RASĀYAN *J. Chem.*
Vol. 19 | No. 1 | 591-604 | January-March | 2026
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

SOLID PHASE SYNTHESIS OF *N*-SUBSTITUTED-4-(TRIFLUOROMETHYL)-*C*-PHENYL ISOXAZOLIDINE, ISOXAZOLINE DERIVATIVES AND THEIR FURTHER APPLICATIONS IN PEPTIDE SYNTHESIS

B. Chakraborty✉, S. Tamang, E. Chettri, and S. Kafley
Organic Chemistry Laboratory, Sikkim Government College (NBBGC),
Gangtok 737102, Sikkim, India
✉Corresponding author: bhaskargtk@yahoo.com

ABSTRACT

N-Substituted-4-(Trifluoromethyl)-*C*-Phenyl nitrones have been employed for the synthesis of some new trifluoromethyl cycloadducts via (3 + 2) cycloaddition reactions using a mechanochemical procedure (ball-milling). This solvent-free procedure in the presence of sodium bicarbonate showed a remarkably faster reaction rate, better yields, as well as easier workup procedure for the isolation of trifluoronitrones and trifluoro cycloadducts compared to microwave-induced cycloaddition procedures. A few trifluoro-cycloadducts (4,5-dicarboxylic acid substituted isoxazolines) have also been successfully employed in the synthesis of new trifluoro di-peptides. This procedure may open a new avenue for the synthesis of dipeptides with excellent yield. We found from the preliminary studies few trifluoro-cycloadducts and di-peptides are capable of exhibiting *anticancer activities*. Though nitrones are usually unstable and utilised in *in situ* reactions, *N*-Substituted-4-(Trifluoromethyl)-*C*-Phenyl nitrones reported here are stable and characterizable.

Keywords: Mechanochemistry; Trifluoro Nitrones; (3+2) Cycloaddition Reaction; Trifluoro Cycloadducts; Enantioselectivity; Dipeptides, Anticancer Activity

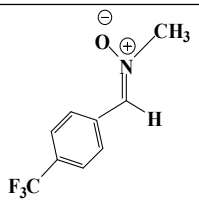
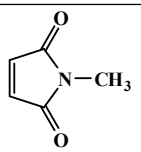
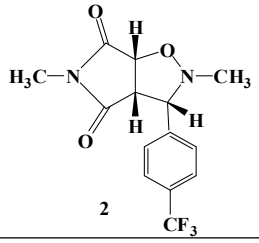
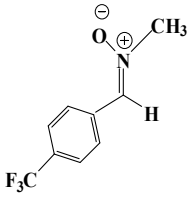
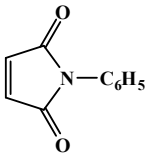
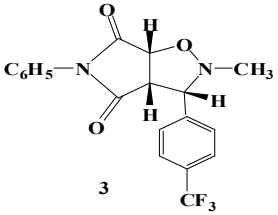
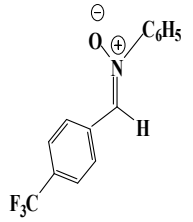
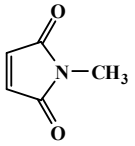
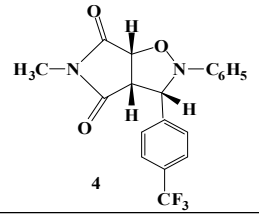
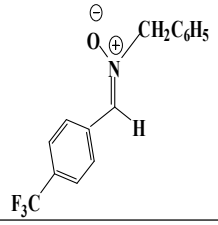
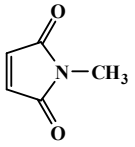
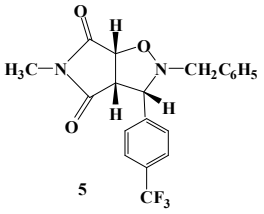
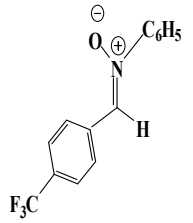
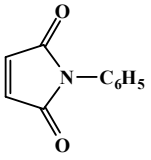
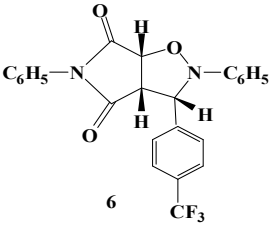
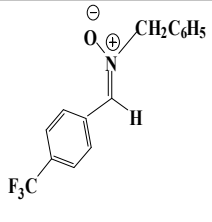
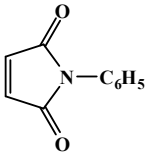
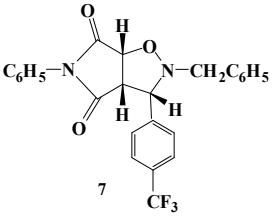
RASĀYAN *J. Chem.*, Vol. 19, No.1, 2026

INTRODUCTION

5-Membered oxygen-nitrogen heterocyclic molecules are most conveniently synthesized using nitrones, which are one of the most suitable reaction intermediates in synthetic organic chemistry.[1] The synthesis, isolation and cycloaddition reactions of nitrones with activated olefins and alkynes are always a point of great interest. This is due to the synthetic applications of cycloadducts (isoxazolidine and isoxazoline derivatives) and also to their significant biological activities.[2-4] There are plenty of methodologies known in this (3 + 2) cycloaddition chemistry, but greener syntheses and cycloaddition reactions of trifluoro nitrones and fluoro cycloadducts have been scarcely reported.[5-7] The introduction of a fluorine atom or atoms in a particular position in an organic molecule is capable of significantly changing the stability and the bioactivity of a molecule.[8] It could be due to three important properties of the fluorine atom, which can play a crucial role in this significant change and bioactivity, and are the high electronegativity of the fluorine, the strong C-F bonding and the same size of the halogen and hydrogen atoms, respectively. These special characters of fluorine, therefore, attract not only the synthetic organic chemists but also the scientists in the field of pharmacology as well.[9] Literature shows plenty of bioactive properties of fluorine atoms and their uses as main ingredients in medicines as well as in cancer studies.[10,11,12] Our research group has plenty of communications in (3 + 2) cycloaddition reactions, which are conducted based on environmentally friendly green chemistry procedures.[13-20] The role of the trifluoromethyl (CF_3) group as a substituent in the phenyl ring is not limited to synthesis, but its active role can also be found in the biological activities of fluoro cycloadducts and peptide derivatives.[10,11,12] In this communication, we report the synthesis of several new stable trifluoro cycloadducts and their further applications in trifluoro di-peptide synthesis using a mechanochemical procedure (Scheme-1; Tables-1 and 2).[21,22] Scheme-1 describes the reaction pathways for the synthesis of trifluoro nitrones, trifluoro isoxazolidines and isoxazoline derivatives, respectively. Scheme-2 shows the synthetic route for the synthesis of trifluoro di-

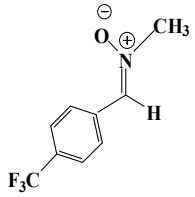
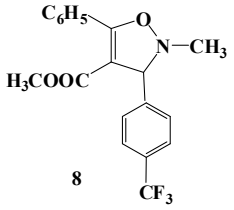
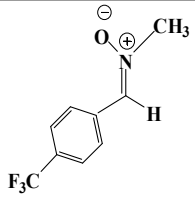
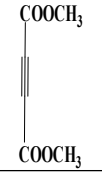
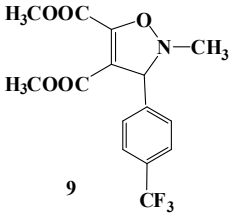
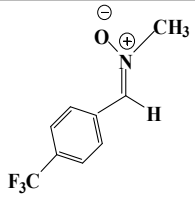
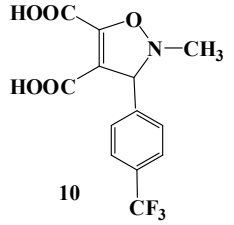
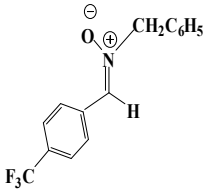
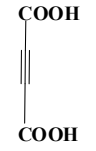
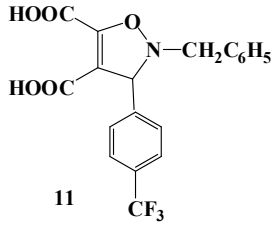
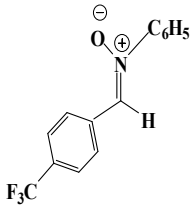
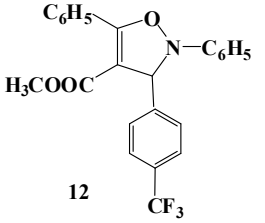
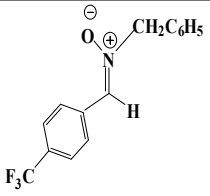
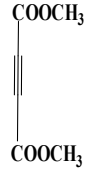
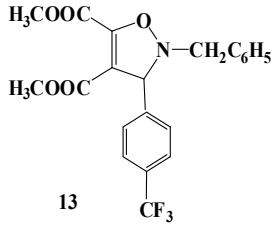
peptides. Tables-1 and 2 present comparative studies of the yields of the products obtained in ball-milling with microwave-mediated (MWI) methodologies, and also the description of the reaction conditions, time required and enantiomeric excess (*ee*) in the footnote. Table-3 shows the preliminary *anti-cancer* studies of few trifluoro cycloadducts and new trifluoro di-peptides synthesized in the present study.

