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Facile Synthesis and Biological Evaluation of Cyclopropyl-Pyrazole Hybrids in [bmim][PF_6]-Water Biphasic System as Antifungal Agents

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ABSTRACT

3-Cyclopropyl-5-(4-substituted)-1-phenyl-4,5-dihydro-1*H*-pyrazoles derived from corresponding chalcones were synthesized and evaluated for their biological activities. A convenient synthesis of a library of these compounds in 1-butyl-3-methylimidazolium hexafluorophosphate-water biphasic system at ambient temperature has been accomplished. The ionic liquid, [bmim][PF_6] and water which are immiscible, has been easily recycled and reused after separation of the products without any noticeable diminution in its activity.

KEYWORDS

Cyclopropyl, Pyrazole, ([bmim][PF_6]), Biphasic, Antifungal activity.

INTRODUCTION

Pyrazoles and their dihydro derivatives, pyrazolines, constitute an interesting heterocyclic family with diverse and defined therapeutic significance. The pyrazolines are used widely in the current decades due to their various biological and pharmacological activities [1]. The α,β-unsaturated ketones can play the role of versatile precursors in the synthesis of the corresponding pyrazoles [2-7]. Numerous pyrazoline derivatives have been found to possess considerable biological activities, which stimulated the research activity in this field. They have several prominent effects, such as antimalarial and antimicrobial [8], antitumor [9], anti-inflammatory [10], anticancer [11], antimycobacterial [12], analgesic [13,14] and antidepressant [15,16] activities. The most convenient syntheses of pyrazoles have usually been in solvents such as dichloromethane, chloroform at 65 °C, methanol-potassium hydroxide at 80 ºC, *N,N*-dimethylformamide and ethanol-potassium hydroxide.

Several synthetic routes have been developed for the preparation of pyrazoles and their derivatives. The synthesis of pyrazoles by [3+2] atom fragments has been relatively well investigated. In this method, β-diketones or their derivatives, such as the three atom fragment, are condensed with hydrazine and its derivatives (by two atom fragment) to close the five-membered ring [17]. Martins and coworkers have reported [18] first an efficient synthesis of 4,5-dihydropyrazoles, through

