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Ionic liquid mediated synthesis of some novel fluoro isoxazolidine and isoxazoline derivatives using *N*-Benzyl fluoro nitrone via cycloaddition reaction and their antimicrobial activities

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Abstract

1-Butyl-3-methylimidazolium based ionic liquids are found to accelerate significantly the intermolecular 1,3-dipolar cycloaddition of *N*-benzyl-fluoro nitrone derived *in situ* from 2,6-difluoro benzaldehyde and *N*-benzylhydroxylamine, with activated alkenes and electron deficient alkynes to afford enhanced rates and improved yields of isoxazolidine, isoxazolines. All the novel isoxazolidine and isoxazoline derivatives have been screened for antimicrobial activities and found to be very active.

Keywords: *N*-Benzyl fluoro nitrone, cycloaddition reaction, fluoro isoxazolidine & isoxazolines, ionic liquid, 1,3-amino alcohol, aldehyde/ketone synthesis, antimicrobial activity

The 1,3-dipolar cycloaddition reactions are among the most important synthetic routes for the construction of five-membered heterocycles, important frameworks of various natural products[1]. In particular the 1,3-dipolar cycloadditions of nitrones with alkenes and alkynes afford isoxazolidines, isoxazolines which are interesting intermediates for the synthesis of *β*-amino alcohols and alkaloids[2,3]. Isoxazolines posses medicinal activities such as antibacterial, anticonvulsant, antibiotic, antituberculer and antifungal activity[4,5]. Despite their potential utility, many of these procedures require high temperature and prolonged reaction times (drastic experimental conditions) and also suffer from poor regioselectivity, and lack of simplicity. In few cases, the yields and selectivities reported are far from satisfactory due to the occurrence of several side reactions[6]. In recent times, ionic liquids have emerged as green solvents with desirable properties such as good solvating ability, wide liquidious range, tunable polarity, high thermal stability, negligible vapour pressure and ease of recyclability[7].

Therefore, classical organic reactions can be performed in these media with great advantages

(yield and selectivity) as compared to conventional conditions. They are referred to as 'designer solvents' as their properties such as hydrophilicity, hydrophobicity, Lewis acidity, viscosity and density can be altered by the fine-tuning of parameters such as the choice of organic cation, inorganic anion and the length of alkyl chain attached to an organic cation

(**Figure 1**).

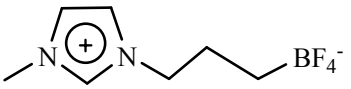


Figure 1- Chemical structure of ionic liquid

These structural variations offer flexibility to the chemist to plan for the most idealized solvent, catering to the needs of any particular process. Since ionic liquids are entirely composed of non-coordinating ions, they can provide an ideal reaction medium for reactions that involve reactive ionic intermediates. Due to the stabilization of charged intermediates by ionic liquids, they can promote unprecedented selectivities and enhanced reaction rates. Consequently, ionic liquids are being used as recyclable solvents for the immobilization of transition metal based catalysts, Lewis acids and enzymes[8]. As a result of their green credentials and potential to enhance reaction rates and selectivities, ionic liquids are finding increasing applications in organic synthesis[9] with an ever-increasing quest for exploration of newer reactions in ionic liquids[10].

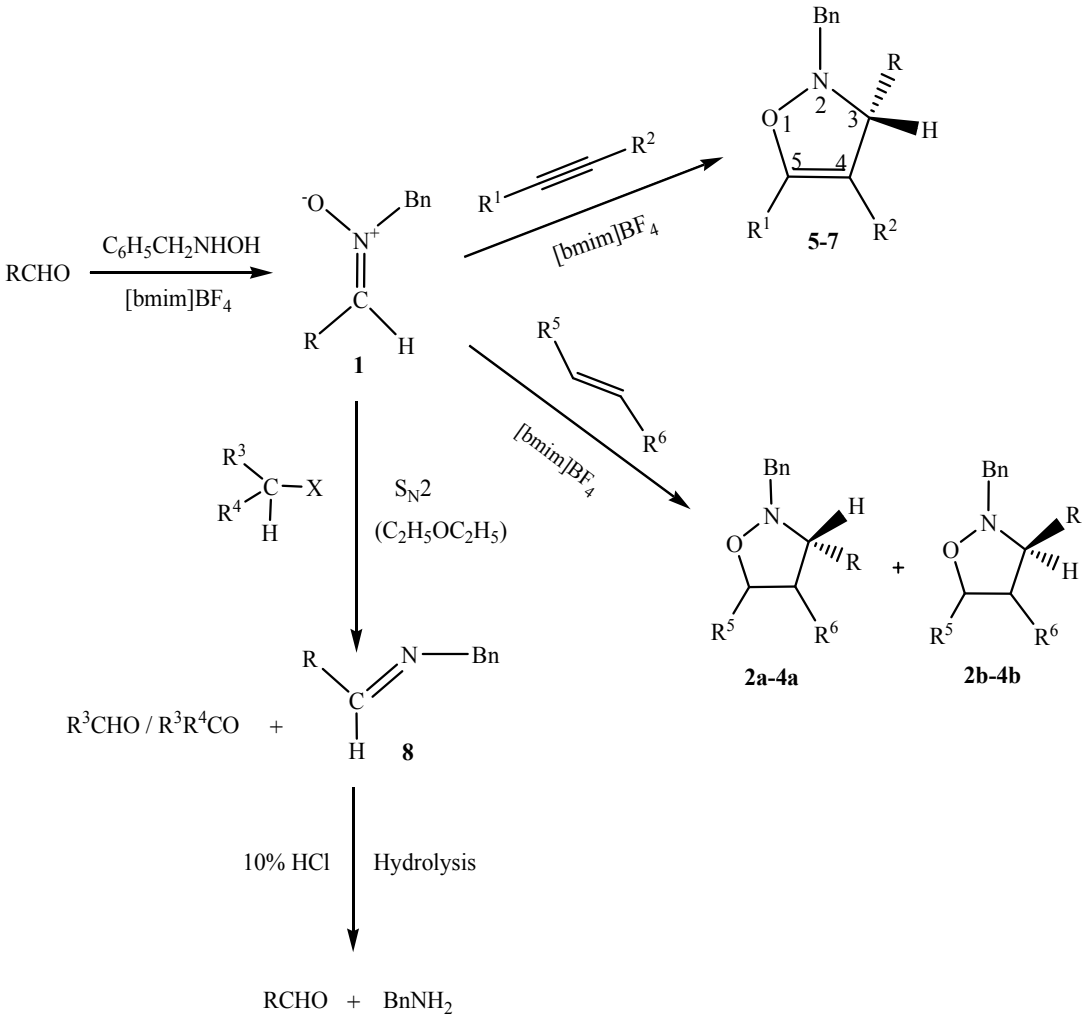
It is known that introduction of fluorine atom into specific position of organic molecule may cause significant changes in the stability, lipophilicity and biological activities of the resulting molecules[11]. This has been attributed to the high electro negativity of the halogen, the strong C-F bond and the similar size of the halogen and hydrogen atoms. For these reason great efforts has been placed on the development and evaluation of biologically active fluorinated materials[12]. The biological properties of multifluorine containing compounds have been recently investigated. Owing to their unique properties, such as high thermal stability and lipophilicity, fluoro-organic compounds have been frequently used as biorelated material, medicine and agrochemicals[13]. The presence of a fluoro group due to a low polarizability and high lipophilicity induces a relative metabolic stability and improves the bioavailability of the modified heterocycles compared to its hydrocarbon analogues[14,15].

