*Plant Archives* Volume 20 No. 2, 2020 pp. 6275-6287 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

# SYNTHESIS, CHARACTERIZATION AND *IN VITRO* ANTICANCER EVALUATION OF 4-(5-(2-(SUBSTITUTED)-4-OXOTHIAZOLIDIN-3-YL)-1,3,4-THIADIAZOL-2-YL)-PHENYL-ACETATE DERIVED FROM PHENOLIC ALDEHYDE

**Sandeep Jain[1], Neetu Chopra[1*] and Sanjeev Kalra[2]**

[1*]Department of Pharmaceutical Chemistry, Guru Jambheshwar University of Science & Technology, Hissar (Haryana), India.

[2]Rajendra Institute of Technology and Sciences, Sirsa (Haryana), India.

## Abstract

A novel series of 4-(5-(2-(substituted)-4-oxothiazolidin-3-yl)-1, 3, 4-thiadiazol-2-yl) -phenyl-acetate from salicaldehyde was (10a-y) synthesized, Characterized and evaluated against *In vitro* five bacterial, two fungal, breast cancer (MCF-7) and cervical carcinoma (HeLa) cell line. Among the entire tested analogs, thiazolidinone ring substituted at 2- position with Methoxy pyrazole (10g), trimethoxy benzaldehyde (10h), P- OH (10s), exhibited promising anticancer results against Human breast cell line (MCF-7) with the value of $IC_{50}$ 7.19 ± 0.16, 8.24 ± 1.36, 9.18 ± 0.24nM as compared to the standard drug doxorubicin ($IC_{50}$ 2.63 ± 0.17) and against gram positive strain B. subtilis (MIC~4.28 ± 0.26 - 4.25 ± 0.16 µg/ml), S. aureus (3.81 ± 0.12- 3.56 ± 0.26 µg/ml) as compared to Ciprofloxacin(MIC~3.47 ± 0.12 µg/ml). While the compound 10k, 10r and 10w having substitution of ethylated indole, p- N, N Dimethyl, 2, 3, 5 Trimethoxy on Thiazolidinone Skelton exhibited significant result of $IC_{50}$ value of 3.61 ± 0.12, 6.12 ± 0.17, 6.29 ± 0.14 µM against Human cervical cancer (HeLa) and against fungal strain C. albicans with MIC value (3.49 ± 0.28µg/ml), 3.64 ± 0.14µg/ml and (MIC~4.13 ± 0.39 µg/ml) as compared to standard Fluconazole drug (3.44 ± 0.28µg/ml) The remaining compounds have shown good to moderate activity against the tested cell lines. Based on results achieved for antimicrobial and anticancer activity, structure activity relationship (SAR) of targeted analogs are discussed.

***Key words:*** 4- Thiazolidinone, Thiadiazole, Salicylaldehyde, Synthesis, Cytotoxic activity, antimicrobial evaluation

## Introduction

Cancer is becoming a major cause of human health concerns with increasing the number of patients worldwide. Cervical cancer and breast cancer is the most identified cancer in the female population (Felix *et al.,* 2016). Despite off many types of chemotherapeutic drugs were used for the treatment, although still, there is a challenge to identify safe and effective drug for the cancers. Drug resistance is the major concern observed during treatment. (Abrahamn, *et al.*, 2002). The design and development of new anticancer agents with increased efficiency, less side effective, cost effective, and time concern for the treatment were the major challenges for present researchers. Considering these facts, the development of new chemotherapeutic targets with selective action has to be identified, as many classes of heterocyclic scaffolds were used for the different types of cancers.

4-Thiazolidine derivatives are an important class of heterocyclic compounds having nitrogen and sulfur heteroatom known for their potential pharmaceutical applications. Recently, this framework containing compounds were effective against antimicrobial (Young *et al.,* 2004) antischistosomal activity (Taha *et al.,* 2007) antifungal (Asati *et al.,* 2005), antiinflammatory (Jain *et al.,*2006), antimalarial (Kristina *et al.,* 2009), herbicidal (Sanemitsu, *et al.,* 2006), antiviral (Eiichi, *et al.,* 2007), antidiabetic (Murugan *et al.,* 2009) and antioxidant activities (Shih *et al.,* 2004), 4-thiazolidinones substituted on 2, 3 position possesses the same spectrum of biological activity including anticancer activity (Gududuru, *et al*., 2004). Fig. 1.

1, 3, 4-Thiadiazoles have been reported to have

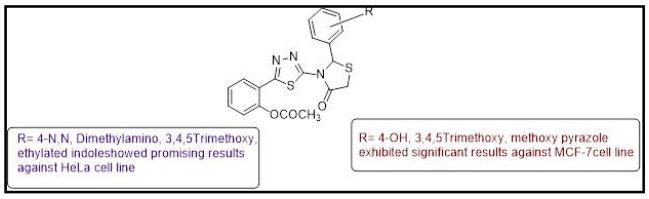
***Author for correspondence :** E-mail : neetushine.chopra@gmail.com

anticancer properties (Ibrahim *et al*., 2009) Additionally, some derivatives also impact DNA synthesis (Juszczak, *et al*., 2011.) Plech and colleagues synthesized a series of 2, 5-substituted 1, 3, 4-thiadiazoles and tested them for activity against cancer cells and Top 2 (Plech *et al.,* 2015) Fig. 2. While some of these compounds do impact cancer cell growth (MCF-7 and MDA-MB231).

Phenolic aldehydes like o, p and m hydroxy-benzaldehydes are biologically very much important because of their use in medication, food industry and cosmetic industry. (Uma *et al*., 1998; Cao *et al*., 2010; Cheng *et al.,* 2010). These phenolic aldehydes comprise a class of molecules that are highly crucial in proton transfer reactions involved in lots of processes ranging from acid base catalysis to enzyme catalysis (Anouar *et al.,* 2013). In this original article we mainly emphasized on Salicylaldehyde (O-Hydoxy-benzaldehyde) having molecular formula $C_6H_4CHO$-2-OH (Chen *et al.,* 2007). Fig. 3.

Encouraged by the promising anticancer activity of Thiazolidinone, Thiadiazole and vanillin we decide to club all rings to make targeted analogs, for the same we combined vanillin thiadiazole and thizolidinone to get better cytotoxic result. Wet lab synthesis was carried out; spectral characterization provides the better information about the synthesized compounds.

**Graphical abstract**




## Materials and Methods

Starting materials were obtained from commercial sources (Central Drug House Private Limited, New Delhi, India; Loba Chemie Private Limited, Mumbai, India and Himedia Laboratories Private Limited, Mumbai, India. The IR spectra of the synthesized compounds were recorded on a Fourier Transform IR spectrometer (Nicolet 380 FT-IR) in the range of 400-4000 using KBr pellets and the values of $\nu_{max}$ are reported in $cm^{-1}$. The proton nuclear magnetic resonance ($^1H$ NMR) and $^{13}C$ NMR spectra of the compounds were recorded on Brooker model DRX-300 NMR spectrometer at I.I.T, Delhi using $CDCl_3$ and DMSO-$d_6$ as solvent in all the cases. Tetramethylsilane (TMS) was used as an internal standard and values of the chemical shift are given in δ scale. Chemical shifts are recorded in parts per million (ppm, δ) and the signals are described as s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). The value of coupling constant (J) is given in Hz.

**Cell culture and medium**

Gram-positive bacteria strains *S. aureus* (MTCC 3160), *B. subtilis* (MTCC 441), Gram-negative bacteria strains *E. coli* (MTCC 443), *K. pneumonia, S. typhi* and fungal strains *C. albicans* (MTCC227) and *A. niger* (MTCC 281) strains, breast cancer cell line (MCF-7) and cervical carcinoma cell line (HeLa) were used.

**General procedure for the synthesis of intermediates and titled compounds (10a-10y)**

The general synthetic scheme for the synthesized titled compound 10a-10y has presented in Fig. 4 (Scheme-1).