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the reaction of enones, with hydrazine derivative in the presence of equimolar quantities of ionic liquid [bmim][BF_4]. These reactions have some advantages over the same experiment carried out in the absence of an ionic liquid. Martins *et al.* [19] have also demonstrated another efficient and mild synthesis of 1-cyanoacetyl-5-hydroxy-5- halomethyl-4,5-dihydro-1*H*-pyrazoles, through the reaction of 4-alkoxy-3-alken-2-ones, with cyanoacetohydrazide using ionic liquid [bmim][BF_4]. Bazgir *et al.* [20] and Chaturvedi [21] have reported an efficient one-pot synthesis of 1*H*-pyrazolo [1,2-*b*]-phthalazine-5,10-dione derivatives, through the three-component reaction between phthalhydrazide, aromatic aldehydes and malononitrile or ethyl cyanoacetate in presence of *p*-toluene sulfonic acid (PTSA) using an ionic liquid, 1-*n*-butyl-3-methyl imidazolium bromide [bmim][Br] as solvent at 100 °C. A new series of pyrazole derivatives was synthesized from cyclic α,β-unsaturated ketones using catalytic amount of bleaching earth pH-12.5 (10 weight %) and PEG-400 as green solvent [22]. These cyclic α,β-unsaturated ketones were synthesized by Claisen-Schmidt Condensation of indan-1-one with different het/araldehydes [23] in the presence of a catalytic amount of bleaching earth (10 mol % of pH 12.5) and PEG-400 as green reaction solvent [24]. The condensations occur smoothly followed by the Michael addition of phenyl hydrazine to corresponding products. Water is recognized as an attractive medium for many organic reactions as it is the cheapest abundantly available solvent. The use of aqueous K_2CO_3 under MWI [25,26] not only gives good yield in less reaction time. The hydrophobic effect, by which nonpolar materials cluster to escape contact with water, can lead to advantages in rates and selectivity, when reactions are performed in water. Water, being polar in nature, is used in the synthesis of benzopyrano[4,3-*c*]pyrazoles under MWI. In this protocol, an active methylene reagent, 4-hydroxy coumarin, has been utilized with aromatic/heteroaromatic aldehydes and phenyl hydrazine hydrochloride taking K_2CO_3 as green base and water as green solvent to furnish a library of benzopyrano[4,3-*c*]pyrazoles [27]. Since water is used as solvent, this completely circumvents the use of other hazardous solvents to get improved yields with purity. The usage of K_2CO_3 also eliminates the requirement of solvent at work-up stages [28]. Hart and Brewbaker, first demonstrated a synthetic route towards pyrazole derivatives, consists of an intramolecular cycloaddition of 3-diazoalkenes generated from the corresponding ethyl alkenylnitrosocarbamates [29]. Doyle and Yan [30] observed formation of pyrazoles during reaction of the corresponding tosylhydrazone salt with $Rh_2(OAc)_4$. A one-pot synthesis of 3(5)-substituted-1*H*-pyrazoles from aldehydes and diethoxyphosphorylacetaldehyde tosylhydrazone is also described in the literature [31]. Another one-pot approach has been proposed by Aggarwal *et al.* [32] using diazo compounds generated *in situ* from tosylhydrazone salts. They studied two different routes to pyrazoles: first, direct 1,3-cycloaddition of diazo compounds onto alkynes and second, employing an olefin bearing a leaving group, which would afford the pyrazole after an elimination/aromatization of the cycloadduct intermediate. Moreover Grandi *et al.* [33] demonstrated that the tosylhydrazones of some acyclic α,β-unsaturated carbonyl compounds containing an hydrogen atom in β position, on treatment with $NaBH_4$ or CH_3ONa or K_2CO_3 in alcoholic solvents (such as CH_3OH) can lead to an intramolecular 1,3-dipolar cycloaddition to give pyrazole derivatives [34]. Nair *et al.* [35] reported 1,3-dipolar cycloaddition of chalcones and arylidene-1,3-dicarbonyls with diazosulfone for the regioselective synthesis of functionalized pyrazoles and pyrazolines.

The hydrobhobic ionic liquid [bmim][PF_6] is well known for its numerous catalytic applications. Different biochemical and chemical reations have been carried out using hydrophobic media containing hexafluorophosphate anions. Reaction conditions have been investigated using model ionic liquid [bmim][PF_6], in the formation of ioxazolines and also illustrated the reuse of [bmim][PF_6] to reactions of diversely substituted substrates [36].

We embarked on the synthesis of cyclopropane-pyrazoline hybrid molecules *via* the cyclocondensation of α,β-unsaturated carbonyl compounds with phenyl hydrazine. Several solvent systems have been explored to set the reaction conditions of these compounds. In the said synthesis, we have selected the fluoro-substituted α,β-unsaturated cyclopropyl ketone as a starting material. We have made efforts in generating a library of pyrazolines using different concentration of [bmim][PF_6]in biphasic solvent system and our observations prompted us to explore the potential use of [bmim][PF_6]/water solvent system.

EXPERIMENTAL

Chemicals used were of analytical reagent grade. All the solvents were dried and freshly distilled prior to use. Thin layer chromatographic analysis was performed on precoated silica gel plates (Alugram® SIL G/UV_{254}, 0.2 mm thickness). Melting points were recorded by open capillary method and are uncorrected. Infrared spectra were recorded on FT-IR spectrometer. Proton magnetic resonance (^{1}H NMR) spectral data were recorded on a Bruker 400 MHz spectrometer in DMSO-d_6 solution. The chemical shifts are reported in δ (ppm) relative to internal standard tetramethylsilane (TMS) and coupling constants *J* are given in Hz. Mass spectrometry was conducted using WATERS, Q-TOF MICROMASS.

