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NOTE

Mechanochemical synthesis and cycloaddition reactions of fluoro nitrone under solvent-free conditions and potential antimicrobial activities of the cycloadducts

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Abstract

Synthesis and cycloaddition reactions of fluoro nitrone under solvent-free conditions using ball-milling technique have been reported. Significant change in rate and yields of the cycloadducts have been noticed compared with solvent-free microwave-induced reactions of fluoro nitrones. The present study reports synthesis of *N*-benzyl fluoro nitrone and its cycloaddition reactions with maleimides and few electron deficient alkynes under solvent-free conditions. The synthesized fluoro cycloadducts were found to exhibit potential antimicrobial activities.

1 | INTRODUCTION

Nitrones are excellent reaction intermediates and easily undergoes 1,3-dipolar cycloaddition reaction, resulting in five-membered heterocyclic compounds (commonly called isoxazolidine and isoxazolines), which are the core group of many natural products.[1] Majority of isoxazolines are found to exhibit medicinal activities such as antibacterial, anticonvulsant, antibiotic, antituberculer, antifungal, and anticancer activities.[2,3] Besides green chemistry methodologies, majority of the reported procedures for the synthesis of isoxazolidines were found to require drastic experimental conditions like high temperature and long reaction times. Moreover, these reactions were found to suffer from selectivity, and there are possibilities of poor yields and developments of side products as well.[4] Mechanochemical procedures using ball-milling technique have become very popular and developed recently in many aspects. The procedure has also attracted the attention of organic chemists in recent years.[5] Conducting organic synthesis under environment-friendly conditions is a challenge nowadays. So, ball-milling technique may be used as a lucrative methodology in the synthesis of important compounds, and this methodology may be considered as an alternative to conventional procedures like heating, microwave irradiation, and sonication. Therefore, synthetic organic chemists should apply mechanochemical procedures like ball-milling as an effective and greener technique for various organic transformations.[6]

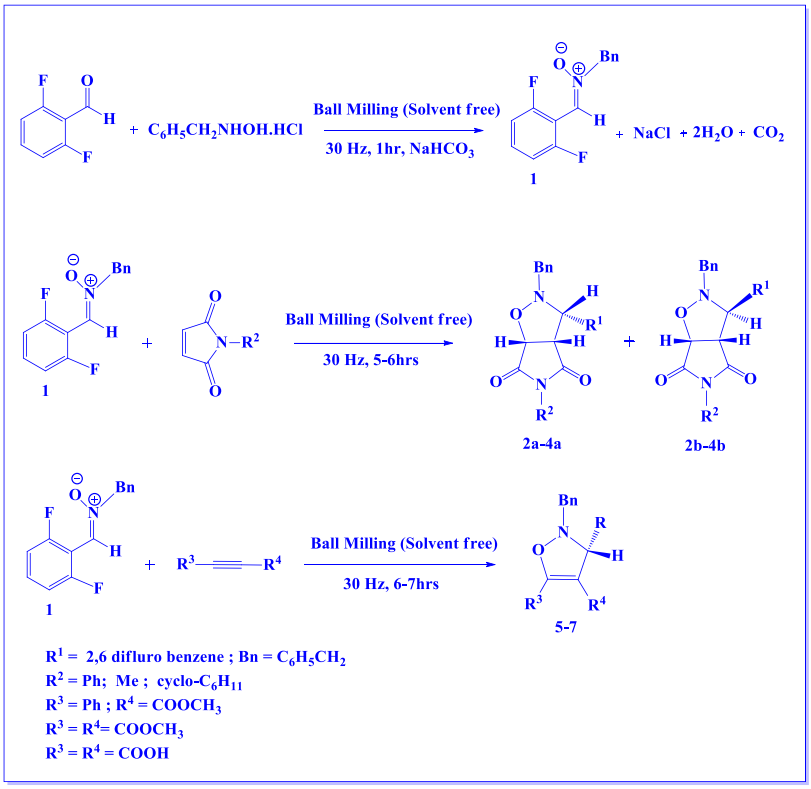
Fluoro nitrones are a less-known group of nitrones, but they are of special interest in organic synthesis for many reasons. It has been observed from various reported works that the introduction of fluorine atom into a particular position of an organic molecule may cause significant changes in the stability and biological activities of the molecules.[7] It could be due to the high electronegativity of the fluorine, the strong C—F bonding, and the same size of the halogen and hydrogen atoms. Because of these factors, syntheses of biologically active fluorinated compounds are in the focus nowadays.[8] Literature survey reveals that synthesized fluorinated compounds not only exhibits excellent biological properties but also

reports how these compounds may be used as medicine and agrochemicals.[9] It is also to be noted that because of low polarizability and high lipophilicity of the fluorine atom, it increases relative metabolic stability and improves the bioavailability of the new heterocyclic compounds compared with its hydrocarbon analogs.[10,11]

In continuation of our efforts to establish various green methodologies in nitrone cycloaddition reactions,[12–17] herein, we wish to report the development of an environment-friendly mechanochemical route to isoxazolidine and isoxazoline derivatives using *N*-benzyl fluoro nitrone via 1,3-dipolar cycloaddition reactions (Scheme 1, Table 1).

