Indian Journal of Chemistry
Vol. 63, November 2024, pp. 1102-1111
DOI: 10.56042/ijc.v63i11.11976

Synthesis, characterization and evaluation of *in vitro* anticancer potential of novel fluorinated 5-benzylidene-3-ethyl-2-(2-methyl-3-trifluoromethyl-phenylimino)-thiazolidin-4-one derivatives: Comparison of reflux and ultrasonic conditions for Knoevenagel reaction

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Received 22 June 2024; accepted (revised) 22 October 2024

A set of novel 5-benzylidene-3-ethyl-2-(2-methyl-3-trifluoromethyl-phenylimino)-thiazolidin-4-one derivatives have been synthesized by Knoevenagel reaction *via* both conventional as well as non-conventional methods on the synthesized iminothiazolidinone core. In terms of yield and reaction time, the ultrasound mediated Knoevenagel reaction method has proved to be more effective than the conventional approach using heat. The ^{1}H NMR spectra have been used to deduce the structure of the compounds, while LC-MS, FTIR, and elemental analysis data have also been utilized for better confirmation. Using a 2D NOESY NMR experiment, the stereochemistry of the final compound has been verified. The synthesized benzylidine compounds have been screened for *in vitro* anticancer potential against Human Hepatoma (Hep-G2) cell line. The compound having *p*-substituted methoxy group on benzylidine moiety is observed to be the most active against the tested cell line as compared to the rest of the compounds.

Keywords: Fluorine, Thiazolidin-4-one, Knoevenagel reaction, Ultrasonic waves, Anticancer activity, Hep-G2 cell line

Since cancer is the main cause of mortality for those under the age of 70 worldwide, it is an important issue that requires the development of new and more potent medications for the treatment of cancer[1,2]. The poor therapeutic index, development of resistance, and limited bioavailability of traditional anticancer chemotherapeutics have forced medicinal chemistry research to investigate other approaches[3,4]. In this regard, small novel molecules are prepared in the present work that could possess the ability to act as an anti-cancer agent.

Certain 5-membered ring heterocycles[5–12] have demonstrated diverse pharmacological activities due to their ability to interact with biological targets. Among 5-membered ring heterocycles, compounds like thiazoles, oxazoles, thiophenes, and furans have received attention in cancer research due to their ability to interfere with various cellular processes involved in cancer development and progression. Researchers continue to explore the potential of various 5-membered ring heterocycles and their derivatives as anticancer agents through synthetic chemistry, computational modelling, and biological testing. These efforts aim to discover novel compounds with improved efficacy, reduced toxicity, and enhanced selectivity for targeting cancer cells. Thiazolidin-4-ones, known as oxo derivatives of thiazolidine, are a significant class of heterocyclic compounds that have a carbonyl group in the fourth position of a five-membered ring together with sulfur and nitrogen[13,14]. These derivatives have gained significant attention in medicinal chemistry due to their diverse biological activities[15] such as anticancer[16–19], cardioprotective[20], anti-ischemic[21], antidiabetic[22,23], cycloxygenase inhibitory[24], anti-platelet activating factor[25], antidiarrheal[26], anticonvulsant[27], antimicrobial[28], 5-antihistaminics[29], anti-HIV[30], Ca^{2+} channel blocker[31], PAF antagonist[32], non-peptide thrombin receptor antagonist[33] and tumour necrosis factor-α antagonist activities[34]. Their involvement in the design and development of new anticancer drugs has been thoroughly investigated. Derivatives of thiazolidinone[35,36] have demonstrated potential in preventing the development of certain

cancer cell lines, such as from the prostate, breast, and lung. There are some of the readily available drugs in the market containing thiazolidinone ring, depicted in Fig. 1 such as Rosiglitazone[37]which is agonist for PPARγ, Lobeglitazone[38] that is agonist for both PPARα and PPARγ, Epalrestat[39] is noncompetitive and reversible Aldose reductase inhibitor, Ponesimod[40] is agonist S1P1 receptor modulator, *etc.*

After a comprehensive analysis of the literature, we were able to determine that the thiazolidinone heterocycle moieties may function as effective anti-cancer drugs[41] by aiding in the treatment of abnormal cell growth[17,18,42–48]. After more investigation, it has been discovered that these heterocycles performed better when tested against the HepG2 cell line[49–51]. Hence in the present work, we have synthesized and characterized a series of novel 5-Benzylidene-3-ethyl-2-(2-methyl-3-trifluoromethyl-phenylimino)-thiazolidin-4-one (4a-n) derivatives using different aldehydes and evaluated their *in vitro* anti-cancer study against HepG2 cell line. The structures of all the 14 novel compounds synthesized have been shown in Fig. 2.

Experimental Section

For the synthesis process, 3-Ethyl-2-(2-methyl-3-trifluoromethyl-phenylimino)-thiazolidin-4-one, ethyl bromoacetate, and aldehydes were procured from Sigma Aldrich Pvt. Ltd., while ethyl isothiocyanate and triethylamine were sourced from Spectrochem Pvt. Ltd. Chemicals such as ethyl acetate, hexane, DMSO, and sodium sulfate were acquired from Thermo Fisher Scientific India Pvt. Ltd, and ethyl alcohol was supplied by Changshu Hongcheng Fine Chemical Co. Ltd., Jiangsu Province, China. Precoated silica gel 60F254 TLC plates were purchased from Merck India. The purification of all final products was accomplished through column

