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Research article

A GREEN PROTOCOL FOR THE SYNTHESIS AND ANTIMICROBIAL EVALUATION OF SOME IMIDAZO [1,2-*A*] PYRAZINES

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ABSTRACT: In this work, we have described a simple and convenient method for the synthesis Imidazo [1,2-*a*] pyrazine derivatives using 2-amino5-bromo pyrazine, and phenacyl bromide in the presence of DABCO as a catalyst and evaluated as antimicrobial agents. The notable features of this protocol are the simple experimental procedure, short reaction time, easy handling and good yields using a catalytic amount of readily available and cost effective catalyst.

Key words: 2-amino 5- bromopyrazine; Antimicrobial activity DABCO; Green Chemistry; Imidazo [1,2-*a*] pyrazine

INTRODUCTION

In recent years, the focus is being shifted towards environmentally green and benign protocols to avoid use of hazardous organic solvents. The organic reactions carried out in aqueous media have attracted many chemists. As a solvent, water offers many economic and environmental advantages including wide availability, cheap in cost, non-hazardous and safe in handling [Li C. J., 2005, Tiwari V., et al., 2011]

Nitrogen bridgehead-fused heterocycles containing an imidazole ring are pharmacologically important molecules as they have a diversified activity on various targets.The chemistry of Imidazo [1,2-a] pyrazines has received wide consideration because of their synthetic and biological importance. Derivatives of Imidazo [1,2-a] pyrazines have been found to have diverse pharmacological activity, for exampleAntibacterial[Rival Y. et al. 1992], Antiinflammatory [Abignente E. et al. 1981], Uterine relaxing activity[Vitse O. et al.1997], Antibronchoplastic [Zimmermann P.J. et al. 2008], Antiulcer[Bonnet P.A. et al. 1992], Cardiac Stimulating [Sablayrolles C. et al. 1984], Antidepressant[Lumma W.C. et al. 1983], Hypoglycemic activity[Meurer L.C. et al. 1992], Antiproliferative [Zurbonsen K. et al. 1999], Smoothmusclerelaxant [Michel A. et al. 1991] etc. There are several methods reported in the literature for the preparation of 2- or 3-substituted imidazo[1,2-a] pyrazines with the majority relying on the condensation of 2-aminopyrazine with α-bromoketones to form the five-membered cyclic system. Most of these methods involve three or more sequential synthetic steps, the use of harsh reaction conditions that give low yields, and in some cases, use of hazardous or expensive starting materials.

With this aspects of clean and green protocols, we have developed a synthesis of imidazo[1,2-a]pyrazine in aqueous media. The synthesis has been carried out by the reaction of 2-amino-5-bromo pyrazine, substituted phenacyl bromide in presence of 1,4-diazabicyclo[2.2.2]octane (DABCO) as base and water as a solvent.

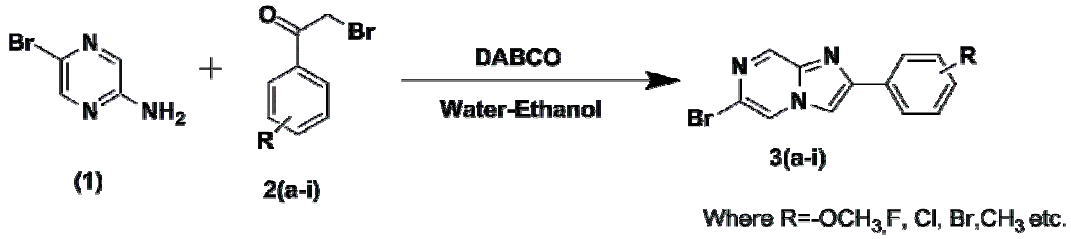

Figure1.Synthesis of Imidazo [1,2-*a*]pyrazine

MATERIALS AND METHODS

All the chemicals and solvents were ofAR grade and used without further purification. Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were identified by iodine and UV light. IR spectra were recorded on Shimadzu FT-IR-8400 instrument using KBr pellet method. Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using direct inlet probe technique.^{1}H NMR and ^{13}C NMR was determined in DMSO-d_6 solvent on a Bruker AC 400 MHz Spectrometer. Single Crystal X-ray was carried out using Rigaku-SCX Mini Single Crystal X-Ray Diffractometer.