2 | RESULTS AND DISCUSSION

According to our proposed plan of work, we have conducted the reactions taking one equivalent each of 2,6-difluoro benzaldehyde and *N*-benzylhydroxylamine hydrochloride along with one equivalent of sodium bicarbonate for the synthesis of fluoro nitrone **1**(Scheme 1). ^{1}H NMR of the product indicated only the development of expected product. The nitrone was used for cycloaddition reactions without further purification. It is to be noted that during the process of ball-milling, slight heat was developed in the reaction vessel and also slight pressure inside the vessel. It was observed while opening the reaction vessel. We have obtained best results when 1:1 ratio of starting materials was used, but when we tried different ratios of starting materials, we have found incomplete conversion to the target molecule. Sodium bicarbonate was added in the mixture during the synthesis of nitrones because it was observed that it could activate the hydroxylamine. It may be due to the fact that because of the addition of sodium bicarbonate, the reaction mixture becomes faintly alkaline and also neutralize the liberated hydrochloric acid. It has been observed under mechanochemistry conditions that pKa data do not make normal sense and actually weaker bases can be used to deprotonate things. We have observed that sometimes, in mechanochemistry, we can form a paste or a gum in the reaction, and things do not mix well unless we had a solid material to help the mixture flow more easily as solid. Since dry-milling procedure has been found to be more efficient, therefore, solid sodium bicarbonate was used, and we received best results. We have also tested the same reaction in absence of sodium bicarbonate, but at the end of the reaction, a gummy crude product was recovered by methanol. It was filtered, the solvent was evaporated off, and we have observed that the reaction was incomplete from the ^{1}H NMR spectrum of the crude product. It has been also observed that if we perform the reactions at a lower frequency (10 or 20 Hz) for a time period between 30 minutes and 2 hours, the aldehyde and hydroxylamine are still present in the crude product. This could be probably due to a lesser amount of energy per impact and indicates that ball-milling procedure with a pause results in lower conversion.



SCHEME 1 Synthesis of fluoro isoxazolidine and isoxazoline derivatives using ball-milling technique [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Physicochemical data of synthesized molecules (**2a**-**4a**; **2b**-**4b** and **5**-**7**)

Entry	Nitrone	Dipolarophile[a]	Time, h	Cycloadducts, m.p.(°C), 2a-4a: *cis*; 2b-4b: *trans*	*Cis/trans* ratio, %	Yield[b], [c] %
1	*N*-benzyl fluoro nitrone	*N*-phenyl Maleimide	5	**2a**: White crystals, 126 **2b**: White solid, 103	**2a**: 65 (57) **2b**: 30 (25)	95 (82)
2	*N*-benzyl fluoro nitrone	*N*-methyl Maleimide	5	**3a**: Yellowish solid, 133 **3b**: Yellowish crystals, 122	**3a**: 65 (50) **3b**: 28 (26)	93 (76)
3	*N*-benzyl fluoro nitrone	*N*-cyclohexyl Maleimide	6	**4a**: Yellow crystals, 140 **4b**: Yellow solid, 111	**4a**: 64 (47) **4b**: 29 (29)	93 (76)
4	*N*-benzyl fluoro nitrone	Methyl phenyl propiolate	6	**5**: Dark red gummy liquid		91 (72)
5	*N*-benzyl fluoro nitrone	Dimethyl acetylene dicarboxylate	7	**6**: Red liquid		91 (70)
6	*N*-benzyl fluoro nitrone	Acetylene dicarboxylic acid	7	**7**: White thick liquid		90 (68)

[a]Reaction conditions: nitrone (1 mmol), dipolarophiles (1 equivalent), ball-milling, 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 yields obtained under MWI & *endo/exo ratio*.