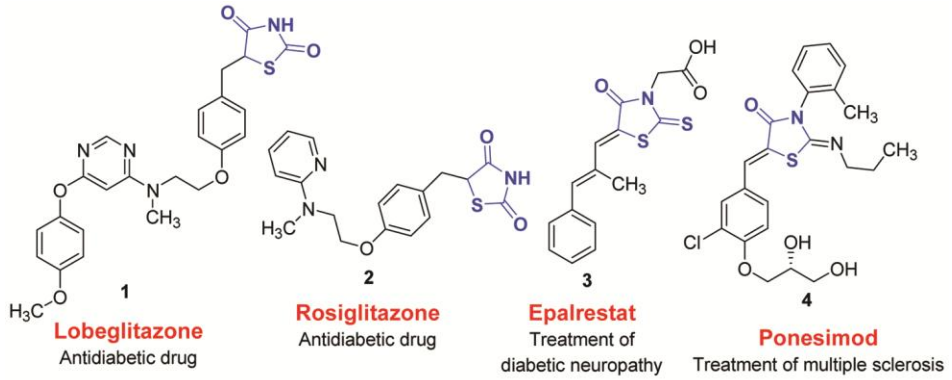


Fig. 1 — Commercially available drugs containing thiazolidinone ring

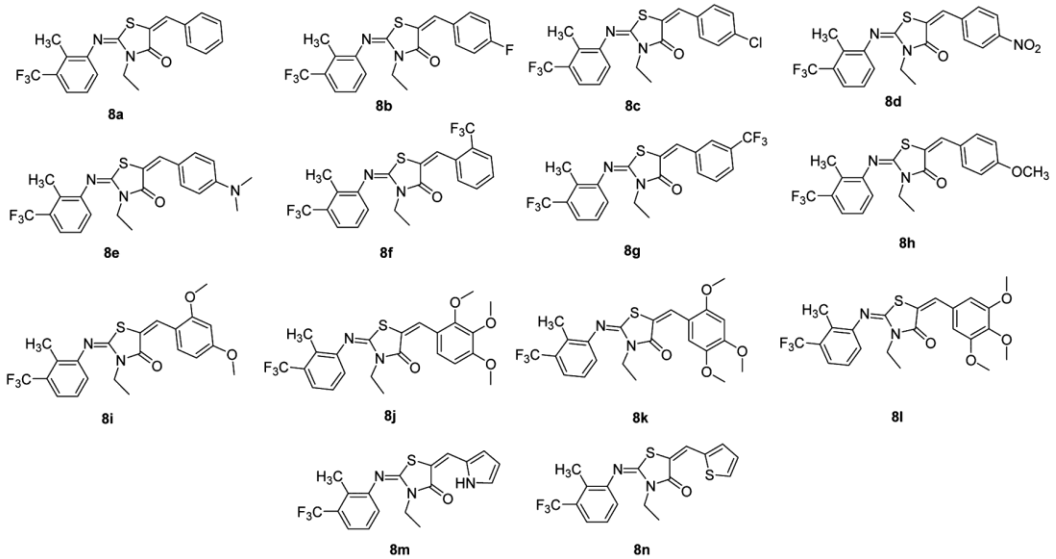


Fig. 2 — Structures of synthesized thiazolidin-4-one derivatives **8(a-n)**

chromatography using 60–120 mesh size silica gel from Thermo Fisher Scientific India Pvt. Ltd.

Characterization of the newly synthesized compounds was conducted through FT-IR, 1H NMR, 2D NOESY, mass spectrometry and elemental analysis. IR spectra were obtained using an Agilent Cary 630 FTIR Spectrophotometer with ATR. Proton and 2D NOESY NMR spectra were recorded on a Bruker Avance III HD 300 MHz NMR Ultra Shield Spectrometer using $CDCl_3$ as solvent. Chemical shift values were expressed in parts per million (ppm) and coupling constant values (*J*) were given in Hz. The stereochemistry of the iminothiazolidinone ring was confirmed through a 2D NOESY experiment using the "noesyphsw" pulse program with a 1.2s NOESY mixing time and 32 scan repetitions. LC-MS spectra were recorded using a Waters Acquity QSM instrument. CHNS elemental analysis was performed using Elementar Vario Micro Cube, with sulfanilamide as the reference standard [Found (%): C, 41.85; H, 4.680; N, 16.26; S, 18.62]. The images of sample tested during anticancer studies (highest drug concentration used) were taken using Ti-S Inverted Research Microscope- Nikon with a magnification of ×20, with Eclipse Image processing software NIS-Elements. The progression of reactions was monitored by thin-layer chromatography (TLC). Chemical names and structures were generated using ChemDraw Ultra 7.0 software.

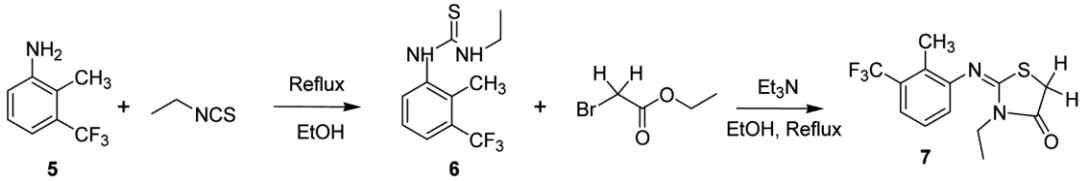
The synthetic route starts with a reaction of 2-methyl-3-trifluoromethyl-phenylamine **(5)** which was stirred in absolute ethanol and further reacted with ethyl isothiocyanate. To get 1-Ethyl-3-(2-methyl-3-trifluoromethyl-phenyl)-thiourea **(6)**, the mixture was refluxed at 90°C. Using triethylamine as a base, the produced intermediate 2 was further refluxed with ethyl bromoacetate in absolute ethanol to generate 3-Ethyl-2-(2-methyl-3-trifluoromethyl-phenylimino)-thiazolidin-4-one **(7)**, a yellow solid chemical with an 82% yield.

1-Ethyl-3-(2-methyl-3-trifluoromethyl-phenyl)-thiourea, 6: Ethylisothiocyanate (4.05 g, 0.046 mol) was added to a solution of 2-methyl-3-trifluoromethyl-phenylamine **(5)** (6 g, 0.041 mol) in absolute ethanol. The reaction mixture was heated at 90–95 ºC for 6 hours. Ethyl acetate (50 mL) and 0.1 N HCl (100 mL) were added to the residue and stirred for five minutes. The organic layer was separated and washed with distilled water (2 × 50 mL). The organic layer was dried over sodium sulphate and evaporated to obtain the desired product 2 as an off-white solid in 92% yield.

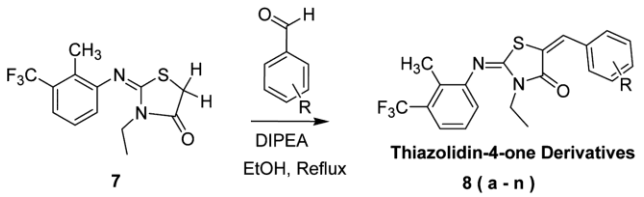
3-Ethyl-2-(2-methyl-3-trifluoromethyl-phenylimino)-thiazolidin-4-one, 7: 1-Ethyl-3-(2-methyl-3-trifluoromethyl-phenyl)-thiourea **(6)** (0.25 g, 0.0010 mol), ethyl bromoacetate (0.225 g, 0.0013 mol), and triethylamine (0.21 g, 0.0015 mol) were added to absolute ethanol, and the reaction mixture was heated for 5 hours at 80–90 ºC. Completion of reaction was monitored using a 4:1 hexane: ethylacetate (v/v) system on TLC. Ethanol was removed under lower pressure once the reaction mixture had cooled to room temperature. After that, 30 mL of distilled water was added to the residue, agitated for 15 minutes, and extracted using ethyl acetate (3 × 20 mL). A sticky red solid was obtained by evaporating the separated organic layer at a lower pressure. An 82% yield of yellow solid was obtained by recrystallizing this product using absolute ethanol. This step completed Scheme 1.