General procedure

Preparation of 3-cyclopropyl-5-(4-fluorophenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazole (4a): [bmim][PF_6] 4 mL, H_2O (20 mmol), biphasic system,1-cyclopropyl-3-(4-fluorophenyl)prop-2-en-1-one (0.01 mol) and phenyl hydrazine (0.01 mol) were mixed in a round bottomed flask and stirred at 70 °C for 2 h. After completion of the reaction was monitored by TLC. The reaction was quenched with H_2O (5 mL) and the immicible ionic liquid layer was separated from the aqueous phase and extracted with Et_2O (10 mL). The extracted layer was dried over Na_2SO_4 and concentrated under reduced pressure to give the crude product. Recrystallization of the crude product from hexane afforded 2.2 g of **4a** as a yellow crystalline solid.

Yield: 90 %, m.p.: 89 °C. IR (KBr, ν_{max}, cm^{-1}): 1599 (C=N); 1329 (C-N); 3011, 3060 (=C-H). ^{1}H NMR (400 MHz, $CDCl_3$/DMSO-d_6): δ (ppm) = 0.82-0.88 (m, 4H, H_{a-d}); 1.87-1.91 (p, 1H, H_e); 3.21-3.28 (m, 1H, H_f); 2.27-2.33 (dd, 1H, *J* = 0.0177 Hz, H_g); 4.90-4.95 (q, 1H, H_h); 6.9-7.19 (m, 5H, Ar-H); 7.22 (s, 4H, Ar-H). ^{13}C NMR (200 MHz $CDCl_3$): δ 7.90, 7.26, 13.15,

40.96, 62.00, 112.99, 118.14, 127.22, 130.00, 131.00, 138.19, 143.89, 158.91. Mass spectrum: *m/z* 280M$^+$, 261, 203, 185, 95.77. CHN calculated (found): C 77.12 (77.10); H 6.11 (6.08); N 9.99 (9.87).

3-Cyclopropyl-5-(4-methoxyphenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazole (4b): Yield: 85 %; m.p.: 93 °C. IR (KBr, ν_{max}, cm^{-1}): 1330 (C-N); 1597 (C=N); 3001-3066 (=C-H). ^{1}H NMR (400 MHz, $CDCl_3$/DMSO-d_6): δ (ppm) = 0.68-0.73 (m, 4H, H_{a-d}); 1.74-1.78 (m, 1H, H_e); 2.28 (s, 3H, CH_3); 2.07-2.13 (dd, 1H, *J* = 0.0178, 0.0182 Hz, H_g); 2.93-3.02 (m, 1H, H_f); 5.32-5.37 (q, 1H, H_h); 6.71-7.18 (m, 5H, Ar-H); 7.22 (s, 4H, Ar-H). ^{13}C NMR (200 MHz $CDCl_3$): δ 7.10, 6.95, 12.95, 40.90, 55.43, 64.00, 114.00, 118.82, 127.92, 130.91, 131.30, 138.20, 146.56, 154.81, 157.96. Mass spectrum: *m/z* 292 M$^+$, 261, 277, 215, 185, 107, 77. CHN calculated (found): C 78.05 (78.01); H 6.89 (6.87); N 9.58 (9.56).

3-Cyclopropyl-1-phenyl-5-(*p*-tolyl)-4,5-dihydro-1*H*-pyrazole (4c): Yield: 82 %; m.p.: 90 °C. IR (KBr, ν_{max}, cm^{-1}):1346 (C-N); 3053, 3084 (=C-H); 1599 (C=N). ^{1}H NMR (400 MHz, $CDCl_3$/DMSO-d_6): δ (ppm) = 0.78-0.83 (m, 4H, H_{a-d}); 1.82-1.86 (p, 1H, H_e); 2.28 (s, 3H, CH_3); 2.40-2.46 (dd, 1H, *J* = 0.0177, 0.0177 Hz, H_g); 3.27-3.34 (m, 1H, H_f); 5.02-5.07 (q, 1H, H_h); 6.60-7.09 (m, 5H, Ar-H); 7.12 (s, 4H, Ar-H). ^{13}C NMR (200 MHz $CDCl_3$): δ 5.70, 5.38, 11.25, 20.61, 39.69, 62.68, 112.49, 117.62, 125.72, 128.61, 129.40, 136.28, 139.89, 145.46, 153.71. Mass spectrum: *m/z* 276M$^+$, 261, 199, 185, 91, 77. CHN calculated (found): C 82.57 (82.54); H 7.29 (7.27); N 10.14 (10.13).