Since the development of nitrone was fast in this technique, the intermolecular cycloaddition reactions of the fluoro nitrone with activated double-bonded dipolarophiles (maleimides) and electron-deficient dipolarophiles (alkynes) were carried out following the same technique only. It was observed that intermolecular cycloaddition reactions of fluoro nitrone with dipolarophiles have been found to be slower than the formation of nitrone and only 56% of cycloadducts (eg, **2a**) were obtained after 2 hours of ball-milling (Table 1, entry 1). We have observed that cycloaddition reactions were completed only after 5 to 7 hours of ball-milling process. We have also tested cycloaddition reactions in presence of polar solvents like acetonitrile and ethanol (1 mL) in the ball-milling process, but we did not observe any notable changes in the rate of cycloaddition reactions. On the basis of these observations, we have conducted all the cycloaddition reactions in ball-milling procedure without using any solvents.

We have also compared the advantages of the ball-milling technology over microwave technology under solvent-free conditions[18–20] and found that lower yields of nitrone and cycloadducts were noted compared with that of the corresponding ball-milling techniques. In microwave technology, the reactions proceeded very slowly (up to 2 to 3 hours) at a high temperature (120°C) and also without achieving a total conversion of the starting material (2,6-difluoro benzaldehyde). It could be due to the degradation of *N*-benzylhydroxylamine, which could be the reason for a low yield. High yields of cycloadducts (up to 90%) could be obtained in dichloromethane solution if we use an excess of hydroxylamine (2 equivalents) under microwave irradiation.[19] Column chromatography was necessary to purify the products. We have also tried a few of these cycloaddition reactions under microwave irradiation in the presence of *N*,*N*-dimethyl formamide (DMF) and noted that the yields were 76% to 72% (which were much lower than that of the ball-milling process).

Significant diastereoselectivity has been observed from the ^{1}H NMR analysis of the crude products when the cycloaddition reaction was conducted between fluoro nitrone **1** with maleimides. The mixture of diastereomers **2a**-**4a** and **2b**-**4b** were obtained in almost 65:35 ratios. The ratio was calculated on the basis of the intensities of two well-separated double doublets at an average value of 6.20 and 2.25 δ ppm, respectively, in the maleimide cycloadducts. These signals are due to 4-H protons of the isoxazolidine moiety of the two isomers. This is also to be noted that three asymmetric centers were generated in a single step of this cycloaddition reaction. It could be due to the *exo* approach of *Z* nitrone towards maleimides for the development of major cycloadducts **2a**-**4a** (transition state **1**, Figure 1), while the *endo* approach of *Z* nitrone towards maleimides develop minor cycloadducts **2b**-**4b** (transition state **2**, Figure 1). Usual separation of the diastereomers were carried out using column chromatography, and the diastereomers were identified considering the multiplicity of the proton signals at 3-H and 4-H protons along with their coupling constant values.[21,22] In isoxazolidines **2a**-**4a**, the values of coupling constant between 3-H and 4-H protons have been measured in the range of 6.00 to 6.26 Hz, implying a *cis* relationship between 3-H and 4-H protons, while in the case of isoxazolidines **2b**-**4b**, the coupling constant has been measured in the range of 2.00 to 2.26 Hz, which implies a *trans* relationship between 3-H and 4-H protons.[21,22]

Electron-deficient alkynes like dimethyl acetylene dicarboxylate, phenyl methyl propiolate, and acetylene dicarboxylic acid (but-2-ynedioic acid) furnishes

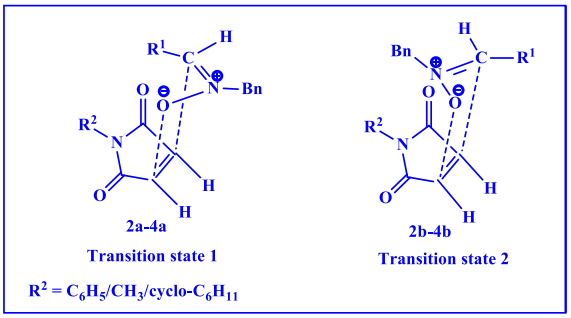


FIGURE 1 *Exo*/*endo* approach of the Z-nitrone to the maleimides in cycloaddition reactions [Color figure can be viewed at wileyonlinelibrary.com]

regioselective isoxazoline derivatives (**5**-**7**) in 1,3-dipolar cycloaddition reaction with *N*-benzyl-fluoro nitrone **1**. These results can be rationalized by an *exo* approach of the *Z* nitrone **1** for the dipolarophiles (alkynes) in the development of fluoro isoxazoline derivatives (**5**-**7**; transition state **3**).[21,23]

