Asian Journal of Chemistry; Vol. 35, No. 8 (2023), 1884-1890

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2023.28052

Synthesis of Novel Fluorinated 5-Benzylidine-3-ethyl-2-(2,3,4-trifluorophenylimino)thiazolidin-4-one Derivatives using Knoevenagel Reaction and Evaluation of their *in vitro* Antimicrobial Potentials

S.D. KADAM[1], D. MAMMEN[1,*], D.S. KADAM[2], S.G. PATIL[2], R.R. BAGUL[3], A. DOSHI[1] and F. PATEL[1]

[1]School of Science, Navrachana University, Vasana-Bhayli Road-391410, India
[2]Organic Chemistry Research Laboratory, Maharashtra Udayagiri Mahavidyalaya, Udgir-413517, India
[3]Department of Chemistry, Career Point University, National Highway 52, Kota-325003, India

*Corresponding author: E-mail: drdenni.mammen@gmail.com

Received: 29 May 2023; *Accepted*: 22 June 2023; *Published online*: 31 July 2023; AJC-21321

A series of novel 5-benzylidine-3-ethyl-2-(2,3,4-trifluoro-phenylimino)-thiazolidin-4-one derivatives were synthesized by Knoevenagel reaction using both conventional as well as non-conventional methods on the synthesized iminothiazolidinone core. The results of an ultrasonic Knoevenagel reaction showed that it was more efficient than the traditional method in terms of yield and reaction time. The compounds were characterized using ^{1}H, ^{19}F and ^{13}C NMR spectra, supported by mass spectrometric and infrared spectroscopic data. The stereochemistry of the final compounds was confirmed using 2D NOESY NMR experiment. The *in vitro* antibacterial potential of the synthesized benzylidine derivatives were also evaluated. Compounds having trifluoromethyl group on benzylidine moiety were observed to be the most active against the tested bacterial strains as compared to the rest of the compounds.

Keywords: Thiazolidin-4-ones, Ultrasonic waves, Knoevenagel reaction, Antibacterial activity.

INTRODUCTION

Recent years have revealed the increase of resistant diseases, prompting researchers to design, chemically synthesize and create novel compounds for use as pharmacological treatments in an effort to combat this growing problem [1,2]. The introduction of the fluorine atom has become an important tool in drug discovery. It is a proven fact that the introduction of the fluorine atom in a drug/drug-like molecule results in a significant influence on its biological and physical properties due to the enhancement of membrane permeability, hydrophobic bonding, stability against metabolic oxidation, *etc*. [3].

Fluorine is known as the 'bioisostere' of the H and OH. Insertion of fluorine atoms in a potential drug molecule can have dramatic effects on the properties of that molecule making them more selective, increasing their efficacy, making them easier to administer, *etc*. Therefore, it is of no great surprise that around 5th position of all commercial drugs, today contain at least one fluorine substituent [4]. Three of the top ten best-sellers drugs contain fluorine atoms like Pfizer's lipid-lowering agent "Lipitor", which has an aromatic fluorine substituent [5]. Linezolid, a commercially available antimicrobial drug also possesses a fluorine atom in the 4-morpholinophenylimino ring [6].

The medicinal potentials of 4-thiazolidinones, sometimes known as 'wonder nucleus' have been documented due to their wide range of biological activities and drug-like properties [7-11]. Nowadays there is growing interest in the synthesis and pharmacological evaluation of benzylidene moiety at the 5th position of the iminothiazolidinone ring. The oxo derivative of thiazolidine is a heterocyclic ring that contains carbonyl bonds, sulfur and nitrogen in a 5-membered ring and is therefore considered as the fundamental component of heterocyclic compounds [12,13]. Thiazolidinone derivatives which attracted great attention due to the diversity of their biological activities [14] such as antidiarrheal [15], antimicrobial [16], antidiabetic [17,18], antihistaminics [19], anticancer [20-23], anti-HIV [24], Ca^{2+} channel blocker [25], cardioprotective [26], anti-ischemic [27], cycloxygenase inhibitory [28] and anti-platelet activating factor [29]. In this study, novel 3-ethyl-2-(2,3,4-trifluorophenylimino)thiazolidin-4-one derivatives have been synthesized on which the *in vitro* antibacterial activity examined.

EXPERIMENTAL

All compounds used during synthesis were procured from Sigma Aldrich Pvt. Ltd., except for ethylisothiocyanate which was procured from Spectrochem Pvt. Ltd. Ethyl acetate, HCl, hexane, DMSO and sodium sulfate were procured from S.D. Fine Chem. Pvt. Ltd. Precoated silica gel $60F_{254}$ TLC plates were procured from Merck India. Nutrient broth for antibacterial activity was purchased from HiMedia Laboratories Pvt. Ltd., India. The cultures for bacterial strains *Escherichia coli* and *Serratia marcescens* have been obtained from the internal repository of Department of Microbiology, The M.S. University, Vadodara, India.

Characterization: Infrared spectra were recorded on Agilent spectrophotometer using zirconium plate. The ^{1}H, ^{19}F and ^{13}C NMR spectra were recorded on Bruker Advance III 300 NMR Ultra Shield spectrometer using DMSO-d_6/$CDCl_3$ as solvent and tetramethyl silane as internal standard. Melting points were determined using the Buchi-M565 melting point apparatus in open capillary tubes and are uncorrected.

Synthesis of 1-ethyl-3-(2,3,4-trifluorophenyl)thiourea (2): To a solution of 2,3,4-trifluoro phenylamine (**1**) (6 g, 0.041 mol) in absolute ethanol, ethylisothiocyanate (4.05 g, 0.046 mol) was added. The reaction mixture was heated at 90-100 ºC for 4 h. After completion of the reaction (monitored using TLC) ethanol was evaporated completely. Ethyl acetate (50 mL) and 0.1 N HCl (100 mL) were added to the residue and stirred for 5 min. The organic layer was separated and washed with distilled water (2 × 50 mL). The organic layer was dried over sodium sulphate and evaporated to get desired product **2** as an offwhite solid in 87% yield.

Synthesis of 3-ethyl-2-(2,3,4-trifluorophenylimino)-thiazolidin-4-ones (3): In absolute ethanol, (2,3,4-trifluorophenyl)-3-ethylthiourea (**2**) (0.25 g, 0.0010 mol), ethyl bromoacetate (0.225 g, 0.0013 mol) and triethylamine (0.21 g, 0.0015 mol) was added and reaction mixture was heated at 80-90 ºC for 3 h. After completion of the reaction, the TLC was checked using the system of 4:1 hexane:ethyl acetate (v/v). The reaction mixture was cooled to room temperature and ethanol was discarded under reduced pressure. Then, the residue was treated with 30 mL distilled water, stirred for 15 min and extracted with ethyl acetate (3 × 20 mL). The separated organic layer was evaporated under reduced pressure to get a sticky red solid (**Scheme-I**). This product was then recrystallized using absolute ethanol to obtain brown solid in 76% yield.