5-(4-Chlorophenyl)-3-cyclopropyl-1-phenyl-4,5-dihydro-1*H*-pyrazole (4d): Yield: 91 %; m.p.: 105 °C. IR (KBr, ν_{max}, cm^{-1}):1597 (C=N); 1324 (C-N); 3011, 3057 (=C-H). ^{1}H NMR (400 MHz, $CDCl_3$/DMSO-d_6): δ (ppm) = 0.69-0.74 (m, 4H, H_{a-d}); 1.74-1.78 (p, 1H, H_e); 3.01-3.11 (m, 1H, H_f); 2.15-2.21 (dd, 1H, *J* = 0.0178, 0.0178 Hz, H_g); 5.09-5.14 (q, 1H, H_h), 6.51-7.58 (m, 5H, Ar-H); 7.61 (s, 4H, Ar-H). ^{13}C NMR (200 MHz $CDCl_3$): δ 7.20, 6.99, 12.95, 41.00, 63.18, 115.09, 120.02, 127.12, 129.91, 130.52, 135.88, 141.59, 146.86, 159.21. Mass spectrum: *m/z* 296M$^+$, 261, 219, 185, 77. CHN calculated (found): C 72.84 (72.80); H 5.77 (5.75); N 9.44 (9.42).

4-(3-Cyclopropyl-1-phenyl-4,5-dihydro-1*H*-pyrazol-5-yl)-*N*,*N*-dimethyl aniline (4e): Yield: 86 %; m.p.: 154 °C. IR (KBr, ν_{max}, cm^{-1}): 1600 (C=N); 1346 (C-N); 3022, 3087 (=C-H). ^{1}H NMR (400 MHz, $CDCl_3$/DMSO-d_6): δ (ppm) = 0.77-0.82 (m, 4H, H_{a-d}); 1.83-1.87 (m, 1H, H_e); 3.92 (s, 6H, CH_3); 2.16-2.22 (dd, 1H, *J* = 0.0180, 0.0175 Hz, H_g); 3.03-3.12 (m, 1H, H_f); 5.43-5.48 (q, 1H, H_h); 6.83-7.31 (m, 5H, Ar-H); 7.34 (s, 4H, Ar-H). ^{13}C NMR (200 MHz $CDCl_3$): δ 7.88, 7.32, 13.10, 40.20, 43.90, 60.50, 113.29, 118.92, 124.99, 128.50, 133.99, 144.00, 148.06, 159.01. Mass spectrum: *m/z* 305M$^+$, 290, 275, 261, 228, 185, 120, 77. CHN calculated (found): C 78.65 (78.62); H 7.59 (7.56); N 13.76 (13.73).

Antifungal activity: All the newly synthesized compounds were screened *in vitro* for their antifungal activity against a fungal strains such as clinically isolated *A. niger, C. albicans* and *A. flavus* by the cup-plate method. The nutrient agar broth were prepared by aseptic inoculation with 0.5 mL of 24 h old subcultures of clinically isolated *A. niger, C. albicans* and *A. flavus* in separate flasks at 40-50 °C and mixing well by gentle shaking.