According to Huisgen's theory,[24] acetylenic dipolarophiles are less reactive then expected on the basis of their ionization potentials. Since alkynes have larger highest occupied molecular orbital (HOMO)–lowest unoccupied molecular orbital (LUMO) gap than alkenes, it is expected that during interactions with alkyne, LUMO plays the most significant part and hence alkynes are less reactive than expected. However, the reactivity of nitrones with electron-deficient alkynes are actually determined by dipole (HOMO)-dipolarophiles (LUMO) interactions and the regiochemistry.[25] In case of alkyne, the dipole (HOMO)-dipolarophile (LUMO) interactions become very important and dominate the reaction for the formation of regioselective adducts. Stereochemistry of the fluoro isoxazoline derivatives (**5**-**7**) could not be determined in detail since two most important protons are absent at 4-H and 5-H positions and the lone singlet signal due to 3-H proton is unable to predict it. The major and minor conformers of the novel isoxazoline ring systems (**5**-**7**) may be represented as follows (Figure 2).

The isolated isoxazolidine and isoxazoline derivatives are stable, and mass spectral analysis shows the presence of all the desired fragmentation peaks like molecular ion peak and base peak. The maleimide cycloadducts (**2**-**4**) have shown a common base peak (B.P), which is due to the fragmentation of benzyl and 2,6-difluoro phenyl ring. Prominent carbonyl group and aromatic C—H absorptions have been noticed in the IR spectral studies. The fragmentation pattern of isoxazoline derivatives (**5**-**7**) are of much attraction as they are found to develop aziridine derivatives. Prominent base peaks are observed because of the fragmentation of PhCO for phenyl methyl propiolate, $COOCH_3$ for dimethyl acetylene dicarboxylate, and

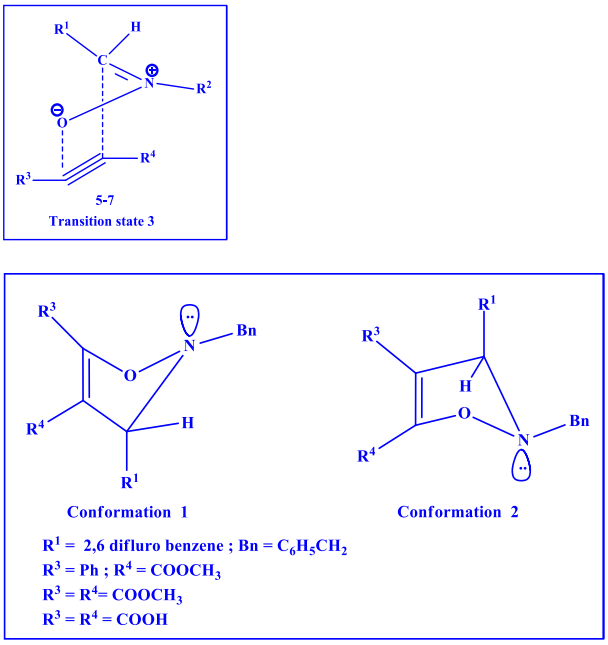


FIGURE 2 Conformations of fluoro isoxazoline derivatives [Color figure can be viewed at wileyonlinelibrary.com]

COOH for acetylene dicarboxylic acid. Therefore, we can confirm that the isoxazoline derivatives underwent rearrangement to aziridine derivatives during mass fragmentation. On the basis of the spectral data of ^{1}H NMR, ^{13}C NMR, MS, and FT-IR, structures of all the synthesized isoxazolidine and isoxazoline derivatives (**2**-**7**) have been confirmed. We have also obtained satisfactory elemental analysis data for all the cycloadducts.

3 | EXPERIMENTAL

Mechanochemical procedure (ball-milling) was performed in Retsch MM500 digital mixer mill, Retsch GmbH, 42781, Hann, Germany. Bruker DRX 300 spectrometer (300 MHz, FT NMR) was used for recording ^{1}H NMR spectra and TMS was used as internal standard. Same instrument was used for recording ^{13}C NMR spectra at 75 MHz. The coupling constants (*J*) are expressed in Hz. Perkin-Elmer RX 1–881 machine was used for recording IR spectra as film or as KBr pellets for all the molecules. Jeol SX-102 (FAB) instrument was used for recording MS spectra. Perkin-Elmer 2400 series CHN analyzer was used for elemental analyses (C,H,N). Progress of all the reactions were monitored by TLC using 0.25 mm silica gel plates (Merck $60F_{254}$ UV indicator). Column chromatography was performed with silica gel (E.Merck Germany) with 60–200 mesh. The starting materials and reagents used in the reactions (*N*-benzylhydroxylamine hydrochloride and 2,6-difluoro benzaldehyde) were obtained commercially from Aldrich,

Lancaster and were used without further purification unless otherwise indicated. All other reagents and solvents were purified before starting reactions or column chromatography.