Table-1: Synthesis of New *N*-Substituted-4-(Trifluoromethyl)-*C*-Phenyl-Isoxazolidine Derivatives

Entry	Nitrone (1)	Dipolarophile[a]	Time(min)	Cycloadduct (2-7)[b]	ee[c]	Yield[d] (%)
1			5 (40)		90	95 (82)
2			5 (45)		88	94 (80)
3			6 (45)		83	93 (76)
4			5 (40)		83	92 (76)
5			6 (45)		84	91(70)
6			5 (50)		84	91 (68)

[a] Reaction conditions: nitrone (1 mmol), dipolarophiles (1 equiv), ball-milling (40-70 Hz)
[b] All products were characterized by IR, ^{1}H NMR, ^{13}C NMR and MS spectral data.
[c] Enantiomeric excess; [d] Isolated yield after purification. The figures in parentheses indicate the yields obtained under the MWI.

Table-2: Synthesis of New *N*-substituted-4-(Trifluoromethyl)-*C*-phenyl-isoxazoline Derivatives

Entry	Nitrone (1)	Dipolarophile[a]	Time(min)	Cycloadduct (8-13)[b]	ee[c]	Yield[d] (%)
1			6 (50)	 **8**	NA	95 (82)
2			6 (50)	 **9**	NA	94 (80)
3			7 (55)	 **10**	NA	93 (76)
4			6 (60)	 **11**	NA	92 (78)
5			6 (60)	 **12**	NA	91(70)
6			7 (60)	 **13**	NA	90 (68)

[a] Reaction conditions: nitrone (1 mmol), dipolarophiles (1 equiv), ball-milling (40-70 Hz)
[b] All products were characterized by IR, ^{1}H NMR, ^{13}C NMR and MS spectral data.
[c] Enantiomeric excess; [d] Isolated yield after purification. The figures in parentheses indicate the yields obtained under the MWI.

EXPERIMENTAL

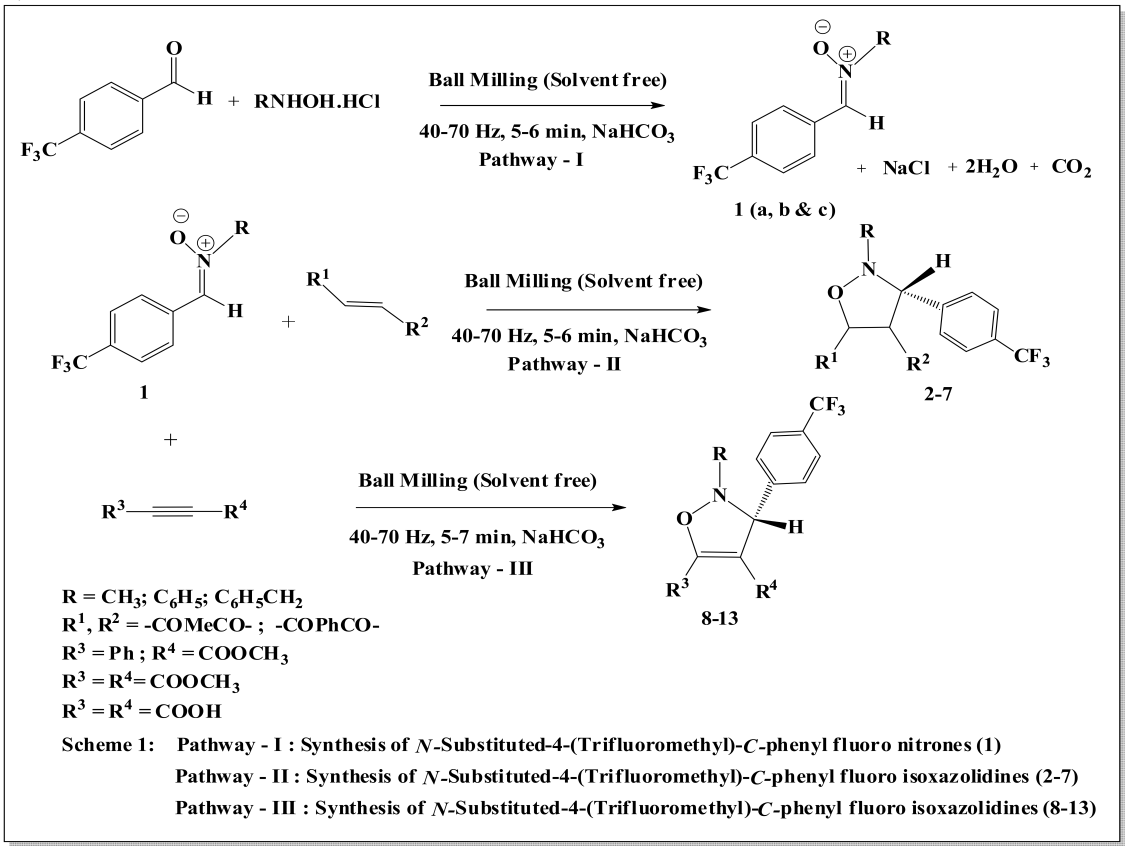
Materials and Methods

All the reagents were obtained from commercial sources (E. Merck, Germany and Sigma-Aldrich, UK) and were used directly. All the reactions were monitored by TLC using 0.25 mm silica gel pre-coated plates (Merck $60F_{254}$, UV indicator). Silica gel (Merck, Germany) with a 60–200 mesh has been used for column chromatography. All laboratory-grade reagents and solvents were purified before we commenced the reactions or column chromatography. A Bruker DRX 300 (300 MHz) spectrometer was used for recording ^1H NMR spectra at ambient temperature, and ^{13}C NMR spectra were recorded on the same instrument at 75MHz, respectively. *J* values (coupling constants) were expressed in Hz. Infra-red spectra (IR) were recorded as a film or KBr pellets on a Perkin-Elmer RX 1-881 machine. Mass-spectrometry (MS) data were recorded using a Joel SX-102 (FAB) instrument. For ball-milling purposes, we have used Retsch MM500 mixer mill digital GmbH, 42781Haan, Germany. Stainless steel jars of size 25 mL steel vessel with 15 mm diameter balls were used for grinding in the solid phase. All the reactions were carried out utilizing this ball-milling equipment.

General Procedure I

General Procedure for the Synthesis of *N*-substituted-4-(Trifluoromethyl)-*C*- phenyl nitrone (1a) in Ball-Milling Procedure

N-methylhydroxylaminehydrochloride (1 mmol), 4-(Trifluoromethyl) benzaldehyde (1 equivalent), and $NaHCO_3$ (1.0 equivalent) were mixed and ball-milled at 40 Hz for 5 min. The progress of the reaction was monitored by thin-layer chromatography (TLC). The R_f value recorded for the development of nitrone was 0.48. After the completion of the reaction, the reaction mixture was dissolved in CH_2Cl_2. It was then filtered using cotton to remove NaCl. The filtrate was evaporated under vacuum to afford *N*-methyl-4-(Trifluoromethyl)-*C*-Phenyl nitrone 1a as a yellowish white crystalline solid with high purity (90%, m.p.; 74^0C). The same methodology was adopted for the synthesis of other trifluoro nitrones (1b and 1c).



Scheme 1: **Pathway - I : Synthesis of *N*-Substituted-4-(Trifluoromethyl)-*C*-phenyl fluoro nitrones (1)**
Pathway - II : Synthesis of *N*-Substituted-4-(Trifluoromethyl)-*C*-phenyl fluoro isoxazolidines (2-7)
Pathway - III : Synthesis of *N*-Substituted-4-(Trifluoromethyl)-*C*-phenyl fluoro isoxazolidines (8-13)

Scheme-1

1a: ***N*-methyl-4-(Trifluoromethyl)-*C*- phenyl nitrone**
Spectroscopic data for nitrone **1a:** White crystals; R_f = 0.48; M.P.: 740. UV λ_{max} 235 nm; IR (KBr): υ_{max} 3034 (m), 2230 (m), 1680 (m), 1620 (s), 1450 (m), 1150 (m), 786 (s) cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.58-7.20 (m, 4H, phenyl protons), 2.34 (s, 3H, CH_3), 1.55 (s, 1H, -CH=N^+); ^{13}CNMR ($CDCl_3$): δ 142.1 (CH=N^+), 134.8, 134.3, 134.1, 133.9 (phenyl carbons), 30.5 (CH_3), 24.5 (CF_3); Calculated for $C_9H_8F_3ON$: C 52.42, H 3.88, N 6.79%; Found: C 52.37, H 3.80, N 6.64%.