General Procedure of 6-bromo-2- substituted phenylmidazo[1,2-a]pyrazine derivatives (3a-3i).

An equimolar mixture of 2-amino 5- bromopyrazine **(1)** (10 mmol) and different phenacyl bromides **(2a-2i)** (10 mmol) were charged into RBF. Then DABCO (1 mmol) was added as a base and Water as a solvent. The reaction mass was heated up to 80-85°C. The completion of reaction was monitored by TLC. After completion of the reaction, the reaction mixture was cooled and the precipitated solid was filtered, washed with water, dried and recrystallized from ethanol. Physical Constants of newly synthesized lmidazo[1,2-a]pyrazines**3a-3i** are recorded in Table 1.

The analytical data of compounds synthesized Imidazo [1,2-*a*]pyridine ***(3a-3i)*** are given as below:

6-bromo-2-(4-bromophenyl)imidazo[1,2-a]pyrazine(3a) IR (ν_{max} cm^{-1}, KBr): 3097 (Ar-C-H str.), 2914 (Alkane-C-H str.), 2358 (C=N str.), 1697 (Alkene C=C str.), 1600 (Ar-C=C str.), 1496(C-H bend.), 1072(=C-H bend.), 819(para substituted). ^{1}H NMR 400 MHz (DMSO-d_6): 8.97-8.96(2H,d), 8.62(1H,s), 8.01-7.99(2H, dd, J=8.0Hz), 7.71-7.69(2H,dd, J=8.0Hz). ^{13}C NMR (100 MHz, DMSO-d_6): 146(C, pyrazine ring), 141(C, pyrazine ring) 139(C, imidazole ring), 131(2C, phenyl ring), 130.5(C, imidazole ring), 128(2CH, phenyl ring), 122(C), 120(C), 111(C, imidazole ring). EI^{+} m/z: 352 Anal. Calcd for $C_{12}H_7Br_2N_3$: C, 40.83%; H, 2.00%; Br, 45.27%; N, 11.90%.

6-bromo-2-(4-chlorophenyl)imidazo[1,2-a]pyrazine(3b) IR (ν_{max} cm^{-1}, KBr): 3097 (Ar-C-H str.), 2914 (Alkane-C-H str.), 2358 (C=N str.), 1697 (Alkene C=C str.), 1600 (Ar-C=C str.), 1496(C-H bend.), 1072(=C-H bend.), 1018(C-O-C str.), 819(para substituted).^{1}H NMR 400 MHz (DMSO-d_6): 8.96-8.93(2H,d), 8.52(1H,s), 7.90-7.88(2H, dd), 7.49-7.47(2H,dd). EI^{+} m/z: 352 Anal. Calcd for $C_{12}H_7Br_2N_3$: C, 40.83%; H, 2.00%; Br, 45.27%; N, 11.90%.

6-bromo-2-(p-tolyl)imidazo[1,2-a]pyrazine(3c)(ν_{max} cm^{-1}, KBr): 3097 (Ar-C-H str.), 2914 (Alkane-C-H str.), 2358 (C=N str.), 1697 (Alkene C=C str.), 1600 (Ar-C=C str.), 1496(C-H bend.), 1072(=C-H bend.), 1018(C-O-C str.), 819(para substituted).^{1}H NMR 400 MHz (DMSO-d_6): 8.96-8.93(2H,d), 8.52(1H,s), 7.63-7.61(2H, dd), 7.25-7.23(2H,dd), 2.1(3H,s). EI^{+} m/z: 352 Anal. Calcd for $C_{12}H_7Br_2N_3$: C, 40.83%; H, 2.00%; Br, 45.27%; N, 11.90%.