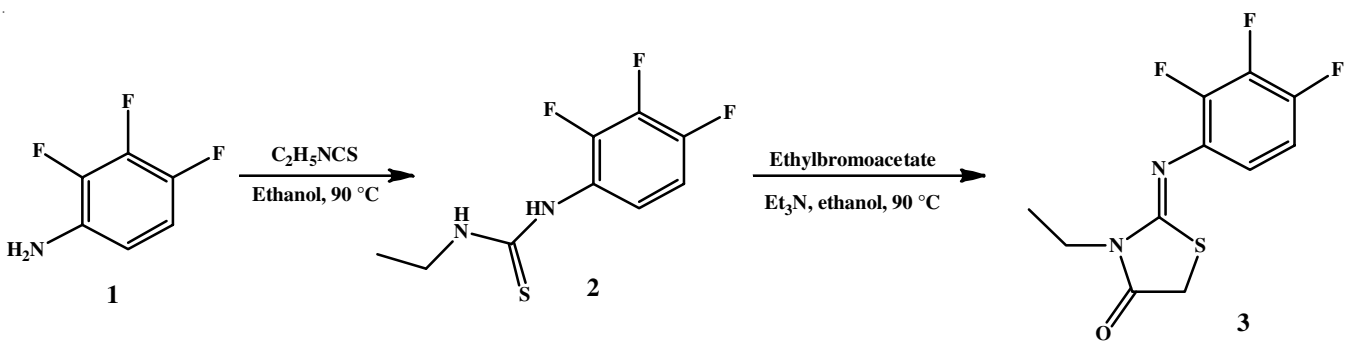
Synthesis of 5-benzylidine 3-ethyl-2-(2,3,4-trifluorophenylimino)thiazolidin-4-one (4a-r)

Conventional method: A mixture of 3-ethyl-2-(2,3,4-trifluorophenylimino)thiazolidin-4-one (0.25 g, 0.91 mmol), benzaldehyde (0.11 g, 1.09 mmol) and diisopropylethylamine (0.158 g, 1.8 mmol) in absolute ethanol was refluxed at 90-95 ºC for 8-10 h. After completion of the reaction (monitored through TLC), ethanol was evaporated, cold water was added to the residue and the compound was extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated under the reduced pressure (**Scheme-II**). The crude product was recrystallized using absolute ethanol to get the compound in good yields (Table-1).

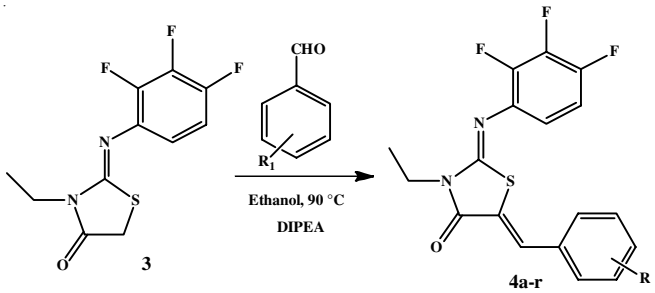
TABLE-1
COMPARISON OF REACTION YIELDS AND TIME USING CONVENTIONAL & NON-CONVENTIONAL METHODS OF KNOEVENAGEL CONDENSATION WITH DIFFERENT SUBSTITUTED ARYL ALDEHYDES

Compd.	Ultrasonic (non-conventional)		Conventional	
	Yield (%)	Time (min)	Yield (%)	Time (h)
4a	86	30	70	8
4b	88	30	72	9
4c	89	30	73	9
4d	89	30	75	6
4e	87	40	76	10
4f	84	40	69	10
4g	85	40	71	10
4h	83	20	66	5
4i	87	40	72	9
4j	80	20	72	4
4k	82	20	71	4
4l	82	20	70	4
4m	86	25	67	8
4n	82	40	67	8
4o	87	20	68	4
4p	91	20	77	4
4q	91	20	69	8
4r	93	20	71	8

Ultrasonic method: A mixture of 3-ethyl-2-(2,3,4-trifluorophenylimino)thiazolidin-4-one (0.25 g, 0.91 mmol) and aldehyde (0.11 g, 1.09 mmol) and diisopropylethylamine (0.158 g, 1.8 mmol) were mixed in 5 mL ethanol in a GC-HS vial, crimped and sonicated for 15 min at room temperature in an ultrasonic bath (LOBALife, 2.5 L, 50 K Hz, Mumbai, India).

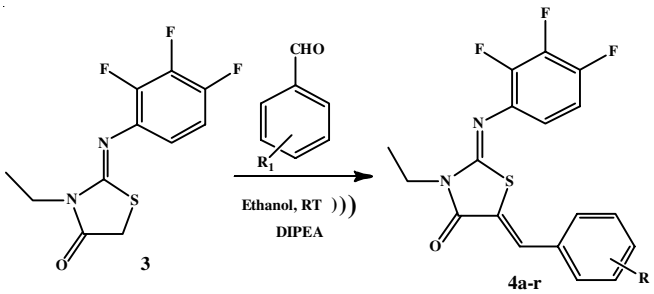


Scheme-I



Scheme-II

After the completion of reaction (as monitored through TLC), the reaction mixture was allowed to stand for 30 min at room temperature in order to precipitate solid in the reaction mixture, later reaction mixture was filtered to get a yellow solid (**Scheme-III**). Solid was washed with absolute ethanol and dried under vacuum to get pure compound (2*E*,5*E*)-5-benzylidene-3-ethyl-2-(2,3,4-trifluorophenylimino)thiazolidin-4-one in good yields (Table-1).



Scheme-III

(2*E*,5*E*)-2-(2,3,4-Trifluorophenylimino)-5-benzylidene-3-ethylthiazolidin-4-one (4a): White solid; m.p.: 135-137 ºC. IR (ATR, cm^{-1}): 2987, 2943 (C-H), 1709 (C=O), 1632 (C=N), 1496 (C=C), 1372 (C-N), 1367 (C-O), 1252. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.37 (t, *J* = 7.11 Hz, 3H), 4.02-4.11 (q, *J* = 7.11 Hz, 2H), 6.71-6.79 (m, 1H), 6.93-7.03 (d t, 1H), 7.36-7.44 (m, 5H), 7.79 (S, 1H); ^{19}F NMR ($CDCl_3$, 282 MHz): δ 138.8 (dd, $^2J_{F\text{-}F}$ = 20.5 &$^3J_{F\text{-}F}$ = 4.1 Hz 1F), 144.5 (dd, $^2J_{F\text{-}F}$ = 20.5 &$^3J_{F\text{-}F}$ = 4.1 Hz 1F), 158.22 (t, $^2J_{F\text{-}F}$ = 20.5 1F). MS (*m/z*): 363.3 [M^+ +1]; $C_{18}H_{13}F_3N_2OS$.

(2*E*,5*E*)-2-(2,3,4-Trifluorophenylimino)-5-(4-fluorobenzylidene)-3-ethylthiazolidin-4-one (4b): Yellow solid; m.p.: 165-167 ºC. IR (ATR, cm^{-1}): 2947 (C-H), 1709 (C=O), 1628 (C=N), 1590 (C=C), 1506 (C-C), 1363 (N-CH_2), 1336 (C-N), 1228 (C-F), 859 (*p*-substituted Ph ring), 819 (C-S-C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.36 (t, *J* = 7.14 Hz, 3H), 4.05 (q, *J* = 7.14 Hz, 2H), 6.74-6.79 (d, t, 1H), 6.94-7.03 (d t, 1H), 7.09-7.15 (dd, 8.7 Hz, 2H), 7.40-7.46 (d, *J* = 8.7 Hz 2H), 7.75 (S, 1H); ^{19}F NMR ($CDCl_3$, 282 MHz): δ -121.6 (s, 1F), -138.8 (dd, $^2J_{F\text{-}F}$ = 20.5 &$^3J_{F\text{-}F}$ = 4.1 Hz 1F), -144.5 (dd, $^2J_{F\text{-}F}$ = 20.5 &$^3J_{F\text{-}F}$ = 4.1 Hz 1F), -158.22 (t, $^2J_{F\text{-}F}$ = 20.5 1F). MS (*m/z*): 381.3 [M^+ +1]; $C_{18}H_{12}F_4N_2OS$.