3.1 | General procedure for the synthesis of *N*-benzyl fluoro nitrone (1) in ball-milling procedure

N-benzylhydroxylamine hydrochloride (1 mmol), 2,6-difluoro benzaldehyde (1 equivalent), and $NaHCO_3$ (1 equivalent) were mixed together and ball-milled at 30 Hz for 1 hour in a 10-mL steel vessel and 15-mm diameter balls at room temperature (16°C). After the completion of reaction, the reaction mixture was taken in CH_2Cl_2. It was filtered on cotton for the removal of NaCl. The filtrate was evaporated under vacuum to afford *N*-benzyl fluoro nitrone **1** as white crystalline solid with high purity (84%, m.p; 82°C).

3.1.1 | Spectroscopic data for nitrone 1

UV λ_{max} 235 nm; IR (KBr): υ_{max} 3022 (m), 2233 (m), 1684 (m), 1624 (s), 1442 (m), 1150 (m), 786 (s) cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.95–7.77 (m, 3H, $C_6H_3F_2$), 7.64–7.34 (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 141.12 (CH═N^+), 134.82, 134.33, 134.15, 133.97 (phenyl carbons), 131.72, 130.23, 129.46, 129.25, 128.76, 128.42 (2,6-difluoro phenyl carbons).

3.2 | General procedure of synthesis of diastereomeric fluoro isoxazolidine derivatives (2-4) in ball-milling procedure

N-benzyl fluoro nitrone **1** (1 equivalent) and *N*-phenyl maleimide (1 equivalent) was mixed together and ball-milled at 30 Hz for 5 hours in a 10-mL steel vessel and 15-mm diameter balls at room temperature (16°C). After the completion of reaction, the reaction mixture was taken in CH_2Cl_2. It was filtered on cotton for the removal of NaCl. The filtrate was evaporated under vacuum to afford mixture of crude fluoro isoxazolidines as white crystals (95%). The crude product was directly charged on silica gel column and eluted with a mixture of ethyl acetate:*n*-hexane (1:8) resulting in pure fluoro isoxazolidines **2a** and **2b** (entry **1**, Table 1, Scheme 1). The same methodology was adopted for the synthesis of other fluoro isoxazolidine derivatives (entries **2** and **3**).

3.2.1 | Spectroscopic data of 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 65%; R_f = 0.66; IR (KBr): υ_{max} 3025 (m), 2924 (m), 2830 (m), 1758 (s), 1686 (s), 1482 (m), 1342 (m), 800 (s), 772 (s) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.73–7.65 (m, 3H, $C_6H_3F_2$), 7.14–6.80 (m, 2X5H, C_6H_5 protons), 5.80 (d, 1H, *J* = 6.68 Hz, C_5H), 3.43 (dd, 1H, *J* = 6.06, 6.16 Hz, C_4H), 3.50 (s, 2H, $C_6H_5CH_2$), 2.92 (d, 1H, *J* = 6.30 Hz, C_3H); ^{13}C NMR ($CDCl_3$): δ 172.38, 172.24 (carbonyl carbons), 138.24, 138.17, 138.10, 137.90, 136.87, 136.68, 136.54, 136.40 (phenyl carbons), 134.40, 134.35, 134.20, 133.90, 133.80 (2,6-difluoro phenyl carbons), 86.17 (C_5), 76.90 (C_3), 58.83 (C_4), 39.88 ($\underline{CH_2}C_6H_5$); 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, 68.40; H, 4.20; N, 6.54.

(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 solid. Yield 30%; R_f = 0.56; IR (KBr): υ_{max} 3015 (m), 2916 (m), 2828 (m), 1766 (s), 1684 (s), 1482 (m), 1340 (m), 862 (s), 778 (s) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.72–7.62 (m, 3H, $C_6H_3F_2$), 7.30–7.14 (m, 2X5H, C_6H_5 protons), 5.74 (d, 1H, *J* = 2.26 Hz, C_5H), 3.62 (dd, 1H, *J* = 2.20, 2.08 Hz, C_4H), 3.32 (s, 2H, $C_6H_5CH_2$), 3.02 (d, 1H, *J* = 3.00 Hz, C_3H); ^{13}C NMR ($CDCl_3$): δ 172.26, 172.17 (carbonyl carbons), 137.46, 137.36, 137.23, 137.20, 137.14, 136.48, 136.30, 136.25 (phenyl carbons), 134.67, 134.48, 134.30, 134.25, 134.10 (2,6-difluoro phenyl carbons), 82.60 (C_5), 76.58 (C_3), 56.66 (C_4), 43.20 ($\underline{CH_2}C_6H_5$); 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, 68.40; H, 4.14; N, 6.56.