**General method of preparation of Acetylated Salicylaldehyde (3)**

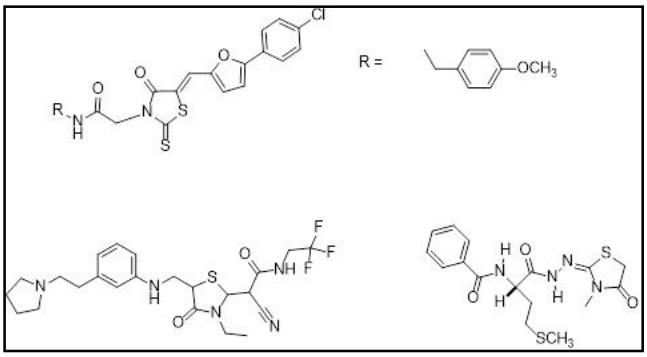



**Fig. 1:** Anticancer activity of 4- Thiazolidinone**.**

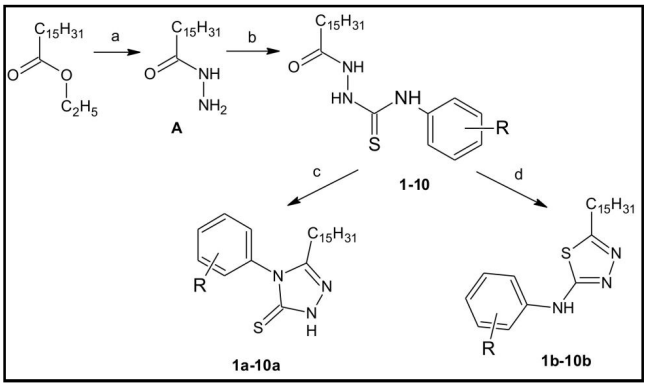



**Fig. 2:** Series of 2, 5-substituted 1, 3, 4-thiadiazoles analogs.

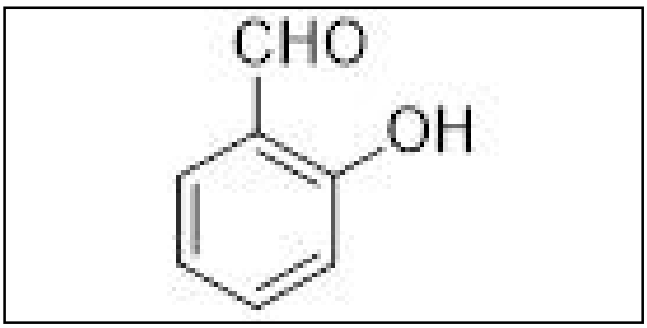



**Fig. 3:** Structure of Salicylaldehyde.

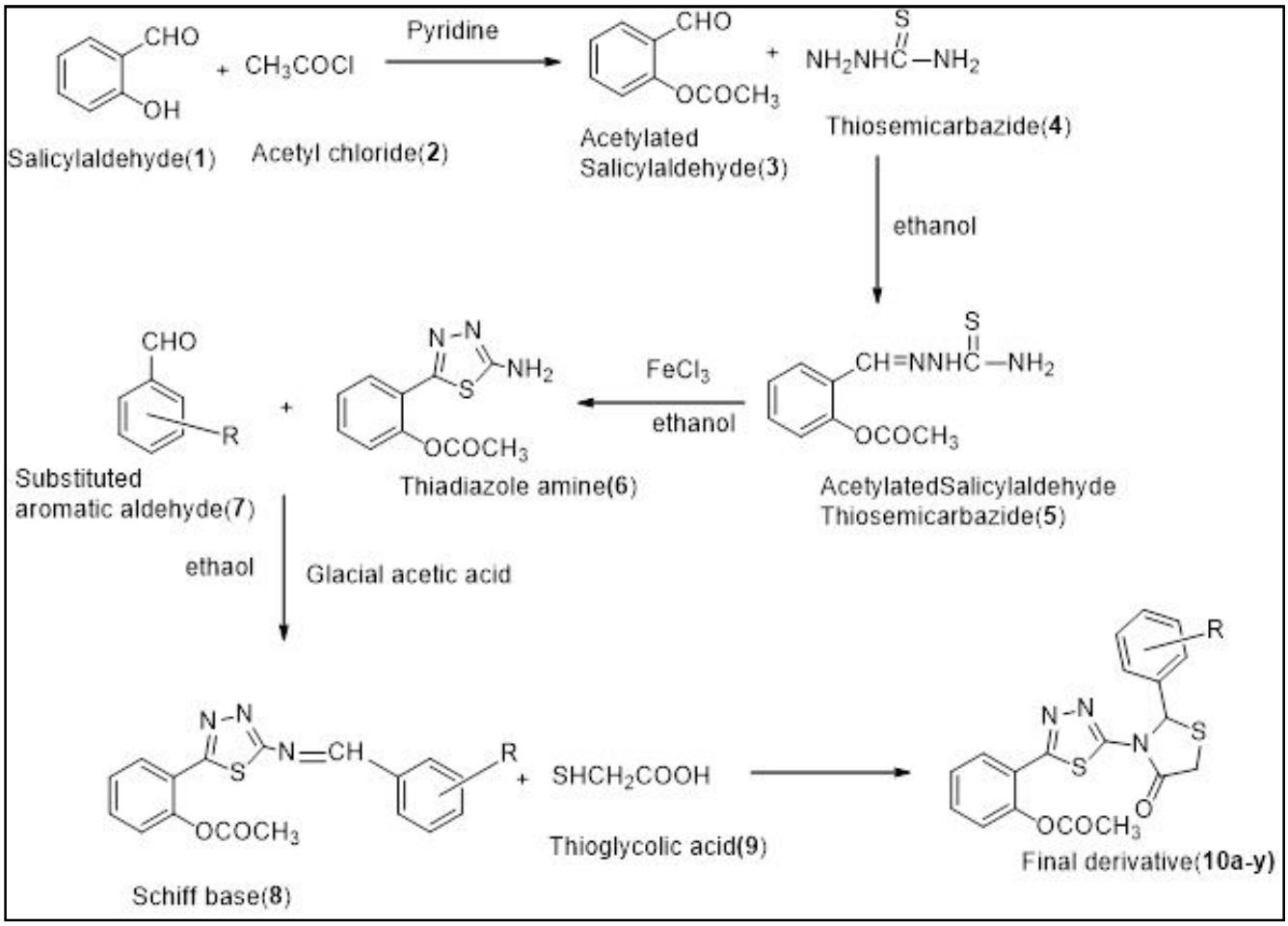



**Fig. 4:** General schemes for the synthesis of final derivatives (10a-y).

Acetylated Salicylaldehyde, was prepared by dissolving 10 gm (0.81mmol) of Salicylaldehyde (1) in dry pyridine 7.14 gm or 7 ml (0.090 mol) in a conical flask, then without delay (since this solution becomes a semi-solid mass on standing), 8.25gm or 7.5 ml (0.11mol) of acetyl chloride (2) was added and the mixture was shaken continuously during the addition. The heat of reaction causes the temperature of the mixture to rise rapidly; therefore the temperature was maintained at 50-60$^0$C throughout the addition. Finally, the mixture was heated on water bath for 5 minutes and after that cool in cold water, the mixture was poured in 300 ml of cold water, with continuous stirring. The crude acetylated Salicylaldehyde either solidify at once or separate as oil which rapidly crystalized as stirring proceeds. Product was filtered; wash thoroughly with water and drain. Recrystallize the product from an equal volume of water and acetic acid. The purity of the product was determined by single spot on the TLC plate. The solvent system used was n-Hexane: ethyl acetate (8 : 2). (Frederick *et al*., 2010).

**General method of preparation of Acetylated Salicylaldehyde thiosemicarbazone (5)**

A solution of Acetylated Salicylaldehyde (3) (1.5g, 0.009 mol) and Thiosemicarbazide (4) (0.83gm, 9mmoL) was prepared in methanol (25ml) was refluxed for 5h. Then the solution was permitted to cool and transferred in cold water. Then the product formed was dried after filtration to get crude product. In the next step, it was recrystallized from hot methanol to achieve pure Acetylated- Salicylaldehyde thiosemicarbazone (5). The purity of the product was determined by single spot on the TLC plate. The solvent system used was n-Hexane: ethyl acetate (8:2). (Suthar *et al.,* 2013.)

**General method of preparation of acetylated Salicylaldehyde thiadiazole amine (6)**

Acetylated Salicylaldehyde thiosemicarbazone (5) (1gm, 4mmoL) was put off in 80 ml of ethanol in an r.b.f. and solution of ferric chloride (0.65 gm, 0.06mol) in water was added with constant stirring. The flask was heated for 3-4 hours minutes the temperature 80-90$^o$C. The crystals solution was filtered, after filtering the amine it was dried and undergoes recrystallization from 50% ethanol. The purity of the compound was determined with the help of TLC by observing single spot. The solvent system selected was n-Hexane: ethyl acetate (8:2)

(Mishra *et al.,* 2006.).

**General method of preparation of Schiff base of thiadiazole amine (8)**

To a solution of 4- (5- amino-1, 3, 4- thiadiazol-2-yl)-phenyl acetate (6, 1gm, 4mmoL) in absolute ethanol (10 mL) was added various aldehydes (7) (4 mmoL) followed by the addition of few drops of glacial acetic acid. The reaction mixture was then refluxed for 5 to 48 hrs. After cooling to room temperature reaction mixture was placed overnight inside the refrigerator and the crude solid product precipitated out was filtered, washed with a solution of cold 80% EtOH in $H_2O$ and dried. The crude product was then recrystallized from absolute ethanol to get the Schiff base. (Kavitha *et al.,* 2006.).

**General procedure for the synthesis of (4-(5-(2-(substituted)-4-oxothiazolidin-3-yl)-1, 3, 4-thiadiazol-2-yl) -phenyl-acetate) (10a-10y)**

A mixture of Schiff base (8) (400 mg, 0.1mmoL) and required amount of thioglycolic acid (9) (0.1mmoL, 0.1 ml) in *N, N* –dimethylformamide (DMF) (10 ml), containing a pinch of anhydrous Zinc Chloride ($ZnCl_2$) was refluxed about 6 hours. The reaction mixture was cooled and poured on to the crushed ice. The solid thus obtained was filtered, washed with water and the product was recrystallized with rectified spirit (Mosmann *et al.,* 1983.). The physicochemical characterization of synthesized analogs are presented in table 1.

**(4-(5-(2-(3-Ethoxy-4-hydroxy-phenyl)-4-oxothiazolidin-3-yl)-1, 3, 4-thiadiazol-2-yl)- phenyl-acetate (10a)**

White solid (Yield 68%); mp: 180-182$^0$C, IR (KBr, cm$^{-1}$): 3560 (OH str), 2982 (C-H Ar str), 2876(C-H str), 1715 (C = O str), 1628, 1534, 1465 (C = C Ar str), 1623 (C = N str), 1514 (C-N str), 667 (C-S bend); $^1$H NMR (DMSO-*d*6, 400 MHz): 9.72 (s, 1 H, OH,) 7.78-7.69 (m, 3 H, Aromatic ring A), 7.57-6.70 (m, 3 H, Aromatic ring B,) 6.62 (s, 1H, N-CH), 4.06(dd), 4.21 (dd, 2H of thiazolidinone), 4.20-4.16 (q, 2H, OCH2), 2.54 (s, 3 H of $OCOCH_3$), 1.42 (s, 5H of $C_2H_5$), 1.39-1.34 (t, 3H, CH3) $^{13}$CNMR (DMSO-$d_6$, 75 MHz, δ ppm 196, 174, 172, 170, 148, 147, 141, 138, 132, 128, 124, 118, 115, 113, 112, 68, 65, 54, 42, 35, 20, 15 MS ES + (ToF): *m/z* (M + 1 458.52).