(2*E*,5*E*)-2-(2,3,4-Trifluorophenylimino)-5-(3-bromo-4-fluorobenzylidene)-3-ethylthiazolidin-4-one (4c): Yellow solid; m.p.: 167-169 ºC. IR (ATR, cm^{-1}): 2987 (C-H), 1710 (C=O), 1631 (C=N), 1497 (C=C), 1366 (C-NCH_2), 1252 (C-N), 1093 (C-F), 905 (trisubstituted Ph ring), 694 (C-S-C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.35 (t, *J* = 7.14 Hz, 3H), 4.06 (q, *J* = 7.11 Hz, 2H), 6.74-6.79 (dt, 1H), 6.95-7.10 (dt, 1H), 7.13-7.18 (t, *J* = 8.31 Hz, 1H), 7.35-7.40 (m, 1H), 7.62-7.64 (dd, *J* = 6.422.01 Hz, 1H), 7.67 (S, 1H). ^{19}F NMR ($CDCl_3$, 282 MHz): δ 108.6 (s, 1F), 138.8 (dd, $^2J_{F\text{-}F}$ = 20.5 &$^3J_{F\text{-}F}$ = 4.1 Hz 1F), 144.5 (dd, $^2J_{F\text{-}F}$ = 20.5 &$^3J_{F\text{-}F}$ = 4.1 Hz 1F), 158.22 (t, $^2J_{F\text{-}F}$ = 20.5 1F). MS (*m/z*): 460.3 [M^+ +1]; $C_{18}H_{11}BrF_4N_2OS$.

(2*E*,5*E*)-5-(2,3-Dichlorobenzylidene)-2-(2,3,4-trifluorophenylimino)-3-ethylthiazolidin-4-one (4d): Yellow solid; m.p.: 177-179 ºC. IR (ATR, cm^{-1}): 2943 (C-H), 1714 (C=O), 1644 (C=N), 1512 (C=C), 1364 (C-NCH_2), 1332 (C-N), 1103 (C-F), 878 (disubstituted Ph ring). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.37 (t, *J* = 7.11 Hz, 3H), 4.06 (q, *J* = 7.11 Hz, 2H), 6.68-6.76 (m, 1H), 6.91-7.08 (m, 1H), 7.27-7.30 (dd, 1H), 7.34-7.37 (d, *J* = 8.4 Hz, 1H), 7.47-7.48 (d, *J* = 2.0 Hz, 1H), 8.00 (s, 1H). ^{19}F NMR ($CDCl_3$, 282 MHz): δ 138.8 (dd, $^2J_{F\text{-}F}$ = 20.5 &$^3J_{F\text{-}F}$ = 4.1 Hz 1F), 144.5 (dd, $^2J_{F\text{-}F}$ = 20.5 &$^3J_{F\text{-}F}$ = 4.1 Hz 1F), 158.22 (t, $^2J_{F\text{-}F}$ = 20.5, 1F). MS (*m/z*): 432.3 [M^+ +1]; $C_{18}H_{11}Cl_2F_3N_2OS$.

(2*E*,5*E*)-2-(2,3,4-Trifluorophenylimino)-5-(2-(trifluoromethyl)benzylidene)-3-ethylthiazolidin-4-one (4e): Yellow solid; m.p.: 125-127 ºC. IR (ATR, cm^{-1}): 2988 (C-H), 1709 (C=O), 1630 (C=N), 1495 (C=C), 1367 (C-NCH_2), 1337 (C-N), 1093 (C-CF_3), 843 (O-substituted Ph ring), 812 (C-S-C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.37 (t, *J* = 7.14 Hz, 3H), 4.06 (q, *J* = 7.11 Hz, 2H), 6.70-6.79 (m, 1H), 6.92-7.02 (m, 1H), 7.45-7.50 (t, *J* = 8.4 Hz 1H), 7.54-7.63 (m, *J* = 8.7 Hz 2H), 7.74-7.76 (d, *J* = 8.4 Hz 1H), 8.05 (S, 1H). ^{19}F NMR ($CDCl_3$, 282 MHz): δ 61.7 (s, 3F), 138.8 (dd, $^2J_{F\text{-}F}$ = 20.5 &$^3J_{F\text{-}F}$ = 4.1 Hz 1F), 144.5 (dd, $^2J_{F\text{-}F}$ = 20.5$^3J_{F\text{-}F}$ = 4.1 Hz 1F), 158.22 (t, $^2J_{F\text{-}F}$ = 20.5 1F). MS (*m/z*): 431.3 [M^+ +1]; $C_{19}H_{12}F_6N_2OS$.

(2*E*,5*E*)-2-(2,3,4-Trifluorophenylimino)-5-(3-(trifluoromethyl) benzylidene)-3-ethylthiazolidin-4-one (4f): Yellow solid; m.p.: 133-135 ºC. IR (ATR, cm^{-1}): 2949 (C-H), 1715 (C=O), 1638 (C=N), 1607 (C=C), 1510 (C-C) 1368 (C-NCH_2), 1333 (C-N), 1106 (C-F_3), 863 (O-substituted Ph ring), 683 (C-S-C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.35 (t, *J* = 7.14 Hz, 3H), 4.06 (q, *J* = 7.11 Hz, 2H), 6.71-6.79 (m, 1H), 6.92-7.02 (m, 1H), 7.54-7.61 (m, 3H), 7.67 (s, 1H), 7.80 (S, 1H). ^{19}F NMR ($CDCl_3$, 282 MHz): δ 62.1 (s, 3F), 138.8 (dd, $^2J_{F\text{-}F}$ = 20.5 &$^3J_{F\text{-}F}$ = 4.1 Hz 1F), 144.5 (dd, $^2J_{F\text{-}F}$ = 20.5 &$^3J_{F\text{-}F}$ = 4.1 Hz 1F), 158.22 (t, $^2J_{F\text{-}F}$ = 20.5 1F). MS (*m/z*): 431.3 [M^+ +1]; $C_{19}H_{12}F_6N_2OS$.

(2*E*,5*E*)-2-(2,3,4-Trifluorophenylimino)-5-(4-(trifluoromethyl) benzylidene)-3-ethylthiazolidin-4-one (4g): Yellow solid; m.p.: 111-113 ºC. IR (ATR, cm^{-1}): 2983 (C-H), 1715 (C=O), 1637 (C=N), 1609 (C=C), 1508 (C-C), 1373 (N-CH_2), 1314 (C-N), 1204 (C-F_3), 878 (*p*-substituted Ph ring), 828 (C-S-C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.36 (t, *J* = 7.14 Hz, 3H), 4.06 (q, *J* = 7.11 Hz, 2H), 6.84-6.93 (d t, 1H), 7.00-7.09 (d t, 1H), 7.53-7.56 (d, 8.4 Hz, 2H), 7.66-7.69 (d, *J* = 8.7 Hz 2H), 7.79 (S, 1H), ^{19}F NMR ($CDCl_3$, 282 MHz): δ 61.7 (s, 3F), 138.8 (dd, $^2J_{F\text{-}F}$ = 20.5$^3J_{F\text{-}F}$ = 4.1 Hz 1F), 144.5 (dd, $^2J_{F\text{-}F}$ = 20.5$^3J_{F\text{-}F}$ = 4.1 Hz 1F), 158.22 (t, $^2J_{F\text{-}F}$ = 20.5 1F). MS (*m/z*): 431.3 [M^+ +1]; $C_{19}H_{12}F_6N_2OS$.