In continuation of our effort to establish green methodologies in nitrone cycloaddition reactions[16-21], herein, we wish to report the use of ionic liquid as recyclable solvent for 1,3-dipolar cycloaddition reactions of *N*-benzyl fluronitrone (having vast synthetic potentials) with active alkenes and electron deficient alkynes to produce fluoro isoxazolidine and isoxazoline derivatives with vast biological activity in a one-pot operation (**Scheme I, Table I**). Compared to conventional conditions the cycloaddition reactions performed in ionic liquids are much faster and selective.

Results and discussion

As an example, the reaction between **1** and alkynes, afforded cycloaddition derivative **5** after 17 h in CH_2Cl_2 in 67% yield and 88% yield (entry **4**) in [bmim]BF_4 at room temperature after

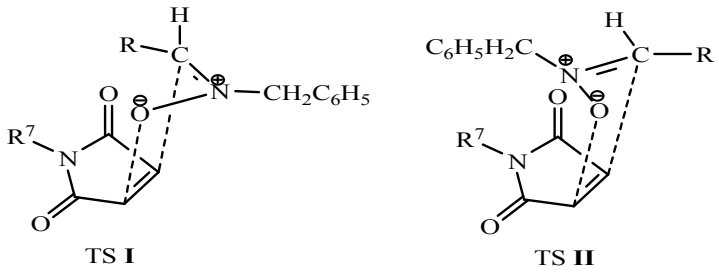
26 min respectively. In a typical procedure 1 mmol of nitrone was mixed with 1 equivalents of alkynes/alkenes in [bmim]BF_4 (2 mL) under stirring, at room temperature. After the development of nitrone (monitored by TLC), 1 mmol of dipolarophile **was** added *in situ* and the progress of the reaction was monitored by TLC. After completion of reaction, the reaction mixture was washed with diethyl ether (3x10mL). The combined ether extracts were concentrated *in vacuo* and the resulting product was directly charged on silica gel column and



R = 2,6 difluro benzene ; Bn = $C_6H_5CH_2$
R^1 = Ph ; R^2 = $COOCH_3$
R^1 = R^2 = $COOCH_3$
R^1 = R^2 = COOH
R^3 = Et ; Ph ; R^4 = H etc
R^3 = R^4 = Ph ; R^3 = Me ; R^4 = Ph
R^5,R^6 = -COPhCO- ; -COMeCO- ; -COCyCO-

Scheme I - Synthesis of fluoro isoxazoline & isoxazolidine derivatives using fluoro nitrone and application of the nitrone in atom efficient aldehyde & ketone synthesis

eluted with a mixture of ethyl acetate:*n*-hexane (1:8) to afford pure isoxazoline. The rest of the viscous ionic liquid was further washed with diethyl ether and dried at 80^0C under reduced pressure to retain its activity in subsequent runs and was reused up to five times without loss of activity nor selectivity after five cycles. We have intentionally stopped the recycle at the fifth cycle, however we are convinced that this process may be carried on many more times. Excellent diastereofacial selectivity and faster reaction reaction rates has been observed when the reaction of nitrone **1** with activated alkenes (maleimides) are carried out in RTIL's. For example, the reaction between **1** with *N*-phenyl maleimide, afforded cycloaddition derivatives **2a & 2b** after 12 h in CH_2Cl_2 in 68% yield and 88% yield (entry **1**) in [bmim]BF_4 at room temperature after 26 min respectively. The addition of nitrone **1** to maleimides results in a mixture of diastereomer **2a–4a** and **2b–4b** (almost 65: 25 ratio in all cases) and generation of as many as three chiral centres in a single step. Studies of organic reactions in ionic liquid shows that there is a higher probability of the formation of mixture of diastereomers when ionic liquid is used as solvent rather than conventional organic solvents. These results can be rationalized by an *exo* approach of nitrone **1** which has *Z* configuration for the formation of major cycloadducts **2a–4a** (transition state **I, Figure 2**). The minor cycloadducts **2b–4b** is formed by the *endo* approach of *Z* nitrone (transition state **II, Figure 2**). The mixture of diastereomers are identified by considering the multiplicity of the proton signals at 3-H and 4-H along with their coupling constant values[22,23]. The most significant differences in the ^{1}H NMR data for the diastereomers are the position and multiplicity of the 3-H signal. In the major adducts **2a–4a**, coupling constant between 3-H & 4-H has been measured as $J_{3,4} \sim 6.26$ Hz implying a *cis* relationship between H-3 and H-4, whilst for minor adducts **2b–4b**, $J_{3,4}$ is ~ 2.26 Hz implies a *trans* relationship between H-3 and H-4[22,23].