**(4-(5-(2-(3, 4-Diethoxy-phenyl)-4-oxothiazolidin-3-yl)-1, 3, 4-thiadiazol-2-yl) -phenyl-acetate (10b)**

White solid (Yield 75%); mp: 150-152$^0$C, IR (ATR, cm$^{-1}$): 3012(C-H Ar str), 2875(C-H str), 1675 (C = O str), 1635, 1545, 1361 (C=C Ar str), 1644 (C = N str), 1521 (C-N str), 6686 (C-S bend); $^1$H NMR (DMSO-*d*6, 400 MHz): 7.79-7.59 (m, 3 H, Aromatic ring A), 6.59-5.89 (m, 3H, Aromatic ring B), 6.68 (s, 1H, N-CH ), 4.17 (s, 5 H, $OC_2H_5$), 4.20, 3.98 (dd of 2 H of thiazolidinone), 3.27 (s, CH of aromatic aldehyde), 4.18-4.14 (q, J = 6.9 Hz, 2H, CH2); 4.08-4.02 (q, J = 6.9 Hz, 2H, CH2), 1.41-1.39 (m, 6H, 2 × CH3 of ethoxy); $^{13}$CNMR (DMSO-$d_6$, 75 MHz, δ ppm 198, 178, 172, 170, 148, 147, 143, 142, 133, 125, 122, 120, 116, 113, 112, 68, 65, 57, 42, 37, 20, 15; MS ES + (ToF): *m/z* (M+1 486.58).

**(4-(5-(2-(4-allyloxy-3-ethoxy-phenyl)-4-oxothiazolidin-3-yl) -1, 3, 4-thiadiazol-2-yl)- phenyl-acetate (10c)**

White solid (Yield 65%), mp: 180-182$^0$C, IR (ATR, cm$^{-1}$): 3018 (C-H Ar str), 2806 (C-H str), 1748 (C = O str), 1687, 1606, 1511, 1441 (C = C Ar str), 1646(C = N str), 634 (C-S bend); $^1$H NMR (DMSO-*d*6, 400 MHz): 7.46-7.42 (m, 3 H, Aromatic ring A), 6.52-6.36 (m, 3 H, Aromatic ring B,), 6.21 (s, 1H, N-CH ), 6.09-6.07 (m,1 H of $CH_2$ = CH-$CH_2$), 5.44-5.32 (m, $CH_2$ of $CH_2$ = CH-$CH_2$), 4.72-4.60 (m, 2H, O-CH2); 4.12, 3.86 (dd, 2 H of thiazolidinone), 2.38 (s, 3 H of $OCOCH_3$), 4.09-3.98 (q, 2H, CH2-CH3); 1.37-1.32 (t, J = 6.9 Hz, 3H, CH2-CH3); $^{13}$CNMR (DMSO-$d_6$, 75 MHz, δ ppm 187, 172, 162, 154, 149, 142, 135, 133, 132, 129, 122, 121, 118, 114, 112, 74, 71, 65, 56, 39; MS ES+ (ToF): *m/z* (M+1 498.59).

**(4-(5-(2-(4-benzyloxy-3-ethoxy-phenyl)-4-oxothiazolidin-3-yl) -1, 3, 4-thiadiazol-2-yl) -acetate (10d)**

White solid (Yield 68%), mp: 180-182$^0$C, IR (ATR, cm$^{-1}$): 3062 (C-H Ar str), 2845 (C-H str), 1745 (C = O str), 1581, 1463 (C = C Ar str), 1684(C = N str), 1536(C-N str), 668 (C-S bend); $^1$H NMR (DMSO-*d*6, 400 MHz): 7.48-7.32 (m, 3 H, Aromatic ring C), 7.5-7.20 (m, 3 H, Aromatic ring A), 7.67-7.48 (m, 3 H of Aromatic ring B), 6.42 (s, 1H, N-CH); 5.12 (s, O-$CH_2$-); 3.8 (s, 3 H of $OCH_3$), 4.19, 3.92 (dd, 2 H of thiazolidinone), 2.4 (s, 3 H of $OCOCH_3$), 4.09-3.98 (q, 2H, $CH_2$-$CH_3$); 1.37-1.32 (t, J = 6.9 Hz, 3H, CH2-CH3), 5.26 (s, 2H, OCH2); 7.46-7.33 (m, 5H, Ar-H); $^{13}$CNMR (DMSO-$d_6$, 75 MHz, δ ppm 164.90, 162.19, 146.69, 142.02, 136.61, 131.10, 130.23, 124.20, 119.52 ,118.58, 111.54, 111.20, 103.70, 103.60, 50.31, 27.32, 16; MS ES+ (ToF): *m/z* M+1 548.65).

**4-(5-(2-Oxo-2-(1-phenyl-3-*p*-tolyl-*1H*-pyrazol-4-yl))-thiazolidin-3-yl) -1, 3, 4-thiadiazol-2-yl)-phenyl-acetate (10e)**

White solid (Yield 75%), mp: 220-222$^0$C; IR (ATR, cm$^{-1}$): 3026 (C-H Ar str), 2845 (C-H str), 1716 (C = O str), 1675, 1532, 1467 (C = C Ar str), 1642 (C = N str), 1521 (C-N str), 672 (C-S bend); $^1$H NMR (DMSO-*d*6, 400 MHz): 7.98-7.92 (d, J = 7.5 Hz, 2H, Ar-H); 7.62-7.40 (m, 7H, Ar-H); 7.29-7.24 (m, 3H, Ar-H), 6.67 (s,

**Table 1:** Physicochemical Characterization of final synthesized analogs(10a-y).

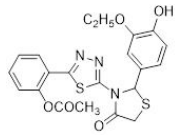
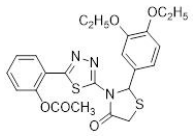
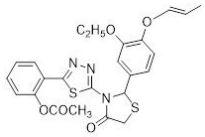
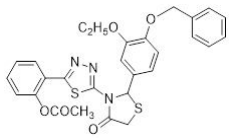
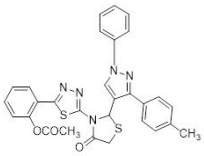
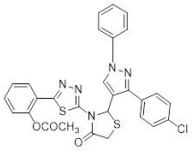
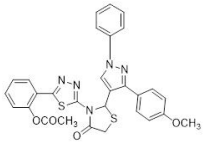
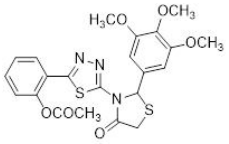
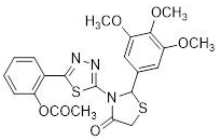
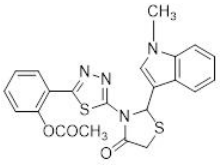
| Sr. No. | Structure | Mol formula | Mol. wt | TLC |
|---|---|---|---|---|
| 1 |  | $C_{21}H_{19}N_3O_5S_2$ | 457.52 | 0.45 (Ethyl acetate: Hexane; 2:5) |
| 2 |  | $C_{23}H_{23}N_3O_5S_2$ | 485.58 | 0.42(Ethyl acetate: Hexane; 2:5) |
| 3 |  | $C_{24}H_{23}N_3O_5S_2$ | 497.59 | 0.5 (Ethyl acetate: Hexane; 2:5) |
| 4 |  | $C_{28}H_{25}N_3O_5S_2$ | 547.65 | 0.6(Ethylacetate: Hexane; 2:5 |
| 5 |  | $C_{29}H_{23}N_5O_3S_2$ | 583.72 | 0.55 (Ethylacetate : Hexane |
| 6 |  | $C_{28}H_{20}N_5O_3S_2Cl$ | 574.07 | 0.6 (Ethylacetate : Hexane |
| 7 |  | $C_{29}H_{23}N_5O_4S_2$ | 569.65 | 0.58 (Ethylacetate : Hexane |
| 8 |  | $C_{22}H_{21}N_3O_6S_2$ | 487.55 | 0.5 (Ethylacetate : Hexane |
| 9 |  | $C_{21}H_{16}N_4O_3S2$ | 436.51 | 0.6 (Ethylacetate : Hexane |
| 10 |  | $C_{22}H_{18}N_4O_3S_2$ | 450.53 | 0.55 (Ethylacetate : Hexane |

***Table 1 Continued.....***

***Table 1 Continued.....***

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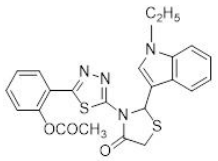
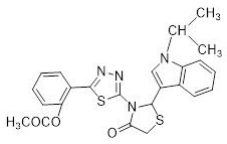
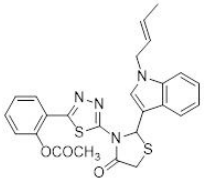
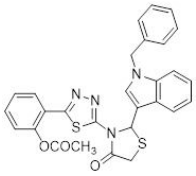
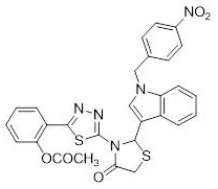
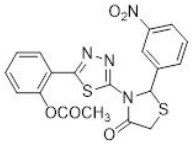
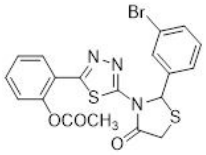
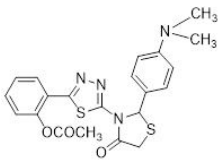
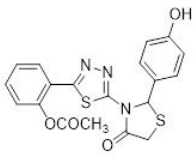
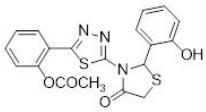
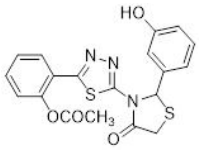
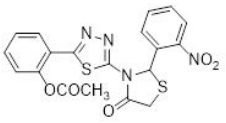
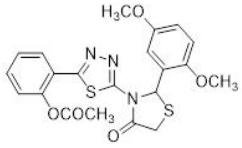
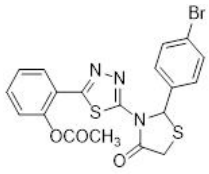
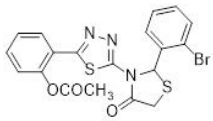
|---|---|---|---|---|
| 11 |  | $C_{23}H_{20}N_4O_3S_2$ | 464.56 | 0.58 (Ethylacetate : Hexane |
| 12 |  | $C_{24}H_{22}N_4O_3S_2$ | 478.59 | 0.56 (Ethylacetate : Hexane |
| 13 |  | $C_{25}H_{22}N_4O_3S_2$ | 490.60 | 0.55 (Ethylacetate : Hexane |
| 14 |  | $C_{28}H_{22}N_4O_3S_2$ | 526.63 | 0.45 (Ethylacetate : Hexane |
| 15 |  | $C_{28}H_{21}N_5O_5S_2$ | 571.63 | 0.5(Ethylacetate : Hexane |
| 16 |  | $C_{19}H_{14}N_4O_5S_2$ | 442.47 | 0.55 (Ethylacetate : Hexane |
| 17 |  | $C_{19}H_{14}N_3O_3S_2Br$ | 506.44 | 0.6 (Ethylacetate : Hexane |
| 18 |  | $C_{21}H_{20}N_4O_3S_2$ | 440.54 | 0.52 (Ethylacetate :Hexane |
| 19 |  | $C_{19}H_{15}N_3O_4S_2$ | 413.47 | 0.53 (Ethylacetate : Hexane |