(2*E*,5*E*)-2-(2,3,4-Trifluorophenylimino)-5-(4-(dimethylamino) benzylidene)-3-ethylthiazolidin-4-one (4h): Yellow solid; m.p.: 171-173 ºC. IR (ATR, cm^{-1}): 2943 (C-H), 1697

(C=O), 1586 (C=N), 1505 (C=C), 1359 (CNCH2), 1333 (N-CH_3), 1109 (C-F), 809 (*p*-substituted Ph ring), 711 (C-S-C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.35 (t, *J* = 7.08 Hz, 3H), 3.02 (s, 6H) 4.03 (q, *J* = 7.08 Hz, 2H), 6.67-6.70 (d, *J* = 8.85 Hz, 2H), 6.72-6.81 (m, 1H), 6.92-7.01 (m, 1H), 7.32-7.35 (t, *J* = 8.85 Hz, 1H), 7.71 (S, 1H). ^{19}F NMR ($CDCl_3$, 282 MHz): δ 138.8 (dd, $^2J_{F\text{-}F}$ = 20.5 &$^3J_{F\text{-}F}$ = 4.1 Hz 1F), 144.5 (dd, $^2J_{F\text{-}F}$ = 20.5$^3J_{F\text{-}F}$ = 4.1 Hz 1F), 158.22 (t, $^2J_{F\text{-}F}$ = 20.5 1F). MS (*m/z*): 406.5 [M$^+$ +1]; $C_{20}H_{18}F_3N_3OS$.

(2*E*,5*E*)-2-(2,3,4-Trifluorophenylimino))-5-(4-fluoro-3-phenoxybenzylidine 3-ethylthiazolidin-4-one (4i): Yellow solid; m.p.: 167-169 ºC. IR (ATR, cm^{-1}): 3056, (SP^{2}C-H) 2938 (C-H), 1708 (C=O), 1636 C=N), 1504 (C=C), 1363 ($CNCH_2$), 1203 (C-F), 905 (*m*-substituted Ph ring), 874 (C-S-C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.33 (t, *J* = 7.08 Hz, 3H), 4.02 (q, *J* = 7.08 Hz, 2H), 6.72-6.79 (m, 1H), 6.90-7.10 (m, 4H), 7.12-7.14 (d, *J* = 8.65 Hz, 1H), 7.15-7.19 (m, 1H), 7.19-7.26 (d, *J* = 8.7 Hz, 1H), 7.27-7.34 (m, 2H) 7.64 (S, 1H). ^{19}F NMR ($CDCl_3$, 282 MHz): δ 103.5, 138.8 (dd, $^2J_{F\text{-}F}$ = 20.5$^3J_{F\text{-}F}$ = 4.1 Hz 1F), 144.5 (dd, $^2J_{F\text{-}F}$ = 20.5$^3J_{F\text{-}F}$ = 4.1 Hz 1F), 158.22 (t, $^2J_{F\text{-}F}$ = 20.5 1F). MS (*m/z*): 473.1 [M$^+$+1]; $C_{24}H_{16}F_4N_2O_2S$.

(2*E*,5*E*)-5-(2,3-Dimethoxybenzylidene)-2-(2,3,4-trifluorophenylimino)-3-ethylthiazolidin-4-one (4j): Yellow solid; m.p.: 171-173 ºC. IR (ATR, cm^{-1}): 2950 (C-H), 1703 (C=O), 1628 (C=N), 1586 (C=C), 1464 (C-NCH_2), 1366 (C-N), 1333 (C-F), 1125 (C-O), 868 (*m*-disubstituted Ph-ring), 687 (C-S-C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.33 (t, *J* = 7.08 Hz, 3H), 3.83 (s, 3H), 3.85 (s, 3H) 4.01 (q, *J* = 7.08 Hz, 2H), 6.44-6.45 (d, *J* = 2.1 Hz, 1H), 6.50-6.54 (d, d *J* = 8.62.1 Hz, 2H), 6.67-6.77 (m, 1H), 6.92-7.01 (m, 1H), 7.29-7.32 (d, *J* = 8.6 Hz, 1H), 8.12 (S, 1H). ^{19}F NMR ($CDCl_3$, 282 MHz): δ 138.8 (dd, $^2J_{F\text{-}F}$ = 20.5 &$^3J_{F\text{-}F}$ = 4.1 Hz 1F), 144.5 (dd, $^2J_{F\text{-}F}$ = 20.5 & $^3J_{F\text{-}F}$ = 4.1 Hz 1F), 158.22 (t, $^2J_{F\text{-}F}$ = 20.5 1F). MS (*m/z*): 423.5 [M$^+$ +1]. $C_{20}H_{17}F_3N_2O_3S$.

(2*E*,5*E*)-5-(2,3,4-Trimethoxybenzylidene)-2-(2,3,4-trifluorophenylimino)-3-ethylthiazolidin-4-one (4k). Yellow solid; m.p.: 171-173 ºC. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.36 (t, *J* = 7.08 Hz, 3H), 3.86 (s, 3H), 3.88 (s, 3H), 3.92 (s, 3H), 4.05 (q, *J* = 7.08 Hz, 2H), 6.69-6.72 (d, *J* = 8.76 Hz, 1H), 6.75-6.78 (m, 1H), 6.91-7.01 (m, 1H), 7.08-7.11 (d, *J* = 8.76 Hz, 1H), 8.04 (S, 1H). ^{19}F NMR ($CDCl_3$, 282 MHz): δ 138.8 (dd, $^2J_{F\text{-}F}$ = 20.5 & $^3J_{F\text{-}F}$ = 4.1 Hz 1F), 144.5 (dd, $^2J_{F\text{-}F}$ = 20.5 & $^3J_{F\text{-}F}$ = 4.1 Hz 1F), 158.22 (t, $^2J_{F\text{-}F}$ = 20.5 1F). IR (ATR, cm^{-1}): 2942 (C-H), 1713 (C=O), 1614 (C=N), 1589 (C=C), 1445 (C-NCH_2), 1366 (C-N), 1128 (C-O), 1090 (C-F), 907(*m*-disubstituted Ph-ring), 782 (C-S-C). MS (*m/z*): 453.1 [M$^+$ +1]; $C_{21}H_{19}F_3N_2O_4S$.