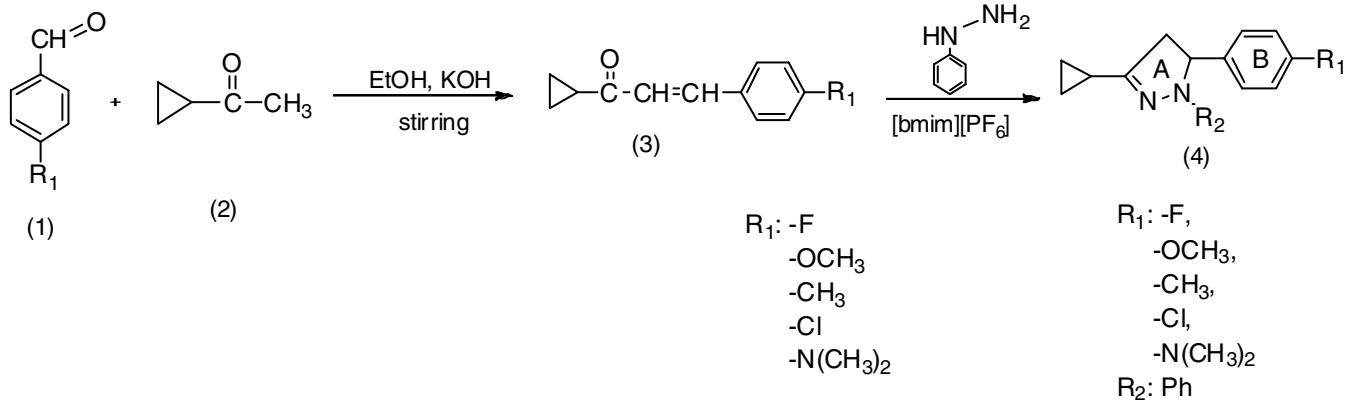
About 25 mL of the contents of the flask were poured and evenly spread in a Petri dish (13 cm in diameter) and allowed to set for 2 h. Each test compound (20 mg) was dissolved in 2 mL of DMSO, which is used as a sample solution. A concentrated (100 µg/mL) solution was prepared by dilution method. Sample size for all the compounds was fixed as 10 µL.

The plates were incubated at 25 °C for 24 h, the control was similarly maintained with 1 mL of DMSO and the zones of inhibition of the fungal growth were measured in mm using zone reader.

Detection method: All solvents were distilled prior to use. TLC was performed on silica gel G (Qualigen). Melting points were determined by open capillary method and are uncorrected. ^{1}H NMR spectra were recorded in $CDCl_3$/DMSO-d_6 solution on a Brucker Avance II 400 NMR spectrometer and ^{13}C NMR spectra was recorded from $CDCl_3$. Chemical shifts are reported in ppm using TMS as an internal standard. IR spectra were obtained on a Shimadzu FT-IR spectrophotometer using KBr discs. Mass spectra were recorded by using Shimadzu gas chromatograph.

RESULTS AND DISCUSSION

Initially compound **3a** was chosen as a model substrate. We have optimized the reaction conditions in various solvents at different time intervals. Compound **3a** was subjected to reaction with phenyl hydrazine to afford 3-cyclopropyl-5-(4-fluorophenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazole (**4a**) (**Scheme-I** and Table-1).



Scheme-I: Synthesis of cyclopropyl-pyrazole hybrids

TABLE-1
OPTIMIZATION OF THE REACTION CONDITIONS FOR THE SYNTHESIS OF 3-CYCLOPROPYL-5-(4-FLUOROPHENYL)-1-PHENYL-4,5-DIHYDRO-1*H*-PYRAZOLE (**4a**)

Entry	Solvent	Temp. (°C)	% of isolated yield at time interval			
			1 h	2 h	3 h	4 h
1	EtOH/KOH (**S_1**)	Reflux	13	15	40	68
2	EtOH/KOH/water (**S_2**)	Reflux	15	21	51	75
3	AcOH (**S_3**)	Reflux	18	28	60	60
4	AcOH/water (**S_4**)	Reflux	12	18	36	65
5	DMF (**S_5**)	Reflux	09	20	35	50
6	DMF/water (**S_6**)	Reflux	11	26	46	55
7	$[Et_3N][HSO_4]$ (**S_7**)	70	20	35	60	80
8	$[bmim][PF_6]$ (**S_8**)	70	30	50	76	88
9	$[bmim][PF_6]$/water (**S_9**)	70	50	90	90	90