***Table 1 Continued.....***

***Table 1 Continued.....***

| | | | | |
|---|---|---|---|---|
| 20 |  | $C_{19}H_{15}N_3O_4S_2$ | 413.47 | 0.52 (Ethylacetate : Hexane |
| 21 |  | $C_{19}H_{15}N_3O_4S_2$ | 413.47 | 0.58 (Ethylacetate : Hexane |
| 22 |  | $C_{19}H_{14}N_4O_5S_2$ | 442.47 | 0.5 (Ethylacetate : Hexane |
| 23 |  | $C_{21}H_{19}N_3O_5S_2$ | 457.52 | 0.6 (Ethylacetate : Hexane |
| 24 |  | $C_{19}H_{14}N_3O_3Br$ | 476.37 | 0.55 (Ethylacetate : Hexane |
| 25 |  | $C_{19}H_{14}N_3O_3S_2Br$ | 476.37 | 0.52 (Ethylacetate : Hexane |

1H, N-CH ), 5.63 (s, 1H, C5 of pyrazol H); 4.21, 3.98 (dd, 2 H of thiazolidinone), 2.34 (s, 3H, $CH_3$), 2.29 (s, 3 H of $OCOCH_3$); $^{13}$CNMR (DMSO-$d_6$, 75 MHz, δ ppm 162.70, 153.66, 149.39, 147.90, 139.72, 133.02, 131.15, 130.942, 130.18, 129.22, 125.98, 105.70, 105.04, 103.84, 63.31, 61.48, 60.82, 59.65, 59.07, 57.39,57.04, 55.73, 55.37, 40.75, 40.47, 40.20, 39..92, 39.64, 39.36, 39.08, MS ES+ (ToF): *m/z* (M+1 584.72).

**4-(5-(2-(3-(4-Chloro-phenyl)-1-phenyl-*1H*-pyrazol-4-yl)-4-oxothiazolidin-3-yl) -1, 3, 4-thiadiazol-2-yl)-phenyl-acetate (10f)**

White solid (Yield 72%), mp: 224-222$^0$C; IR (ATR, cm$^{-1}$): 3062 (C-H Ar str), 2838 (C-H str), 1721 (C = O str), 1675, 1535, 1452 (C = C Ar str), 1642 (C = N str), 1521 (C-N str), 741 cm$^{-1}$ (C-Cl); 666 (C-S bend), $^1$H NMR (DMSO-*d*6, 400 MHz): 7.96-7.91 (d, J = 7.5 Hz, 2H, Ar-H); 7.58-7.46 (m, 7H, Ar-H); 7.26(m, 3 Ar-H), 6.65 (s, 1H, N-CH ), 5.21 (s, 1H, $C_5$ of pyrazol H); 4.11, 3.96 (dd, 2 H of thiazolidinone), 2.28 (s, 3 H of $OCOCH_3$); $^{13}$CNMR (DMSO-$d_6$, 75 MHz, δ ppm 166.11, 161.11, 154.68, 144.97, 136.98, 135.33, 131.51, 129.95, 129.17, 128.81, 128.59, 128.40, 128.08, 127.85, 127.61, 125.07, 122.64, 120.04, 119.43, 117.64, 115.73, 40.55, 40.27, 40.00, 39.72,39.44, 39.16, 38.88, 36.09; MS ES+ (ToF): *m/z* (M+1 575.07).

**4-(5-(2-(3-(4-Methoxy-phenyl)-1-phenyl-1H-pyrazol-4-yl)4-oxo-thiazolidin-3-yl) -1, 3, 4-thiadiazol -2-yl)-phenyl-acetate (10g)**

White solid (Yield 71%), mp: 200-202$^0$C; IR (ATR, cm$^{-1}$): 3028 (C-H Ar str), 2835 (C-H str), 1712 (C = O str), 1685, 1526, 1456 (C = C Ar str), 1640(C = N str), 1519 (C-N str), 1267 (C-O). 673(C-S bend), $^1$H NMR (DMSO-*d*6, 400 MHz): 7.85-7.05 (m, 9H, Ar-H); 3.85 (s, 3H, -OCH3); 7.29-7.24(m, 3 Ar-H), 6.67 (s, 1H, N-CH ), 5.70 (s, 1H, C5 of pyrazole H); 4.21, 3.98 (dd, 2 H of thiazolidinone), 2.29 (s, 3 H of $OCOCH_3$); $^{13}$CNMR (DMSO-$d_6$, 75 MHz, δ ppm 164.25, 161.74, 147.61, 145.92 , 144.23, 142.54, 136.78, 129.99, 128.89, 124.73, 124.42, 123.92, 118.82, 112.46, 111.30, 104.60, 104.05, 50.23 27.27 ; MS ES+ (ToF): *m/z* (M+1 570.65)

**4-(5-(4-Oxo-2-(3,4,5-trimethoxy—phenyl)-thiazolidin-3-yl) -1, 3, 4-thiadiazol-2-yl)-phenyl-**

**acetate (10h)**

Yellow solid (yield 80%), mp: 160-163$^0$C; IR (ATR, cm$^{-1}$): 3140 (aromatic C-H), 2981 (aliphatic C-H), 1710 (C = O), 1543 (C = C), 1267 (C-O), 678(C-S bend); ), $^1$H NMR (DMSO-*d*6, 400 MHz): 7.29-7.24 (m, 3 Ar-H), 7.15-7.13(m, 2H, Aromatic H), 6.28(s, 1H, N-CH), 3.89, 3.81-3.76(m,9H, OCH3), 4.21, 4.11 (dd, 2 H of thiazolidinone), 2.29 (s, 3 H of $OCOCH_3$), $^{13}$CNMR (DMSO-$d_6$, 75 MHz, δ ppm 164.22, 158.31, 156.66, 149.82, 146.7, 133.34, 131.10, 131.8, 129.38, 129.20, 128.56, 128.28, 127. 91, 124.37, 124.09, 123.810, 121.99, 121.493, 121.125, 119.31, 119.19, 118.90, 110.23, 1088.90, 40.58, 40.30, 40.02, 39.74, 39.46, 39.18, 38.90, 34.31; MS ES+ (ToF): *m/z* (M+1 488.55).

**4-(5-(2-(1*H*-indol-3yl)-4-oxo-thiazolidin-3-yl)-1, 3, 41, 3, 4-thiadiazol-2-yl)-phenyl-acetate (10i)**

White solid (75%), mp: 172-174$^0$C; IR (KBr, cm$^{-1}$): 3321 (indole NH), 2995 (aromatic C-H), 1712 (C = O), 1545 (C = C), 1265 (C-O), 654 (C-S bend); $^1$H NMR (DMSO-*d*6, 400 MHz): 10.6 (s, 1H, NH of indole), 9.22 (s, 1H, Ar-H); 7.86-7.82 (m, 1H, Ar-H); 7.58-7.54 (m, 1H, Ar-H); 7.30-7.29 (m, 2H, Ar-H); 7.29-7.27 (m, 3 Ar-H), 6.28 (s, 1H, N-CH), 4.15, 3.9 (dd, 2 H of thiazolidinone), 2.29 (s, 3 H of $OCOCH_3$), $^{13}$CNMR (DMSO-$d_6$, 75 MHz, δ ppm 162.65, 162.59, 131.71, 131.20, 130.35, 130.12, 129.69, 116.40, 115.95, 115.49, 115.02, 114.57, 114.11, 113.64, 113.18, 112.72, 112.27, 111.80, 77.42, 77.01, 76.58, 67.05, 66.11, 65.13, 58.12, 57.62, 57.52, 57.11, 55.89, 54.98, 54.06, 26.90, 26.34, 26.19, 25.81, 25.02, 24.76, 24.03, 23.12; MS ES+ (ToF): *m/z* (M+1 437.51).

**4-(5-(2-(1-methyl-indol-3yl)-4-oxo-thiazolidin-3-yl) -1, 3, 4-thiadiazol-2-yl)-phenyl-acetate (10j)**

White solid (72%), mp 187-189$^0$C; IR (KBr, cm$^{-1}$): 3152 (aromatic C-H), 2967 (aliphatic C-H), 1740 (C = O), 1575 (aromatic C = C), 1146 (C-O). $^1$H NMR (DMSO-*d*6, 400 MHz): 9.22 (s, 1H, Ar-H); 7.86-7.84 (m, 1H, Ar-H); 7.59-7.57 (m, 1H, Ar-H); 7.30-7.29 (m, 2H, Ar-H); 7.28-7.24 (m, 3 Ar-H),6.29(s, 1H, N-CH), 4.12 (s, 3H, N-CH3); 4.15, 3.9 (dd, 2 H of thiazolidinone), 2.28 (s, 3 H of $OCOCH_3$); $^{13}$CNMR (DMSO-$d_6$, 75 MHz, δ ppm 160.19, 138.06, 130.87, 129.49, 129.16, 128.65, 128.47, 128.19, 122.91, 121.43, 113.44, 55.85, 40.58, 40.31, 40.03, 39.75, 39.47, 39.19, 38.91; MS ES+ (ToF): *m/z* (M+1 451.53).