(2*E*,5*E*)-5-(2,4,6-Trimethoxybenzylidene)-2-(2,3,4-trifluorophenylimino)-3-ethylthiazolidin-4-one (4l): Yellow solid; m.p.: 171-173 ºC. IR (ATR, cm^{-1}): 2942 (C-H), 1713 (C=O), 1614 (C=N), 1589 (C=C), 1445 (NCH_2), 1366 (C-N), 1128 (C-O), 1090 (C-F), 907 (*m*-disubstituted Ph-ring) 782 (C-S-C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.36 (t, *J* = 7.14 Hz, 3H), 3.84-388, (s, 9H), 4.04 (q, *J* = 7.14 Hz, 2H), 6.65 (s, 2H), 6.71-6.79 (m, 1H), 6.92-7.01 (m, 1H), 7.72 (s, 1H). ^{19}F NMR ($CDCl_3$, 282 MHz): δ 138.8 (dd, $^2J_{F\text{-}F}$ = 20.5 & $^3J_{F\text{-}F}$ = 4.1 Hz 1F), 144.5 (dd, $^2J_{F\text{-}F}$ = 20.5 & $^3J_{F\text{-}F}$ = 4.1 Hz 1F), 158.22 (t, $^2J_{F\text{-}F}$ = 20.5 1F). MS (*m/z*): 453.1 [M+ $^{+1}$]; $C_{21}H_{19}F_3N_2O_4S$.

(2*E*,5*E*)-2-(2,3,4-Trifluorophenylimino))-5-(4-hydroxy-3-methoxybenzylidine 3-ethylthiazolidin-4-one (4m): White solid; m.p.: 132-134 ºC. IR (ATR, cm^{-1}): 3350 (OH), 2944 (C-H), 1714 (C=O), 1598 (C=C), 1504 (C-O-C), 1363 (C-N), 1127 (C-O), 872 (disubstituted Ph ring), 730 (C-S-C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.34 (t, *J* = 7.14 Hz, 3H), 3.88 (s, 3H) 4.05 (q, *J* = 7.14 Hz, 2H), 4.08 (bs, 1H) 6.72-6.77 (m, 3H), 6.94-7.03 (m, 2H), 7.72 (S, 1H). ^{19}F NMR ($CDCl_3$, 282 MHz): δ 138.8 (dd, $^2J_{F\text{-}F}$ = 20.5 & $^3J_{F\text{-}F}$ = 4.1 Hz 1F), 144.5 (dd, $^2J_{F\text{-}F}$ = 20.5 & $^3J_{F\text{-}F}$ = 4.1 Hz 1F), 158.22 (t, $^2J_{F\text{-}F}$ = 20.5 1F). MS (*m/z*): 409.5 [M$^+$+1]; $C_{19}H_{15}F_3N_2O_3S$.

(2*E*,5*E*)-2-(2,3,4-Trifluorophenylimino)-3-ethyl-5-((furan-2-yl)methylene)thiazolidin-4-one (4n): Yellow solid; m.p.: 171-173 ºC. IR (KBr): 3100 (C=C-O), 2943 (C-H), 1714 (C=O), 1644 (C=C), 1512 (C-O-C), 1364 (C-N), 1332 (C-C-F), 1103 (C-O), 878 (disubstituted furan ring). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.35 (t, *J* = 7.14 Hz, 3H), 4.03 (q, *J* = 7.14 Hz, 2H), 6.54-6.55 (dd, 1H), 6.73-6.74 (d, 1H), 6.75-6.97 (m, 1H), 7.02-7.11 (m, 1H), 7.56 (s, 1H), 7.61-7.62 (d, 1H). ^{19}F NMR ($CDCl_3$, 282 MHz): δ 138.8 (dd, $^2J_{F\text{-}F}$ = 20.5 & $^3J_{F\text{-}F}$ = 4.1 Hz 1F), 144.5 (dd, $^2J_{F\text{-}F}$ = 20.5 & $^3J_{F\text{-}F}$ = 4.1 Hz 1F), 158.22 (t, $^2J_{F\text{-}F}$ = 20.5 1F). IR (ATR, cm^{-1}): 353.4 [M$^+$ +1]; $C_{16}H_{11}F_3N_2O_2S$.

(2*E*,5*E*)-5-((1*H*-Pyrrol-2-yl)methylene)-2-(2,3,4-trifluorophenylimino)-3-ethyl thiazolidin-4-one (4o): Yellow solid; m.p.: 215-217 ºC. IR (ATR, cm^{-1}): 3742 (C-C=N), 2978 (C-H), 1705 (C=O), 1633 (C=C), 1598 (C-O-C), 1503 (C-N), 1377 (C-C-F), 1200 (C-O), 962 (disubstituted phenyl ring). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.35 (t, *J* = 7.14 Hz, 3H), 4.03 (q, *J* = 7.11 Hz, 2H), 6.38-6.39 (m, 1H), 6.54 (bs, 1H), 6.76-6.81 (m, 1H), 6.92-7.02 (m, 1H), 7.69 (s, 1H), 8.89 (bs, 1H, exchangeable with D_2O). ^{19}F NMR ($CDCl_3$, 282 MHz): δ 138.8 (dd, $^2J_{F\text{-}F}$ = 20.5 &$^3J_{F\text{-}F}$ = 4.1 Hz 1F), 144.5 (dd, $^2J_{F\text{-}F}$ = 20.5 & $^3J_{F\text{-}F}$ = 4.1 Hz 1F), 158.22 (t, $^2J_{F\text{-}F}$ = 20.5 1F). MS (*m/z*): 352.5 [M$^+$ +1]; $C_{16}H_{12}F_3N_3OS$.

(2*E*,5*E*)-2-(2,3,4-Trifluorophenylimino)-5-(4-methoxybenzylidene)-3-ethylthiazolidin-4-one (4p): White solid; m.p.: 132-134 ºC. IR (ATR, cm^{-1}): 2954 (C-H), 1700 (C=O), 1628 (C=N), 1592 (C=C), 1508 (C-C), 1363 (N-CH_2), 1253 (C-O), 1026, 818 (*p*-substituted Ph ring), 691 (C-S-C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.36 (t, *J* = 7.11 Hz, 3H), 3.83 (s, 3H) 4.05 (q, *J* = 7.11 Hz, 2H), 6.71-6.80 (m, 1H), 6.91-6.94 (d, 8.7 Hz 2H), 6.95-7.02 (m, 1H), 7.38-7.42 (d, *J* = 8.7 Hz 2H), 7.75 (S, 1H). ^{19}F NMR ($CDCl_3$, 282 MHz): δ 138.8 (dd, $^2J_{F\text{-}F}$ = 20.5 & $^3J_{F\text{-}F}$ = 4.1 Hz 1F), 144.5 (dd, $^2J_{F\text{-}F}$ = 20.5 & $^3J_{F\text{-}F}$ = 4.1 Hz 1F), 158.22 (t, $^2J_{F\text{-}F}$ = 20.5 1F). MS (*m/z*): 393.3 [M$^+$+1]. $C_{19}H_{15}F_3N_2O_2S$.

(2*E*,5*E*)-2-(2,3,4-Trifluorophenylimino)-3-ethyl-5-((thiophene-2-yl)methylene)thiazolidin-4-one (4q): White solid; m.p.: 133-135 ºC. IR (ATR, cm^{-1}): 2954 (C-H), 1700 (C=O), 1628 (C=N), 1592 (C=C), 1508 (C-C), 1363 (N-CH_2), 1253 (C-O), 1026, 818 (*p*-substituted Ph ring), 691 (C-S-C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.36 (t, *J* = 7.14 Hz, 3H), 4.05 (q, *J* = 7.11 Hz, 2H), 6.74-6.79 (m, 1H), 6.93-6.96 (d, 8.7 Hz 2H), 6.94-7.03 (m, 1H), 7.14-7.15 (d, *J* = 3.84 Hz, 1H), 7.32-7.33 (d, 3.5 Hz, 1H), 7.54-7.56 (d, *J* = 4.98 Hz, 1H), 7.94 (S, 1H). ^{19}F NMR ($CDCl_3$, 282 MHz): δ 138.8 (dd, $^2J_{F\text{-}F}$ = 20.5 & $^3J_{F\text{-}F}$ = 4.1 Hz 1F), 144.5 (dd, $^2J_{F\text{-}F}$ = 20.5 & $^3J_{F\text{-}F}$ = 4.1 Hz 1F), -158.22 (t, $^2J_{F\text{-}F}$ = 20.5 1F). MS (*m/z*): 393.3 [M$^+$+1]. $C_{19}H_{15}F_3N_2O_2S$.

