2,4 (1H,3H) - dione.(1a)

2,4,5-tribromoimidazole was dissolved (0.304 g, 0.1mmol) in Methanol(25 ml) at room temperature, K_2CO_3 (1 mmol) and thymine (0.126 g,0.1 mmol)/uracil (0.112 g,0.1mmol) [thymine/uracil dissolved in water (25 ml) at 50^0 C] were placed into a 250 ml two neck round bottom flask which was fitted with condenser and thermometer. The reaction mixer was refluxed at 35^0 C for 4 h later it was cooled at room temperature. The progress of the reaction was monitored by TLC [Methanol / DCM, 1:9]. The reaction mixture was kept overnight at room temperature. The content was then poured over crushed ice and the solid obtained was filtered, dried and crystallized with ethanol. Physical data of compounds (1a-1c&2a-2c) are presented in **Table-1.** . White solid, Yield (85%); mp 240^0C (dec); IR (KBr) 3355(NH Str)**,** 3064(-CH Str), 2922, 1734((-C=O), 1443, 1393, 1244, 1027 (C-Br), 939,837cm^{-1}; ^{1}H NMR (500 MHz, DMSO-*d6*) δ: 11.01(s, 1H) 10.596 (s,1H), 7.256 (q, 1H),1.732 (d,3H);^{13}C NMR (125.76 MHz, DMSO-

d6) δ:165.3 (-C=O), 151.9, 150.3, 140.2,138.3,138.1,117.8 (C4&C5,-C-Br), 108.1,12.2 (-$CH_{3)}$; HRMS (m/z): 349.711; Anal. calcd for $C_8H_6Br_2N_4O_2$: C,27.6; H, 1.73; N,16.01**.** found: C,27.71; H,1.71; N, 16.13

1- (4,5-dibromo - 1H-imidazol-2-yl) pyrimidine-2,4 (1H,3H)-dione.(2a):
White solid, Yield (74%); mp 349^{0}C (dec); IR (KBr) 3273 (-NH Str), 3167,3062 (-CH Str), 2731, 1733 (-C=O), ,1445, 1393, 1244,1199, 1028 (C-Br), 934,841(C-N)cm^{-1}; ^{1}H NMR (500 MHz, DMSO-*d6*) δ: 11.00 (s, 1H) 10.8 (s, 2H), 7.39 (d,1H), 5.46 (d,1H); ^{13}C NMR (125.76 MHz, DMSO-*d6*) δ:163.5(-C=O), 149.8,146.0,137.67,116.15 (C4&C5,-C-Br), 107.63.;HRMS(m/z): 335.98; Anal. calcd for: $C_7H_4Br_2N_4O_2$**:** C,25.03; H,1.20; N,16.68**.** found: C,25.09; H,1.25; N, 16.80

General procedure for synthesis of compounds 1b-2b
1,1'-(5-bromo-1H-imidazole-2,4-diyl) bis(5-methylpyrimidine -2,4 (1H,3H) - dione) (1b):
2,4,5-tribromoimidazole was dissolved (0.304 g, 0.1 mmol) in Methanol (25 ml) at room temperature, K_2CO_3 (1 mmol) and thymine (0.252g, 0.2 mmol)/uracil(0.224 g, 0.2 mmol) [thymine/uracil dissolved in water (25 ml) at 50^0 C] were placed into a 250 ml two neck round bottom flask which was fitted with condenser and thermometer. The reaction mixer was refluxed at 35^0 C for 12-16 h later it was cooled at room temperature. The progress of the reaction was monitored by TLC [Methanol / DCM, 1:9]. The reaction mixture was kept overnight at room temperature. The content was then poured over crushed ice and the solid obtained was filtered, dried and crystallized with ethanol White solid, Yield (79%); mp 281^{0}C (dec); IR (KBr) 3356 (-NH Str)**,** 3087 (-CH Str), 2928, 1733(-C=O), 1443, 1394, 1251, 1028 (C-Br), 939,837cm^{-1}; ^{1}H NMR (500 MHz, DMSO-*d6*) δ: 11.03 (s, 1H) 10.61(s, 2H), 7.26 (q,2H),1.73 (d,6H); ^{13}C NMR (125.76MHz, DMSO-*d6*) δ:165.4 (-C=O), 151.9, 150.3, 138.3, 138.1, 127.9, 117.8(C5,-C-Br),108.1,93.01,12.2(-$CH_{3)}$; HRMS (m/z): 395.029; Anal.calcd for $C_{13}H_{11}BrN_6O_4$**:** C,36.60; H,3.07; N,21.34**.** found: C,36.67; H,3.07; N, 21.45 **.** .