We have implemented the optimization model, which was earlier reported in the synthesis of isoxazolines in our laboratory by Lanjewar *et al.* [36]. The reaction of compound **3a** and phenyl hydrazine in ethanol with catalytic amount of KOH **S_1** at reflux was completed within 4 h, giving compound **4a** in 68 % isolated yield. The reaction time was cautiously controlled to avoid the disintegration of products and formation of byproducts. It was examined that the reaction progressed well by addition of a small amount of water, the yield was improved to 75 %. The effectiveness of reaction was strikingly influenced by the nature of solvents, when we increased the hydrophilicity of the reaction mixture **S_2**. Further reaction in acetic acid **S_3** and acetic acid/water **S_4** at reflux for 4 h reduced the yield. In **S_4**, the yield obtained was slightly more than in **S_3**. The yield of compound **4a** was considerably reduced in DMF **S_5** and DMF/water **S_6** at reflux for 4 h. The reaction in DMF/water **S_6** system showed 55 % yield indicating a preference protic conditions.

Furthermore, the reactions in ionic liquid $[Et_3N][HSO_4]$ **S_7**, $[bmim][PF_6]$ **S_8**, $[bmim][PF_6]$/water **S_9** were monitored at 70 °C after various time intervals. The reaction took place at ambient temperature in ionic liquids **S_7**, **S_8** and **S_9**. The use of $[bmim][PF_6]$/water **S_9** resulted in much elevated reaction rates than those performed with **S_7-S_8** solvent systems. It was observed that the reaction of compound **3a** proceeded better in all ionic liquids as compared to common organic solvents **S_1-S_6**. Reaction in **S_9** showed the utmost levels of efficacy and atom economy when compared to solvents **S_1-S_8**. In addition, use of water as a secondary solvent in all protic, aprotic and ionic liquid systems improved isolated yields. Table-1 summarizes our results, clearly showing the superiority of ionic liquid-water biphasic system over organic solvent-water system.

The course of the reaction was monitored by TLC but we found that for all early experiments, TLC or HPLC was not an optimal choice for evaluation of yields or for a quantitative in-process assay; the viscous solution containing water and ionic liquids was not conductive to TLC. We resorted to quenching the reaction by adding little amount of water and furthermore, the product was isolated by extraction with Et_2O in 4 installments. All elemental analysis was conducted on isolated compounds and yields were calculated on purified compounds.

We extended our investigation to other substrates by varying the substituents on ring B (Table-2).

TABLE-2
PREPARATION OF CYCLOPROPYL INCORPORATED PYRAZOLES AND THEIR DERIVATIVES

Entry	Product	R_1	R_2	Yield[a] (%)
1	**4a**	F	Ph	90
2	**4b**	OCH_3	Ph	85
3	**4c**	CH_3	Ph	82
4	**4d**	Cl	Ph	91
5	**4e**	$-N(CH_3)_2$	Ph	86

[a]Isolated yield

The incorporation of electron donating substituents at the *para* position of ring B (compounds **4b, 4c, 4e**) resulted in decreased yield. Table-2 reveals that the substrates with electron withdrawing groups at *para* positions of ring B (R_1) result in increased efficiency of the reaction.

To verify the impact of concentration of $[bmim][PF_6]$ on the efficiency of the reaction, we gradually increased the concentration of $[bmim][PF_6]$/water from 1-15 mmol keeping 20 mmol of water constant at 70 °C for 2 h (Table-3). It was observed that as concentration of $[bmim][PF_6]$ in water increased, the efficiency of the reaction also increased. On completion, variations in yield were observed, prompted us to reproduce this procedure several times; proving that variations in yield were not attributable to work-up losses. Beyond 10 mmol of $[bmim][PF_6]$ the efficiency was decreased, suggesting that the nonpolar organic hydrophobic tail of ionic liquid may interfere with the reaction mechanism.