6-bromo-2-(4-methoxyphenyl)imidazo[1,2-a]pyrazine(3d)IR (ν_{max} cm^{-1}, KBr): 3097 (Ar-C-H str.), 2914 (Alkane-C-H str.), 2358 (C=N str.), 1697 (Alkene C=C str.), 1600 (Ar-C=C str.), 1496(C-H bend.), 1072(=C-H bend.), 1018(C-O-C str.), 819(para substituted).^{1}H NMR 400 MHz (DMSO-d_6): 8.96-8.93(2H,d), 8.52(1H,s), 7.99-7.97(2H, dd, J=8.0Hz), 7.07-7.05(2H,dd, J=8.0Hz), 3.16(1H,s). ^{13}C NMR (100 MHz, DMSO-d_6): 160(O-CH_3), 146(C, pyrazine ring), 138(C, pyrazine ring), 127(2C, phenyl ring), 123(C, imidazole ring),120(C, phenyl ring) 114(2CH, phenyl ring), 122(C, pyrazine ring), 120(C, pyrazine ring), 110(C,imidazole ring), 55.2(CH_3).^{1}H NMR 400 MHz (DMSO-d_6): 8.96-8.93(2H,d), 8.52(1H,s), 7.99-7.97(2H, dd, J=8.0Hz), 7.07-7.05(2H,dd, J=8.0Hz), 3.16(1H,s).EI^{+} m/z: 303 Anal. Calcd for $C_{13}H_{10}BrN_3O$: C, 51.34%; H, 3.31%; Br, 26.27%; N, 13.82%, O, 5.26%.

6-bromo-2-(4-fluorophenyl)imidazo[1,2-a]pyrazine(3e)(ν_{max} cm^{-1}, KBr): 3097 (Ar-C-H str.), 2914 (Alkane-C-H str.), 2358 (C=N str.), 1697 (Alkene C=C str.), 1600 (Ar-C=C str.), 1496(C-H bend.), 1072(=C-H bend.), 1018(C-O-C str.), 819(para substituted).^{1}H NMR 400 MHz (DMSO-d_6): 8.96-8.93(2H,d), 8.52(1H,s), 7.99-7.97(2H, dd), 7.25-7.05(2H,dd, J=8.0Hz). EI^{+} m/z: 352 Anal. Calcd for $C_{12}H_7Br_2N_3$: C, 40.83%; H, 2.00%; Br, 45.27%; N, 11.90%.

6-bromo-2-(4-nitrophenyl)imidazo[1,2-a]pyrazine(3f)(ν_{max} cm^{-1}, KBr): 3097 (Ar-C-H str.), 2914 (Alkane-C-H str.), 2358 (C=N str.), 1697 (Alkene C=C str.), 1600 (Ar-C=C str.), 1496(C-H bend.), 1072(=C-H bend.), 1018(C-O-C str.), 819(para substituted).^{1}H NMR 400 MHz (DMSO-d_6): 8.96-8.93(2H,d), 8.52(1H,s), 8.35-8.33(2H, dd, J=8.0Hz), 8.03-8.01(2H,dd). EI^{+} m/z: 352 Anal. Calcd for $C_{12}H_7Br_2N_3$: C, 40.83%; H, 2.00%; Br, 45.27%; N, 11.90%.

6-bromo-2-(3-nitrophenyl)imidazo[1,2-a]pyrazine(3g)(ν_{max} cm^{-1}, KBr): 3097 (Ar-C-H str.), 2914 (Alkane-C-H str.), 2358 (C=N str.), 1697 (Alkene C=C str.), 1600 (Ar-C=C str.), 1496(C-H bend.), 1072(=C-H bend.), 1018(C-O-C str.), 819(para substituted).^{1}H NMR 400 MHz (DMSO-d_6): 8.96-8.93(2H,d), 8.52(1H,s), 8.3(1H,s), 7.99-7.97(2H,m),7.49(1H,t). EI^{+} m/z: 352 Anal. Calcd for $C_{12}H_7Br_2N_3$: C, 40.83%; H, 2.00%; Br, 45.27%; N, 11.90%.