$R^7 = CH_3/C_6H_5/C_6H_{11}$

Figure 2 - Exo/endo approach of the nitrone to the maleimides in cycloaddition reactions

Several butylmethylimidazolium based ILs, [bmim]X, with varying anions (X=PF_6^-, Br^-, BF_4^-) were screened for this reaction. Evidently, [bmim]BF_4 was found to be superior in terms of yield (88%) and reaction time (26 min) as compared with [bmim]PF_6 (84%; 43 min; entry **4**). For optimizing the conditions, we used the substrates in different ratios. It was found that best results were obtained using 1:1 reactant ratio. The reaction in [bmim]BF_4 was also conducted at elevated temperatures for optimizing the conditions and no significant improvements were observed in yields and reaction times. We examined the reaction under neat condition also, without using IL, to demonstrate catalytic ability of [bmim]BF_4. This result clearly indicates that [bmim]BF_4 has significant catalytic role in this reaction (**Table I**).

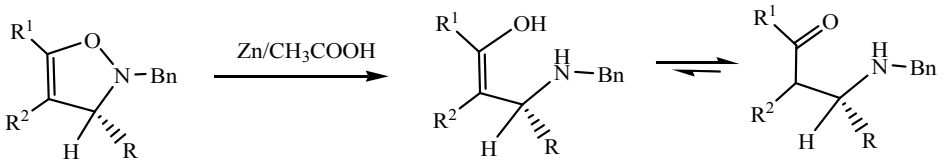
Table I — Physicochemical data of synthesized compounds **2a-4a ; 2b-4b & 5-7**

Entry	Nitrone	Dipolarophile[a]	Time (min)	Cycloadduct, m.p.(°C), **2a-4a: *cis*** ; **2b–4b: *trans***	*Cis/trans ratio (%)*	Yield[b] (%)
1	*N*-benzyl fluoro nitrone	*N*-phenyl maleimide	26 (12h)	**2a**: White crystals, 128 **2b**: White crystals, 102	**2a**: 66 **2b**: 22	88 (68)
2	*N*-benzyl fluoro nitrone	*N*-methyl maleimide	30 (13h)	**3a**: White solid, 135 **3b**: White solid, 120	**3a**: 65 **3b**: 21	86 (66)
3	*N*-benzyl fluoro nitrone	*N*-cyclohexyl Maleimide	36 (13h)	**4a**: Yellow crystals, 142 **4b**: Yellow crystals, 113	**4a**: 63 **4b**: 22	85 (66)
4	*N*-benzyl fluoro nitrone	Methyl phenyl propiolate	26 (17h)	**5**: Dark red thick liquid		88 (67)
5	*N*-benzyl fluoro nitrone	Dimethyl acetylene dicarboxylate	30 (19h)	**6**: Red viscous liquid		86 (65)
6	*N*-benzyl fluoro nitrone	Acetylene dicarboxylic acid	32 (18h)	**7**: Colourless thick liquid		86 (66)

[a] Reaction conditions: nitrone (1 mmol), dipolarophile (1 equivalent), [bmim]BF_4 (2 mL), N_2 atmosphere, RT
[b] All products were characterized by IR, ^{1}H NMR, ^{13}C NMR and MS spectral data.
[c] Isolated yield after purification. Figures in parentheses indicate reactions performed in conventional methods.

All the novel fluoro cycloadducts are stable and prominent molecular ion peak, base peaks are obtained in the mass spectrum as expected. All the maleimide cycloadducts have shown a common mass fragmentation pattern leading to the development of a base peak (B.P) due to the fragmentation of benzyl and 2,6 difluro phenyl ring from these molecules and thereby showing a correlation in their structures. IR spectral studies of the maleimide cycloadducts also supports the structural correlation as far as the carbonyl group and aromatic C-H absorptions are concerned. In case of fluoro isoxazoline derivatives (**5-7**), we have also obtained expected fragmentation peaks due to the development of different aziridine derivatives. Base peaks are obtained due to loss of PhCO for phenyl methyl propiolate, $COOCH_3$ for dimethyl acetylene dicarboxylate and COOH for acetylene dicarboxylic acid cycloadducts respectively. Hence it is confirmed that during mass fragmentation, the isoxazoline cycloadducts underwent rearrangement to aziridine derivatives. Structures of all the isoxazolidine and isoxazoline derivatives (2-7) have been confirmed on the basis of expected signals obtained in ^{1}H NMR, ^{13}C NMR, MS and FT-IR spectrum. Satisfactory elemental analysis values were also obtained for all the novel cycloadducts.

Furthermore, synthetic potential of the novel fluoro isoxazoline derivatives (**5-7**) are tremendous as they could be converted into 1,3 difunctional amino alcohols (**Scheme II**). Studies are in progress.



Scheme II - Synthesis of 1,3 amino alcohols from isoxazoline derivatives

Synthetic potentiality of nitrone **1** has been tested successfully as an oxidizing reagent in the conversion of alkyl halides to aldehydes and ketones (**Scheme I**) following a pattern of atom efficient reactions reported by our group[16]. Studies are in progress in our laboratory. We have already reported synthesis of various aldehydes and ketones from alkyl halides using α-chloro nitrones in atom efficient reactions[16,24].