TABLE-3
EFFECT OF VARYING CONCENTRATION OF THE IONIC LIQUID $[bmim][PF_6]$/WATER FOR THE SYNTHESIS OF 3-CYCLOPROPYL-5-(4-FLUOROPHENYL)-1-PHENYL-4,5-DIHYDRO-1*H*-PYRAZOLE (**4a**)

Entry	$[bmim][PF_6]$ (mmol)	Water (mmol)	Temp. (°C)	Yield (%)
1	1	20	70	84
2	3	20	70	85
3	7	20	70	87
4	10	20	70	90
5	13	20	70	82
6	15	20	70	81

Balanced hydrophobic-hydrophilic solvent interactions have been observed. Increased yields were observed by addition of measured amount of water to all solvent systems, suggested

that an environment of polar solvents or protic solvents is more conducive to the reaction.

Interestingly, the ionic liquid-water biphasic system such as [bmim][PF_6]-water proved to be exceptionally effective in enhancing the efficiency of the reaction. [bmim][PF_6] was utilized in a biphasic system as substrate reservoir for substrates that are poorly soluble in water/organic solvent mixtures. A plausible explanation of the reaction mechanism [36] in ionic liquid-water biphasic system is as follows:

1-Butyl-3-methyl imidazolium hexafluorophosphate [bmim][PF_6] is hydrophobic and immiscible with water. One of the reactants **3a** is hydrophobic while phenyl hydrazine is hydrophilic in nature. Different solvation affinities of these two reactants retard the reaction. **S_9** solvent system provides a platform in which the non-polar organic counterpart $[bmim]^+$ easily makes compound **3a** soluble; $[PF_6]^-$ water counterpart makes phenyl hydrazine soluble. The phenyl hydrazine in aqueous phase is transported to ionic liquid phase where reaction occurs at the junction of two immiscible solvents (Fig. 1). This suggests that ionic liquid biphasic system effectively delivers the substrates at the interface of two immiscible liquids.

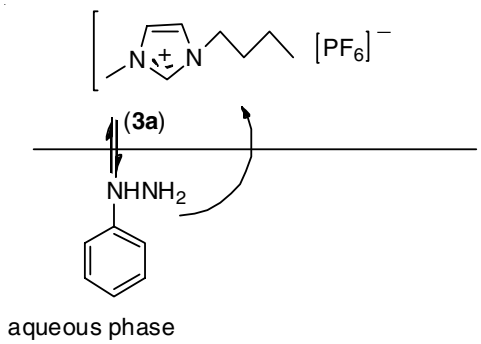


Fig. 1. A plausible mechanism in ionic liquid-water biphasic system [Ref. 36]

On completion of the reaction, we have optimized the extraction and isolation of the resulting product in ionic phase by using various solvents such as diethyl ether, di-chloromethane, ethyl acetate and *n*-hexane. The best results were obtained with diethyl ether and this was the solvent of choice for further experiments. Inorganic salts, formed as byproduct dissolved in water. The separated ionic liquid was flushed out with water and reused directly without further purification without diminution of the yields up to the 10 cycle but there is noticeable drop in yield after 10^{th} cycle suggested that the catalyst may have contaminated or degraded or exhausted. Protocol has the merit of being environmentally benign, possessing simple operation, convenient work-up, reduced time and proceeding in good yields.