1,1'-(5-bromo-1H-imidazole-2,4-diyl) dipyrimidine-2,4 (1H,3H) - dione.(2b):
White solid; 71% yield; mp 368^{0}C (dec); IR (KBr) 3273 (-NH Str)**,** 3189,3062 (-CH Str), 2737, 1735 (-C=O), ,1445, 1393, 1244,1201, 1027 (C-Br), 934,841 (C-N Str)cm^{-1}; ^{1}H NMR (500 MHz, DMSO-*d6*) δ: 11.16 (s, 1H) 10.9 (s, 2H), 7.39 (d,2 H), 5.46 (d,2H); ^{13}C NMR (125.76 MHz, DMSO-*d6*)δ: 163.5 (-C=O), 153.5,146.0,137.6,126.5,107.6,93.13; HRMS (m/z): 376.99; Anal.calcd for**:** $C_{11}H_7BrN_6O_4$:C,35.99; H,1.92; N,22.89**.** found: C,34.01 H,2.01; N, 23.01

General procedure for synthesis of compounds 1c-2c:
1,1'1''-(1H-imidazole-2,4,5-triyl)tris(5-methylpyrimidine-2,4(1H,3H)-dione) (1c):
2,4,5-tribromoimidazole was dissolved (0.304 g, 0.1 mmol) in Methanol(25 ml) at room temperature, K_2CO_3 (1 mmol) and thymine(0.378g,0.3mmol)/uracil(0.336g,0.3mmol) [thymine/uracil dissolved in water (25 ml) at 50^0 C] were placed into a 250 ml two neck round bottom flask which was fitted with condenser and thermometer. The reaction mixer was refluxed at 35^0 C for 16-21 h later it was cooled at room temperature. The progress of the reaction was monitored by TLC [Methanol / DCM, 1:9]. The reaction mixture was kept overnight at room temperature. The content was then poured over crushed ice and the solid obtained was filtered, dried and crystallised with ethanol. White solid, Yield (85%); mp 342^{0}C (dec); IR (KBr) 3373 (-NH Str),3062 (-CH Str),2928, 1735 (-C=O), 1527,1444, 1393, 1244, 1027 (C-Br), 934,841cm^{-1}; ^{1}H NMR (500 MHz, DMSO-*d6*) δ:11.09(s,1H)10.98(s,3H),7.24(q,3H),1.72(d,9H);^{13}CNMR(125.76MHz,DMSO-*d6*)δ:165.3(C=O),151.9, 150.3, 138.3, 138.1, 117.8, 108.1, 93.01,12. (-$CH_{3)}$;HRMS(m/z):440.07; Anal. calcd for **$C_{18}H_{16}N_8O_6$:C**,49.09; H,3.66; N,25.45**.** found: C,49.08; H,3.70; N, 25.55.

1,1'1''-(1H-imidazole-2,4,5-triyl)tripyrimidine-2,4(1H,3H)-dione.(2c):
White solid; 61% yield;mp 393^{0}C (dec); IR (KBr) 3180 (N-H, Str), 3057, 2962, 2737,1735 (-C=O), 1527,1444, 1393, 980, 841(C-N Str)cm^{-1}; ^{1}H NMR (500 MHz, DMSO-*d6*) δ: 11.16(s, 1H) 10.98 (s,3H), 7.393 (d,3H)5.46 (d,3H);^{13} CNM R(125.76MHz, DMSO-*d6)*δ:164.9 (-C=O), 154.2,144.2,137. 6,119.47,116.15,107.65; HRMS (m/z): 398.85; Anal. calcdfor**:** $C_{15}H_{10}N_8O_6$**:**C45. 23; H,2.53; N,28.13**.** found: C,45.24 H,2.50; N,28.21.