(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

Yellowish solid. Yield 65%; R_f = 0.60; IR (KBr): υ_{max} 3010 (m), 2938 (m), 2822 (m), 1767 (s), 1672 (s), 1466 (s), 1342 (m), 810 (s), 776 (s) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.90–7.84 (m, 3H, $C_6H_3F_2$), 7.65–7.42 (m, 5H, C_6H_5 protons), 6.53 (d, 1H, *J* = 6.12 Hz, C_5H), 3.84 (s, 2H, $C_6H_5CH_2$), 3.76 (dd, 1H, *J* = 6.04, 5.98 Hz, C_4H), 3.46 (s, 3H, N—CH_3), 2.96 (d, 1H, *J* = 6.74 Hz, C_3H); ^{13}C NMR ($CDCl_3$): δ 170.48, 170.35 (carbonyl carbons), 136.36, 136.28, 136.20, 136.16 (phenyl carbons), 132.67, 132.60, 132.53, 132.40, 132.25 (2,6-difluoro phenyl carbons), 83.90 (C_5), 75.57 (C_3), 59.80 (C_4), 39.52 ($\underline{CH_2}C_6H_5$), 37.59 (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, 63.37; H, 4.28; N, 7.50.

(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

Yellowish crystals. Yield 28%; $R_f = 0.54$; IR (KBr): υ_{max} 3018 (m), 2900 (m), 2835 (s), 1763 (s), 1680 (s), 1455 (s), 1350 (m), 820 (s), 780 (s) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.86–7.80 (m, 3H, $C_6H_3F_2$), 7.64–7.47 (m, 5H, C_6H_5 protons), 6.53 (d, 1H, $J = 3.20$ Hz, C_5H), 3.86 (s, 2H, $C_6H_5CH_2$), 3.70 (dd, 1H, $J = 1.94$, 2.10 Hz, C_4H), 3.46 (s, 3H, N—CH_3), 2.90 (d, 1H, $J = 1.94$ Hz, C_3H); ^{13}C NMR ($CDCl_3$): δ 171.40, 171.34 (carbonyl carbons), 135.90, 135.78, 135.67, 135.60 (phenyl carbons), 133.38, 133.26, 132.20, 132.18, 132.15 (2,6-difluoro phenyl carbons), 84.77 (C_5), 73.46 (C_3), 54.80 (C_4), 41.36 ($\underline{CH_2}C_6H_5$), 39.20 (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, 63.37; H, 4.30; N, 7.55.

(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 64%, $R_f = 0.62$; IR (KBr): υ_{max} 3014 (m), 2910 (s), 2836 (m), 1765 (s), 1666 (br, s), 1470 (s), 1330 (m), 800 (s), 780 (s) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.64–7.55 (m, 3H, $C_6H_3F_2$), 7.18–7.04 (m, 5H, C_6H_5 protons), 6.30 (d, 1H, $J = 6.76$ Hz, C_5H), 3.66 (s, 2H, $C_6H_5CH_2$), 3.44 (dd, 1H, $J = 6.20$, 6.20 Hz, C_4H), 2.80 (d, 1H, $J = 6.74$ Hz, C_3H), 1.94–1.50 (m, 11H, cyclohexyl protons); ^{13}C NMR ($CDCl_3$): δ 168.40, 168.34 (carbonyl carbons), 131.60, 131.54, 131.50, 131.42 (phenyl carbons), 129.48, 129.30, 128.26, 128.12, 128.05 (2,6-difluoro phenyl carbons), 85.60 (C_5), 75.72 (C_3), 57.20 (C_4), 38.80 ($\underline{CH_2}C_6H_5$), 27.60, 27.54, 26.40, 26.26, 26.16, 26.06 (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, 67.37; H, 5.43; N, 6.30.

(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 solid. Yield 29%, $R_f = 0.54$; IR (KBr): υ_{max} 3008 (m), 2900 (s), 2842 (m), 1758 (s), 1672 (s), 1460 (s), 1334 (m), 813 (s), 783 (s) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.55–7.47 (m, 3H, $C_6H_3F_2$), 7.26–7.15 (m, 5H, C_6H_5 protons), 6.14 (d, 1H, $J = 2.20$ Hz, C_5H), 3.50 (s, 2H, $C_6H_5CH_2$), 3.52 (dd, 1H, $J = 2.08$, 2.08 Hz, C_4H), 2.85 (d, 1H, $J = 2.75$ Hz, C_3H), 1.93–1.40 (m, 11H, cyclohexyl protons); ^{13}C NMR ($CDCl_3$): δ 168.84, 168.74 (carbonyl carbons), 132.50, 132.30, 130.26, 130.22 (phenyl carbons), 128.58, 128.47, 128.43, 128.40, 128.27 (2,6-difluoro phenyl carbons), 82.58 (C_5), 77.60 (C_3), 58.36 (C_4), 36.17 ($\underline{CH_2}C_6H_5$), 26.80, 26.58, 26.37, 26.25, 24.80, 24.77 (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, 67.40; H, 5.28; N, 6.22.