Antimicrobial screening test

In vitro antimicrobial evaluation of all the synthesized novel compounds (2-7) were tested on fifteen (15) bacterial strains (depicted in Table II) with amoxicillin as reference[25]. Nutrient agar was used as culture medium and the strains were grown at 37°c for 24 hrs. The suspension was prepared by matching a 0.5 McFarland standard[26]. The compounds were dissolved in 4% dimethyl sulphoxide solution along with sterile distilled water for screening using Agar dilution[27]. The susceptible organisms were screened for minimum inhibitory concentration (MIC) using standard Cup plate assay method[28]. 0.1 mL of bacterial solution (2 x 10^6 CFU/mL) was transferred to nutrient agar plates and uniformly spread by a sterile glass spreader. The sensitivity was evaluated by measuring the presence of clear zone of inhibition on agar surface around the wells observed after 24 hrs of incubation at 37ºC[29]. From the antimicrobial study, we have obtained the MIC values (Table II) of the synthesized novel compounds (2-7) and their corresponding zone of inhibition using amoxicillin as reference antimicrobial agent (Table III). Detailed study confirms that compound 6 has no antimicrobial activity, compound 7 has its effect specifically on *Salmonella typhi* 62 and *Vibrio cholerae* 20 which gives an indication of new enteric drug. Compound 2a, 3b, 4a, 4b, 5 and 7 have been found to be very effective against gram positive and gram negative organisms which gives an opportunity to develop new broad spectrum antimicrobial agents. Screening study on compound 2b and 3a are going on at present.

Experimental Section

^{1}H NMR spectra were recorded with a Bruker DRX 300 spectrometer (300 MHz, FT NMR) using TMS as internal standard. ^{13}C NMR spectra were recorded on the same instrument at 75 MHz. The coupling constants (*J*) are given in Hz. IR spectra were obtained with a Perkin-Elmer RX 1-881 machine as film or as KBr pellets for all the products. MS spectra were recorded with a Jeol SX-102 (FAB) instrument. Elemental analyses (C,H,N) were performed with a Perkin-Elmer 2400 series CHN analyzer. All the reactions were monitored by TLC using 0.25 mm silica gel plates (Merck $60F_{254}$ UV indicator) while column chromatography was performed with silica gel (E.Merck India) 60–200 mesh. Starting materials and reagents used in the reactions (*N*-benzylhydroxylamine, 2,6 difluoro benzaldehyde) were obtained commercially from Aldrich, Lancaster, and were used without purification, unless otherwise indicated. All other reagents and solvents were purified after receiving from commercial suppliers. All the bacterial strains were obtained from the Division of Microbiology, Department of Pharmaceutical technology , Jadavpur University, Kolkata, India.

Table II - MIC values (µg/mL) of novel fluoro isoxazolidine and isoxazolines (**2-7**)

Organisms/compounds	**2a**	**3b**	**4a**	**4b**	**5**	**6**	**7**
Escherichia coli ATCC 25938	600	600	600	400	_	_	_
Salmonella.typhi 62	600	600	200	400	_	_	600
Vibrio cholerae 20	600	600	200	600	1000	_	600
Klebsiella pneumoniae 10031	600	600	_	600	_	_	_
Shigella dysentery 1	800	600	400	600	1000	_	_
Pseudomonas AMRI 100	800	600	400	600	_	_	_
Salmonella typhimurium NTCC 74	600	600	600	400	_	_	_
Staphylococcus aureus 29737	600	600	400	600	1000	_	_
Bacillus cereus 11778	600	400	400	600	1000	_	_
Bacillus subtilis 6633	600	600	400	600	1000	_	_
Streptococcus epidermidis 1222	600	400	400	600	1000	_	_
Micrococcus luteus 10240	800	600	400	600	600	_	_
Pseudomonas aeruginosa 2561	600	200	400	400	_	_	_
Bacillus pumilus 14884	600	400	600	600	1000	_	_
Bordetella bronchiseptica NCTC 4617	600	600	400	600	_	_	_

'-' represents no antimicrobial activity of the compounds.

General procedure of synthesis of *N*-Benzyl fluoro nitrone (1) in ionic liquid

2,6-difluoro benzaldehyde (1 mmol) and *N*-benzylhydroxylamine (1 equivalent) was added to [bmim]BF_4 (2 mL) in a 10 mL conical flask, mixed thoroughly and stirred at room temperature for 60 min. The formation of nitrone was monitored by TLC (R_f=0.40). After completion of reaction, the reaction mixture was washed with diethyl ether (3x10mL) and the combined ether extract was concentrated *in vacuo* to obtain nitrone (**1**) as white crystalline solid (m.p 42^0C, uncorrected). As the nitrone decomposes on keeping at room temperature, *in situ* reactions were performed with alkene and alkynes.

Spectroscopic data for nitrone **1:** UV λ_{max} 238 nm; IR (KBr): υ_{max} 3025 (m), 2235 (m), 1680

(m), 1610 (s), 1440 (m), 1154 (m), 784 (s) cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.96-7.79 (m, 3H, $C_6H_3F_2$), 7.67-7.35 (m, 5H, $C_6H_5CH_2$), 6.98 (s, 1H,-CH=N^+), 3.37 (s, 2H, $C_6H_5CH_2$). ^{13}CNMR($CDCl_3$): δ 142.04 (CH=N^+), 134.80, 134.34, 134.12, 133.93 (phenyl carbons), 131.60, 130.00, 129.55, 129.46, 128.67, 128.22 (2,6 difluoro phenyl carbons).