Antimicrobial studies:
The antimicrobial activity of synthesized compounds (1a-2c) was determined by serial dilution method. The compounds were tested at a concentration of 100 µg/ml in Dimethyl sulfoxide. The antibacterial activities in terms of minimum inhibitory concentration (MIC) of compounds (1a-2c) are depicted in Table-2

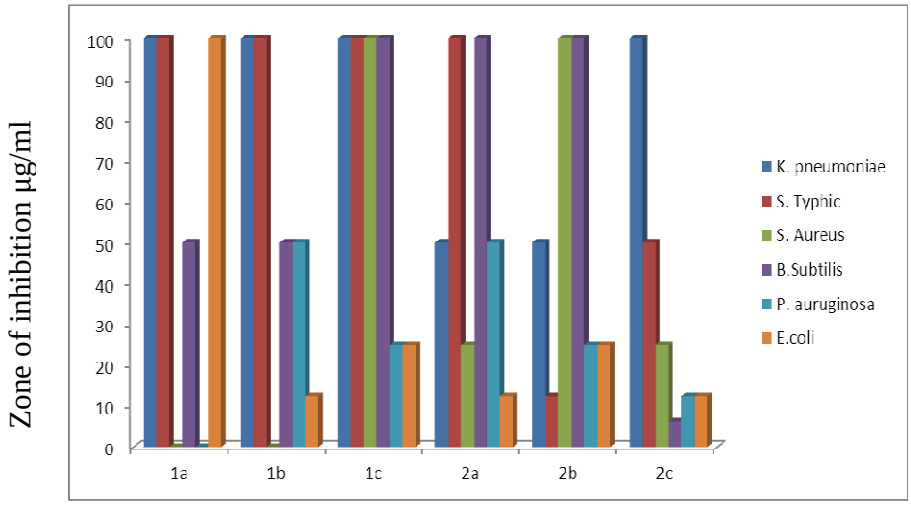
Table – 2 Antibacterial activities of compounds 1a – 1c, 2a-2c, for bacterial strains in MIC (µg/ml)

Bacterial Strains (MIC)	Compounds						
	Streptomycin	1a	1b	1c	2a	2b	2c
K. pneumoniae	50	100	100	100	50	50	100
S. Typhic	50	100	100	100	100	12.5	50
S. Aureus	50	-	-	100	25	100	25
B.Subtilis	12.5	50	50	100	100	100	6.25
P. auruginosa	25	-	50	25	50	25	12.5
E.coli	12.5	100	12.5	25	12.5	25	12.5

Note:-no inhibition

And their MIC's were compared with streptomycin standarad drug[14].MIC values in Table-2 revealed that compound 2c exhibited two fold increased activity against *B.substilis* at MIC 6.25 µg/ml_than the streptomycin standard. In addition compounds 1b, 1c, 2a exhibited superior activity against *E.coli* than the reference streptomycin drug.

ANTIBACTERIAL ACTIVITIES AGAINST STREPTOMYCIN



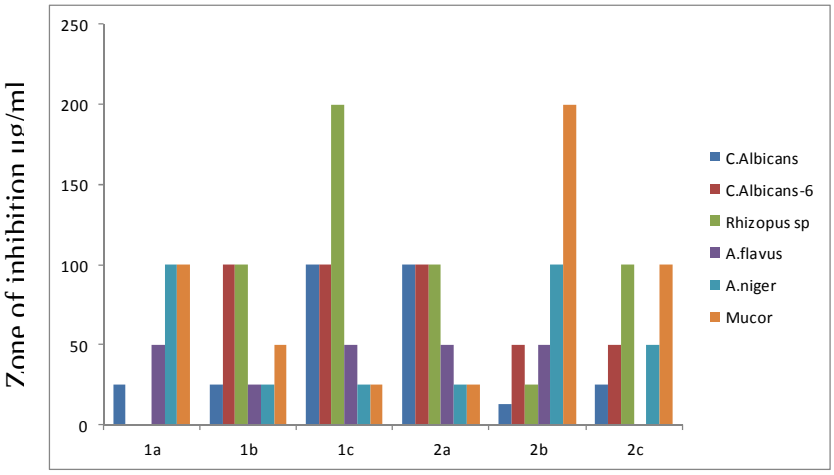
Antifungal activity of compound (1a-1c) and (2a-2c) were also screened and their MIC values are listed in **Table-3**.Here Amphotericin-B was used as standard drug. The compound 2a showed activity against *A.niger* at MIC 12.5 µg/ml than the standard Amphotericin –B drug.