Synthesis of 5-benzylidene-3-ethyl-2-(2-methyl-3-trifluoromethyl-phenylimino)-thiazolidin-4-one 8a-n

Conventional approach: A mixture of 3-Ethyl-2-(2-methyl-3-trifluoromethyl-phenylimino)-thiazolidin-4-one **(7)** (0.25 g, 0.91 mmol), aldehyde (0.11 g, 1.09 mmol) and diisopropylethylamine (0.158 g, 1.8 mmol) in absolute ethanol was refluxed at 90-95 ºC for 8-9 hrs. Following TLC monitoring, ethanol was removed, cold water was added to the residue, and ethyl acetate (3 × 20 mL) was used to extract the product. The pooled organic layers were subjected to a brine wash, dried on anhydrous sodium sulfate, filtered, and concentrated at a lower pressure (Scheme 2). The chemical was obtained in good



Scheme 1 — Synthesis of 3-Ethyl-2-(2-methyl-3-trifluoromethyl-phenylimino)-thiazolidin-4-one



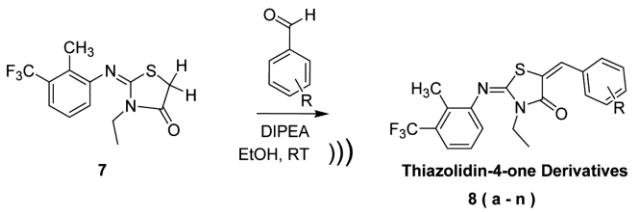
Scheme 2 — Synthesis of thiazolidin-4-one derivatives *via* conventional method 8 (a-n)

Table 1 — Calculated yields and reaction time for the synthesized compounds

Sr. No.	Compd	Non-conventional method (using ultrasound)		Conventional method (using heat)	
		Yield (%)	Time(min)	Yield (%)	Time(hrs)
1	**8a**	85	30	70	8
2	**8b**	87	30	72	9
3	**8c**	90	30	73	9
4	**8d**	89	30	75	6
5	**8e**	88	40	76	10
6	**8f**	83	40	69	10
7	**8g**	86	40	71	10
8	**8h**	84	35	66	5
9	**8i**	85	30	72	9
10	**8j**	81	20	72	4
11	**8k**	83	20	71	4
12	**8l**	83	20	70	4
13	**8m**	87	25	67	8
14	**8n**	81	35	67	8

yields by recrystallizing the crude product with absolute ethanol (Table 1)[46,52].

Non-Conventional approach: A mixture of 3-Ethyl-2-(2-methyl-3-trifluoromethyl-phenylimino)-thiazolidin-4-one**(7)** (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).The reaction mixture was allowed to remain at room temperature for 30 minutes after the reaction was completed (as determined by TLC), during which time a solid precipitated out and the reaction mixture was filtered to produce a yellow solid (Scheme 3). In order to get a pure compound, the solid was cleaned with 100% ethanol and vacuum-dried to get(2E, 5E)-5-Benzylidene-3-ethyl-2-(2-methyl-3-trifluoromethyl-phenylimino)-thiazolidin-4-one in good yields (Table 1).[46,52]



Scheme 3 — Synthesis of thiazolidin-4-one derivatives *via* ultrasonic method 8 (a-n)

Anticancer Activity

The *in vitro* anti-cancer analysis of the synthesized compounds on HEPG2 cells were analyzed using SRB Assay.[53–57]The cell lines were grown in an appropriate medium containing 10% fetal bovine serum and 2 mM L-glutamine. For the present screening experiment, 5000 cells/well were inoculated into 96 well microtiter plates in 100 µL. After cell inoculation, the microtiter plates were incubated at 37°C, 5% CO_2, 95% air and 100% relative humidity for 24 hours prior to the addition of experimental drugs. Experimental drugs were solubilized in appropriate solvent at 100mg/mL and diluted to 1mg/ml using water and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate (1mg/mL) was thawed and diluted to 100 µg/mL, 200 µg/mL, 400 µg/mL and 800 µg/mL with complete medium containing test article. Aliquots of 10 µL of these different drug dilutions were added to the appropriate microliter wells already containing 90 µL of the medium, resulting in the required final drug concentrations *i.e.*10 µg/mL, 20 µg/mL, 40 µg/mL, 80 µg/mL. After compound addition, plates were incubated at standard conditions for 48 hours and the assay was terminated by the addition of cold TCA. Cells were fixed *in situ* by the gentle addition of 50 µL of cold 30% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 minutes at 4°C. The supernatant was discarded; the plates were washed five times with tap water and air-dried. Sulforhodamine B (SRB) solution (50 µL) at 0.4% (w/v) in 1% acetic acid was added to each of the wells, and plates were incubated for 20 minutes at room temperature. After staining, the unbound dye was recovered, and the residual dye was removed by washing five times with 1% acetic acid. The plates were air-dried. The bound stain was subsequently eluted with 10 mM trizma base, and the absorbance was read on a plate reader at a wavelength of 540 nm, using 690 nm as reference wavelength. Percent growth was calculated on a plate-by-plate basis for

test wells relative to control wells. Percent Growth was expressed as the ratio of average absorbance of the test well to the average absorbance of the control wells * 100.Using the six absorbance measurements [time zero (Tz), control growth (C), and test growth in the presence of drug at the four concentration levels (Ti)], the percentage growth was calculated at each of the drug concentration levels. Growth inhibition of 50% (GI50) drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. Values were calculated for each of these three parameters if the level of activity was reached; however, if the effect was not reached or was exceeded, the values for that parameter were expressed as greater or less than the maximum or minimum concentration tested.

Results and Discussion

Spectroscopic Data

5-Benzylidene-3-ethyl-2-(2-methyl-3-trifluoromethyl-phenylimino)-thiazolidin-4-one (8a)

Orange solid IR (ATR): 2942(C-H), 1710(C=O), 1632(C=N), 1496(C=C), 1372(C-N), 1367(C-O) cm^{-} ^{11}H NMR ($CDCl_3$, 300 MHz): δ 1.42 (t, *J* = 7.08 Hz, 3H), 2.32 (s, 3H), 4.11 (q, *J* = 7.11 Hz, 2H)

7.12 (d, *J* = 8.01 Hz, 1H), 7.28-7.52 (m, 7H), 7.80 (s, 1H).MS (*m/z*): 391.14 [M^+ +1]. Elemental analysis calculated for $C_{20}H_{17}F_3N_2OS$ (%): C, 61.53; H, 4.39; N, 7.18; S, 8.21. Found (%): C, 61.43; H, 4.41; N, 7.16; S, 8.23.