1b: ***N*-benzyl-4-(Trifluoromethyl)-*C*- phenyl nitrone**
Spectroscopic data for nitrone **1b:** White crystals; R_f = 0.50; M.P.: 820. UV λ_{max} 234nm; IR (KBr): υ_{max} 3010 (m), 2215 (m), 1670 (m), 1625 (s), 1430 (m), 782 (s) cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.90-7.40 (m, 5H), 7.39-6.96 (m, 4H), 3.36 (s, 2H, CH_2Ph), 1.48 (s, 1H, -CH=N^+); ^{13}CNMR ($CDCl_3$): δ 143.0 (CH=N^+), 134.7, 133.8, 133.5, 133.2 (C-phenyl carbons), 130.6, 130.4, 130.3, 129.8, 128.7 (phenyl carbons of benzyl group), 26.0 (CF_3), 24.1 (CH_2Ph); Calculated for $C_{15}H_{12}F_3ON$: C 64.28, H 4.28, N 5.00%; Found: C 64.17, H 4.15, N 4.86%.

1c: ***N*-phenyl-4-(Trifluoromethyl)-*C*- phenyl nitrone**
Spectroscopic data for nitrone **1c:** White crystals; R_f = 0.52; M.P.: 880. UV λ_{max} 238nm; IR (KBr): υ_{max} 3036 (m), 2220 (m), 1660 (m), 1635 (s), 1436 (m), 1200 (m), 780 (s) cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.82-7.20 (m, 5H and m, 4H merged signals of phenyl ring protons), 1.78 (s, 1H, -CH=N^+); ^{13}CNMR ($CDCl_3$): δ 141.8 (CH=N^+), 137.6, 137.4, 137.2, 136.9, 134.5, 134.4, 134. 1, 133.9, 133.7 (two phenyl carbons), 26.5 (CF_3); Calculated for $C_{14}H_{10}F_3ON$: C 62.04, H 3.73, N 5.22%; Found: C 61.90, H 3.66, N 5.16%.

General Procedure II
Mechanochemical Syntheses of Trifluoro Isoxazolidine and Isoxazoline Derivatives from *N*-substituted-4-(Trifluoromethyl)-*C*-Phenyl Nitrone (1) (Table-1; entry 1)
N-methyl-4-(Trifluoromethyl)-*C*-phenyl nitrone (**1a**; 1 equivalent), *N*-methyl maleimide (1 equivalent) and $NaHCO_3$ (1 equivalent) were mixed and ball-milled at 40 Hz for 5 min. The progress of the reaction was monitored by thin-layer chromatography (TLC). The R_f value recorded for the molecule was 0.62. After the completion of the reaction, the reaction mixture was dissolved in CH_2Cl_2. It was filtered using cotton to remove NaCl. The filtrate was evaporated under vacuum to afford the crude trifluoro isoxazolidine derivative as white crystals (yield: 95%). The crude product was directly charged to a silica gel column and eluted with a mixture of ethyl acetate and *n*-hexane (1:8), resulting in pure isoxazolidine **2** (entry-1, Table-1, Scheme-1). The same methodology was adopted for the synthesis of other trifluoro isoxazolidine and isoxazoline derivatives (**3-13)**.

(3*S*)-2-methyl-3-(4-trifluoromethyl)-dihydro-5-methyl-2*H*-pyrrolo[3,4-*d*]isoxazole-4,6(5*H*,6a-*H*)-dione, 2
White crystals. M.P 126^{0}C; Yield 95%; R_f= 0.62; IR (KBr): υ_{max} 3310 (m), 2940 (m), 2830 (m), 1760 (s), 1670 (s), 1485 (m), 1320 (m), 780 (s) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.76 – 7.28 (m, 4H, aromatic protons), 6.38 (d, 1H, *J* = 2.00 Hz, C_5H), 6.34 (d, 1H, *J* = 2.00 Hz, C_3H), 3.40 (s, 6H, 2XCH_3), 2.24 (dd, 1H, *J* = 2.00, 2.00 Hz, C_4H); ^{13}C NMR ($CDCl_3$): δ 169.0 (carbonyl carbons), 138.4, 138.1, 137.2, 131.4, 130.8. 128.1 (aromatic carbons carbons), 85.4 (C_5), 76.8 (C_3), 62.2 (C_4), 30.2, 28.0 (CH_3 carbons), 24.5 (CF_3); FAB-MS: *m/z* 314 ($M^{+)}$, 245, 169 (BP, 100%), 145, 69; Calculated for $C_{14}H_{13}F_3O_3N_2$: C 53.48, H 4.16, N 8.91%; Found: C 53.34, H 4.06, N 8.70%.

(3*S*)-2-phenyl-3-(4-trifluoromethyl)-dihydro-5-methyl-2*H*-pyrrolo[3,4-*d*]isoxazole-4,6(5*H*,6a-*H*)-dione, 3
White solid. M.P 140^{0}C; Yield 92%; R_f = 0.60; IR (KBr): υ_{max} 3236 (m), 2980 (m), 2775 (m), 1760 (s), 1685 (s), 1440 (s), 1262 (m), 784 (s) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.68 – 7.20 (m, 5H; m, 4H merged signals of phenyl ring protons), 6.42 (d, 1H, *J* = 2.10 Hz, C_5H), 6.36 (d, 1H, *J* = 2.10 Hz, C_3H), 3.36 (s, 3H, N-CH_3), 2.34 (dd, 1H, *J* = 2.00, 2.00 Hz, C_4H); ^{13}C NMR ($CDCl_3$): δ 170.5, 170.0 (carbonyl carbons), 134.6, 134.3, 134.2, 134.0, 130. 4, 130.2, 129.0, 128.5, 128.3, 127.4 (phenyl carbons), 85.5 (C_5), 77.5 (C_3), 61.0 (C_4), 28.3 (N-CH_3), 23.5 (CF_3); FAB-MS: *m/z* 376 (M^+),361, 307, 299, 231 (BP, 100%), 231, 77, 69; Calculated for $C_{19}H_{15}F_3O_3N_2$: C 60.14, H 3.98, N 7.38%; Found: C 60.02, H 3.85, N 7.27%.

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

White crystals. M.P 136^{0}C; Yield 91%; R_f= 0.58; IR (KBr): υ_{max} 3230 (m), 2975 (m), 2770 (m), 1760 (s), 1680 (s), 1440 (s), 1260 (m), 780 (s) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.68 – 7.20 (m, 5H; m, 4H merged signals of phenyl ring protons), 6.42 (d, 1H, *J* = 2.10 Hz, C_5H), 6.36 (d, 1H, *J* = 2.10 Hz, C_3H), 3.36 (s, 3H, N-CH_3), 2.34 (dd, 1H, *J* = 2.00, 2.00 Hz, C_4H); ^{13}C NMR ($CDCl_3$): δ 170.5, 170.0 (carbonyl carbons), 134.6, 134.3, 134.2, 134.0, 130. 4, 130.2, 129.0, 128.5, 128.3, 127.4 (phenyl carbons), 85.0 (C_5), 76.5 (C_3), 60.0 (C_4), 28.5 (N-CH_3), 23.0 (CF_3); FAB-MS: *m/z* 376 (M^+),361, 307, 299, 231 (BP, 100%), 231, 77, 69; Calculated for $C_{19}H_{15}F_3O_3N_2$: C 60.14, H 3.98, N 7.38%; Found: C 60.05, H 3.70, N 7.20%.

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

White solid. Yield 92%; R_f= 0.60; IR (KBr): υ_{max} 3310(m), 2910 (m), 2840 (m), 1765 (s), 1678 (s), 1460 (s), 1330 (m), 785 (s) cm^{-1};^{1}H NMR ($CDCl_3$): δ 7.67 – 7.23 (m, 5H and m, 4H, merged phenyl ring protons), 6.42 (d, 1H, *J* = 2.00 Hz, C_5H), 6.24 (d, 1H, *J* = 2.00 Hz, C_3H), 3.38 (s, 3H, N-CH_3), 2.38 (dd, 1H, *J* = 2.00, 2.00 Hz, C_4H), 1.20 (s,2H, CH_2); ^{13}C NMR ($CDCl_3$): δ 170.0, 168.2 (carbonyl carbons), 138.0, 137.8, 137.2, 136.8, 130.5, 130.1, 129.8, 127.6, 126.3 (phenyl carbons), 80.1 (C_5), 77.2 (C_3), 60.2 (C_4), 28.5 (N-CH_3), 25.0 (CF_3), 22.5 (CH_2); FAB-MS: *m/z* 390 (M^+),284, 245 (BP, 100%), 145, 106, 91, 77; Calculated for $C_{20}H_{17}O_3F_3N_2$: C 61.51%, H 4.39%, N 7.18%. Found C 61.32%, H 4.26%, N 7.04%.