Table III - Represents Zone of Inhibition of novel compounds (**2-7**) at MIC value (in mm)

Organisms/compounds	**2a**	**3b**	**4a**	**4b**	**5**	**6**	**7**	Amoxicillin
Escherichia coli ATCC 25938	12	19	19	20	_	_	23	38
Salmonella.typhi 62	14	20	18	17	_	_	28	40
Vibrio cholerae 20	10	30	16	23	16	_	_	35
Klebsiella pneumoniae 10031	14	13	_	22	_	_	_	21
Shigella dysentery 1	18	22	25	20	24	_	_	23
Pseudomonas AMRI 100	10	11	18	21	_	_	_	20
Salmonella typhimurium NTCC 74	19	28	20	19	_	_	_	24
Staphylococcus aureus 29737	13	18	21	22	20	_	_	36
Bacillus cereus 11778	17	28	22	22	21	_	_	31
Bacillus subtilis 6633	12	30	24	23	24	_	_	40
Streptococcus epidermidis 1222	20	18	18	21	22	_	_	32
Micrococcus luteus 10240	24	20	14	19	13	_	31	39
Pseudomonas aeruginosa 2561	21	29	12	18	_	_	_	40
Bacillus pumilus 14884	37	21	19	20	12	_	_	40
Bordetella bronchiseptica NCTC 4617	30	24	23	21	_	_	_	40

'-' represents no measurable zone of diameter at MIC value of supplied compounds.

General procedure of synthesis of novel diastereomeric fluoro isoxazolidine derivatives (2-4) in ionic liquid

N-phenyl maleimide (1 equivalent) was added *in situ* at the time of development of nitrone **1** and the reaction mixture was further stirred at room temperature for an appropriate time (**Table I**). After completion of reaction, as indicated by TLC (R_f= 0.58, 0.64), the reaction mixture

was washed with diethyl ether (3x10mL). The combined ether extracts were concentrated *in vacuo* and the resulting product mixture was directly charged on silica gel column and eluted with a mixture of ethyl acetate:*n*-hexane (1:8) to afford pure fluoro isoxazolidines **2a & 2b** as white crystals (88%, entry **1**, **Table I, Scheme I**). The rest of the viscous ionic liquid was further washed with ether and dried at 80°C under reduced pressure to retain its activity in subsequent runs. Same methodology was adopted for the synthesis of other novel fluoro isoxazolidine derivatives (entry **2** & **3**).

Spectroscopic data of novel fluoro isoxazolidine derivatives (2-4)

(3*S*)-2-Benzyl-3-(2,6-difluorophenyl)-dihydro-5-phenyl-2*H*-pyrrolo[3,4-*d*]isoxazole-4,6(5*H*,6 a-*H*)-dione, 2a

White crystals. Yield 66%; R_f = 0.58; IR (KBr): υ_{max} 3020 (m), 2920 (m), 2835 (m), 1758 (s), 1690 (s), 1480 (m), 1346 (m), 805 (s), 770 (s) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.74 – 7.68 (m, 3H, $C_6H_3F_2$), 7.12 – 6.83 (m, 2X5H, C_6H_5 protons), 5.84 (d, 1H, *J* = 6.70 Hz, C_5H), 3.40 (dd, 1H, *J* = 6.06, 6.18 Hz, C_4H), 3.54 (s, 2H, $C_6H_5CH_2$), 2.95 (d, 1H, *J* = 6.32 Hz, C_3H); ^{13}C NMR ($CDCl_3$): δ 173.42, 173.10 (carbonyl carbons), 138.10, 138.06, 138.02, 137.97, 136.86, 136.81, 136.78, 136.75 (phenyl carbons), 134.34, 134.14, 134.06, 133.76, 133.65 (2,6 difluoro phenyl carbons), 85.22 (C_5), 77.20 (C_3), 58.46 (C_4), 39.55 ($\underline{CH_2}C_6H_5$); FAB-MS: *m/z* 420 (M^+,100%), 343, 329, 306, 252, 216 (B.P), 113, 91, 77; Anal. Calcd. for $C_{24}H_{18}O_3N_2F_2$: C, 68.57; H, 4.28; N, 6.66%. Found: C, 68.44; H, 4.19; N, 6.52.

(3*R*)-2-Benzyl-3-(2,6-difluorophenyl)-dihydro-5-phenyl-2*H*-pyrrolo[3,4-*d*]isoxazole-4,6(5*H*,6 a-*H*)-dione, 2b

White crystals. Yield 22%; R_f = 0.64; IR (KBr): υ_{max} 3010 (m), 2915 (m), 2830 (m), 1764 (s), 1685 (s), 1486 (m), 1340 (m), 864 (s), 783 (s) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.70 – 7.66 (m, 3H, $C_6H_3F_2$), 7.30 – 7.12 (m, 2X5H, C_6H_5 protons), 5.76 (d, 1H, *J* = 2.24 Hz, C_5H), 3.63 (dd, 1H, *J* = 2.26, 2.08 Hz, C_4H), 3.28 (s, 2H, $C_6H_5CH_2$), 3.06 (d, 1H, *J* = 3.04 Hz, C_3H); ^{13}C NMR ($CDCl_3$): δ 172.40, 172.24 (carbonyl carbons), 137.80, 137.74, 137.72, 137.57, 137.36, 136.34, 136.26, 136.18 (phenyl carbons), 134.80, 134.60, 134.44, 134.22, 134.13 (2,6 difluoro phenyl carbons), 80.65 (C_5), 76.52 (C_3), 57.90 (C_4), 41.24 ($\underline{CH_2}C_6H_5$); FAB-MS: *m/z* 420 (M^+,100%), 343, 329, 306, 216 (B.P), 113, 91, 77; Anal. Calcd. for $C_{24}H_{18}O_3N_2F_2$: C, 68.57; H, 4.28; N, 6.66%. Found: C, 68.49; H, 4.17; N, 6.50.