3-Ethyl-5-(4-fluoro-benzylidene)-2-(2-methyl-3-trifluoromethyl-phenylimino)-thiazolidin-4-one (8b)

Yellow solid IR (ATR): 2948(C-H), 1708(C=O), 1625(C=N), 1593(C=C), 1507(C-C), 1365 (N-CH_2), 1340(C-N), 1229(C-F), 859(*p*-substituted Ph ring), 819(C-S-C) cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.40 (t, *J* = 7.05 Hz, 3H), 2.32 (s, 3H), 4.11 (q, *J* = 7.11 Hz, 2H), 6.89 (d, *J* = 8.82 Hz, 2H), 7.13 (d, *J* = 7.83 Hz, 1H), 7.32 (m, 3H), 7.50 (d, 1H), 7.70 (s, 1H). MS (*m/z*): 409.21 [M^+ +1].Elemental analysis calculated for $C_{20}H_{16}F_4N_2OS$ (%): C, 58.82; H, 3.95; N, 6.86; S, 7.85. Found (%): C, 58.80; H, 3.93; N, 6.85; S, 7.87.

5-(4-Chloro-benzylidene)-3-ethyl-2-(2-methyl-3-trifluoromethyl-phenylimino)-thiazolidin-4-one (8c)

Yellow solid IR (ATR): 2945(C-H), 1712(C=O), 1645(C=N), 1510(C=C), 1363(C-NCH_2),1331(C-N), 1103(C-F), 878 (disubstituted Ph ring cm^{-1}). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.41 (t, *J* = 7.08 Hz, 3H), 2.32 (s, 3H), 4.13 (q, *J* = 7.05 Hz, 2H), 7.11 (d, *J* = 7.86 Hz, 1H), 7.28-7.41 (m, 5H), 7.52 (d, *J* = 7.95 Hz, 1H), 7.7 (s, 1H). MS (*m/z*): 425.01 [M^+ +1].Elemental analysis calculated for $C_{20}H_{16}ClF_3N_2OS$ (%): C, 56.54; H, 3.80; N, 6.59; S, 7.55. Found (%): C, 56.46; H, 3.81; N, 6.62; S, 7.54.

3-Ethyl-2-(2-methyl-3-trifluoromethyl-phenylimino)-5-(4-nitro-benzylidene)-thiazolidin-4-one (8d)

Yellow solid IR (ATR): ,2941(C-H), 1716(C=O), 1630(C=N), 1497(C=C), 1373(C-N), 1368(C-O) cm^{-} ^{11}H NMR ($CDCl_3$, 300 MHz): δ 1.43 (t, *J* = 7.08 Hz, 3H), 2.33 (s, 3H), 4.13 (q, *J* = 7.06 Hz, 2H), 7.10 (d, *J* = 7.86 Hz, 1H), 7.31 (m, 1H), 7.58 (m, 3H), 7.81 (s, 1H), 8.29 (d, *J* = 7.86 Hz, 2H). MS (*m/z*): 436.34 [M^+ +1].Elemental analysis calculated for $C_{20}H_{16}F_3N_3O_3S$ (%): C, 55.17; H, 3.70; N, 9.65; S, 7.36. Found (%): C, 55.15; H, 3.69; N, 9.62; S, 7.38.

5-(4-Dimethylamino-benzylidene)-3-ethyl-2-(2-methyl-3-trifluoromethyl-phenylimino)-thiazolidin-4-one, 8e: Yellow solid IR (ATR): 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) cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.40 (t, *J* = 7.08 Hz, 3H), 2.33 (s, 3H), 3.03 (s, 6H), 4.11 (q, *J* = 7.11 Hz, 2H), 6.70 (d, *J* = 8.82 Hz, 2H), 7.13 (d, *J* = 7.92 Hz, 1H), 7.28-7.36 (m, 3H), 7.50 (d, *J* = 7.74 Hz, 1H), 7.77 (s, 1H). MS (*m/z*): 434.02 [M^+ +1].Elemental analysis calculated for $C_{22}H_{22}F_3N_3OS$ (%): C, 60.96; H, 5.12; N, 9.69; S, 7.40. Found (%): C, 60.98; H, 5.10; N, 9.71; S, 7.43.

3-Ethyl-2-(2-methyl-3-trifluoromethyl-phenylimino)-5-(2-trifluoromethyl-benzylidene)-thiazolidin-4-one, 8f: Yellow solid IR (ATR): 2989(C-H), 1710(C=O), 1627(C=N), 1498(C=C), 1369(C-NCH2), 1339(C-N), 1095(C-CF_3), 842(*O*-substituted Ph ring), 768(C-S-C) cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.43 (t, *J* = 7.08 Hz, 3H), 2.31 (s, 3H), 4.11 (q, *J* = 7.11 Hz, 2H), 7.06 (d, *J* = 7.83 Hz, 1H), 7.28 (m, 2H), 7.44-7.60 (m, 3H), 7.76 (d, *J* = 7.68 Hz, 1H), 8.06 (s, 1H). MS (*m/z*): 458.95 [M^+ +1].Elemental analysis calculated for $C_{21}H_{16}F_6N_2OS$ (%): C, 55.02; H, 3.52; N, 6.11; S, 6.99. Found (%): C, 55.04; H, 3.49; N, 6.09; S, 6.98.

3-Ethyl-2-(2-methyl-3-trifluoromethyl-phenylimino)-5-(3-trifluoromethyl-benzylidene)-thiazolidin-4-one, 8g: Yellow solid IR (ATR): 2948(C-H), 1711(C=O), 1640(C=N), 1609(C=C), 1513(C-C) 1370(C-NCH_2), 1339(C-N), 1114(C-F_3),

865(*O*-substituted Ph ring), 686 (C-S-C) cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.42 (t, *J* = 7.08 Hz, 3H), 2.33 (s, 3H), 4.14 (q, *J* = 7.11 Hz, 2H), 7.11 (d, *J* = 7.83 Hz, 1H), 7.30 (m, 1H), 7.50-7.68 (m, 5H), 7.80 (s, 1H). MS (*m/z*): 459.02 [M^+ +1].

Elemental analysis calculated for $C_{21}H_{16}F_6N_2OS$ (%): C, 55.02; H, 3.52; N, 6.11; S, 6.99. Found (%): C, 55.05; H, 3.57; N, 6.08; S, 7.03.