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

Yellow solid. M.P 117^{0}C; Yield 91%; R_f= 0.66; IR (KBr): υ_{max} 3250 (m), 2960 (m), 2835 (m), 1760 (s), 1685 (s), 1460 (s), 1360 (m), 782 (s) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.68 – 7.20 (m, 2x5H; m, 4H, merged phenyl ring protons), 6.42 (d, 1H, *J* = 2.00 Hz, C_5H), 6.24 (d, 1H, *J* = 2.00 Hz, C_3H), 2.34 (dd, 1H, *J* = 2.00, 2.00 Hz, C_4H); ^{13}C NMR ($CDCl_3$): δ 170.2, 169.5 (carbonyl carbons), 135.9, 135.7, 135.3, 135.0, 134.8, 134.0, 133.7, 133.4, 133.2, 132.7, 131.6, 131.3, 130.2, 129.5, 128.4, 128.1 (phenyl ring carbons), 82.6 (C_5), 75.6 (C_3), 55.5 (C_4), 24.0 (CF_3); FAB-MS: *m/z* 441 (M^+), 364, 287, 256 (BP,100%), 145, 77; Calculated for $C_{24}H_{17}F_3O_3N_2$: C 65.02, H 3.88, N 6.35%; Found: C 64.90, H 3.66, N 6.13%.

(3*S*)-2-benzyl-3-(4-trifluoromethyl)-dihydro-5-phenyl-2*H*-pyrrolo[3,4-*d*]isoxazole-4,6(5*H*,6a-*H*)-dione, 7

Brown crystals. M.P 108^{0}C; Yield 91%; R_f= 0.60; IR (KBr): υ_{max} 3240 (m), 2940 (m), 2830 (m), 1760 (s), 1680 (s), 1462 (s), 1366 (m), 786 (s) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.76 – 7.20 (m, 2x5H; m, 4H, merged phenyl ring protons), 6.40 (d, 1H, *J* = 2.00 Hz, C_5H), 6.32 (d, 1H, *J* = 2.00 Hz, C_3H), 2.34 (dd, 1H, *J* = 2.00, 2.00 Hz, C_4H), 1.24 (s, 2H, CH_2); ^{13}C NMR ($CDCl_3$): δ 171.3, 168.5 (carbonyl carbons), 136.8, 135.8, 135.3, 135.1, 134.8, 134.2, 133.7, 133.2, 133.0, 132.4, 131.9, 131.3, 130.2, 128.5, 128.4, 128.0 (phenyl ring carbons), 80.5 (C_5), 74.6 (C_3), 57.5 (C_4), 26.0 (CH_2), 24.0 (CF_3); FAB-MS: *m/z* 455 (M^+), 364, 287, 219 (BP,100%), 145, 91, 77; Calculated for $C_{25}H_{19}F_3O_3N_2$: C 65.93, H 4.18, N 6.15%; Found : C 65.75, H 4.06, N 6.05%.

(*S*)-methyl-2-methyl-3-(4-trifluoromethyl)-2,3-dihydro-5-phenylisoxazole-4-carboxylate, 8

Red thick liquid. Yield 91%; R_f = 0.70; IR (KBr): υ_{max} 3380 (m), 2250 (m), 1760 (s), 1700 (s), 1680 (s),14820(s),1210 (s), 780 (s) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.80 – 7.30 (m, 5H), 7.30-7.20 (m, 4H), 3.88 (s, 3H, -$COOCH_3$), 2.64 (s, 3H, N-CH_3), 1.26 (s, 1H, C_3H); ^{13}C NMR ($CDCl_3$): δ 170.0 (-<u>CO</u>OCH_3), 138.4, 138.0, 137.4 137.1, 136.4, 136.3, 135.7, 135.2 (phenyl carbons), 90.0 (C_5), 79.4 (C_3), 63.0 (C_4), 30.0 (-COO<u>CH$_3$</u>), 26.2 (N-CH_3), 24.0 (CF_3); FAB - MS (*m/z*): 363 (M^+), 304, 286, 218 (BP), 145, 77, 59; Calculated for $C_{19}H_{16}F_3O_3N$: C 62.27, H 4.40, N 3.82%; Found: C 62.13, H 4.10, N 3.66%.

(*S*)-dimethyl-2-methyl-3-(4-trifluoromethyl)-2,3-dihydroisoxazole-4,5-dicarboxylate, 9

Red liquid. Yield 92%; R_f= 0.60; IR (KBr): υ_{max} 3290 (m), 2180 (m), 1740 (s), 1670 (s), 1620 (s), 1260 (s), 870 (m), 776 (s) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.76 – 7.24 (m, 4H, phenyl protons), 3.84 (s, 6H, 2X -$COOCH_3$), 1.84 (s, 3H, N-CH_3), 1.28 (s, 1H, C_3H); ^{13}C NMR ($CDCl_3$): δ 172.0, 170.0 (-<u>CO</u>OCH_3,

carbonyl carbons of the ester group), 134.0, 133.4, 130.0, 129.5, 79.0 (C_5H), 66.5 (C_3H), 54.5 (C_4H), 32.0 (N-CH_3), 26.5 (CF_3); FAB - MS (*m/z*): 345 (M^+), 330, 296, 200 (BP), 145, 59; Calculated for $C_{15}H_{14}F_3O_5N$: C 51.72, H 4.02, N 4.12%; Found: C 51.60, H 3.90, N 3.97%.

(*S*)-2-methyl-3-(3-trifluoromethyl)-2,3-dihydroisoxazole-4,5-dicarboxylic acid, 10

Colourless thick liquid. Yield 91%; R_f = 0.64; IR (KBr): υ_{max} 3180 (m), 2990 (br), 1760 (s), 1485 (s), 1360 (s), 1210 (s), 790 (s) cm^{-1}; ^1H NMR ($CDCl_3$): δ 10.00 (s, 2H, 2XCOOH), 7.68 – 7.40 (m, 4H, phenyl protons), 2.36 (s, 1H, C_3H), 1.24 (s,3H, N-CH_3); ^{13}C NMR ($CDCl_3$): δ 173.2, 172.5 (carboxyl carbons), 138.5, 137.0, 135.0, 134.5, 130. 0 (phenyl carbons), 79.5 (C_5), 56.0 (C_3), 46.8 (C_4), 28.0 (N-CH_3 carbon), 25.0 (CF_3); FAB - MS (*m/z*): 317 (M^+), 302, 272, 172 (BP), 145, 45; Calculated for $C_{13}H_{10}F_3O_5N$: C 48.74, H 3.14, N 4.37%; Found: C 48.58, H 3.07, N 4.18%.

(*S*)-2-benzyl-3-(3-trifluoromethyl)-2,3-dihydroisoxazole-4,5-dicarboxylic acid, 11

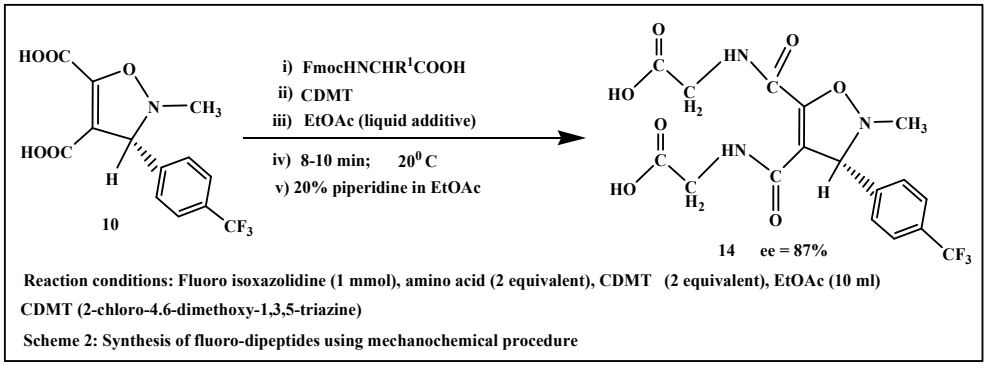
Colourless thick liquid. Yield 90%; R_f = 0.68; IR (KBr): υ_{max} 3280 (m), 2980 (br), 1760 (s), 1485 (s), 1350 (s), 1220 (s), 780 (s) cm^{-1}; ^1H NMR ($CDCl_3$): δ 10.02 (s, 2H, 2XCOOH), 7.70 – 7.20 (m, 5H and m, 4H, merged phenyl protons), 2.36 (s,1H, C_3H), 1.26 (s,2H, CH_2); ^{13}C NMR ($CDCl_3$): δ 170.0, 160.0 (carboxyl carbons), 137.5, 137.0, 136.5, 135.5, 135.0, 134.0, 133.5, 130.0, 128.5 (phenyl carbons), 77.0 (C_5), 64.0 (C_3), 54.0 (C_4), 29.0 (CH_2 carbon), 27.0 (CF_3); FAB - MS (*m/z*): 396 (M^+), 305, 160 (BP), 145, 91, 77; Calculated for $C_{19}H_{14}F_3O_5N$: C 57.57, H 3.53, N 3.53%; Found: C 57.450, H 3.40, N 3.34%.