**4-(5-(2-(1-ethyl-indol-3yl)-4-oxo-thiazolidin-3-yl) -1, 3, 4-thiadiazol-2-yl)-phenyl-acetate (10k)**

White solid (75%), mp 180-182$^0$C; IR (KBr, cm$^{-1}$): 3028 (aromatic C-H), 2982 (aliphatic C-H), 1721 (C = O), 1556 (C = C), 1246 (C-O), 664(C-S bend); $^1$H NMR (DMSO-*d*6, 400 MHz): 9.28 (s, 1H, Ar-H); 7.83-7.80 (m, 1H, Ar-H); 7.54-7.52 (m, 1H, Ar-H); 7.37-7.34 (m, 2H, Ar-H); 7.29-7.26 (m, 3 Ar-H), 7.29-7.24 (m, 3 Ar-H), 6.29 (s, 1H, N-CH), 4.46-4.44 (q, J = 6.9 Hz, 2H, $CH_2$); 4.15, 3.9 (dd, 2 H of thiazolidinone), 2.27 (s, 3 H of $OCOCH_3$), 1.49-1.44 (t, J = 6.9 Hz, 3H, $CH_3$). $^{13}$CNMR (DMSO-$d_6$, 75 MHz, δ ppm 162.52, 151.41, 149.75, 140.73, 136.33, 133.01, 131.18, 130.95, 130.43, 130.16, 129.19, 128.42, 126.64, 125.96, 40.76, 40.48, 40.21, 39.93, 39.65, 39.37, 39.10. MS ES+ (ToF): *m/z* (M+1 465.56).

**4-(5-(2-(1-isopropyl-indol-3yl)-4-oxo-thiazolidin-3-yl) -1, 3, 4-thiadiazol-2-yl)-phenyl-acetate (10l)**

White solid (75%), mp 165-167$^0$C; IR (KBr, cm$^{-1}$): 3131 (aromatic C-H stretching), 2875 (aliphatic C-H stretching), 1679 (C = O), 1596 (C = C), 1265 (C-O), 658(C-S bend); $^1$H NMR (DMSO-*d*6, 400 MHz): 9.48 (s, 1H, indole H); 7.93-7.92 (m, 1H, Ar-H); 7.78-7.76 (m, 1H, Ar-H); 7.38-7.36(m, 2H, Ar-H); 7.28-7.24 (m, 3 Ar-H), 4.95-4.93 (m, 1H, CH); 1.58-1.54 (m, 6H, 2 × $CH_3$ of isopropyl), 5.93 (s, 1H, N-CH), 4.15, 3.8 (dd, 2 H of thiazolidinone), 2.29 (s, 3 H of $OCOCH_3$). $^{13}$CNMR (DMSO-$d_6$, 75 MHz, δ ppm 167.16, 151.84, 151.07, 147.41, 146.90, 140.47, 134.88, 131.10, 129.16, 128.68, 128.26, 127.86, 124.69, 124.46, 124.15, 123.81, 123.81, 123.56, 121.92, 61.08, 41.86, 40.57, 40.29, 40.08, 39.73, 39.46, 39.18, 38.90, 15.52 MS ES+ (ToF): *m/z* (M+1 479.59).

**4-(5-(2-(1-allyl-1-*H*-indol-3yl)-4-oxo-thiazolidin-3-yl) -1, 3, 4-thiadiazol-2-yl)- -phenyl-acetate (10m)**

White solid, (Yield 65%), mp 185-187$^0$C; IR (KBr, cm$^{-1}$): 3154 (aromatic C-H), 2971 (aliphatic C-H), 1718 (C=O), 1548 (aromatic C=C), 1276 (C-O), 664(C-S bend) $^1$H NMR (DMSO-*d*6, 400 MHz): 9.21 (s, 1H, Ar-H); 7.95-7.94 (m, 1H, Ar-H); 7.69-7.68 (m, 1H, Ar-H); 7.42-7.40 (m, 2H, Ar-H); 7 .28-7.22 (m, 3 Ar-H),. 6.09-6.06 (m, 1H, -CH = $CH_2$); 5.30-5.20 (m, 2H, = $CH_2$); 5.10-5.09 (m, 2H, -N-$CH_2$); 7.69-7.67 (m, 3H, Ar-H) 5.92(s, 1H, N-CH), 4.12, 3.8 (dd, 2 H of thiazolidinone), 2.74 (s, 3 H of $OCOCH_3$). $^{13}$CNMR (DMSO-$d_6$, 75 MHz, δ ppm 162.51, 149.76, 146.98, 144.65, 138.96, 137.56, 133.04, 132.81, 131.04, 129.23, 126.03, 115.46, 113.50, 111.95, 40.74, 40.46, 40.18, 39.91, 39.63, 39.35, 39.07, 34.89.MS ES+ (ToF): *m/z* (M+1 491.60).

**4-(5-(2-(1-Benzyl-1-*H*-indol-3yl)-4-oxo-thiazolidin-3-yl) -1, 3, 4-thiadiazol-2-yl)- -phenyl-acetate (10n)**

White solid, (yield 68%), mp 222-224$^0$C; IR (KBr, cm$^{-1}$): 3128 (aromatic C-H), 2968, 2922 (aliphatic C-H), 1723, 1696 (C = O), 1565 (C = C), 1272 (C-O). $^1$H NMR (DMSO-*d*6, 400 MHz): 9.29 (s, 1H, Ar-H); 7.92-7.90 (d,

J = 7.6 Hz, 1H, Ar-H); 7.36-7.32 (m, 6H, Ar-H); 7.24-7.20 (m, 2H, Ar-H); 7.29-7.26 (m, 3 Ar-H), 5.42 (s, 2H, -N-CH2-); 5.96(s, 1H, N-CH, 4.12, 3.92 (dd, 2 H of thiazolidinone), 2.79 (s, 3 H of $OCOCH_3$). $^{13}$CNMR (DMSO-$d_6$, 75 MHz, δ ppm 169.54, 163.69, 151.25, 149.49, 148.54, 146.73, 140.97, 140.55, 139.18, 138.63, 137.89, 136.20, 134.29, 132.77, 132.22, 131.77, 131.20, 130.47, 130.11, 129.02, 128.417, 127.33, 126.76, 126.08, 124.90, 124.53, 119.88, 119.32, 40.78, 40.50, 40.22, 39.94, 39.66, 39.39, 39.11; MS ES+ (ToF): *m/z* (M+1 527.63).

**4-(5-(2-(1-(4-nitro-benzyl)-1-*H*-indol-3yl)-4-oxo-thiazolidin-3-yl) -1, 3, 4-thiadiazol-2-yl)- -phenyl-acetate (10o)**

Orange solid, (Yield 65%), mp 220-222$^0$C; IR (KBr, cm$^{-1}$): 3129 (aromatic C-H), 2953 (aliphatic C-H), 1740 (C = O), 1575 (C = C), 1264 (C-O). $^1$H NMR (DMSO-*d*6, 400 MHz): 9.23 (s, 1H, Ar-H); 8.22-8.20 (d, J = 8.4, 2H, nitrophenyl); 7.96-7.94 (m, 1H, Ar-H); 7.60-7.58 (d, 1H Ar-H); 7.53-7.52 (d, J = 8.4, 2H, nitrophenyl), 7.37-7.34 (m, 2H, Ar-H); 7.26-7.24(m,3H, Ar-H); 5.78 (s, 2H, -N-$CH_2$-); 5.84(s, 1H, N-CH, 4.15, 3.8 (dd, 2 H of thiazolidinone), 2.76 (s, 3 H of $OCOCH_3$). $^{13}$CNMR (DMSO-$d_6$, 75 MHz, δ ppm 164.43 (C = O), 161.964 (C = O), 145.820, 139.05, 136.56, 130.00, 124.46, 123.91, 118.72, 112.53, 111.08, 103.84, 103.24, 49.34, 37.22 34.4; MS ES+ (ToF): *m/z* (M+1 572.63).

**4-(5-(2-(3-Nitro-phenyl)-4-oxo-thiazolidin-3-yl) -1, 3, 4-thiadiazol-2-yl)- -phenyl-acetate (10p)**

Yellow solid, (Yield 75%), mp 165-168$^0$C; IR (KBr, cm$^{-1}$): 1565 (C = N str., N = CH), 1318 (C-N str.), 2830 (C-H str., aliphatic), 1432 (C = C str., Ar), 3023 (C-H str., Ar), [1672 (C = O str.), 1214 (C-O str.), 1322(N-O str., sym.), 1274 (C-O), 663 (C-S bending); $^1$H NMR (DMSO-*d*6, 400 MHz): 8.62 (s, 1H, Ar-H); 8.27-8.23 (m, 1H, Ar-H); 8.15-8.13 (d, *J* = 7.8 Hz, 1H, Ar-H); 7.64-7.61 (t, *J* = 7.8 Hz, 1H, Ar-H); 7.24-7.21 (m, 3H, Ar-H); 5.95(s, 1H, N-CH, 4.12, 3.8 (dd, 2 H of thiazolidinone), 2.69 (s, 3 H of $OCOCH_3$). $^{13}$CNMR (DMSO-$d_6$, 75 MHz, δ ppm 167.13, 146.03, 143.52, 135.52, 134.38, 132.74, 132.11, 131.70, 130.02, 129.49, 129.17, 128.68, 128.27, 122.65, 100.44, 97.56, 95.01, 93.09, 67.49, 40.61, 40.34, 40.06, 39.78, 39.50, 39.22, 38.95, 38.60, 27.10; MS ES+ (ToF): *m/z* (M+1 443.47).