For elucidation of the structure **4a-e**, the IR spectra showed a peak at 1599 cm^{-1} due to C=N stretching of pyrazoline ring, peak at 1329 cm^{-1} because of C-N stretching of pyrazoline ring respectively. Disappearence of peak at 1657 cm^{-1} associated with α,β-unsaturated carbonyl compound **3**, confirmed the formation of compound **4**. Furthermore ^{1}H NMR spectra of compounds **4a-e** showed a singlet of 3 protons at δ 2.25-2.28 due to Ar-CH_3 protons. In pyrazoline ring, there are three types of hydrogens, one attached to C_3 carbon *i.e.* H_h and (H_f and H_g) two at C_4 carbon of pyrazoline ring. The two hydrogens attached to C_4 position are juxtaposed *cis* and trans H_f and H_g. ^{1}H NMR spectra showed for H_f, multiplets at δ 3.27-3.36 due to H_g proton and H_h. H_h coupled with both H_g and H_f to give a double doublet at δ 5.02-5.07. The multiplet at δ 2.40-2.51 due to H_h and H_f proton, confirms the structure of pyrazoline ring. In addition, -OCH_3 group of compounds **4b** resonated as singlet at δ 3.89-3.91 integrating for three protons (**Scheme-I**). The ^{13}C NMR spectra also support the structure; signals at δ 40, 55, 127, 146 and 154.96 indicate the presence of pyrazoline ring. Compounds **4a-e** gave satisfactory elemental analysis; mass spectra also lend credence to the structures.

Stereochemical aspects: The structure of the compounds displayed the stereochemical centre at C_3 position of pyrazoline ring. The reactants used are non stereochemical components. Thus the reactions are not stereochemically controlled reactions. To reveal the stereospeficity of the compounds, they were tested for specific rotation. It was observed that all the compounds were found to be optically inactive with no specific rotation. Thus we have not atempted to resolve the chiral centres: this was referred to later date pending obtaintion of possible activity and subsequent optimization.

***in vitro* Antifungal activity:** All cyclopropyl-pyrazole derivatives were screened against clinically isolated *A. niger, C. albicans* and *A. flavus* as antifungal agents. The cytotoxicity of all scaffolds was compared with griesofulvin for antifungal study. The antifungal screening was carried out by cup-plate method at different levels of concentration (12.5, 25, 40, 50 µg/mL) in solvent DMSO. No zones of inhibition were observed at concentrations 12.5 and 25 µg/mL. It was found that, *C. albicans* were highly sensitive to compounds **4a-d** and showed strong zones of inhibition. However, compound **4e** showed moderate sensitivity towards *C. albicans*. The inhibitory effects of compounds **4a-e** against *A. niger* were evaluated and it was observed that except compound **4e**, the rest of the compounds displayed moderate sensitivity against the microbs. *A. flavus*

TABLE-4
ANTIFUNGAL ACTIVITY OF COMPOUNDS **4a-e**

Compound	Zone of inhibition (mm)					
	C. albicans		*A. niger*		*A. flavus*	
	40 µg/mL	50 µg/mL	40 µg/mL	50 µg/mL	40 µg/mL	50 µg/mL
4a	15	18	9	10	-	-
4b	16	18	8	9	15	20
4c	14	18	8	9	-	15
4d	14	16	9	12	-	-
4e	10	7	10	15	-	-
Gresiofulvin 50 µg	18		20		20	

were found to be resistant to all compounds, showed poor zones of inhibition. The results are depicted in Table-4. The results were compared with gresiofulvin 50 µg/mL as standard drugs.

Conclusion

In conclusion, the ionic liquid [bmim][PF_6]/water proved to be an exceptionally efficient biphasic solvent system for the synthesis of isoxazolines at ambient temperature within 2 h. The protocol has the merit of environment friendliness, simple operation, convenient work-up, reduced time and good yield. [bmim][PF_6] can be reused up to 10^{th} cycle but after 10^{th} the loss of its activity has been observed. The reaction is influenced by aqueous-[bmim][PF_6] biphasic system which stabilizes the hydrophobic reactant and water stabilizes hydrazine hydrate: the reaction presumably occurs at the junction of two immiscible phases. The study was finally completed by performing their *in vitro* antifungal activities of all compounds. The compounds **4a-e** showed exceptional antifungal activity at 50 µg/mL against *C. albicans* strain. However, no activity has been detected against *A. flavus*. The moderate antifungal activity has been revealed against *A. niger* at 50 µg/mL.

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