(3*S*)-2-Benzyl-3-(2,6-difluorophenyl)-dihydro-5-methyl-2*H*-pyrrolo[3,4-*d*]isoxazole-4,6(5*H*,6 a-*H*)-dione, 3a

White solid. Yield 65%; R_f = 0.54; IR (KBr): υ_{max} 3005 (m), 2935 (m), 2820 (m), 1760 (s), 1675 (s), 1465 (s), 1340 (m), 814 (s), 778 (s) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.89 – 7.86 (m, 3H, $C_6H_3F_2$), 7.64 – 7.46 (m, 5H, C_6H_5 protons), 6.56 (d, 1H, *J* = 6.10 Hz, C_5H), 3.89 (s, 2H, $C_6H_5CH_2$), 3.79 (dd, 1H, *J* = 6.00, 5.90 Hz, C_4H), 3.49 (s, 3H, N-CH_3), 2.95 (d, 1H, *J* = 6.76 Hz, C_3H); ^{13}C NMR ($CDCl_3$): δ 170.58, 170.50 (carbonyl carbons), 136.44, 136.40, 136.32, 136.25 (phenyl carbons), 132.70, 132.64, 132.51, 132.43, 132.18 (2,6 difluoro phenyl carbons), 82.98 (C_5), 76.66 (C_3), 59.70 (C_4), 39.60 ($\underline{CH_2}C_6H_5$), 37.54 (N-CH_3); FAB-MS: *m/z* 358 (M^+,100%), 345, 267, 252, 244, 154 (B.P), 113, 91; Anal. Calcd. for $C_{19}H_{16}O_3N_2F_2$: C, 63.68; H, 4.46; N, 7.82%. Found: C, 63.49; H, 4.36; N, 7.57.

(3*R*)-2-Benzyl-3-(2,6-difluorophenyl)-dihydro-5-methyl-2*H*-pyrrolo[3,4-*d*]isoxazole-4,6(5*H*,6 a-*H*)-dione, 3b

White solid. Yield 21%; R_f = 0.60; IR (KBr): υ_{max} 3015 (m), 2905 (m), 2828 (s), 1760 (s), 1680 (s), 1460 (s), 1355 (m), 820 (s), 783 (s) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.88 – 7.84 (m, 3H, $C_6H_3F_2$), 7.60 – 7.49 (m, 5H, C_6H_5 protons), 6.52 (d, 1H, *J* = 3.22 Hz, C_5H), 3.84 (s, 2H, $C_6H_5CH_2$), 3.76 (dd, 1H, *J* = 1.96, 2.12 Hz, C_4H), 3.47 (s, 3H, N-CH_3), 2.96 (d, 1H, *J* = 1.96 Hz, C_3H); ^{13}C NMR ($CDCl_3$): δ 171.34, 171.27 (carbonyl carbons), 135.98, 135.94, 135.82, 135.75 (phenyl carbons), 133.12, 133.04, 132.91, 132.83, 132.77 (2,6 difluoro phenyl carbons), 84.08 (C_5), 73.80 (C_3), 54.95 (C_4), 41.42 ($\underline{CH_2}C_6H_5$), 39.05 (N-CH_3); FAB-MS: *m/z* 358 (M^+,100%), 345, 267, 252, 154 (B.P), 113, 91, 77; Anal. Calcd. for $C_{19}H_{16}O_3N_2F_2$: C, 63.68; H, 4.46; N, 7.82%. Found: C, 63.42; H, 4.32; N, 7.62.

(3*S*)-2-Benzyl-5-cyclohexyl-3-(2,6-difluorophenyl)-dihydro-2*H*-pyrrolo[3,4-*d*]isoxazole-4,6(5*H*,6 a-*H*)-dione, 4a

Yellow crystals. Yield 63%, R_f = 0.50; IR (KBr): υ_{max} 3015 (m), 2900 (s), 2840 (m), 1760 (s), 1674 (br, s), 1470 (s), 1330 (m), 805 (s), 786 (s) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.60 – 7.56 (m, 3H, $C_6H_3F_2$), 7.17 – 7.06 (m, 5H, C_6H_5 protons), 6.30 (d, 1H, *J* = 6.74 Hz, C_5H), 3.60 (s, 2H, $C_6H_5CH_2$), 3.42 (dd, 1H, *J* = 6.20, 6.10 Hz, C_4H), 2.83 (d, 1H, *J* = 6.76 Hz, C_3H), 1.95-1.52 (m, 11H, cyclohexyl protons); ^{13}C NMR ($CDCl_3$): δ 168.54, 168.50 (carbonyl carbons), 131.66, 131.60, 131.55, 131.50 (phenyl carbons), 129.15, 129.06, 128.80, 128.73, 128.68 (2,6 difluoro phenyl carbons), 83.60 (C_5), 74.55 (C_3), 58.24 (C_4), 38.78 ($\underline{CH_2}C_6H_5$), 27.40, 27.29, 26.87, 26.70, 26.58, 26.46 (cyclohexyl carbons); FAB-MS: *m/z* 426 (M^+,100%), 343, 335, 312, 252, 222 (B.P), 113, 91, 83; Anal. Calcd. for $C_{24}H_{24}O_3N_2F_2$: C, 67.60; H, 5.63; N, 6.57%. Found: C, 67.46; H, 5.35; N, 6.37.