3.3 | General procedure of synthesis of fluoro isoxazoline derivatives (5-7) in ball-milling procedure

N-benzyl fluoro nitrone **1** (1 equivalent) and methyl phenyl propiolate (1 equivalent) was mixed together and ball-milled at 30 Hz for 6 hours in a 10-mL steel vessel and 15-mm diameter balls at room temperature (16°C). After the completion of reaction, the reaction mixture was taken in CH_2Cl_2. It was filtered on cotton for the removal of NaCl. The filtrate was evaporated under vacuum to afford crude fluoro isoxazoline as dark red gummy liquid (91%). The 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** (entry **4**, Table 1, Scheme 1). The same methodology was adopted for the synthesis of other fluoro isoxazoline derivatives (entries **5** and **6**).

3.3.1 | Spectroscopic data of fluoro isoxazoline derivatives (5-7)

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

Dark red gummy liquid. Yield 91%; $R_f = 0.70$; IR (KBr): υ_{max} 3020 (m), 2246 (m), 1748 (s), 1714 (s), 1688 (s), 1616 (s), 1482 (s), 1324 (s), 1214 (s), 816 (m), 787 (s) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.85–7.78 (m, 3H, $C_6H_3F_2$), 7.65–7.30 (m, 2x5H, C_6H_5), 3.34 (s, 3H, —$COOCH_3$), 2.65 (s,2H, $C_6H_5CH_2$), 1.23 (s,1H, C_3H); ^{13}C NMR ($CDCl_3$): δ 169.50 (—$\underline{CO}OCH_3$), 136.28, 136.25, 136.20, 136.15, 135.10, 135.05, 134.73, 134.48 (aromatic carbons), 133.60, 133.52, 133.47, 133.38, 133.28, 133.17 (2,6-difluoro phenyl carbons), 87.44 (C_5), 74.90 (C_3), 59.66 (C_4), 45.28 (—$COO\underline{CH_3}$), 35.25 (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, 70.48; H, 4.53; N, 3.31%.

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

Red liquid. Yield 91%; $R_f = 0.72$; IR (KBr): υ_{max} 3015 (m), 2254 (m), 1726 (s), 1682 (s), 1610 (s), 1445 (s), 1264 (s), 1226 (s), 800 (m), 783 (s) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.44–7.37 (m, 3H, $C_6H_3F_2$), 7.12–6.95 (m, 5H, C_6H_5),

3.37 (s, 3H, —$COOCH_3$), 3.28 (s, 3H, —$COOCH_3$), 2.60 (s, 2H, $C_6H_5CH_2$), 1.74 (s,1H, C_3H); ^{13}C NMR ($CDCl_3$): δ 170.66, 170.56 (—$\underline{CO}OCH_3$, carbonyl carbons of the ester group), 136.70, 136.65, 136.47, 136.36 (aromatic carbons), 132.70, 132.48, 132.40, 132.18, 132.05, 132.00 (2,6-difluoro phenyl carbons), 86.20 (C_5), 75.68 (C_3), 57.80 (C_4), 47.38, 47.26 (—$COO\underline{CH_3}$, methyl carbons of the ester methyl group), 39.57 (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, 61.52; H, 4.21; N, 3.34%.

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

White thick liquid. Yield 90%; $R_f = 0.74$; IR (KBr): υ_{max} 3012 (m), 2990 (br), 2245 (m), 1765 (s), 1610 (s), 1470 (s), 1326 (s), 1210 (s), 1108 (s), 808 (m), 788 (s) cm^{-1}; ^1H NMR ($CDCl_3$): δ 10.10 (s, 2H, 2XCOOH), 7.92–7.84 (m, 3H, $C_6H_3F_2$), 7.68–7.40 (m, 5H, C_6H_5), 2.93 (s,2H, $C_6H_5CH_2$), 2.85 (s,1H, C_3H); ^{13}C NMR ($CDCl_3$): δ 172.80, 172.74 (carboxyl carbons), 137.68, 137.60, 137.53, 137.45 (aromatic carbons), 134.38, 134.27, 134.23, 134.17, 134.06, 134.00 (2,6-difluoro phenyl carbons), 87.80 (C_5), 75.90 (C_3), 57.53 (C_4), 34.22 (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, 59.72; H, 3.33; N, 3.55%.