3-Ethyl-5-(4-methoxy-benzylidene)-2-(2-methyl-3-trifluoromethyl-phenylimino)-thiazolidin-4-one, 8h: Orange solid IR (ATR): 2956(C-H), 1702(C=O), 1631(C=N), 1591(C=C), 1513(C-C), 1361(N-CH_2), 1248(C-O), 818(*p*-substituted Ph ring), 691(C-S-C) cm^{-1}.^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.41 (t, *J* = 7.08 Hz, 3H), 2.32 (s, 3H), 3.84 (s, 3H), 4.12 (q, *J* = 7.11 Hz, 2H), 6.96 (d, *J* = 8.73 Hz, 2H), 7.12 (d, *J* = 7.83 Hz, 1H), 7.30 (t, 1H), 7.42 (d, *J* = 8.7 Hz, 2H), 7.51 (d, *J* = 7.71 Hz, 1H), 7.75 (s, 1H).MS (*m/z*): 421.12 [M^+ +1]. Elemental analysis calculated for $C_{21}H_{19}F_3N_2O_2S$ (%): C, 59.99; H, 4.55; N, 6.66; S, 7.63. Found (%): C, 60.05; H, 4.48; N, 6.70; S, 7.66.

5-(2,4-Dimethoxy-benzylidene)-3-ethyl-2-(2-methyl-3-trifluoromethyl-phenylimino)-thiazolidin-4-one, 8i: Yellow solidIR (ATR): 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) cm^{-1}.^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.40 (t, *J* = 7.05 Hz, 3H), 2.32 (s, 3H), 3.89 (s, 7H), 4.11 (q, *J* = 7.11 Hz, 2H), 6.46 (s, 1H), 6.52 (d, *J* = 8.64 Hz, 1H), 7.11 (d, *J* = 7.89 Hz, 1H), 7.31 (t, *J* = 8.13 Hz, 1H), 7.49 (d, *J* = 7.71 Hz, 1H), 8.14 (s, 1H).MS (*m/z*): 450.98 [M^+ +1]. Elemental analysis calculated for $C_{22}H_{21}F_3N_2O_3S$ (%): C, 58.66; H, 4.70; N, 6.22; S, 7.12. Found (%): C, 58.61; H, 4.81; N, 6.11; S, 7.07.

3-Ethyl-2-(2-methyl-3-trifluoromethyl-phenylimino)-5-(2,3,4-trimethoxy-benzylidene)-thiazolidin-4-one, 8j: Yellow solid IR(ATR): 2945(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) cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.34 (t, *J* = 7.05 Hz, 3H), 2.22 (s, 3H), 3.82 (s, 7H), 3.85 (s, 3H), 4.03 (q, *J* = 7.11 Hz, 2H), 6.62 (d, *J* = 8.88 Hz, 1H), 7.05 (d, *J* = 8.89 Hz, 2H), 7.43 (d, *J* = 7.83 Hz, 1H), 7.96 (s, 1H). MS (*m/z*): 481.09 [M^+ +1].Elemental analysis calculated for $C_{23}H_{23}F_3N_2O_4S$ (%): C, 57.49; H, 4.82; N, 5.83; S, 6.67. Found (%): C, 57.44; H, 4.86; N, 5.86; S, 6.61.

3-Ethyl-2-(2-methyl-3-trifluoromethyl-phenylimino)-5-(2,4,5-trimethoxy-benzylidene)-thiazolidin-4-one, 8k: Yellow solid IR (ATR): 2942(C-H), 1713(C=O), 1614(C=N), 1589(C=C), 1445(NCH2), 1366(C-N), 1128(C-O), 1090 (C-F), 907(*m*-disubstituted Ph-ring), 782(C-S-C) cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.40 (t, *J* = 7.05 Hz, 3H), 2.32 (s, 3H), 3.77 (s, 3H), 3.9 (s, 3H), 4.11 (q, *J* = 7.11 Hz, 2H), 6.51 (s, 1H), 6.88 (s, 1H), 7.12 (d, *J* = 8.81 Hz, 2H), 7.30 (s, 3H), 7.48 (d, *J* = 7.83 Hz, 1H), 8.12 (s, 1H). MS (*m/z*): 481.02 [M^+ +1].Elemental analysis calculated for $C_{23}H_{23}F_3N_2O_4S$ (%): C, 57.49; H, 4.82; N, 5.83; S, 6.67. Found (%): C, 57.41; H, 4.89; N, 5.79; S, 6.71.

3-Ethyl-2-(2-methyl-3-trifluoromethyl-phenylimino)-5-(3,4,5-trimethoxy-benzylidene) thiazolidin-4-one, 8l: Yellow solid IR(ATR): 2945(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) cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.41 (t, *J* = 7.11 Hz, 3H), 2.33 (s, 3H), 3.84 (s, 6H), 3.89 (s, 3H), 4.13 (q, *J* = 7.05 Hz, 2H), 6.51 (s, 1H), 6.66 (s, 1H), 7.12 (d, *J* = 7.74 Hz, 2H), 7.50 (d, *J* = 7.68 Hz, 1H), 7.72 (s, 1H). MS (*m/z*): 480.98 [M^+ +1].Elemental analysis calculated for $C_{23}H_{23}F_3N_2O_4S$ (%): C, 57.49; H, 4.82; N, 5.83; S, 6.67. Found (%): C, 57.41; H, 4.86; N, 5.75; S, 6.74.

3-Ethyl-2-(2-methyl-3-trifluoromethyl-phenylimino)-5-(1H-pyrrol-2-ylmethylene)-thiazolidin-4-one, 8m: Green Solid IR (ATR): 3742(C-C=N), 2978(C-H), 1705(C=O), 1633(C=C), 1503(C-N), 1377(C-C-F),1200(C-O), 962 (disubstituted phenyl ring) cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.41 (t, *J* = 7.08 Hz, 3H), 2.33 (s, 3H), 4.13 (q, *J* = 7.05 Hz, 2H), 6.37 (s, 1H), 6.52 (s, 1H), 7.02 (s, 1H), 7.13 (d, *J* = 7.80 Hz, 2H), 7.51 (d, *J* = 7.86 Hz, 1H), 7.71 (s, 1H), 8.86 (s, 1H).MS (*m/z*): 379.96 [M^+ +1]. Elemental analysis calculated for $C_{18}H_{16}F_3N_3OS$ (%): C, 56.98; H, 4.25; N, 11.08; S, 8.45. Found (%): C, 56.91; H, 4.21; N, 11.13; S, 8.51.