6-bromo-2-phenylimidazo[1,2-a]pyrazine(3h)(ν_{max} cm^{-1}, KBr): 3097 (Ar-C-H str.), 2914 (Alkane-C-H str.), 2358 (C=N str.), 1697 (Alkene C=C str.), 1600 (Ar-C=C str.), 1496(C-H bend.), 1072(=C-H bend.), 1018(C-O-C str.), 819(para substituted).^{1}H NMR 400 MHz (DMSO-d_6): 8.96-8.93(2H,d), 8.52(1H,s), 8.09-8.07(2H, dd), 7.49-7.44(3H,m). EI^{+} m/z: 352 Anal. Calcd for $C_{12}H_7Br_2N_3$: C, 40.83%; H, 2.00%; Br, 45.27%; N, 11.90%.

6-bromo-2-(2,4-dichlorophenyl)imidazo[1,2-a]pyrazine(3i)(ν_{max} cm^{-1}, KBr): 3097 (Ar-C-H str.), 2914 (Alkane-C-H str.), 2358 (C=N str.), 1697 (Alkene C=C str.), 1600 (Ar-C=C str.), 1496(C-H bend.), 1072(=C-H bend.), 1018(C-O-C str.), 819(para substituted).^{1}H NMR 400 MHz (DMSO-d_6): 8.96-8.93(2H,d), 8.52(1H,s), 7.81-7.79(1H, d), 7.50-7.48(1H,d), 7.01(1H,s). EI^+ m/z: 352 Anal. Calcd for $C_{12}H_7Br_2N_3$: C, 40.83%; H, 2.00%; Br, 45.27%; N, 11.90%.

BIOLOGICAL ACTIVITY

All the synthesized compounds 3(a-i) were tested in vitro for their antibacterial and antifungal activity. All the glass apparatus used were sterilized before use. The broth dilution technique was used to determine the minimum inhibitory concentration (MIC) of the synthesized compounds. Bacterial strain of *Staphylococcus aureus*MTCC87, Bacillus Subtillius MTCC 441 as a gram positive, *Escherichia coli* MTCC1302, *shigella* MTCC 11947 as a gram negative used in a present study. Fungal strains of *Aspergillusniger* MTCC 1344and*Candida albicans* MTCC 227were taken. DMSO was used as the solvent for the compounds. A blank test was carried out to check the antimicrobial activity of DMSO. Ampicillin, Ciprofloxacin and Norfloxacin were used as the standard drugs for antibacterial activity and Clotrimazole and Terbinafeine were used as the standard drug for antifungal activity. Activity results are depicted in Table 2.

RESULT AND DISCUSSION

As part of our efforts on the development of new route for the preparation of biologically active molecules and considering the important biological properties of imidazo[12-*a*]pyrazines herein, we described an efficient, green, benign and simple synthesis of the nuclei.

Reaction was successfully performed using equivalent ratios of 2-amino5-bromo pyrazine and various phenacyl bromides. The structures of imidazo [1,2-a]pyrazines3(a-i) were confirmed by IR, ^{1}H NMR, ^{13}C NMR spectroscopy, mass spectrometry and elemental analysis. The mass spectrum of 3a displayed the molecular ion peak (Mþ) peak at m/z 353, which was consistent with the product structure. The ^{1}H NMR spectrum of 3a exhibited two doublets, arising from the two equivalent phenyl ring protons at (d 8.97 and 7.71 ppm) and other 1H singlet and 2H doublet with appropriate chemical shifts for the three H atoms of the imidazopyrazine moiety. The ^{13}C NMR spectrum of 3d showed C-O peak at δ 160 ppm and O-CH_3 peak at 55 ppm even proton NMR comfirmed the presence of methoxy group by sharp singlet at 3.16 ppm. The IR spectrum of 3d exhibited characterstic absorption band at 1018 cm^{-1} for (C-O linkage) and all other general frequency band are in good agreement with the structure.