(*S*)-2-phenyl-3-(4-trifluoromethyl)-2,3-dihydro-5-phenylisoxazole-4-carboxylate, 12

Red thick liquid. Yield 92%; R_f = 0.66; IR (KBr): υ_{max} 3230 (m), 2240 (m), 1740 (s), 1715 (s), 1680 (s),1320 (s), 780 (s) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.87 – 7.40 (m, 2x5H, phenyl protons), 7.39-7.20 (m, 4H, phenyl protons), 3.86 (s, 3H, -$COOCH_3$), 1.25 (s,1H, C_3H); ^{13}C NMR ($CDCl_3$): δ 168.5 (-$\underline{CO}OCH_3$), 137.0, 136.0, 135.0, 134.3, 134.00, 132.0, 131.0, 130.5, 127.0, 126.5, 125.0, 123.7 (phenyl carbons), 82.5 (C_5), 66.0 (C_3), 55.0 (C_4), 31.0 (-$COO\underline{CH_3}$), 28.0 (CF_3); FAB - MS (*m/z*): 425 (M^+), 348, 280 (BP), 271, 154, 77; Calculated for $C_{24}H_{18}F_3O_3N$: C 67.28, H 4.20, N 3.27%; Found: C 67.07, H 4.05, N 3.13%.

(*S*)-dimethyl-2-benzyl-3-(4-trifluoromethyl)-) 2,3-dihydroisoxazole-4,5-dicarboxylate, 13

Yellow liquid. Yield 91%; R_f= 0.70; IR (KBr): υ_{max} 3310 (m), 2190(m), 1740 (s), 1680 (s), 1660 (s), 1440 (s), 1265(s), 1220 (s), 788 (s) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.68 – 7.30 (m, 5H and m, 4H, merged signals of phenyl protons), 3.88 (s, br, 2X3H, -$COOCH_3$), 1.70 (s, 3H, C_3H), 1.25 (s,2H, CH_2); ^{13}C NMR ($CDCl_3$): δ 172.0, 162.0 (-$\underline{CO}OCH_3$, carbonyl carbons of the ester group), 136.0, 134.0 133.5, 133.0, 132.5, 131.5, 130.0 129.0 (phenyl carbons), 82.5 (C_5), 74.0 (C_3), 58.0 (C_4), 29.0 (CH_2), 26.5 (CF_3); FAB - MS (*m/z*): 421 (M^+), 344, 330, 276 (BP), 91, 77; Calculated for $C_{21}H_{18}F_3O_5N$: C 59.43, H 4.24, N 3.30%; Found: C 59.26, H 4.10, N 3.15%.



Scheme-2: Synthesis of Fluoro-dipeptide using Mechanochemical Procedure

General Experimental Procedure for Trifluoro Dipeptide Synthesis (Dipeptide 14) Using Mechanochemical Procedure

A mixture of the trifluoro isoxazoline derivative (**10**; 1 mmol), Fmoc-glycine (2 equivalents) and CDMT (2 equivalents) was added to EtOAc (10 ml). The mixture was ball-milled at 60 Hz for 8 min. The progress of the reaction was monitored by thin-layer chromatography (TLC). The R_f value recorded for

the molecule was 0.70. After the completion of the reaction, the reaction mixture was dissolved in CH_2Cl_2. Deprotection of Fmoc was accomplished using 20% piperidine and EtOAc (2:8; 2 x 3 min, 1 x 4 min, and 1 x 5 min). Fmoc was washed, and deprotection was carried out with 10 ml of solvent for each wash, followed by evaporation under vacuum to afford the crude trifluoro-dipeptide derivative as white crystals (95%; m.p.: 90 °C). The crude product was directly charged on a silica gel column and eluted with a mixture of ethyl acetate and n-hexane (1:8), resulting in pure trifluoro-dipeptide **14** (Scheme-2). Usually, (dicyclohexylcarbodimide) DCC has been employed as a coupling reagent for peptide synthesis in solid-phase peptide synthesis (SPPS), but it has certain drawbacks that we identified during peptide synthesis. We observed that due to the development of an insoluble byproduct (*N, N*-dicyclohexylurea), purification of the synthesized peptide became tedious. Therefore, in the present study, we have used CDMT (2-chloro-4.6-dimethoxy-1,3,5-triazine) as a coupling reagent for the trifluoro-dipeptide synthesis and found an excellent outcome.

***(S)*-2-methyl-*N*-((3*R*)-2-methene-4,5-dicarboxylic acid-2H-pyrrolo[3-phenyl,4-trifluorod]-isoxazol-3-yl) propanamide**

White crystals; R_f = 0.70; Yield 95%; m.p.: 90^0 C. IR (KBr): υ_{max} 3380 (s, N-H), 3152 (aromatic C-H protons), 1740 (s, C=O), 1685 (s, -NHC=O), 1665 (s, C=O), 1420 (m, isoxazoline ring C-C), 786 (s, aromatic C-C) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 10.70 (s, br, 2H, 2x-COOH), 7.62-7.22 (m, 4H, C_6H_5 protons), 3.70 (br, 2H, 2x-NH), 2.64 (s, 2xCH_2, 4H), 2.46 (s, 1H, C_3H), 2.31 (s, 1H, C_3H), 1.24 (s, 3H, N-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.4 (C=O of 2xCOOH), 170.2 (C=O of 2xHN-C=O), 134.5 ,132.0, 130.0, 129.6, 128.5.5, 128.0 (aromatic carbons), 94.5 (C_5), 66.5 (C_4), 45.0 (C_3), 44.5 (CH_3), 33.4, 29.3, 28.2 (CF_3). FAB-MS (m/z): 399 (M^+), 313, 254, 239, 145. Anal. Calcd. for $C_{17}H_{16}O_5N_3F_3$: C, 51.12; H, 4.01; N, 10.52%. Found: C, 50.45; H, 3.80; N, 10.30%.

RESULTS AND DISCUSSION

The experiences of utilizing almost all the greener procedures and from the recent literature studies, we decided to conduct our present synthesis using the ball-milling technique. While conducting our synthesis employing a ball-milling procedure (mechanochemistry), we noticed a significant acceleration in the reaction rate, yield of the cycloadducts and dipeptides, as we also conducted the same synthesis using microwave irradiation methodology (MWI) and compared the reaction rate and yields of the products obtained (Tables-1 and 2). We could understand why mechanochemical procedures involving the ball-milling technique are so popular and regarded as one of the lucrative methodologies in green organic synthesis in today's scenario.[21,22] In addition to faster reaction rates and high yield of products ball-milling procedure has many environmentally friendly aspects as well. *N*-substituted-4-(Trifluomethyl)-*C*-Phenyl nitrones (**1a-c**) synthesized in our laboratory are very stable (melting point ranges between 68 °C and 85 °C respectively). Therefore, utilization of these nitrones in cycloaddition reactions becomes very easy and conventional *in situ* reactions are not needed. A few new cycloadducts and the fluoro dipeptide (**14**) were found to exhibit significant *anticancer* activities. Hence, we may consider these *N*-substituted-4-(Trifluoromethyl)-*C*-Phenyl nitrones as important precursors for the synthesis of new *anticancer drugs*. Detailed studies on the cell cycle are currently in progress.