(2*E*,5*E*)-2-(2,3,4-Trifluorophenylimino)-5-(4-cyanobenzylidene)-3-ethylthiazolidin-4-one (4r): White solid; m.p.: 141-143 ºC. IR (ATR, cm^{-1}): 2954 (C-H), 1700 (C=O), 1628 (C=N), 1592 (C=C), 1508 (C-C), 1363 (N-CH_2), 1253 (C-O), 1026, 818 (*p*-substituted Ph ring), 691 (C-S-C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.37 (t, *J* = 7.11 Hz, 3H), 4.06 (q, *J* = 7.11 Hz, 2H), 6.76-6.81 (m, 1H), 6.95-6.98 (d, 8.7 Hz 2H), 6.97-7.11 (m, 1H), 7.46-7.49 (d, *J* = 8.7 Hz, 2H), 7.78 (S, 1H). ^{19}F NMR ($CDCl_3$, 282 MHz): δ 138.8 (dd, $^2J_{F\text{-}F}$ = 20.5 & $^3J_{F\text{-}F}$ = 4.1 Hz, 1F), 144.5 (dd, $^2J_{F\text{-}F}$ = 20.5 &$^3J_{F\text{-}F}$ = 4.1 Hz, 1F), 158.22 (t, $^2J_{F\text{-}F}$ = 20.5 Hz, 1F). MS (*m/z*): 393.3 [M^+ +1]. $C_{19}H_{15}F_3N_2O_2S$.

Antibacterial activity: To investigate the impact of the synthesized iminothiazolidinone moieties on the bacterial growth, a broth dilution assay was used to test the antibacterial activity of the synthesized compounds (**4a-r**). The Gram-negative bacteria, *Escherichia coli* and *Serratia marcescens* were used for the experiment. Both the organisms were challenged with different concentrations of compounds ranging from 50 ng/mL to 20 µg/mL. Nutrient broth (peptic digest of animal tissue 20 g/L, potassium sulfate 10 g/L, magnesium chloride 1.4 g/L, pH 7.0 ± 0.2) was used as a growth medium. The culture of both organisms was used to prepare inoculum, which was standardized to 0.5 McFarland turbidity standard [30]. The amount of compound and/or DMSO was kept constant at 0.2% v/v for all the experiments. Appropriate controls such as vehicle control (containing DMSO), abiotic control (containing compound and growth medium, but no inoculum), positive control (DMSO and culture) and negative control (only growth medium) were included in the experiment. Treated and untreated cultures were incubated at 37 ºC for 24 h followed by measuring their optical density (OD) at 600 nm on Shimadzu 1900i spectrophotometer. The growth profile of the cultures was compared with the positive control. The synthesized iminothiazolidinone derivatives (**4a-r**) were tested for its potential to inhibit the growth of different bacterial and fungal species at doses of 100 µg/mL in DMSO as a solvent, against bacterial and fungal cultures.

RESULTS AND DISCUSSION

The designed synthetic route starts with a reaction of 2,3,4-trifluorophenylamine (**1**) which was stirred in absolute ethanol and further reacted with ethyl isothiocyanate. The mixture was refluxed at 90 ºC to obtain 1-ethyl-3-(2,3,4-trifluorophenyl)-thiourea (**2**). The synthesized intermediate **2** was further refluxed with ethyl bromoacetate in ethanol using triethylamine as a base to obtain 3-ethyl-2-(2,3,4-trifluorophenylimino)thiazolidin-4-one (**3**) as a brownish solid compound showing yield of 81%. Knoevenagel reaction was performed on molecule **3** by condensation with various aldehydes using both conventional reflux, as well as non-conventional sonication methods. The various derivatives (**4a-r**) synthesized have been shown in Fig. 1. Knoevenagel products (**4a-r**) synthesized using different aldehydes *via* conventional method showed yields between 62-78% as compared to non-conventional method which showed yields between 82-95% as shown in Table-1.

3-Ethyl-2-(2,3,4-trifluorophenylimino)thiazolidin-4-one (**3**) was characterized by strong absorption bands at 1651 cm^{-1} (C=O) and at 1734 cm^{-1} (C=N), which confirms the presence of C=O and C=N functional groups, respectively. The ^{1}H NMR spectrum of compound **3** showed a triplet at δ 1.30 ppm with a coupling constant of δ 7.08 Hz for three protons, which were attributed to the methyl protons of N-CH_2-$\underline{CH_3}$. Another triplet resonated at δ 3.91 ppm with a coupling constant of 7.08 Hz due to the integration of two protons methylene groups attached to the N-atom N-$\underline{CH_2}$-CH_3. The formation of iminothiazolidinone ring structure was confirmed due to the observance of a singlet at δ 3.86 ppm integrating two protons of the methylene group attached to the S-atom of iminothiazolidin-4-one ring. Two aromatic protons of the trifluorinated benzene ring exhibited multiplets between δ 6.73-6.74 ppm and δ 6.90-6.98 ppm integrating for one proton, respectively. These multiplets are due to H{F} coupling constants are 6.0, 8.010 Hz respectively. ^{19}F NMR spectrum shows three peaks integrating for 1 fluorine each for the three fluorine atoms of the aromatic ring at 139.37 ppm (dd, 1F, $^2J_{F\text{-}F}$ = 20 Hz, $^3J_{F\text{-}F}$ = 4.1 Hz), 144.8 ppm (dd, 1F, $^2J_{F\text{-}F}$ = 20 Hz, $^3J_{F\text{-}F}$ = 4.1 Hz) and 158.5 ppm (t, 1F, $^2J_{F\text{-}F}$ = 20.4 Hz). ^{13}C NMR spectrum shows showed a peak at 12.3 ppm for the methyl carbon of N-CH_2-$\underline{CH_3}$. Another peak resonated at 38.5 ppm due to the carbon of the methylene group attached to the N-atom N-$\underline{CH_2}$-CH_3. The formation of the iminothiazolidinone ring structure was confirmed due to the presence of a peak at δ 33.0 ppm of the carbon of the methylene group, which is attached to the S-atom of iminothiazolidin-4-one ring. Carbons of 2,3,4-trifluorinated benzene ring exhibited multiplets at δ 111.5 ppm (dd, $^2J_{C\text{-}F}$ = 20 Hz, $^3J_{C\text{-}F}$ = 4.4 Hz), while the signal δ 116.0 ppm (dd, $^3J_{C\text{-}F}$ = 30 Hz), was assigned to the protonated carbon of benzene ring. Three sets of multiplets with $^1J_{C\text{-}F}$ = 255 Hz, $^2J_{C\text{-}F}$ = 20 Hz and $^3J_{C\text{-}F}$ = 8 Hz were observed for the three carbon atoms attached to fluorine between 130.0 to 150.0 ppm. The peaks at 96.0 and 152.0 can be attributed to the C=N carbon of the iminothiazolidinone ring, while the peak at 166.8 ppm is due to the carbonyl carbon of the iminothiazolidinone ring. Thus, with the help of ^{1}H, ^{19}F and ^{13}C NMR spectra, the formation of desired iminothiazolidinone pharmacophore is confirmed. The mass spectrum showed a peak at *m/z* = 274.53 (M^++1) was accordance with the molecular formula $C_{11}H_9F_3N_2OS$. All the spectral values and analysis data confirmed the core structure of the key intermediate.