(3*R*)-2-Benzyl-5-cyclohexyl-3-(2,6-difluorophenyl)-dihydro-2*H*-pyrrolo[3,4-*d*]isoxazole-4,6(5*H*,6 a-*H*)-dione, 4b

Yellow crystals. Yield 22%, R_f = 0.62; IR (KBr): υ_{max} 3010 (m), 2905 (s), 2835 (m), 1764 (s), 1675 (s), 1466 (s), 1336 (m), 815 (s), 783 (s) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.52 – 7.85 (m, 3H, $C_6H_3F_2$), 7.25 – 7.14 (m, 5H, C_6H_5 protons), 6.14 (d, 1H, *J* = 1.88 Hz, C_5H), 3.55 (s, 2H, $C_6H_5CH_2$), 3.38 (dd, 1H, *J* = 2.08, 2.04 Hz, C_4H), 2.80 (d, 1H, *J* = 1.80 Hz, C_3H), 1.90-1.38 (m, 11H, cyclohexyl protons); ^{13}C NMR ($CDCl_3$): δ 169.88, 169.83 (carbonyl carbons), 130.54, 130.49, 130.45, 130.32 (phenyl carbons), 128.77, 128.68, 128.56, 128.53, 128.48 (2,6 difluoro phenyl carbons), 80.44 (C_5), 77.50 (C_3), 58.97 (C_4), 37.05 ($\underline{CH_2}C_6H_5$), 25.30, 25.22, 25.17, 25.06, 24.88, 24.76 (cyclohexyl carbons); FAB-MS: *m/z* 426 (M^+,100%), 343, 312, 252, 222 (B.P), 113, 91, 83, 77; Anal. Calcd. for $C_{24}H_{24}O_3N_2F_2$: C, 67.60; H, 5.63; N, 6.57%. Found: C, 67.37; H, 5.40; N, 6.33.

General procedure of synthesis of novel fluoro isoxazoline derivatives (5-7) in ionic liquid

Methyl phenyl propiolate (1 equivalent) was added *in situ* at the time of development of nitrone **1** and the reaction mixture was further stirred at room temperature for an appropriate time (**Table I**). After completion of reaction, as indicated by TLC (R_f = 0.66), the reaction mixture was washed with diethyl ether (3x10mL). The combined ether extract was concentrated *in vacuo* and the resulting crude product was directly charged on silica gel column and eluted with a mixture of ethyl acetate:*n*-hexane (1:8) to afford pure fluoro isoxazoline **5** as dark red thick

liquid (88%, entry **4**, **Table I, Scheme I**). The rest of the viscous ionic liquid was further washed with ether and dried at 80°C under reduced pressure to retain its activity in subsequent runs. Same methodology was adopted for the synthesis of other novel fluoro isoxazoline derivatives (entry **5** & **6**).

Spectroscopic data of novel fluoro isoxazoline derivatives (5-7)

(*S*)-methyl-2-benzyl-3-(2,6-difluorophenyl-2,3-dihydro-5-phenylisoxazole-4-carboxylate, 5

Dark red thick liquid. Yield 88%; R_f = 0.66; IR (KBr): υ_{max} 3010 (m), 2246 (m), 1740 (s), 1710 (s), 1690 (s), 1610 (s), 1480 (s), 1324 (s), 1215 (s), 810 (m), 782 (s) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.87 – 7.80 (m, 3H, $C_6H_3F_2$), 7.68-7.31 (m, 2x5H, C_6H_5), 3.38 (s, 3H, -$COOCH_3$), 2.68 (s,2H, $C_6H_5CH_2$), 1.25 (s,1H, C_3H); ^{13}C NMR ($CDCl_3$): δ 168.52 (-$\underline{CO}OCH_3$), 137.20, 137.04, 136.87, 136.66, 135.65, 135.48, 135.20, 134.93 (aromatic carbons), 132.77, 132.35, 132.08, 131.78, 130.80, 129.90 (2,6 difluoro phenyl carbons), 88.16 (C_5), 73.60 (C_3), 58.45 (C_4), 45.17 (-COO$\underline{CH_3}$), 36.80 (benzylic carbon); FAB - MS (*m/z*): 407 (M^+), 330, 294, 211 (B.P), 203, 113, 105, 91, 77. Anal. Calcd. for $C_{24}H_{19}O_3F_2N$: C, 70.76; H, 4.66; N, 3.43. Found: C, 70.63; H, 4.61; N, 3.35%.

(*S*)-dimethyl-2-benzyl-3-(2,6-difluorophenyl)-2,3-dihydroisoxazole-4,5-dicarboxylate, 6

Red viscous liquid. Yield 86%; R_f = 0.60; IR (KBr): υ_{max} 3015 (m), 2250 (m), 1725 (s), 1685 (s), 1610 (s), 1440 (s), 1260 (s), 1225 (s), 805 (m), 780 (s) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.44 – 7.36 (m, 3H, $C_6H_3F_2$), 7.10-6.98 (m, 5H, C_6H_5), 3.30 (s, 3H, -$COOCH_3$), 3.24 (s, 3H, -$COOCH_3$), 2.55 (s, 2H, $C_6H_5CH_2$), 1.72 (s,1H, C_3H); ^{13}C NMR ($CDCl_3$): δ 169.74, 169.58 (-$\underline{CO}OCH_3$, carbonyl carbons of the ester group), 135.80, 135.73, 135.54, 135.47 (aromatic carbons), 133.30, 133.28, 133.24, 133.15, 133.12, 133.05 (2,6 difluoro phenyl carbons), 85.25 (C_5), 77.80 (C_3), 56.90 (C_4), 45.74, 44.82 (-COO$\underline{CH_3}$, methyl carbons of the ester methyl group), 39.23 (benzylic carbon); FAB - MS (*m/z*): 389 (M^+), 358, 330, 302, 276, 271 (B.P), 185, 113, 91, 77; Anal. Calcd. for $C_{20}H_{17}O_5F_2N$: C, 61.69; H, 4.37; N, 3.59. Found: C, 61.58; H, 4.26; N, 3.35%.