For the synthesis of *N*-substituted-4-(Trifluoromethyl)-*C*-Phenyl nitrones (**1a-c**), we have conducted the reactions taking one equivalent each of 4-(Trifluoromethyl)-benzaldehyde and *N*-substitutedhydroxylaminehydrochlorides along with one equivalent of sodium bicarbonate (solid phase support) for the synthesis of **1a-c** (Scheme 1; R = Me, Ph, Bz). The synthesized trifluoro nitrones were used for the cycloaddition reactions without further purification. Since all the nitrones (**1a-c**) were stable (m.p: 68^o to 85 °C), we conducted cycloaddition reactions with dipolarophiles in a 1:1 ratio. We also observed in the ball-milling procedure that the heat and the pressure were usually low in the reaction vessel. During the synthesis, the best results were obtained when a 1:1 ratio of starting materials was used. We also observed incomplete conversion to target molecules when different ratios (1:2 and 2:1) of starting materials were used. The purpose of the addition of solid sodium bicarbonate to the reaction mixture for the synthesis of trifluoro nitrones was to activate *N*-substitutedhydroxylamines because we also studied the same reaction without sodium bicarbonate and observed a slower reaction rate. Moreover, ball-milling with solid support is always favourable as far as reaction rate and yields are concerned. So,

we had decided to add sodium bicarbonate. Another reason could be that with the addition of sodium bicarbonate, the reaction mixture became faintly alkaline, and thus the liberated HCl was neutralized. We also observed that if we don't add sodium bicarbonate in the ball-milling procedure for our synthesis, the reaction would have developed a paste or a gum. This could be the fact that the ingredients could not mix well within the vessel. Also, solid material support helps in free-flow proceedings in ball-milling techniques. So, we would like to recommend the use of solid sodium bicarbonate for more efficiency in the process of ball-milling to obtain the best results.

In our study, we aimed to study and characterize the *N*-substituted-4-(Trifluoromethyl)-*C*-Phenyl nitrones first, followed by (3 + 2) cycloaddition reactions. After a successful study by using various reaction conditions and trials, we decided to conduct the cycloaddition reactions at a frequency of 40-70Hz. We found excellent developments of trifluoro-cycloadducts in 5-7 minutes of ball-milling and fluoro-dipeptide in 7 minutes. Out of curiosity, we tried the reactions using a liquid medium, which is polar (acetonitrile, 1 ml), in a ball-milling procedure. As expected, we observed slower developments and lower yields of trifluoro cycloadducts and dipeptides. So, finally, we could decide to conduct the reactions in ball-milling using solid phase and compare with microwave-induced methodologies (Tables-1 and 2) in solvent-free conditions.[23,24] Our observations clearly show that in microwave-induced methodology, high temperature (120-130 ^{o}C) was required for the synthesis of both trifluoro nitrones and trifluoro cycloadducts. Also, the average time required for the syntheses was found to be 40 – 60 minutes, respectively. Moreover, there were traces of starting materials along with our target molecules, indicating incomplete conversion. Probably there was a possibility of degradation of *N*-substitutedhydroxylaminehydrochlorides. We have tested the reactions at a lower frequency (10-20Hz), but we did not observe any indications of target molecules. This observation was checked in the ^{1}H NMR spectrum of the crude products. We have also found the presence of starting materials at this frequency. Also, the reaction rates were very slow (almost 40-45 minutes). It could be the fact that at a lower frequency lesser amount of energy per impact is involved, and the ball-milling procedure results lower conversion rate from the starting materials to the products.

^{1}H NMR spectroscopy was the main experimental procedure used to confirm the structures of the newly synthesized molecules, viz, *N*-substituted-4-(Trifluoromethyl)-*C*-Phenyl nitrones, trifluoro isoxazolidine, isoxazoline and trifluoro-dipeptide derivatives, respectively.[25,26,27] The other spectroscopic techniques used were ^{13}C NMR, MS and IR for the same purpose. From the nature of coupling patterns, values of coupling constants, as well as the absorptions of the protons in the desired fields (low and high) in the ^{1}H NMR spectrum, we could assume that trifluoro isoxazolidine and isoxazoline derivatives (**2-7 and 8-13**) are expected to be symmetrical in nature. All the trifluoro-nitrones (**1a-c**) exist exclusively in the *Z* configuration, and expectedly *syn* trifluoro-cycloadducts were developed from *Z* trifluoro-nitrones via an *exo* transition state geometry. Comparing *exo* vs *endo* transition states as intermediates in our cycloaddition reactions, we believed that in the *endo* transition state, substituents on the dipolarophiles might experience unfavourable steric interactions with the substituents of the 1,3-dipole. These interactions would have increased the activation energy if the cycloaddition reaction went via the *endo* pathway. Whereas, the *exo* transition state avoided these steric interactions, as the substituent of the 1,3-dipole points away from the larger ring system, and thus developed to lower activation energy and faster reaction rate. In addition, we have also found in literature[28] that secondary orbital interactions are not as prominent in the *exo* transition state as in the *endo* transition state and often make it the more favourable pathway. Though we have not performed DFT study regarding this issue, computational studies using density functional theory (DFT) have shown that the activation energy for the *exo* transition state is lower than that of the *endo* transition state. However, this is not a universal rule because preference for *exo* transition state over *endo* depends on few factors related to nitrones and dipolarophiles (mainly substituents and solvents).[29] Another study on fluoro nitrones and fluoro cycloadducts by B. Rachid and coworkers[30] reveals that using "*Conceptual Density Function Theory*" (CDFT), this kind of one-step mechanism could be considered as kinetically controlled and also the potential energy surface of the reaction pathways can be mapped. The corresponding *Gibbs free energy* may reveal that the *exo* fluoro cycloadducts are kinetically and thermodynamically more favoured than *endo* cycloadducts. Therefore,

both the theoretical concepts are in good agreement with our reported *exo* TS for the development of trifluoro cycloadducts (Fig.-1).

The configurations of the protons at C_3, C_4, and C_5 positions of the trifluoro cycloadducts are *syn* in nature because the coupling constant (J ~ 2.00-3.00Hz, for C_4-C_5 and J ~ 2.00-3.00 Hz, for C_3-C_4) values are in good agreement with the values found in literature and also in our previously published research articles.[25,26,28] Therefore, we may have an opinion based on coupling constant values that the dipolarophiles with *syn* configuration produced *syn* fluoro cycloadducts, and the addition of fluoro nitrones to electron-rich alkenes, viz, maleimides are stereo stereospecifically *syn* in nature. The 3-H and 4-H protons in maleimide cycloadducts are *syn-orientated* and also *enantioselective* in nature.

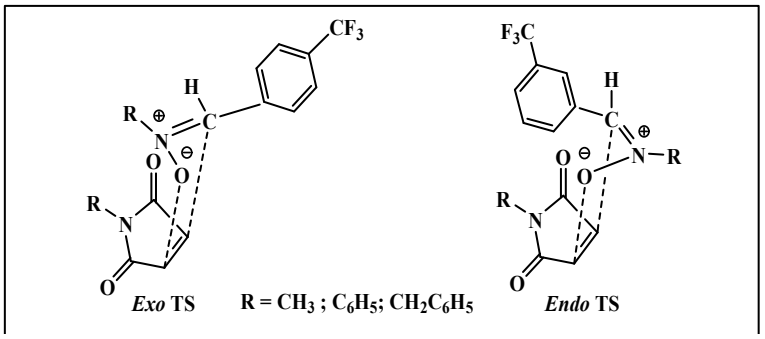


Fig.-1: *Exo/Endo* Approach of the Nitrone 1 to the Maleimides in Cycloaddition Reaction

The J values (coupling constant) of the 3-H and 4-H protons ($J_{3,4}$ ~ 2.00 Hz)[25,26,30] are also in good agreement with the *cis*-oriented structure. Due to the absence of 4-H protons, the exact geometry of trifluoro-isoxazoline derivatives could not be determined. In the Mass spectrum (MS), we have observed and obtained expected fragmentation peaks, which include the molecular ion (M^+) and base peaks (BP) in all the trifluoro-cycloadducts and trifluoro dipeptide. The mass fragmentation pattern of trifluoro isoxazoline derivatives is interesting. In addition to M^+, the prominent peaks observed could be due to the fragmentations of the $COOCH_3$, Ph and COOH groups. The possible reason is the development of *aziridine* derivatives after the fragmentation of the said groups. In the case of the ^{13}C NMR spectrum of many new molecules, we did not obtain markings of the absorptions of couplings of C and F atoms, though we have received signals in the spectrum which was in the range of 100 – 119 MHz (usually meant for C-F couplings). This could be due to the very weak couplings of Carbon and Fluorine atoms. Since further studies are ongoing with a variety of fluoro nitrones, we will be able to identify these C-F couplings in the near future.