**4-(5-(2-(3-Bromo-phenyl)-4-oxo-thiazolidin-3-yl) -1, 3, 4-thiadiazol-2-yl)-phenyl-acetate (10q)**

Yellow solid, (Yield 78%), mp 175-178$^0$C; IR (KBr, cm$^{-1}$): 1646 (C = N str.,), 1272 (C-N str.), , 2968 (C-H str., aliphatic), 1532 (C = C str., Ar), 3065 (C-H str., Ar), 667 (C-Br str., Ar). $^1$H NMR (DMSO-*d*6, 400 MHz): 8.21-7.81 (m, 2H, Ar-H); 7.59-7.57 (d, *J* = 7.8 and 8.1Hz, 1H, Ar-H); 7.40-7.38 (dd, *J* = 7.8 Hz, 1H, Ar-H); 7.29-7.25 (m, 3H), 5.97 (s, 1H, N-CH, 4.15, 4.03 (dd, 2 H of thiazolidinone), 2.69 (s, 3 H of $OCOCH_3$). $^{13}$CNMR (DMSO-$d_6$, 75 MHz, δ ppm 164.11, 160.62, 158.26, 154.19, 148.54, 136.04, 132.13, 128.68, 128.14, 127.01, 125.26, 117.52, 112.81, 110.64, 104.13, 77.50, 77.08, 76.65, 70.75, 64.66, 27.46, 14.63; . MS ES+ (ToF): *m/z* (M+1 507.44).

**2-Methoxy-4-(5-(2-(4-dimethylamino-phenyl)-4-oxo-thiazolidin-3-yl) -1, 3, 4-thiadiazol-2-yl)- -phenyl-acetate (10r)**

Orange solid, (Yield 78%), mp 166-162$^0$C; IR (KBr, cm$^{-1}$): 1652 (C = N str.), 1343 (C-N str.), 2842 (C-H str., aliphatic), 1422 (C = C str., Ar), 3046 (C-H str., Ar), 1734 (C = O str.), 1282 (C-O str.), 657 (C-S bending), $^1$H NMR (DMSO-*d*6, 400 MHz): 7.67-7.53 (d, *J* = 8.4 Hz, 2H, Ar-H); 6.75-6.72 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.04-7.02 (m, 2H, Ar-H), 6.96-6.95 (m, 1H, Ar-H), 5.92 (s, 1H, N-CH, 3.84, 4.14 (dd, 2 H of thiazolidinone), 2.97 (bs, 6H, 2 × $CH_3$); 2.62(s, 3 H of $OCOCH_3$). $^{13}$CNMR (DMSO-$d_6$, 75 MHz, δ ppm 161.68, 160.52, 150.51, 147.54, 133.96, 132.14, 127.67, 126.48, 125.94, 124.68, 122.80, 112.54, 64.25, 41.34, 40.77, 40.49, 40.21, 39.3, 39.66, 39.38, 39.11, 14.54. MS ES+ (ToF): *m/z* (M+1 441.54).

**4-(5-(2-(4-Hydroxy-phenyl)-4-oxo-thiazolidin-3-yl) -1, 3, 4-thiadiazol-2-yl)-phenyl-acetate (10s)**

White solid (Yield 65%), mp 182-184$^0$C; IR (KBr, cm$^{-1}$):1656 (C = N str., N = CH), 1375 (C-N str.), 2826 (C-H str., aliphatic), 1584 (C = C str., Ar), 3076 (C-H str., Ar), 3362 (O-H str., Ar); $^1$H NMR (DMSO-*d*6, 400 MHz): 9.64 (s, 1H, OH); 7.36-7.34 (m, 2H, Ar-H); 7.32-7.28 (m, 3H, Ar-H); 6.84-6.820 (d, *J* = 7.5 Hz, 2H, Ar-H); 5.92 (s, 1H, N-CH, 3.92, (dd), 4.14 (dd, 2 H of thiazolidinone), 2.64 (s, 3 H of $OCOCH_3$). $^{13}$CNMR (DMSO-$d_6$, 75 MHz, δ ppm, 169, 139, 156, 57, 114, 135, 123, 120, 166, 138, 167, 37, 49, 125, 155, 57, 56, 114, 156, 116, 16; MS ES+ (ToF): *m/z* (M+1 414.47).

**4-(5-(2-(2-Hydroxy-phenyl)-4-oxo-thiazolidin-3-yl) -1, 3, 4-thiadiazol-2-yl)-phenyl-acetate (10t)**

White solid (Yield 62%), mp 178-180$^0$C; IR (KBr, cm$^{-1}$): 1653 (C = N str.,), 1354 (C-N str.), 2828 (C-H str., aliphatic), 1586 (C = C str., Ar), 3072 (C-H str., Ar), 3365 (O-H str., Ar); $^1$H NMR (DMSO-*d*6, 400 MHz): 9.48 (s, 1H, OH); 6.83-6.81(d, *J* = 7.5 Hz, 1H, Ar-H); 7.04-7.02 (d, 1H, Ar-H), 6.96-6.94 (m, 2H, Ar-H), 7.31-7.28 (m, 3H, Ar-H), 5.96 (s, 1H, N-CH, 3.98, (dd), 4.12 (dd, 2 H of thiazolidinone), 2.29 (s, 3 H of $OCOCH_3$). $^{13}$CNMR (DMSO-$d_6$, 75 MHz, δ ppm 169, 139, 156, 57, 114, 135, 123, 120, 166, 138, 167, 37, 49, 125, 155, 57, 56,

114, 156, 116, 16; MS ES+ (ToF): *m/z* (M+1 414.47).

**4-(5-(2-(3-Hydroxy-phenyl)-4-oxo-thiazolidin-3-yl) -1, 3, 4-thiadiazol-2-yl)-phenyl-acetate (10u)**

White solid, Yield 75%), mp 170-173$^0$C; $^1$H NMR (DMSO-*d*6, 400 MHz): 9.47 (s, 1H, OH); 6.82-6.81 (d, *J* = 7.5 Hz, 2H, Ar-H); 7.02-7.01 (d, 1H, Ar-H), 6.96-6.94 (m, 1H, Ar-H), 7.31-7.28 (m, 3H, Ar-H), 5.96 (s, 1H, N-CH, 3.98, (dd), 4.13 (dd, 2 H of thiazolidinone), 2.29 (s, 3 H of $OCOCH_3$). $^{13}$CNMR (DMSO-$d_6$, 75 MHz, δ ppm 161.68, 160.52, 150.51, 147.54, 133.96, 132.14, 127.67, 126.48, 125.94, 124.68, 122.80, 112.54, 64.25, 41.34, 40.77, 40.49, 40.21, 39.93, 39.66, 39.38, 39.11, 14.5; MS ES+ (ToF): *m/z* (M+1 414.47).

**4-(5-(2-(2-Nitro-phenyl)-4-oxo-thiazolidin-3-yl) -1, 3, 4-thiadiazol-2-yl)- -phenyl-acetate (10v)**

White solid, Yield (75%), mp 180-182$^0$C; IR (KBr, cm$^{-1}$): 1665 (C = N str.,), 1342 (C-N str.), 2965 (C-H str., aliphatic), 1424 (C = C str., Ar), 3088 (C-H str., Ar), 1529 (N-O str, $^1$H NMR (DMSO-*d*6, 400 MHz): 8.64-8.63 (d, 1H, Ar-H); 8.29- 8.25 (m, 2H, Ar-H); 8.13-8.10 (d, *J* = 7.8 Hz, 1H, Ar-H); 7.25-7.22 (m, 3H, Ar-H); 5.93 (s, 1H, N-CH, 4.15, 3.8 (dd, 2 H of thiazolidinone), 2.64 (s, 3 H of $OCOCH_3$); $^{13}$CNMR (DMSO-$d_6$, 75 MHz, δ ppm 162.01, 149.51, 148.22, 140.56, 136.01, 135.89, 133.86, 132.55, 131.91, 131.46, 130.88, 130.44, 129.72, 129.11, 128.36, 128.18, 126.87, 126.41, 122.18, 40.76, 40.49, 40.21, 39.93, 39.65, 39.37, 39.09, 22.39, 21.50, 20.59, 13.37; MS ES+ (ToF): *m/z* (M+1 443.47).

**4-(5-(2-(2, 5-Dimethoxy-phenyl)-4-oxo-thiazolidin-3-yl) -1, 3, 4-thiadiazol-2-yl)-phenyl-acetate (10w)**

White solid, Yield (75%), mp 160-162$^0$C; IR (KBr, cm$^{-1}$): 1582 (C = N str., N = CH), 1384(C-N str.), 3318(N-H str.), 2834 (C-H str., aliphatic), 1428 (C = C str., Ar), 3028 (C-H str., Ar), 1126 (C-O-C str., -$OCH_3$). , $^1$H NMR (DMSO-*d*6, 400 MHz): 7.80-7.77 (m, 1H, Ar-H); 7.29-7.26 (m, 3H, Ar-H); 7.06-6.98 (m, 2H, Ar-H); 3.81-3.75 (m, 6H, 2 × $OCH_3$); 5.97(s, 1H, N-CH, 3.98, (dd), 4.13 (dd, 2 H of thiazolidinone), 2.57 (s, 3 H of $OCOCH_3$). $^{13}$CNMR (DMSO-$d_6$, 75 MHz, δ ppm 164.11, 160.62, 158.26, 154.19, 148.54, 136.04, 132.13, 128.68, 128.14, 127.01, 125.26, 117.52, 112.81, 110.64, 104.13, 77.50, 77.08, 76.65, 70.75, 64.66, 27.46, 14.63; MS ES+ (ToF): *m/z* (M+1 458.52).