3-Ethyl-2-(2-methyl-3-trifluoromethyl-phenylimino)-5-thiophen-2-ylmethylene-thiazolidin-4-one, 8n: Green Solid IR (ATR): 2956(C-H), 1702(C=O), 1630(C=N), 1595(C=C), 1513(C-C), 1366(N-CH_2), 1253(C-O), 818(*p*-substituted Ph ring), 691(C-S-C) cm^{-1}.^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.41 (t, *J* = 7.11 Hz, 3H), 2.33 (s, 3H), 4.12 (q, *J* = 7.14 Hz, 2H), 7.13 (s, 2H), 7.33

(d, 2H), 7.49-7.56 (m, 2H), 7.97 (s, 1H).MS (*m/z*): 397.12 [M^+ +1]. Elemental analysis calculated for $C_{18}H_{15}F_3N_2OS_2$ (%): C, 54.53; H, 3.81; N, 7.07; S, 16.18. Found (%): C, 54.42; H, 3.87; N, 7.12; S, 16.11.

The FT-IR analysis of 3-Ethyl-2-(2-methyl-3-trifluoromethyl-phenylimino)-thiazolidin-4-one **(7)** gave strong absorption bands at 1651 cm^{-1} (C=O) and 1734 cm^{-1} (C=N), indicating the presence of C=O and C=N functional groups, respectively.The ^{1}H NMR spectrum of compound **7** showed a triplet of three protons at 1.34 ppm with a coupling constant of 7.08 Hz for three protons and quartet of two protons resonated at 3.98 ppm with a coupling constant of 7.08 Hz which were attributed to the ethyl group protons of N-CH_2-CH_3.The formation of iminothiazolidinone ring structure was confirmed due to the observance of a singlet at 3.83 ppm integrating two protons of the methylene group attached to the S-atom of iminothiazolidin-4-one ring. Three protons of CH_3 group attached to benzene ring gave singlet at 2.29 ppm. *Ortho* proton attached to benzene ring gave doublet at 7.06ppm, *meta* proton gave triplet at 7.26 ppm and *para* proton gave doublet at 7.45 ppm. Thus, with the help of FT-IR and ^{1}H NMR spectra, confirmed the formation of desired iminothiazolidinone core. The mass spectrum showed a peak at *m/z* = 303.03 (M^++1). Elemental analysis calculated for $C_{13}H_{13}F_3N_2OS$ (%):C, 51.65; H, 4.33; N, 9.27; S, 10.61. Found (%): C, 51.52; H, 4.23; N, 9.35; S, 10.67. All the spectral values and analysis data confirmed the core structure of the key intermediate.

Molecule **7** was later subjected to the Knoevenagel reaction by condensation with several aldehydes utilizing both traditional reflux and unconventional sonication techniques. The reaction of 3-Ethyl-2-(2-methyl-3-trifluoromethyl-phenylimino)-thiazolidin-4-one**(7)** with various aryl aldehydes in the presence of Diisopropylethylamine as a base in absolute ethanol yielded 5-Benzylidene-3-ethyl-2-(2-methyl-3-trifluoromethyl-phenylimino)-thiazolidin-4-one **(8a-n)**. Fig. 2 displays the many derivatives **8a-n** that were synthesized. All the Knoevengel reaction products are characterized by using mass analysis, IR, and NMR spectroscopy. Spectral data of representative compound **(8b)** (2E,5E)-3-Ethyl-5-(4-fluoro-benzylidene)-2-(2-methyl-3-trifluoromethyl-phenylimino)-thiazolidin-4-oneshowed strong IR absorption bands at 2949cm^{-1} (C=C), 1709cm^{-1} (C=O) and at 1630 cm^{-1} (C=N) confirms the presence of C=C, C=O and C=N functional groups respectively.

The ^{1}H NMR spectrum shows protons of N-CH_2-CH_3 resonating at 1.40 ppm (t, J=7.05 Hz,3H) and N-CH_2-CH_3 at 4.11ppm (q, J=7.11 Hz,2H). The absence of the signal of methylene protons of thiazolidin-4-one ring of starting core moiety at 3.83 ppm and the presence signal of the methine proton as a singlet at 7.70 ppm confirms the formation of the C=C bond across thiazolidinone rings. The CH_3 proton of 2-methyl-3-trifluoromethyl-phenyl ring resonate at 2.32 ppm (s,3H),while protons attached to phenyl ring are observed between 7.10 to 7.50 ppm. The 4-Fluoro benzylidene ring protons resonated between 6.86 to 7.35 ppm.

The mass spectrum exhibited a peak at m/z = 408.98 (M^++1). Elemental analysis calculated for $C_{20}H_{16}F_4N_2OS$ (%): C, 58.82; H, 3.95; N, 6.86; S, 7.85. Found (%): C, 58.73; H, 4.02; N, 6.87; S, 7.84. All the spectral values and analysis data confirmed the core structure of the key intermediate.

The stereochemistry of compound **8b** was confirmed by conducting a 2D NOESYNMR experiment. The compound shows no interaction of N-CH_2-CH_3 protons with benzylidine proton. Benzylidine proton at 7.70 ppm shows correlation with the 4-fluorobenzene 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 alsoobserved that there is no interaction of N-CH_2-CH_3 protons ofethyl group with protons of the 2-methyl-3-trifluoromethyl-phenyl ring as well asthe protons of the benzylidine group, which confirms that the stereochemistry about C=N and C=C groups is E, E. As indicated in Table 1, the yields of the novel synthesized products **8(a-n)** utilizing various aldehydes using the conventional heating technique ranged from 66 to 76%, whereas the yields from the non-conventional ultrasound method were found to be between 81 to 90%. It was concluded that the non-conventional method is better than the conventional method as reflux uses excess ethanol as solvent and requires heating. The formation of impurities is another disadvantage of the process.

Anti-Cancer Activity

The effect of the synthesized on the growth of the HEPG2 cells has been depicted in Fig. 3. The activities of the compounds show growth quite high as compared to the anticancer drug doxorubicin

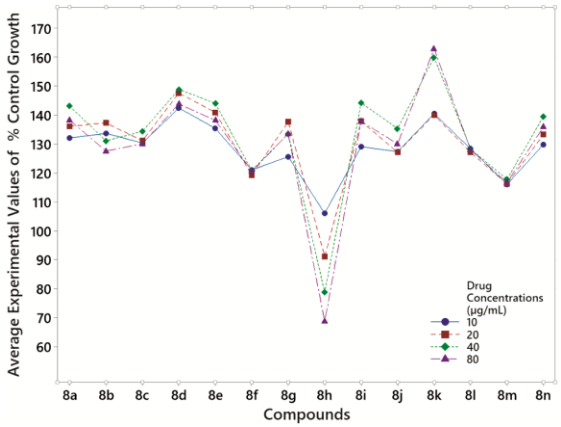


Fig. 3 — Graph showing% growth control of the synthesized compounds on Human Hepatoma cell line (HEPG2) at four different concentration levels