3.4 | Antimicrobial study

All the synthesized fluoro isoxazolidine and isoxazoline derivatives (**2**-**7**) were screened for antimicrobial activities against 11 bacterial strains (Table 2) with Gentamycin as

TABLE 2 MIC values (μg/mL) of fluoro isoxazolidine and isoxazoline derivatives (**2**-**7**)

Organisms/Molecules	2a	3b	4a	4b	5	6	7
Escherichia coli ATCC 25930	600	400	600	400	_	_	_
Salmonella typhi 60	600	600	200	400	_	600	-
Vibrio cholerae 21	600	400	200	600	1000	600	-
Klebsiella pneumoniae 10030	600	600	_	600	_	_	_
Shigella dysentery 12	800	600	400	600	1000	_	_
Pseudomonas AMRI 102	800	600	400	400	_	_	_
Salmonella typhimurium NTCC 70	600	600	400	400	_	_	_
Staphylococcus aureus 29735	800	600	400	600	1000	_	_
Bacillus cereus 11770	600	400	400	600	1000	_	_
Bacillus subtilis 6634	800	600	400	600	1000	_	_
Streptococcus epidermidis 1220	600	400	400	400	1000	_	_

Note. "-" represents no antimicrobial activity of the molecules.

TABLE 3 Represents zone of inhibition of molecules (**2**-**7**) and MIC value (in mm)

Organisms/Compounds	2a	3b	4a	4b	5	6	7	Gentamycin
Escherichia coli ATCC 25930	12	18	19	22	_	23		38
Salmonella typhi 60	13	22	18	16	_	27		40
Vibrio cholerae 21	10	28	16	23	16	_	_	35
Klebsiella pneumoniae 10030	14	13	_	22	_	_	_	21
Shigella dysentery 12	18	23	24	20	24	_	_	22
Pseudomonas AMRI 102	12	14	18	21	_	_	_	20
Salmonella typhimurium NTCC 70	18	27	20	19	_	_	_	25
Staphylococcus aureus 29735	14	18	22	23	20	_	_	36
Bacillus cereus 11770	17	27	22	22	21	_	_	30
Bacillus subtilis 6634	16	23	20	23	24	_	_	40
Streptococcus epidermidis 1220	20	18	17	20	22	_	_	32

Note. "-" represents no measurable zone of diameter at MIC value of molecules under study.

reference.[26] For culture medium, nutrient agar was used, and the strains were grown at 23°C for 24 hours. Following McFarland standard,[27] the suspension was prepared. All the compounds under study were dissolved in 4% dimethyl sulfoxide (DMSO) solution and sterile distilled water was added for screening using Agar dilution.[28] Standard cup plate assay method[29] was used for finding minimum inhibitory concentration (MIC) of the organisms to be screened. Bacterial solution (2×10^6 CFU/mL) of 0.1 mL was transferred to nutrient agar plates and spread uniformly with a sterile glass spreader. The clear zone of inhibition on agar surface around the wells was the area that was measured for evaluation of sensitivity after observing for 24 hours of incubation at 23°C.[30] We have observed the MIC values (Table 2) from antimicrobial study of the synthesized molecules (**2**-**7**) and also the corresponding zone of inhibition of the molecules using gentamycin as reference antimicrobial agent (Table 3). Our study shows that compound **7** has no antimicrobial activity and compound **6** has effect on specific organisms like *Salmonella typhi* 62 and *Vibrio cholerae* 20. It gives an indication of a new enteric drug. The molecules **2a**, **3b**, **4a**, **4b**, **5**, and **6** have been found to be very effective against both Gram-positive and Gram-negative organisms, and it gives us an opportunity to develop new "broad-spectrum antimicrobial agents." Antimicrobial screening study of the molecules **2b** and **3a** are going on at present.

4 | CONCLUSION

In summary, fluoro nitrone and fluoro cycloadducts were synthesized for the first time in a ball-mill under mechanochemical procedure and solvent-free conditions with excellent yield and selectivity. This protocol is promising and therefore should attract the attention of organic chemists working in the field of green chemistry. This methodology also is very simple, cost efficient, and is safe for the environment as well. Majority of the synthesized molecules have been found to have potential antimicrobial activity against both Gram-positive and Gram-negative organisms, and hence it creates an opportunity for further biological study (antitumor and anticancer activity) for these molecules.

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