Anticancer Study

Recent research studies have mainly focused on the possibilities of trifluomethyl-substituted phenyl rings which can be one of the key factors and may be active ingredients in the manufacturing of cancer drugs in the pharmaceutical industries. The high electronegativity, metabolic stability, and ability of fluorine atoms to penetrate cells are the most effective and useful to arrest and kill the cancer cells, and consequently, trifluoromethyl-cycloadducts and dipeptides are expectedly to act more effectively against cancer cells. In the recent advancements of cancer vaccine studies[31], where primary ingredients are fluorinated peptides and O-N heterocycles, we had decided to conduct a few preliminary *anticancer studies* with our new trifluoromethyl cycloadducts (selected in purity-wise) and dipeptide. We conducted the study by screening with selective human cells.[12,32] Cytotoxicity of the new molecules was determined based on measurement of *in vitro* growth inhibition of tumour cell lines[32,33] using the MTT assay. The cells utilized were A549 derived from human alveolar adenocarcinoma epithelial cells (ATCC No.CCL-180), HeLa derived from human cervical cancer cells (ATCC No. CCL-14), MDA-MB-167 from human breast adenocarcinoma cells (ATCC No. HTB-35) and MCF7 cells from human breast adenocarcinoma cells (ATCC No.HTB-40) respectively, in this study. IC_{50} values (50% inhibitory concentration in μM) are expressed as the average value of the two independent experiments. The growth of cancer cell lines after the treatment and interactions with trifluoromethyl-cycloadducts, dipeptide (**2-13** and **14**), was

determined following the general procedure used by the National Cancer Institute for *in vitro* anticancer drug studies. Protein-binding dye sulforhodamine B was utilized in this procedure for the estimation of cell growth.[34,35] The cell growth was counted (95 cells per well in 100 mL medium) using 90 microtitre plates. Incubation study of the cells was conducted for 40 hours at 200 °C. Our experimental setup comprised three different wells where the cells were maintained and kept for 36 hrs. After that, the cells were treated with 30% cold (5-10 °C) TCA and left for 2 hrs at 20 °C. It was then washed and dried in air. The staining of the cells was conducted with sulforhodamine B dye, which was dissolved in Tris-buffer solution. Plates used in this experimental procedure were placed in a shaker and kept for 20-25 minutes. Calculation of cell growth was done using the optical density (OD) method, and the results were reported in IC_{50} values. Doxorubicin and epirubicin was used as the standard reference to compare the anti-*cancer* activities of our molecules. The results reported (Table-3) are preliminary information only. IC_{50} values obtained from our selected new molecules indicate that six (6) trifluoromethyl-cycloadducts and fluoro-dipeptide showed significant cytotoxicity against human alveolar adenocarcinoma epithelial cells, human cervical cancer cells, human breast adenocarcinoma cells and human breast adenocarcinoma cells, respectively. The trifluoromethyl-cycloadducts **2, 4, 7,** and fluoro-dipeptide **14** showed comparatively more potent IC_{50} values against (ATCC No.CCL-180), HeLa derived from human cervical cancer cells (ATCC No. CCL-14), and MDA-MB-167 derived from human breast adenocarcinoma cells (ATCC No. HTB-35), as well as MCF7 cells derived from human breast adenocarcinoma cells (ATCC No. HTB-40), compared to other new trifluoro cycloadducts. We therefore concluded that, two most potent trifluoro isoxazolidine derivatives **2, 4** and dipeptide **14** will be used for cell cycle analysis. The process is currently underway.

Table-3: IC_{50} Values (μM) of Various Fluoro Isoxazolidine, Isoxazoline and Peptide Derivatives

Compound (μg mL^{-1})	HeLa (Cervical)	MDA-MB-231 (Breast)	MCF-7 (Breast)	A549 (Lung cancer)
2	98	85	97	95
3	55	93	82	56
4	38	80	42	32
5	70	94	60	80
14	97	95	85	83
15	88	76	75	70
Doxorubicin (Standard)	0.8	2.00	0.4	0.6
Epirubicin	1.1	1.48	0.7	0.3

CONCLUSION

We have adopted one of the most favourable green chemistry methodologies (mechanochemistry: ball-milling) for the synthesis of *N*-substituted-4-(Trifluoromethyl)-*C*-Phenyl nitrones and trifluoro cycloadducts with excellent yields in a minimum time frame in solid phase. We believe that this simple, cost-efficient, and time-saving methodology can be adopted by many more researchers in synthetic organic chemistry. We have also successfully conducted simultaneous synthesis of new trifluoro di-peptides employing trifluoro isoxazoline derivatives with 4,5-disubstituted carboxylic acid groups. Finally, we found promising *anticancer* activities in a few newly synthesized trifluoromethyl-substituted cycloadducts and peptides in preliminary cancer studies. We believe that, in the near future, we will be able to establish these molecules as "*anti-cancer drugs*" after proper applications of cell cycle studies.

ACKNOWLEDGEMENTS

The authors are grateful to CSIR-Central Drug Research Institute (CDRI), Lucknow, India, for recording and providing the spectroscopic data of the synthesized molecules. Also, BC is grateful to the Department of Biotechnology, Government of India, New Delhi, India, for the *"Overseas Associateship Programme"* at Cardiff University, Cardiff, UK, in 2019 for collaborative research work in new research methodologies and also administrative support and help for conducting the research work at the School of Chemistry, Cardiff University, Wales, UK.

CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-7: Affordable and Clean Energy*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

B. Chakraborty http://orchid.org/0000-0002-0642-9794
S. Tamang http://orchid.org/0009-0002-1218-6415
E. Chettri http://orchid.org/0009-0005-9238-8659
S. Kafley http://orchid.org/0009-0001-8200-2506

Cite this article as: B. Chakraborty *et al. et al.*, *Rasayan J. Chem.*, 19(1), 591-604(2026), https://doi.org/10.317 88/RJC.2026.1919594.

REFERENCES

1. (a) S. Thakur, A. Das, T. Das, *New Journal of Chemistry*, **45**, 11420(2021), https://doi.org/10.1039/D1NJ02023D; (b) X. Tian, T. Xuan, J. Gao, J. Zhang, T. Liu, Y. Wang, *Nature Communication*, **15**, 6429 (2024), https://doi.org/10.1038/s41467-024-50509-4
2. W. Patterson, P.S. Cheung, M.J. Ernest, *Journal of Medicinal Chemistry*, **35**, 507(1992), https://doi.org/10.1021/jm00081a011
3. E. Wagner, L. Becan, E. Nowakowska, *Bioorganic and Medicinal Chemistry*, **12**, 265(2004), https://doi.org/10.1021/jm00171a015
4. (a) P.N. Confalone, E.M. Huie, *Organic Reactions*, **36**, 1(1988), https://doi.org/10.1002/0471264180.or036.01 ; (b) I.A. Grigor'ev, H. Feuer, Ed. *Nitrile Oxides, Nitrones and Nitronates in Organic Synthesis. Nitrones: Novel Strategies in Synthesis* (Wiley-Interscience, Hoboken, NJ), **2**, 129(2008); (c) R.C.F. Jones, A. Padwa and W.H. Pearson, Ed. *Synthetic Applications of 1,3-Dipolar Cycloaddition Chemistry Toward Heterocycles and Natural Products* (John Wiley and Sons, Inc., NY), *The Chemistry of Heterocyclic Compounds,* **59**, 1(2002), https://doi.org/10.1002/0471221902
5. S. Cao, X. H Qian, G.H. Song, Q.C Huang, *Journal of Fluorine Chemistry*, **63**, 117(2002), https://doi.org/10.1002/slct.201800123
6. A. Arnone, R. Bernardi, F. Blasco, R. Cardillo, I.I Gerus, V.P. Kukhar, *Tetrahedron*, **54**, 2809 (1998), https://doi.org/10.51521/WJMRR.2025.3207
7. (a) R.D. Chambers, *Fluorine in Organic Chemistry*, (Blackwell Publishing: Oxford, UK), 2004, https://doi.org/10.1007/8363; (b) P. Kirsch, *Modern Fluoroorganic Chemistry*, (Wiley-VCH: Weinheim, Germany) 2004, https://doi.org/10.1002/352760393X
8. D. See Bach, *Angewandte chemie (International English Edition)*, **29**, 1320(1990), https://doi.org/10.1002/anie.199013201
9. J.T. Welch, *Tetrahedron*, **43**, 3123 (1987), https://doi.org/10.1016/S0040-4020(01)90286-8.
10. W.Wu, Y. You, Z. Weng, *Chinese Chemistry Letters,* **33 (10)**, 4517 (2022), https://doi.org/10.1016
11. M.Rahaman, A.K. Bagdi, D.S. Kopchuk, I.S. Kovaley, G.V. Zyvanoy, O.N. Chupakhin, A. Majee, A. Hajra, *Organic and Biomolecular Chemistry,* **47**, 9562 (2020) https://doi.org/10.1039/D0OB01638A
12. D.N. Binjawhar, F.A. Al-Salmi, M.A.A Alqahtani, E. Fayad, R.M Saleem, I. Zaki, *ACS Omega,* **9 (16)**, 18505 (2024) https://doi.org/10.1039/D4RA03157A
13. B. Chakraborty, M.S. Chettri, G.P. Luitel, *Journal of Heterocyclic Chemistry*, **54 (2)**, 1611(2017), https://doi.org/10.1002/jhet.. 2181
14. B. Chakraborty, G.P. Luitel, *Journal of Heterocyclic Chemistry*, **52 (3)**, 726(2015), https://doi.org/10.1002/jhet.2181