**4-(5-(2-(4-Bromo-phenyl)-4-oxo-thiazolidin-3-yl) -1, 3, 4-thiadiazol-2-yl)-phenyl-acetate (10x)**

Yellow solid, Yield 75%, mp 170-172$^0$C; IR (KBr, cm$^{-1}$): 1646 (C = N str.,), 1272 (C-N str.), 2984 (C-H str., aliphatic), 1558 (C = C str., Ar), 3086 (C-H str., Ar), 659 (C-Br str.Ar). $^1$H NMR (DMSO-*d*6, 400 MHz): 7.58-7.54 (d, *J* = 7.8 and 8.1Hz, 2H, Ar-H); 7.38-7.36 (d, *J* = 7.8 Hz, 2H, Ar-H); 7.29-7.26(m,3H), 5.96(s, 1H, N-CH, 4.15, 4.01 (dd, 2 H of thiazolidinone), 3.72 (s, 3H of OCH3), 2.29 (s, 3 H of $OCOCH_3$). $^{13}$CNMR (DMSO-$d_6$, 75 MHz, δ ppm 164.25, 161.74, 147.61, 145.91, 144.23, 142.53, 136.77, 129.99, 128.89, 128.40, 124.73, 124.42, 123.92, 118.82, 112.46, 111.29, 104.60, 104.04, 50.23, 40.82, 40.54, 40.26, 39.99, 39.71, 39.43, 39.15, 27.27. MS ES+ (ToF): *m/z* (M+1 477.37).

**4-(5-(2-Bromo-phenyl)-4-oxo-thiazolidin-3-yl) -1, 3, 4-thiadiazol-2-yl)- phenyl-acetate (10y)**

White solid, Yield 65%, mp 165-167$^0$C; IR (KBr, cm$^{-1}$): 1592 (C = N str.,), 1321 (C-N str.), 3330 (N-H str.), 2893 (C-H str., aliphatic), 1511 (C = C str) Ar), 3042 (C-H str., Ar), 658(C-Br str-Ar). $^1$H NMR (DMSO-*d*6, 400 MHz): 7.38-7.35 (d, 1H, Ar-H), 7.29-7.26 (m, 3H) 7.06-7.01 (m, 2H, Ar-H), 6.98- 6.96 (d, 1H, Ar-H), 5.92 (s, 1H, N-CH, 3.98, (dd), 4.13 (dd, 2 H of thiazolidinone), 2.29 (s, 3 H of $OCOCH_3$). $^{13}$CNMR (DMSO-$d_6$, 75 MHz, δ ppm 163.72, 160.63, 157.39, 154.04, 148.56, 131.78, 124.79, 116.86, 112.38, 111.47, 104.49, 64.70, 55.93, 40.80, 40.52, 40.25, 39.97, 39.69, 39.41, 39.13, 27.32, 14.88 MS ES+ (ToF): *m/z* (M+1 477.37).

## Results and Discussion

The synthesis of the final analogs (5-(2-substituted)-4-oxothiazolidin-3-yl) -1, 3, 4-thiadiazol-2-yl)-phenyl-acetate derivatives 10a-10y was outlined in experimental section, starting from the readily available Salicylaldehyde (1) which is firstly protected with acetyl chloride (2) in the presence of pyridine to give up acetylated Salicylaldehyde (3) that undergoes in reaction with thiosemicarbazide (4) to synthesize thiosemicabazone (5), the formed thiosemicarbazone (6) on cyclization with ferric chloride to form thiadiazole amine (7), the free amine group available on thiadiazole moiety is treated with different substituted pyrazole, Indole and benzaldehydes to synthesize Schiff base (8), the synthesized Schiff base (8) on further cyclization with thioglycolic acid (9) give 4-thiazolidinone substituted derivatives or final derivatives (10a-10y). All the synthesized derivatives were characterized by different spectral means nuclear magnetic resonance (NMR), infrared [IR], and mass spectroscopy (ESI-MS). All the spectral data of the synthesized compounds were in full agreement with the proposed structures.

The IR spectra of synthesized derivatives (10a-y) exhibited the characteristic N-H, C-N and C = NH absorption bands at 1535-1656 cm$^{-1}$, 1214-1392 cm$^{-1}$ and 3298-3398 cm$^{-1}$ respectively. The appearance of IR stretching vibrations at 1415-1620cm$^{-1}$, 3028-3098 cm$^{-1}$

and 2814-2975 $cm^{-1}$. The appearance of C = O, C-O and O-H group in synthesized compounds may be confirmed by the appearance of IR vibrations at 1658-1735 $cm^{-1}$, 1210-1318 $cm^{-1}$ and 3419-3566 $cm^{-1}$, respectively. The presence of aromatic -Br group in compounds 10x and 10y can be verified by the appearance of C-Br stretching at 652-675 $cm^{-1}$. The Ar-$NO_2$ stretch in compound 10o, 10p, 10v appeared at 1320-1346 $cm^{-1}$ and asymmetric stretch at 1520-1533 $cm^{-1}$ in IR spectra further, the presence of halogen group in the spectra of compounds 10f suggested the presence of Ar-Cl stretching vibrations at 713-743 $cm^{-1}$. The presence of Ar-$OCH_3$ group was confirmed by IR in compound 10g, 10h and 10w around 1120-1124 $cm^{-1}$ in the synthesized compounds The existence of multiplet signals at 8.32-7.90 δ ppm indicated the presence of aromatic protons in the synthesized compounds. the formation of doublets of doublets (dd) at 3.80and 4.16 due to ($CH_2$) group of thiazolidinone confirmed the formation of thiazolidinone ring in the synthesized compounds The appearance of singlet signals around 2.69-2.72 δ ppm pointed out the presence of $OCOCH_3$ group of acetylated Salicylaldehyde . The presence of singlet 9.72 δ ppm indicates the presence of OH group in compound 10a. On the other hand broad signal appeared at 10.8 δ ppm showed the presence of NH of indole ring. In $^{13}$C-NMR peaks due to carbonyl carbons appeared at δ 164.44. Signal due to methylene group ($CH_2$) of thiazolidinone was appeared at δ 145.78. The peaks due to carbons of ethyl group attached to the nitrogen of indole ring were appeared at δ 42.67 and 15.28 respectively. All the spectral characterization of final derivatives confirmed the synthesis of proposed analogs. This derivatives exhibited significant *In vitro* anticancer and antimicrobial results. One of the most remarkable analysis was observed that the group which is electron donating groups attached to the aromatic aldehydes showed more promosing results as comapred to the electron withdrwawing groups.

## Antimicrobial results

Antimicrobial activity of synthesized derivatives was measured in terms of minimum inhibitory concentration (MIC) and presented in table 2.

**Table 2:** Antimicrobial activity of synthesized analogs with MIC value (µg/ml) (10a-y).

| Comp. | Bacterial strains | | | | | Fungal strains | |
|---|---|---|---|---|---|---|---|
| | Gram positive | | | Gram negative | | | |
| | *S. aureus* | *B. subtilis* | *E. coli* | *K. pneumonia* | *S.typhi* | *C. albicans* | *A. niger* |
| 10a | 9.08±0.14 | 5.17± 0.19 | 4.24± 0.18 | 4.24± 0.25 | 4.14± 0.21 | 6.13± 0.39 | 4.40± 0.17 |
| 10b | 3.71±0.16 | 5.89± 0.14 | 3.81± 0.23 | 3.81± 0.27 | 3.81± 0.24 | 5.10± 0.27 | 4.81± 0.19 |
| 10c | 3.78±0.17 | 5.69± 0.23 | 3.69± 0.17 | 3.69± 0.32 | 3.69± 0.18 | 4.69± 0.53 | 4.69± 0.29 |
| 10d | 3.56±0.19 | 5.56± 0.25 | 3.66± 0.13 | 3.56± 0.37 | 3.56± 0.19 | 4.36± 0.34 | 4.36± 0.23 |
| 10e | 4.48±0.17 | 5.48±0.17 | 3.81± 0.21 | 3.81± 0.19 | 3.81± 0.72 | 4.81± 0.40 | 4.81± 0.27 |
| 10f | 3.69±0.24 | 3.69± 0.18 | 3.69± 0.15 | 3.69± 0.27 | 3.69± 0.11 | 4.69± 0.49 | 7.39±0.18 |
| 10g | *3.81± 0.12* | *4.28±0.26* | 3.70± 0.29 | 3.70± 0.21 | 7.61± 0.17 | 4.70± 0.57 | 4.70± 0.34 |
| 10h | *3.61± 0.14* | *4.27±0.12* | 3.47±0.27 | 3.47±0.23 | 3.72± 0.14 | 4.13± 0.39 | 5.10±0.27 |
| 10i | 3.66±0.23 | 5.64± 0.12 | 3.49± 0.27 | 5.47± 0.23 | 5.47± 0.26 | 4.26± 0.29 | 5.10± 0.27 |
| 10j | 4.48±0.21 | 5.48±0.21 | 3.69± 0.14 | 3.69± 0.28 | 3.69± 0.22 | 4.69± 0.37 | 4.69± 0.33 |
| 10k | 4.26±0.19 | 6.26± 0.16 | 4.26± 0.16 | 4.26± 0.15 | 4.26± 0.14 | *3.49±0.28µg* | 4.26± 0.16 |
| 10l | 5.08±0.26 | 6.00± 0.18 | 4.00± 0.30 | 4.00± 0.19 | 4.00± 0.22 | 4.90± 0.47 | 4.90± 0.13 |
| 10m | 3.88±0.29 | 5.88± 0.25 | 3.88± 0.32 | 3.88± 0.22 | 3.88± 0.26 | 4.88± 0.54 | 4.88± 0.25 |
| 10n | 3.61±0.16 | 5.51± 0.24 | 3.71± 0.35 | 3.71± 0.16 | 3.71± 0.31 | 4.51± 0.56 | 4.51± 0.37 |
| 10o | 4.00±0.28 | 7.00± 0.29 | 4.00± 0.27 | 4.00± 0.17 | 4.00± 0.14 | 5.00± 0.52 | 8.01± 0.39 |
| 10p | 7.76±0.17 | 7.88± 0.17 | 3.88± 0.16 | 3.88± 0.13 | 3.88± 0.18 | 4.88± 0. 44 | 5.88± 0.28 |
| 10q | 7.76±0.17 | 6.88± 0.17 | 3.88± 0.16 | 3.88± 0.13 | 3.88± 0.18 | *3.64±0.14* | 5.88± 0.28 |
| 10r | 7.76±0.17 | 5.88± 0.17 | 3.88± 0.16 | 3.88± 0.13 | 3.88± 0.18 | 4.10± 0.39 | 5.88± 0.28 |
| 10s | *3.56±0.26* | *4.25±0.16* | 3.88± 0.16 | 3.88± 0.13 | 3.88± 0.18 | 6.28± 0. 44 | 5.88± 0.28 |
| 10t | 5.76±0.17 | 4.68± 0.12 | 4.68± 0.18 | 4.68± 0.15 | 4.68± 0.12 | 7.38± 0. 26 | 5.68± 0.12 |
| 10u | 6.56±0.24 | 5.88± 0.17 | 3.88± 0.16 | 3.88± 0.13 | 3.28± 0.18 | 4.83± 0. 42 | 7.88± 0.21 |
| 10v | 7.16±0.14 | 6.82± 0.26 | 3.88± 0.16 | 3.88± 0.13 | 4.87± 0.12 | 4.82± 0. 41 | 6.38± 0.26 |
| 10w | 5.76±0.12 | 6.85± 0.25 | 3.88± 0.16 | 3.88± 0.13 | 3.88± 0.14 | *4.13 ± 0.39* | 5.88± 0.24 |
| 10x | 7.12±0.19 | 6.88± 0.17 | 3.88± 0.16 | 3.88± 0.13 | 3.78± 0.16 | 428± 0. 24 | 8.88± 0.21 |
| 10y | 7.26±0.17 | 6.82± 0.27 | 3.88± 0.16 | 3.88± 0.13 | 3.83± 0.28 | 4.21± 0. 34 | 5.28± 0.23 |
| Standard | *3.47±0.12*[a] | *3.47±0.12* [a] | *3.47±0.12*[a] | *3.47±0.12* | *3.47±0.12* [a] | *3.44±0.28* | *3.44±0.28* |