(*S*)-2-Benzyl-3-(2,6-difluorophenyl)-2,3-dihydroisoxazole-4,5-dicarboxylic acid, 7

Colourless thick liquid. Yield 66%; R_f = 0.66; IR (KBr): υ_{max} 3010 (m), 2995 (br), 2246 (m), 1760 (s), 1610 (s), 1480 (s), 1324 (s), 1215 (s), 1105 (s), 800 (m), 782 (s) cm^{-1}; ^1H NMR ($CDCl_3$): δ 10.02 (s, 2H, 2XCOOH), 7.90 – 7.87 (m, 3H, $C_6H_3F_2$), 7.66-7.44 (m, 5H, C_6H_5), 2.91 (s,2H, $C_6H_5CH_2$), 2.88 (s,1H, C_3H); ^{13}C NMR ($CDCl_3$): δ 173.69, 172.04 (carboxyl carbons), 138.50, 138.44, 138.37, 138.26 (aromatic carbons), 135.44, 135.40, 135.28, 134.93, 134.87, 134.75 (2,6 difluoro phenyl carbons), 88.20 (C_5), 74.43 (C_3), 58.60 (C_4), 37.87 (benzylic carbon); FAB - MS (*m/z*): 361 (M^+), 344, 316, 288, 271 (B.P), 248, 157, 113, 91, 77. Anal. Calcd. for $C_{18}H_{13}O_5F_2N$: C, 59.83; H, 3.60; N, 3.87. Found: C, 59.75; H, 3.40; N, 3.58%.

Conclusion

In conclusion, we have shown that 1,3-dipolar cycloadditions of fluoro nitrones with activated alkenes and electron deficient alkynes may be conveniently carried out in RTIL's with the obtainment of corresponding novel fluoro isoxazolidine & isoxazolines in good conversions and yields with tremendous synthetic potentiality. The ionic liquid may be recycled several times without loss of activity nor selectivity. Majority of the synthesized compounds have been found to have potential activity against both gram positive and gram negative organisms and thereby showing an opportunity to behave as broad spectrum antimicrobial agents.

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References

1 Frederickson M, *Tetrahedron*, 53, **1997**, 403.

2 For a general review of this area see: (a) Tufariello J J, *1,3-Dipolar cycloaddition chemistry*. Edited by Padwa A, (Wiley: New York) **1984**; (b) Torssell K B G, *Nitrile Oxides, Nitrones and Nitronates in Organic Synthesis*, (VCH: New York) **1988**.

3 (a) Seerden J P G, Boeren M M M & Scheeren H W, *Tetrahedron*, 53 (34), **1997**, 11843; (b) Gothelf K V & Jørgensen K A, *Chem. Rev*, 98, **1998**, 863.

4 Patterson W, Cheung P S & Ernest M J, *J Med Chem*, 35, **1992**, 507.

5 Wagner E, Becan L & Nowakowska E, *Bio-Org Med Chem,* 12, **2004**, 265.

6 Confalone P N & Huie E M*, Org React*, 36, **1988**, 1–173, Chapter. 1.

7 (a) Welton T, *Chem Rev*, 99, **1999**, 2071; (b) Wasserscheid P & Keim W, *Angew Chem Int, Ed Engl*, 39, **2000**, 3772.

8 Sheldon R, *Chem Commun*, **2001**, 2399.

9 Gordon C M, *Appl Catal. A: Gen*, 222, **2001**, 101.

10 (a) Yadav J S, Reddy B V S & Baishya G, *J Org Chem*, 68, **2003**, 7098; (b) Yadav J S, Reddy B V S, Reddy J S S & Srinivas Rao R, *Tetrahedron,* 59, **2003**, 1599.

11 See Bach D, *Angew Chem*, *Int Engl*, 29, **1990**, 1320.

12 Welch J T, *Tetrahedron*, 43, **1987**, 3123.

13 Cao S, Qian X H, Song G H & Huang Q C, *J Fluorine Chem,* 63, **2002**, 117.

14 Arnone A, Bernardi R, Blasco F, Cardillo R, Gerus I I & Kukhar V P, *Tetrahedron,* 54, **1998**, 2809.

15 (a) Chambers R D, *Fluorine in Organic Chemistry*, (Blackwell Publishing: Oxford, UK), **2004**; (b) Kirsch P, *Modern Fluoroorganic Chemistry*, (Wiley-VCH: Weinheim, Germany) **2004**.

16 Chakraborty B & Sharma P K, *Synth Commun,* 42, **2012**, 1804.

17 Chakraborty B, Sharma P K, Rai N, Kafley S & Chhetri M S, *J Chem Res (RSC),* 34 (3), **2010**,

18 Chakraborty B, Sharma P K, Rai N & Sharma C D, *J Chem Sci*, 124(3), **2012**, 679.

19 Chakraborty B, Sharma P K & Chhetri M S, *J Het Chem*, 49, **2012**, 1260.

20 Chakraborty B, Sharma P K & Kafley S, *Green Chem Lett Rev*, 6 (2), **2013,** 141.

21 Chakraborty B, Luitel G P, *Tetrahedron Lett*, 54, **2013**, 765.

22 Deshong P, Li W, Kennington J W & Ammon H L, *J Org Chem*, 56, **1991**, 1364.

23 Gandolfi R & Grunanger P, *The chemistry of heterocyclic compounds*, (Wiley Interscience), 49 (2), **1999**, 774.

24 Chakraborty B, Sharma C D, *Tetrahedron Lett*, 54, **2013**, 5532-5536.

25 Sahu K B, Ghosh S, Banerjee M, Maity A, Mondal S, Paira R, Saha P, Naskar S, Hazra A, Banerjee S, Samanta A & Mondal N B, *Med Chem Res*, 22, **2013**, 94.

26 McFarland J, *J Am Med Assoc*, 14, **1907**, 1176.

27 Paira M K, Mondal T K, Ojha D, Slawin A M Z, Tiekink E R T, Samanta A & Sinha C, *Inorg Chim Acta*, 370, **2011**, 175.

28 Andrews J M, *J Antimicrobial Chemother*, 48, **2001**, 43.

29 National Committee for Clinical Laboratory Standards (NCCLS), Approved Standard M7-A3, 3rd Edn. NCCLS, Villanova, PA, **1993**.

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