15. B. Chakraborty, *Journal of Heterocyclic Chemistry,* **57**, 477(2020), https://doi.org/10.1002/jhet.3804
16. B. Chakraborty, E. Chhetri, *Journal of Heterocyclic Chemistry*, **55**, 1157(2018), https://doi.org/10.1002/jhet.3148
17. B. Chakraborty, P. K. Sharma, S. Kafley, *Green Chemistry Letters and Reviews*, **6(2)**, 141(2013), https://doi.org/10.1080/17518253.2012.720719
18. B. Chakraborty, G. P. Luitel, *Tetrahedron Letters*, **54**, 765(2013), https://doi.org/10.1016/j.tetlet.2012.11.111
19. B. Chakraborty, P. K. Sharma, *Synthetic Communications*, **42**, 1804(2012), https://doi.org/10.1080/17518253.2012.720719
20. B. Chakraborty, N. Rai, *Journal of Heterocyclic Chemistry*, **55**, 1053(2018), https://doi.org/10.1002/jhet.3137
21. (a) B. Ranu, A. Stolle, *Ball Milling Towards Green Synthesis: Applications, Projects and Challenges* (Royal Society of Chemistry, UK), 2015, https://doi.org/10.51521/WJMRR.2025.3207 ; **(**b) I. Huskic, I. Halasz, T. Friscic, H. Vancik, *Green Chemistry*, **14**, 1597(2012), https://doi.org/10.1039/C2GC35410A
22. For selected recent examples of organic synthesis in ball mills, see: (a) J.G. Hernandez, E. Juaristi, *Journal of Organic Chemistry*, **76**, 1464 (2011), https://doi.org/10.4000/annuaire-cdf.19055; (b) J.G Hernandez, V. Garcıa-Lopez, E. Juaristi, *Tetrahedron*, **68**, 92(2012), https://doi.org/10.1016/j.tet.2011.10.093 ; (c) Y.F Wang, R.X Chen, K. Wang, B.B Zhang, B.Z Lib, D.Q Xu, *Green Chemistry*, **14**, 893(2012); https://doi.org/10.51521/WJMRR.2025.3207 ; (d) M. Jorres, S. Mersmann, G. Raabe, Green *Chemistry*, **15**, 612 (2013), https://doi.org/10.1039/C2GC36906K ; (e) R. Trotzki, O. Hoffmann, B. Ondruschka, Green *Chemistry*, **10**, 873 (2008), https://doi.org/10.1039/b808265k ; (f) J. Mack, M. Shumba, *Green Chemistry*, **9**, 328 (2007), https://doi.org/10.1002/chem200700188 ; (g) D.C Waddell, J. Mack, *Green Chemistry*, **11**, 79 (2009), https://doi.org/10.1039/b810714a ; (h) F. Schneider, T. Szuppa, A. Stolle, B. Ondruschka, H. Hopf, *Green Chemistry*, **11**, 1894 (2009), https://doi.org/10.1039/B915744C ; (i) G. Cravotto, D. Garella, D. Tagliapietra, A. Stolle, S. Schusler, S.E.S. Leonhardt and B. Ondruschka, *New Journal of Chemistry*, **36**, 1304(2012), https://doi.org/10.51521/WJMRR.2025.3207 ; (j) D.A. Fulmer, W.C Shearouse, S.T. Medonza, J. Mack, *Green Chemistry*, **11**, 1821 (2009), https://doi.org/10.1039/B915669K ; (k) R. Thorwirth, A. Stolle, B. Ondruschka, *Green Chemistry,* **12***,* 985 (2010), https://doi.org/10.1039/c000674b ; (l) R. Thorwirth, A. Stolle, B. Ondruschka, A. Wild, U.S. Schubert, *Chemical Communications,* **47**, 4370(2011), https://doi.org/10.1039/C0CC05657J; (m) T.L. Cook, J.A Walker, J. Mack, *Green Chemistry*, **15**, 617(2013), https://doi.org/10.1039/C3GC36720G ; (n) V. Estevez, M. Villacampa, C.J Menendez, *Chemical Communication*, **49**, 591 (2013), https://doi.org/10.1039/C3CS60015G ; (o) W. Su, J. Yu, Z. Li, Z. Jiang, *Journal of Organic Chemistry*, **76**, 9144(2011), https://doi.org/10.1021/jo2015533 ; (p) V. Strukil, B. Bartolec, T. Portada, I. Đilovic, I. Halasz, D. Margetic, *Chemical Communication*, **48**, 1210 (2012), https://doi.org/10.1039/C2CC36613D ; (q) G.J. Hernandez. G, E. Juaristi, *Journal of Organic Chemistry*, **75**, 7107(2010), https://doi.org/10.1351/pac200375050589 ; (r) D. Tan, V. Strukil, C. Mottillo, C.T. Friscic, *Chemical Communication,* **50**, 5248 (2014), https://doi.org/10.1039/C3CC47905F ; (s) K. Crossey, K. R.N Cunningham, P. Redpath, M.E Migaud, *RSC Advances*, **5**, 58116(2015), https://doi.org/10.1039/C5RA12239B ; (t) I. IDokli, M. Gredicak, *European Journal of Organic Chemistry*, 2727 (2015), https://doi.org/10.51521/WJMRR.2025.3207 ; (u) T.X Metro, J. Bonnamour, T. Reidon, A. Duprez, J. Sarpoulet, J. Martinez, F. Lamaty, *Chemistry-An European Journal*, **21**, 12787 (2015), https://doi.org/10.1002/chem.201501325; (v) T. K Achar, S. Maiti, P. Mal, *RSC Advances*, **4**, 12834 (2014), https://doi.org/10.1039/C4RA00415A
23. A. Loupy, Ed: *Microwaves in Organic Synthesis* (Wiley-VCH: Weinheim), 2006, https://doi.org/10.1002/9783527619559
24. A. Banerji, P.K. Biswas, P. Sengupta, S. Dasgupta, M. Gupta, *Indian Journal of Chemistry*, **43B**, 880 (2004).

25. P. Deshong, W. Li, J.W. Kennington, H.L Ammon*, Journal of Organic Chemistry.*, **56**, 1364 (1991), https://doi.org/10.1016/S0957-4166(00)86129-7
26. R. Gandolfi, P. Grunanger, *The Chemistry of Heterocyclic Compounds* (Wiley Interscience), **49(2),** 774 (1999), https://doi.org/10.51521/WJMRR.2025.3207
27. Y. Yu, M. Ohno, S. Eguchi, *Tetrahedron*, **49**, 824(1993), https://doi.org/10.47485/2832-9384.1075
28. R. Huisgen, *Journal of Organic Chemistry*, **41**, 403(1976), https://doi.org/10.1016/j.inoche.2025.115537
29. K.N. Houk, K. Yamaguchi, *1,3-Dipolar Cycloaddition Chemistry* (Wiley, New York), **2**, 407(1984), https://doi.org/10.1016/B978-0-08-034712-7.50011-4
30. B. Rachid, K. Abbiche, M.D Mellaoui, A. Imjjad, *Journal of Computational Chemistry,* **5**, 45(2023), https://doi.org/10.1002/jcc.27235
31. (a) C. Sanjai, S.S Hakkimane, B.R Guru, S.L Gaonkar, *A Comprehensive Review on Anticancer Evaluation,* **142** 106973(2024), https://doi.org/10.1016/j.bioorg.2023.106973 ; (b) S. Srivastava, D. Singh, S. K. Verma, M. Pandey, A. Sharma, H. Pandey, A. Mishra, A. *Vacunas* (Elsevier), **25 (1)**, 97(2024), https://doi.org/10.1016/j.vacun.2023.10.005
32. D. Campoccia, S. Ravaioli, S. Santi, V. Mariani, C.R. Arciola, M. Daglia, *Biomedicine and Pharmacotherapy,* **141**, 111895(2021), https://doi.org/10.1016/j.biopha.2021.111895
33. L. Cai, X. Qin, Y. Song, H. Jiang, Y. Wu, H. Ruan, *ACS-Omega,* **4**,12036(2019), https://doi.org/10.1021/acsomega.9b01142
34. B. Chakraborty, M.N. Roy, *ACS-Omega*, **7**, 4457(2022), https://doi.org/10.1021/acsomega.2c05989
35. T.M. El-Dine, M. Lavayssiere, H. Adihou, G. Subra, T.X. Metro, F. Lamaty, *Chemistry Europe*, **2**, 3, (2024).

[RJC-9594/2025]