The examination of the data of the Table 2 revealed that many of the compounds shown good activity against Bacterial strain and Fungi strain when compared with the standard drugs like Ampicillian, Norfloxacin, Ciprofloxacin, Clotrimazole and Terbenafeine.

Table1. Characterization data of compounds prepared.

Sr.No.	Substitution R	M. F.	M. W.	Melting Range (°C)	Yield (%)
3a	4-Br	$C_{12}H_7Br_2N_3$	353.01	161-163	80
3b	4-Cl	$C_{12}H_7ClN_3$	308.56	152-154	80
3c	4-Me	$C_{13}H_{10}BrN_3$	288.14	159-161	72
3d	4-OMe	$C_{13}H_{10}BrN_3O$	303.66	179-181	78
3e	4-F	$C_{13}H_{10}BrN_3$	303.11	154-156	68
3f	4-NO_2	$C_{12}H_7BrN_4O_2$	319.11	178-180	65
3g	3-NO_2	$C_{12}H_7BrN4O_2$	319.11	168-170	62
3h	H	$C_{12}H_7Br_2N_3$	353.01	161-163	75
3i	2,4-diCl	$C_{12}H_6BrCl_2N_3$	343.00	174-176	66

From the results of antimicrobial activity we can say that 3a is most active with MIC of 50µg/ml against *Bacillus Subtillius* with respect to standard Ampicillin, Ciprofloxacin and Norfloxacin having the same MIC, With excellent result of 3a in gram positive bacteria it also having MIC of 50µg/ml against Escherichia coli as a gram negative bacteria with respect to standard Ciprofloxacin having the same MIC, While compound 3f is moderately active.

Compound 3h is moderately active against and *Staphylococcus aureus* and *Shigella*. Looking at the antifungal results, compounds 3a, 3c, 3f, 3g, 3h are showing equally good activity having MIC of 100µg/ml against *Aspergillus niger* with respect to standard antifungal drugs like Clotrimazole and Terbinafein. While Compounds 3d, 3g& 3i are moderately active with respect to standard against Candida albicans with respect to standard and compounds 3b &3e are moderately active against *Aspergillus niger*. Overall we can say that 3a is most active antibacterial agent and 3f and 3h are moderate active against specific bacteria growth. While compounds 3a, 3c, 3f, 3g, 3h are excellent antifungal agents.

Table 2. Antimicrobial activity of compounds prepared.

Sr. No.	Antibacterial Activity MIC(µg/mL)				Antifungal activity MIC(µg/mL)	
	S.aureus	B. Subtillius	Shigella	E.Coli	C. Albicans	A.Niger
3a	500	**50**	250	**50**	250	**100**
3b	500	200	500	200	250	125
3c	200	500	500	500	250	**100**
3d	250	200	500	200	125	250
3e	500	250	500	250	250	125
3f	250	200	250	125	250	**100**
3g	200	250	250	200	125	**100**
3h	100	500	125	250	250	**100**
3i	200	500	500	250	125	250
A.	**50**	**50**	**50**	**25**	**-**	**-**
C.	**50**	**50**	**25**	**50**	**-**	**-**
N.	**50**	**50**	**10**	**10**	**-**	**-**
Cl.	**-**	**-**	**-**	**-**	**100**	**100**
T.	**-**	**-**	**-**	**-**	**100**	**100**

A: Ampiciliin, C: Ciprofloxacin, N: Norfloxacin, Cl: Clotrimazole, T: Terbinafeine

CONCLUSION

In summary, we have developed a facile and green reaction for the synthesis of imidazo [1,2-*a*] pyrazines and tested them as antimicrobial agents.

CONFLICT OF INTEREST

Authors have no conflict of interest for publication of the present work.

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