The stereochemistry of compound **4j** was confirmed by running a 2D NOESY NMR experiment. The compound shows no interaction of N-CH_2-CH_3 protons with benzylidine proton. Benzylidine proton at δ 8.13 ppm shows corelation with the 3,4-dimethoxybenzene ring of the benzylidine moiety, which means that benzylidine bond formation at thiazolidinone rings is *trans*- to the S-atom of thiazolidinone ring. It was also observed that there is no interaction of N-CH_2-CH_3 protons of ethyl group with protons of the trifluorophenyl ring as well as the protons of the benzylidine group, which confirms that the stereochemistry about C=N and C=C groups is E, E.

The reaction yield of the Knoevenagel reaction formed using ultrasonic condition is better than the conventional Knoevenagel reaction (Table-1). All the compounds formed in 82-95% yields, which are better than the conventional method that uses excess ethanol as solvent and requires heating. During

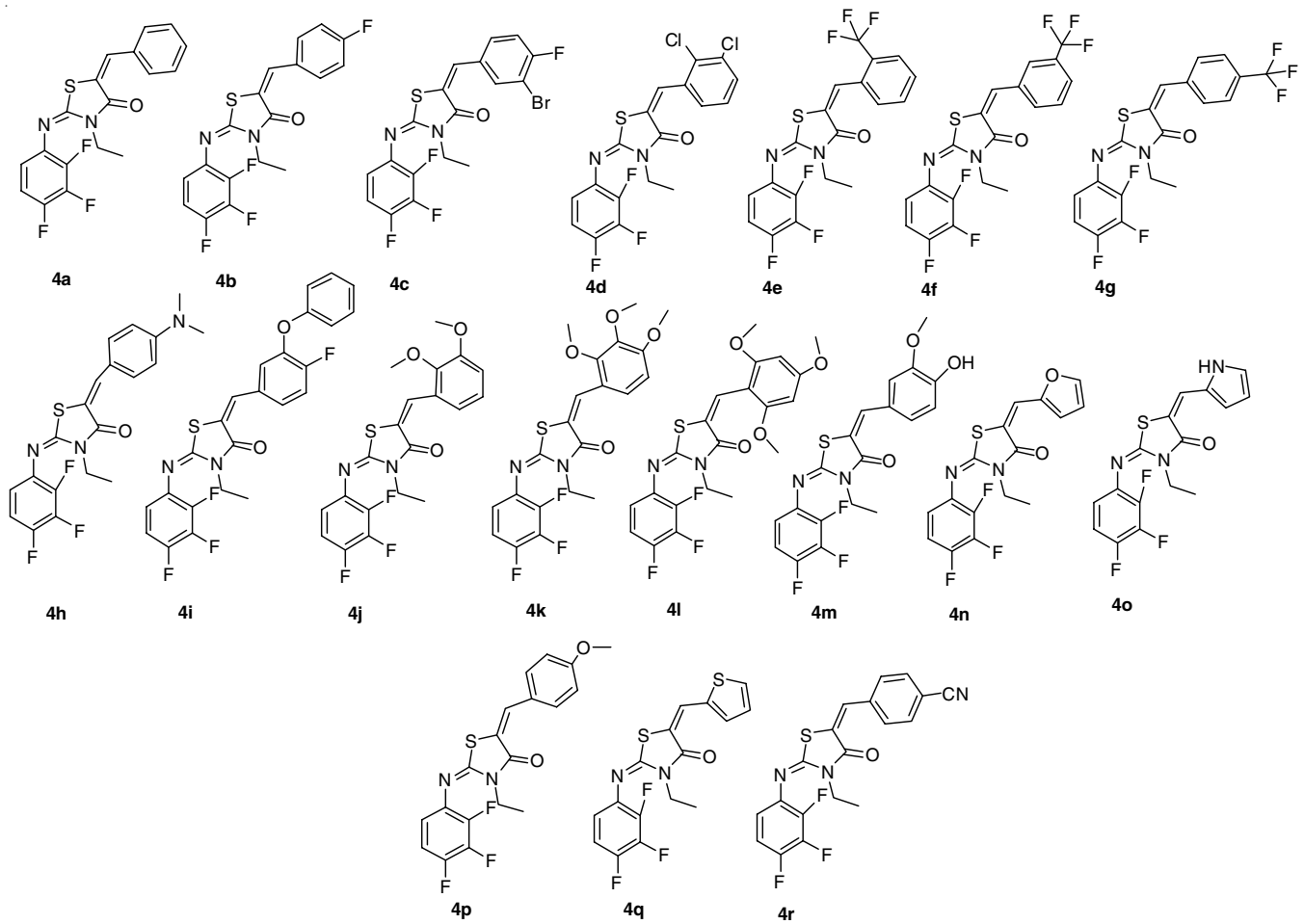


Fig. 1. Synthesized novel thiazolidin-4-one derivatives (**4a-r**)

heating, there is the formation of impurities, which is another disadvantage of the process.

Antibacterial activity: Synthesized compounds (**4a-r**) were tested to evaluate their *in vitro* antibacterial potential and found to exhibit excellent antibacterial against *Escherichia coli* and *Serratia marcescens*. Compounds **4m**, **4n**, **4o**, **4q** and **4r** showed the excellent activity among which compound **4r** with -CN functional group at the *para*-position of benzaldehyde showed the best activity among all the tested compounds against *Escherichia coli*. Whereas, compounds **4m**, **4n**, **4o**, **4q** and **4r** showed excellent activity out of which compound **4n** showed the best activity against *Serratia marcescens* (Table-2).

Conclusion

In this work, novel fluorinated 5-benzylidine-3-ethyl-2-(2,3,4-trifluorophenylimino)thiazolidin-4-one derivatives (**4a-r**) were synthesized and characterized using conventional as well as ultrasonic process using Knoevenagel method. The present work aimed at decreasing reaction time and increasing the yield, without the use of heat and only employing sonication.