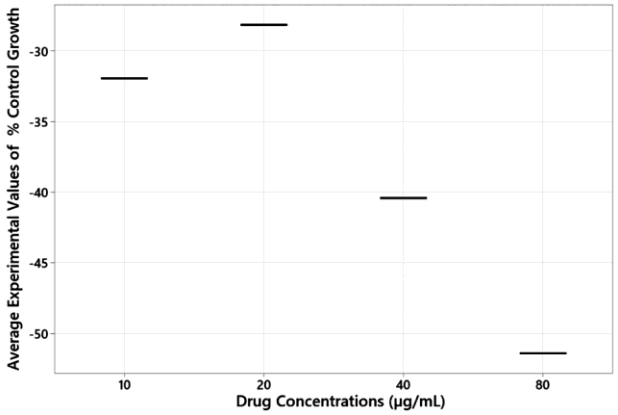


Fig. 4 — Graph showing% growth control of doxorubicin on Human Hepatoma cell line (HEPG2) at four different concentration levels

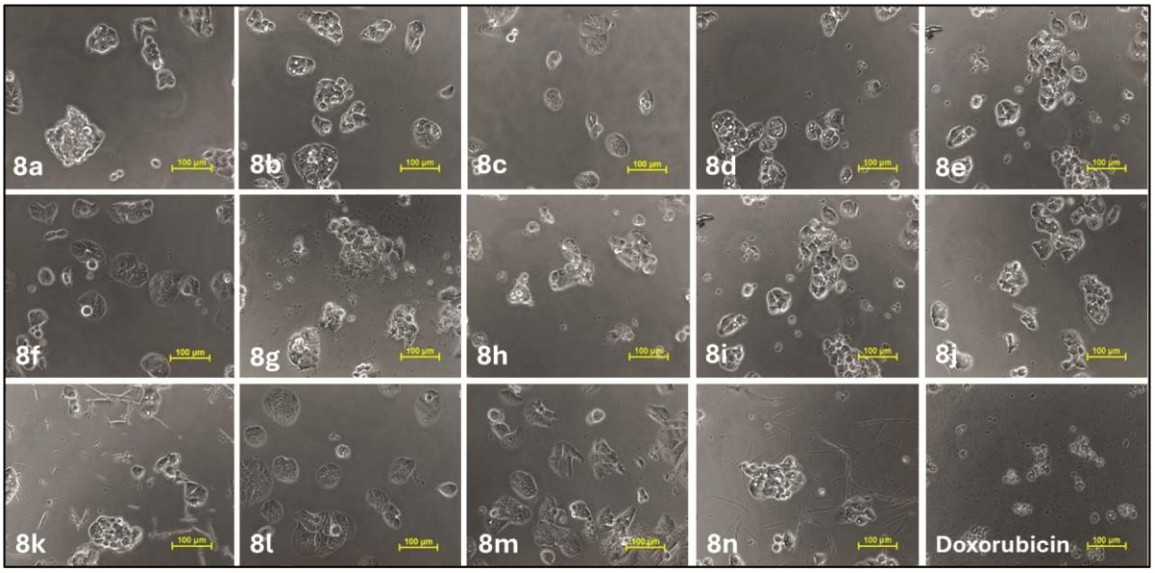


Fig. 5 — Images ofHEPG2 cells after treatment with **s**ynthesized thiazolidin-4-one derivatives **8(a-n)**, and doxorubicin as positive control

(Fig. 4). The molecule 8h having a single methoxy group at *p*-position of the benzylidene ring exhibited the best activity among all the synthesized molecules. The presence of halogen atoms, heterocyclic rings, trifluoromethyl groups, dimethylamino as well as higher number of methoxy groups at different positions of the ring did not show much effect in controlling the growth of the cells. Even if the ring is unsubstituted there is no profound inhibition in the growth of cells. The presence of pyrrole ring shows similar growth control at all the analyzed concentrations, indicating that it is effective even at low levels. However, replacement of the same by thiophene ring shows diminished growth control potential. The images of the cells after incubation post treatment with the synthesized compounds, as well as doxorubicin have been shown in Fig. 5.

Conclusion

The present work describes the synthesis of novel fluorinated 5-benzylidene-3-ethyl-2-(2-methyl-3-trifluoromethyl-phenylimino)-thiazolidin-4-one derivatives**.** In addition to this, the study also describes the effect of ultrasound on the yield and reaction time for successful Knoevenagel reaction was also carried out. This nonconventional method was found to give better yields as compared to the conventional heating method. Also, the reaction was carried out at room temperature, which is an added advantage. A small amount of solvent is required

when sonication is employed, as compared to reflux method. These compounds when tested against HEPG2 cell line, showed that presence of *p*-substituted methoxy group in the benzylidene ring showed best anticancer potential.

Acknowledgements

The authors gratefully acknowledge the guidance and support of Dr. Ajit Borhade, PI Industry Limited and Mr. Tushar Patil, Bharat Rasayan Limited in executing the work. The authors are also thankful to Anti-Cancer Drug screening facility (ACDSF) at ACTREC, Tata Memorial Centre, Navi Mumbai for the *in vitro* anti-cancer evaluation of synthesized compounds.