Table-3 Antifungal activities of compounds 1a – 1c, 2a-2c, for bacterial strains in MIC (µg/ml)

Fungal Strains (MIC)	Compounds						
	Amphotericin-B	1a	1b	1c	2a	2b	2c
C.Albicans	25	25	25	100	100	12.5	25
C.Albicans-6	25	-	100	100	100	50	50
Rhizopus sp	25	-	100	200	100	25	100
A.flavus	50	50	25	50	50	50	-
A.niger	25	100	25	25	25	100	50
Mucor	25	100	50	25	25	200	100

Note : - No inhibition

ANTIFUNGAL ACTIVITIES AGAINST AMPHOTERICIN-B



RESULTS AND DISCUSSION

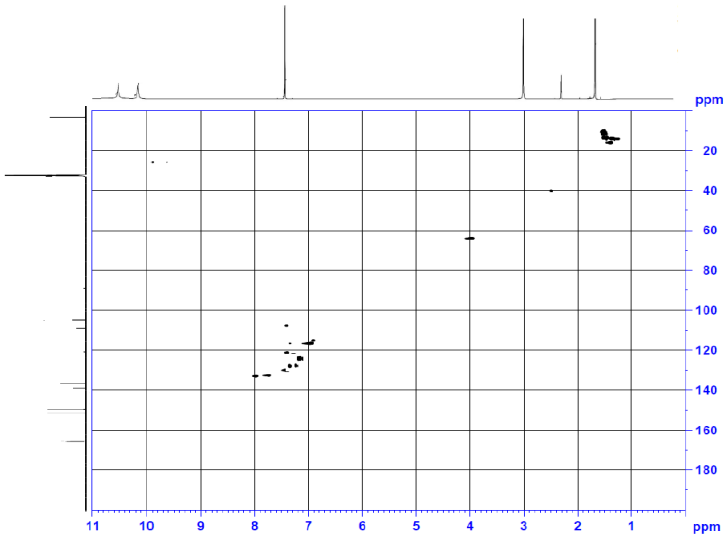


Fig.1 HSQC spectrum of 1c

The synthesis of various imidazole derivatives were carried out as depicted in **scheme-1.** The target molecules (1a-1c,2a-2c) were synthesized by stoichiometric addition of nucleophiles to 2,4,5-tribromoimidazole in the presence of K_2CO_3 were used as the nucleophiles for the synthesis of the corresponding derivatives. Using Triethylamine and K_2CO_3 base predominantly N1 substituted pyrimidine[15] as the predominant products. In the present investigation, a heteroaromatic halide like 2,4,5-tribromoimidazole has been choosen instead of alkyl halides. Imidazole is amphoteric, i.e. it can function as both an acid and as a base .Here it is interesting to note that nucleobases can act as nucleophiles instead of acting as substrates in aromatic nucleophilic substitutions. A broad band at 3167-3273cm^{-1} is ascribed to N-H stretching frequency of the amide(-NH-C=O) moiety.A strong band at 1735cm^{-1} is due to the amide carbonyl (C=O) stretching frequencies. Imidazole derivatives show another important band in the region 1526cm^{-1} is ascribed to C=N stretching vibrations. Hence the IR data illustrate the formation of the 2,4,5-imidazole nucleobase derivatives. In ^{1}H NMR spectrum of compound 1a-1c & 2a-2c show broad singlet in the region of 11.01-11.16ppm and is assigned for free- NH group present in 2,4,5-tribrmoimidazole.A sharp singlet at 10.59-11.00 ppm is assignable to amide –NH protons. On focusing the ^{13}CNMR spectral assignments, the signals at 165.4ppm is due to amide carbonyl carbon of pyrimidine based imidazole compounds 1a-1c & 2a-2c whereas (C-2) carbon of C-N in imidazole resonates at 151.9ppm. The regioselectivity and other structure features of compound 1c were analyzed by

1D NMR (^{1}H, ^{13}C) and 2D NMR (HSQC,HMBC) spectral techniques. In ^{1}H NMR spectrum, the doublet at 1.72ppm with nine integral values for methyl group presence in three thymine moieties.C-4 proton and C-9 proton of the thymine moiety resonates closely at 7.24 and 1.72ppm respectively. Further, this assignment was substantiated by HSQC analysis **(Fig.1)** .In ^{13}C spectra, peals at 138 and 12.2 ppm were unambiguously assigned to C-4 and C-9 carbons respectively. In HSQC spectra, chemical shift at 138 ppm (C-4 carbon of thymine) shows one band correlation with signal at 7.24 ppm and hence peak at 1.72 ppm was attributed to C-9 proton signal at 12.2ppm.

CONCLUSION

Six new imidazole derivatives were synthesized in reasonably good yields. They were characterized by IR, ^{1}H, ^{13}C NMR(1D,2DNMR), HRMASS and elemental analysis. All the newly synthesized compounds were tested for antimicrobial activity by serial dilution method. Among the screened samples, compound 2c exhibited as most active against *B.subsitils* compared to the standard drug.

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