**Table 3:** *In vitro* anticancer activity of the final derivatives (10a-y).

| Analogs | MCF-7 | HeLa |
|---|---|---|
| 10a | 33.62 ± 1.31 | 37.52 ± 1.12 |
| 10b | 31.63 ± 1.34 | 34.12 ±2.14 |
| 10c | 61.88 ± 1.29 | 57.45 ± 1.52 |
| 10d | 29.15 ± 0.92 | 7.44 ± 0.32 |
| 10e | 15.28±0.12 | 29.41 ± 0.67 |
| 10f | 25 ± 0.49 | 18.44 ± 0.22 |
| 10g | 7.19±0.16 | 25 ± 1.35 |
| 10h | 8.24±1.36 | 25 ± 1.35 |
| 10i | 25 ± 1.14 | 13.12 ± 0.38 |
| 10j | 17.18±0.28 | 37.66 ±1.92 |
| 10k | 30.62 ± 1.43 | *3.61±0.12* |
| 10l | 13.12 ± 0.38 | 12.77 ± 0.64 |
| 10m | 15.19 ± 0.31 | 12.42 ± 0.31 |
| 10n | 28.11 ± 0.64 | 9.84 ± 0.82 |
| 10o | 84.85 ± 2.62 | 96.16 ± 1.05 |
| 10p | 96.16 ± 1.05 | 67.80 ± 1.65 |
| 10q | 48.19 ± 1.12 | 61.58 ± 1.84 |
| 10r | 12.19 ± 1.01 | *6.12±0.17* |
| 10s | 9.18±0.24 | 12.42 ± 0.31 |
| 10t | 11.18 ± 1.01 | 31.42 ± 1.64 |
| 10u | 28.11 ± 0.64 | 37.84 ± 0.82 |
| 10v | 68.16 ± 0.83 | 50.14 ± 2.06 |
| 10w | 28.11 ± 0.64 | *6.29±0.14* |
| 10x | 63.65 ± 0.31 | 41.36 ± 0.82 |
| 10y | 18.19 ± 1.32 | 33.15 ± 2.14 |
| Doxorubicin | 2.63 ± 0.37 | 2.63 ± 0.37 |

Compounds 10g (*MIC~4.28 ± 0.26µg/ml*), 10h (*MIC~4.27 ± 0.12µg/ml*), 10s (*MIC~4.25 ± 0.16 µg/ml*) exhibited significant antimicrobial activity against *B. subtilis,* while against *S. aureus* compounds showed identical results with no significant (Unpaired t test, P>0.05) difference as compared to ciprofloxacin (*MIC~3.47 ± 0.12 µg/ml*). On the other hand, compound 10k (*MIC~3.94 ± 0.28 µg/ml*) 10r (*MIC~3.64 ± 0.14 µg/ml*) and 10w (*MIC~4.13 ± 0.39µg/ml*) also exhibited somewhat similar antifungal activity against *C. albicans* as compared to *3.44 ± 0.28µg/ml* of fluconazole with no significantly difference (Unpaired t test, P>0.05). The result disguised that the electron donating group substituted on the ortho, para position of benzene, substituted. Indole and pyrazole aldehydes exhibited promising results as compared to the electron withdrawing substituted group which didn't demonstrated significant results.

### Anticancer results

*In vitro* anticancer activity was performed against two cancer lines (MCF-7and HeLa) and normal cell line (HEK-293) by MTT assay (25) using doxorubicin as a positive control, and the results are tabulated in table 3. From the screening results in table 3 (compounds 10a-10y), it have been seen that majority of compounds exhibited good to moderate anticancer activity against MCF-7 and HeLa. Among all compounds 10g, 10h and 10s exhibited potent activity against MCF-7 with significant $IC_{50}$ values ranging from *7.19 ± 0.16*, *8.24 ± 1.36*, *9.18 ± 0.24* µM Remaining compounds have shown good to moderate ranging from 15.19 ± 1.06 to 34.12 ± 2.14 µM and moderate to poor activity against MCF-7, $IC_{50}$ values ranging from 28.11 ± 0.64 to 95.16 ± 1.04µM, respectively. However compounds 10k, 10r and 10w significant result of $IC_{50}$ value of *3.61 ± 0.12, 6.12 ± 0.17, 6.29 ± 0.14* µM.

## Conclusion

In conclusion, a new series of 4-(5-(2-(substituted)-4-oxothiazolidin-3-yl) -1, 3, 4-thiadiazol-2-yl)-phenyl-acetate) (10a-y) was synthesized from Salicylaldehyde. From the synthesized derivatives three compounds 10g, 10h and 10s exhibited significant activity against MCF- 7 cell lines and gram positive bacterial strains and three compounds 10k, 10r and 10w displayed promising results against HeLa cell line and fungal strains. From the results acquired it can be concluded that 4- thiazolidinone substituted with electron donating group exhibited significant results as compared to the electron withdrawing group. Further structural modifications of the titled Compounds at different unoccupied position which could provide more interesting results and new lead molecules can be generated for good results.

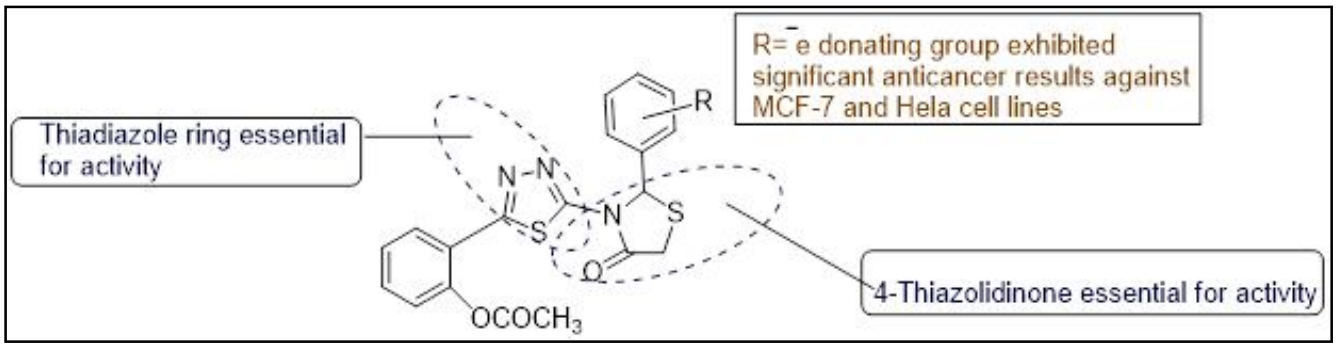



**Fig. 5:** SAR of synthesized derivatives.

**Structure activity relationship (SAR)**

The SAR of synthesized analogs illustrated that thiazolidinone having thiadiazole ring is essential for antimicrobial and anticancer activity. Compounds having Methoxy pyrazole (10g), trimethoxy benzaldehyde (10h), *P*- OH have better antibacterial activity against *B. subtilis* and against MCF-7 cell lines whereas the presence of electron donating group such ethylated indole, *P*- N,N-Dimethyl, 2,5 Trimethoxy on thiazolidinone enhanced therapeutic potential against fungal strains and HeLa cell lines. Fig. 5.

## Acknowledgement

The authors are thankful to the Principal of Rajendra Institute of Technology and Sciences for providing laboratory facilities. The authors are thankful to the head, Department of Pharmaceutics, NIPER, Hyderabad for data of biological activity.

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