References

1 Sung H, Ferlay J, Siegel R L, Laversanne M, Soerjomataram I, Jemal A & Bray F, *CA Cancer J Clin*, 71 (2021) 209.
2 Meunier B, *Acc Chem Res*, 41 (2008) 69.
3 Kayl A E & Meyers C A, *Curr Opin Obstet Gynecol*, 18 (2006) 24.
4 Siegel R L, Miller K D, Wagle N S & Jemal A, *CA Cancer J Clin*, 73 (2023) 17.
5 Kumar S & Choudhary M, *Indian J Chem*, 62 (2023) 472.
6 Stuart J G & Jebaraj J W, *Indian J Chem*, 62 (2023) 1061.
7 Hien N, Van Dat N, Du N D, Mai N T N, Hien N T T & Hoan D Q, *Indian J Chem*, 62 (2023) 1276.
8 Shamili S, Siddoju K, Chandrakala G & Farheen S I, *Indian J Chem*, 62 (2023) 445.
9 Harsha J & Reji T F A F, *Indian J Chem*, 62 (2023) 1247.
10 Jiang Y R, Su D, Wang M Z, Jia A Q & Zhang Q F, *Indian J Chem*, 62 (2023) 425.
11 Misra S, Paul S, Pakrashy S, Ghosh S, Naskar S, Maurya P K, Sardar P S, Venkateswarlu K & Bose A, *Indian J Chem*, 62 (2023) 1001.
12 Rani R M & Kavitha P, *Indian J Chem*, 63 (2024) 281.
13 Kumar R, Patil S & Pharm M, *Hygeia J D Med*, 9 (2017) 3590.
14 Abhinit M, Ghodke M & Pratima NA, *Int J Pharm Pharm Sci*, (2009) 47.
15 Tripathi AC, Ji S, Naz G, Kumar P & Verma A, *Eur J Med Chem*, 72 (2014) 52.
16 Gavade S N, Markad V L, Kodam K M, Shingare M S & Mane D V, *Bioorg Med Chem Lett*, 22 (2012) 5075.
17 Türe A, Ergül M, Ergül M, Altun A & Küçükgüzel İ, *Mol Divers*, 25 (2021) 1025.
18 Szychowski K A, Leja M L, Kaminskyy D V, Binduga U E, Pinyazhko O R, Lesyk R B & Gmiński J, *Chem Biol Interact*, 262 (2017) 46.
19 Senkardes S & Kucukguzel S, *Mini Rev Org Chem*, 13 (2016) 377.
20 Kato T, Ozaki T & Ohi N, *Tetra Asymmetry*, 10 (1999) 3963.
21 Adachi Y, Suzuki Y & Homma N, *Eur J Pharmacol*, 367 (1999) 267.
22 Abeed A A O, Youssef M S K & Hegazy R, *J Braz Chem Soc*, 28 (2017) 2054.
23 Agrawal N, Upadhyay P K, Shah K & Mishra P, *J Indian Chem Soc*, (2017).
24 Ottanaá R, Mazzon E, Dugo L, Monforte F, Maccari R, Sautebin L, Luca G D, Vigorita G M, Alcaro S, Ortuso F, Caputi A P & Cuzzocrea S, *Eur J Pharmacol*, 448 (2002) 71.
25 Yamamoto S H, Okumura H & Suzukamoc G, *J Chem Soc Perkin Trans*, (1995). https://doi.org/10.1039/P19950000935
26 Diurno M V, Izzo AA, Mazzoni O, Bolognese A & Capasso F, *J Pharm Pharmacol*, 48 (1996) 760.
27 Ergene N & Capan G, *Farmaco*, 49 (1994) 133.
28 Chavan A A & Pai N R, *Arkivoc*, 2007 (2007) 148.
29 Diurno M V, Mazzoni O, Piscopo E, Calignano A, Giordano F & Bolognese A, *J Med Chem*, 35 (1992) 2910.
30 Rawal R K, Prabhakar Y S, Katti S B & De Clercq E, *Bioorg Med Chem*, 13 (2005) 6771.
31 Kato T, Ozaki T, Tamura K, Suzuki Y, Akima M & Ohi N, *J Med Chem*, 42 (1999) 3134.
32 Tanabe Y, Suzukamo G, Komuro Y, Imanshi N, Morooka S, Enomoto M, Kojima A, Sanemitsu Y & Mizutani M, *Tetrahedron Lett*, 32 (1991) 379.
33 Kato Y, Kita Y, Nishio M, Hirasawa Y, Ito K, Yamanaka T, Motoyama Y & Seki J, *Eur J Pharmacol*, 384 (1999) 197.
34 Voss M E, Carter P H, Tebben A J, Scherle P A, Brown G D, Thompson L A, Xu M, Lo Y C, Yang G, Liu R-Q, Strzemienski P, Everlof J G, Trzaskos J M & Decicco C P, *Bioorg Med Chem Lett*, 13 (2003) 533.
35 Srivastava K, Srivastava A, Tiwari R P, Tripathi A, Peta S & Verma M, *Indian J Chem*, 62 (2023) 770.
36 Ghayalkar R B & Deshmukh S P, *Indian J Chem*, 62 (2023) 746.
37 Balfour J A B & Plosker G L, *ADIS NEW DRUG P*, 57 (1999) 921.
38 Bae J, Park T, Kim H, Lee M & Cha B S, *Diabetes Metab J*, 45 (2021) 326.
39 Steele J W, Faulds D & Goa K L, *Drugs Aging*, 3 (1993) 532.
40 D'ambrosio D, Freedman M S & Prinz J, *Ther Adv Chronic Dis*, 7 (2016) 18.
41 Kadam S, Bhatt R, Mammen D & Nikam L, *Interwoven An Interdiscip J Navrachana Univ*, 6 (2023) 115.
42 Kobylinska L I, Boiko N M, Panchuk R R, Grytsyna I I, Klyuchivska O Y, Biletska L P, Lesyk R B, Zimenkovsky B S & Stoika R S, *Croat Med J*, 57 (2016) 151.
43 Kulabaş N, Özakpinar ÖB, Özsavcı D, Leyssen P, Neyts J, & Küçükgüzel İ, *Marmara Pharm J*, 21 (2017) 371.
44 Parameswari P & Devika R, *Res J Pharm Tech*, 12 (2019) 5305.
45 Shaty M H, Al-Ezzi M I, Arif I S & Basil D, *Res J Pharm Technol*, 12 (2019) 5815.
46 Kadam S D, Mammen D, Kadam D S, Patil S G, Bagul R R, Doshi A & Patel F, *Asian J Chem*, 35 (2023) 1884.
47 Kadam D S, Mammen D, Kadam D S & Patil S G, *Asian J Res Chem*, 16 (2023) 55.
48 Kadam D S, Patil G S, Mammen D, Kadam S D & More V, *J Appl Organomet Chem*, 3 (2023) 13.
49 George R F, *Eur J Med Chem*, 47 (2012) 377.
50 Keil A M, Frederick D M, Jacinto E Y, Kennedy E L, Zauhar R J, West N M, Tchao R & Harvison V J, *Toxicol Vitro*, 29 (2015) 1887.
51 Tang S Q, Lee Y Y I, Packiaraj D S, Ho H K & Chai C L L, *Chem Res Toxicol*, 28 (2015) 2019.
52 Kadam D, Patil S, Kadam S, Doshi A & Patel F, *JETIR*, 9 (2022) 691.

53 Kholiya F, Chatterjee S, Bhojani G, Sen S, Barkume M, Kasinathan N K, Kode J & Meena R, *Carbohydr Polym*, 240 (2020) 116282.
54 Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren J T, Bokesch H, Kenney S & Boyd M R, *J Natl Can Inst*, 82 (1990) 1107.
55 Vichai V & Kirtikara K, *Nat Protoc*, 1 (2006) 1112.
56 Kode J, Maharana J, Kumar KN, Pradhan T, Ingle A, Barkume M, Patkar M, Thampi N, Patil A, Vaibhaw A, Kovvuri J & Kamal A, *Cancer Res*, 82 (2022) 4222.
57 Kode J, Kovvuri J, Nagaraju B, Jadhav S, Barkume M, Sen S, Kasinathan N K, Chaudhari P, Mohanty B S, Gour J, Sigalapalli D K, Kumar C G, Pradhan T, Banerjee M & Kamal A, *Bioorg Chem*, 105 (2020) 104447.