TABLE-2
IC_{50} VALUES OF 5-BENZYLIDINE 3-ETHYL-2-(2,3,4-TRIFLUORO-PHENYLIMINO)-THIAZOLIDIN-4-ONE DERIVATIVES (**4a-r**) BASED ON ANTIMICROBIAL ACTIVITY STUDY

Compd.	*Escherichia coli* NCIM No. 2602	*Serratia marcescens* NCIM No. 2919	Compd.	*Escherichia coli* NCIM No. 2602	*Serratia marcescens* NCIM No. 2919
4a	5.2	6.0	**4k**	10.1	8.7
4b	8.9	7.7	**4l**	6.9	6.1
4c	5.3	5.8	**4m**	3.2	3.1
4d	7.9	7.8	**4n**	3.1	2.4
4e	10.1	8.2	**4o**	2.8	2.5
4f	11.9	10.6	**4p**	7.1	6.2
4g	10.8	10.2	**4q**	3.2	2.4
4h	7.2	7.2	**4r**	2.5	2.5
4i	7.4	7.8	Standard (Ampicillin)	1.3	2.2
4j	5.6	5.1			

The new method required reaction times ranging from 20-40 min, as well as giving excellent yields ranging from 80-93%. The method also requires a lesser amount of solvent. Thus, the decrease in time energy and solvent for reaction, as well as the increase in reaction yield makes this modified method a better option than the tedious conventional Knoevenagel method.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. F. Mohajer, G.M. Ziarani and A. Badiei, *J. Appl. Organomet. Chem.*, **1**, 143 (2021); https://doi.org/10.22034/jaoc.2021.301405.1032
2. B. Khameneh, M. Iranshahy, V. Soheili and B.S.F. Bazzaz, *Antimicrob. Resist. Infect. Control*, **8**, 118 (2019); https://doi.org/10.1186/s13756-019-0559-6
3. P. Shah and A.D. Westwell, *J. Enzyme Inhib. Med. Chem.*, **22**, 527 (2007); https://doi.org/10.1080/14756360701425014
4. M. Inoue, Y. Sumii and N. Shibata, *ACS Omega*, **5**, 10633 (2020); https://doi.org/10.1021/acsomega.0c00830
5. A. Graul and J. Castañer, *Drugs Future*, **22**, 956 (1997); https://doi.org/10.1358/dof.1997.022.09.423212
6. C. Roger, J.A. Roberts and L. Muller, *Clin. Pharmacokinet.*, **57**, 559 (2018); https://doi.org/10.1007/s40262-017-0601-x
7. N. Kaushik, N. Kumar, A. Kumar and U.K. Singh, *Immunol. Endocr. Metab. Agents Med. Chem.*, **18**, 3 (2018); https://doi.org/10.2174/1871522218666180525100850
8. R. Ottanà, R. Maccari, M.L. Barreca, G. Bruno, A. Rotondo, A. Rossi, G. Chiricosta, R. Di Paola, L. Sautebin, S. Cuzzocrea and M.G. Vigorita, *Bioorg. Med. Chem.*, **13**, 4243 (2005); https://doi.org/10.1016/j.bmc.2005.04.058
9. A. Dandia, R. Singh, S. Khaturia, C. Mérienne, G. Morgant and A. Loupy, *Bioorg. Med. Chem.*, **14**, 2409 (2006); https://doi.org/10.1016/j.bmc.2005.11.025
10. W. Cunico, C.R.B. Gomes and W.T. Vellasco Jr., *Mini Rev. Org. Chem.*, **5**, 336 (2008); https://doi.org/10.2174/157019308786242232
11. A.C. Tripathi, S.J. Gupta, G.N. Fatima, P.K. Sonar, A. Verma and S.K. Saraf, *Eur. J. Med. Chem.*, **72**, 52 (2014); https://doi.org/10.1016/j.ejmech.2013.11.017
12. R. Kumar and S. Patil, *Med (N. Y.)*, **9**, 3590 (2017); https://doi.org/10.15254/H.J.D.Med.9.2017.166
13. O.M.A. Elhafez, E.E.D.A.M.E. Khrisy, F. Badria and A.E.D.M. Fathy, *Arch. Pharm. Res.*, **26**, 686 (2003); https://doi.org/10.1007/BF02976675
14. A.K. Jain, A. Vaidya, V. Ravichandran, S.K. Kashaw and R.K. Agrawal, *Bioorg. Med. Chem.*, 20, 3378 (2012); https://doi.org/10.1016/j.bmc.2012.03.069
15. M.V. Diurno, A.A. Izzo, O. Mazzoni, A. Bolognese and F. Capasso, *J. Pharm. Pharmacol.*, **48**, 760 (2011); https://doi.org/10.1111/j.2042-7158.1996.tb03966.x
16. A.A. Chavan and N.R. Pai, *ARKIVOC*, **2007**, 148 (2007); https://doi.org/10.3998/ark.5550190.0008.g16
17. A.A.O. Abeed, M.S.K. Youssef and R. Hegazy, *J. Braz. Chem. Soc.*, **28**, 2054 (2017); https://doi.org/10.21577/0103-5053.20170050
18. S.A. Khan and M. Yusuf, *Eur. J. Med. Chem.*, **44**, 2597 (2009); https://doi.org/10.1016/j.ejmech.2008.09.004
19. M.V. Diurno, O. Mazzoni, E. Piscopo, A. Calignano, F. Giordano and A. Bolognese, *J. Med. Chem.*, **35**, 2910 (1992); https://doi.org/10.1021/jm00093a025
20. S.N. Gavade, V.L. Markad, K.M. Kodam, M.S. Shingare and D.V. Mane, *Bioorg. Med. Chem. Lett.*, **22**, 5075 (2012); https://doi.org/10.1016/j.bmcl.2012.05.111
21. A. Türe, M. Ergül, M. Ergül, A. Altun and I. Küçükgüzel, *Mol. Divers.*, **25**, 1025 (2020); https://doi.org/10.1007/s11030-020-10087-1
22. K.A. Szychowski, M.L. Leja, D.V. Kaminskyy, U.E. Binduga, O.R. Pinyazhko, R.B. Lesyk and J. Gmiński, *Chem. Biol. Interact.*, **262**, 46 (2017); https://doi.org/10.1016/j.cbi.2016.12.008
23. S. Senkardes and S. Kucukguzel, *Mini Rev. Org. Chem.*, **13**, 377 (2016); https://doi.org/10.2174/1570193X13666160826154159
24. R.K. Rawal, Y.S. Prabhakar, S.B. Katti and E. De Clercq, *Bioorg. Med. Chem.*, **13**, 6771 (2005); https://doi.org/10.1016/j.bmc.2005.07.063
25. T. Kato, T. Ozaki, K. Tamura, Y. Suzuki, M. Akima and N. Ohi, *J. Med. Chem.*, **42**, 3134 (1999); https://doi.org/10.1021/jm9900927
26. T. Kato, T. Ozaki and N. Ohi, *Tetrahedron Asymm.*, **10**, 3963 (1999); https://doi.org/10.1016/S0957-4166(99)00441-3
27. Y. Adachi, Y. Suzuki, N. Homma, M. Fukazawa, K. Tamura, I. Nishie and O. Kuromaru, *Eur. J. Pharmacol.*, **367**, 267 (1999); https://doi.org/10.1016/S0014-2999(98)00938-8
28. R. Ottaná, E. Mazzon, L. Dugo, F. Monforte, R. Maccari, L. Sautebin, G. De Luca, M.G. Vigorita, S. Alcaro, F. Ortuso, A.P. Caputi and S. Cuzzocrea, *Eur. J. Pharmacol.*, **448**, 71 (2002); https://doi.org/10.1016/S0014-2999(02)01888-5
29. Y. Tanabe, H. Yamamoto, M. Murakami, K. Yanagi, H. Okumura, Y. Kubota, Y. Sanemitsu and G. Suzukamo, *J. Chem. Soc., Perkin Trans. 1*, **1**, 935 (1995); https://doi.org/10.1039/p19950000935
30. S.J. Brickner, D.K. Hutchinson, M.R. Barbachyn, P.R. Manninen, D.A. Ulanowicz, S.A. Garmon, K.C. Grega, S.K. Hendges, D.S. Toops, C.W. Ford and G.E. Zurenko, *J. Med. Chem.*, **39**, 673 (1996); https://doi.org/10.1021/jm9509556