Egypt. J. Chem. **Vol. 65**, No. 6 pp. 621 - 645 (2022)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Synthesis, characterization and biological Activityof *β*-Lactam and Thiazolidinone Derivatives Based on Sulfonamide

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Abstract

Several new and know sulfonamide Schiff bases were prepared by the condensation reaction of sulfonamide (*i.e.*2-amino-4-chlorobenzenesulfonamide, sulfamerazine, sulfanilamide, sulfamethazine, sulfathiazole and sulfadiazine) with vanillin and salicylaldehyde, respectively in an acidic medium. These Schiff bases were used to a new series of *β*-lactam (azetidin-2-one) compounds (*i.e.* 4-chloro-2-(2-(4-hydroxy-3-methoxyphenyl)-3-mercapto-4-oxoazetidin-1-yl)benzenesulfonamide , 4-[2-aryl-3-mercapto (or 3-hydroseleno)-4-oxoazetidin-1-yl]-N-substituted benzenesulfonamide; **Z5A$_1$-Z5A$_6$, Z5A$_9$-Z5A$_{12}$, Z5A$_{2'}$, Z5A$_{9'}$-Z5A$_{11'}$**) by their reactions with thioglycolic acid and 2-seleno-glycolic acid, respectively, in presences of phosphorus oxychloride and triethylamine. Cyclocondensation of the Schiff bases with 2-mercaptobutanoic acid in presence of zinc chloride afforded 4-thiazolidinone derivatives (*i.e.* 4-[5-ethyl-2-aryl-4-oxothiazolidin-3-yl]-N-substituted benzenesulfonamide; **ZZ5A$_2$-ZZ5A$_6$, ZZ5A$_9$-ZZ5A$_{12}$**). All new azetidin-2-one and 1,3-thiazolidin-4-onederivatives were characterized by IR, ^{1}H NMR, ^{13}C NMR, mass spectroscopic techniques and elemental analysis. The toxicity of new compounds was assayed via the determination of their LD_{50} value by using Dixon's up and down method. The antibacterial activity of azetidin-2-onecompounds were tested *in vitro* against *Staphylococcus aureus*, *Bacillus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Furthermore, the antioxidant and anticancer efficiency of compounds were evaluated.

Keywords: Antibacterial activity; Anticancer activity; Antioxidant;Acute toxicity; Azetidin-2-one;Sulfonamide; Thiazolidin-4-one.

1. Introduction

Sulfonamides are the first effective chemotherapeutic agents used for bacterial disease in humans. They are widely used for prophylaxis and treatment of bacterial infections although they are bacteriostatic rather than bactericidal. Their value lies in the ability to slow down or prevent growth in wounds or infected organs without appreciable toxicity to normal tissues.[1] A large number of sulfonamide derivatives were synthesized, which made it possible to establish a correlation between specific structural characteristics and the antimicrobial activity of newly synthesized molecules. A free aromatic NH_2 group in the para position, relative to the sulfonamide group, is essential for the activity of sulfonamides.[2] The presence of the additional substituent in the ortho and meta position of the benzene ring reduces the sulfonamide activity. On the other hand, the N1-monosubstituted derivatives of sulfanilamide produce active compounds. The activity degree of such compounds increased by introducing heteroaromatic substituents. The introduction of various substituents resulted in the products with different physicochemical, pharmacokinetic (a degree of protein binding, metabolism, excretion), and pharmacodynamic properties.[3] Recent studies demonstrated that sulphonamides are ready to prevent cancerous cells.[4]

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Receive Date: 20 November, Revise Date: 10 December 2021, Accept Date: 12 December 2021

DOI: 10.21608/EJCHEM.2021.106965.4912

Beta-lactams (2-azetidinones) are Saturated four-membered ring heterocyclic compounds containing three carbon atoms, nitrogen atom and carbonyl group.[5] The name "*β*-Lactam" is given to cyclic amides because the nitrogen atom is associated with the *β*-carbon atom relative to the carbonyl group.

β-Lactams, being a structural unit found in the most widely used antibiotics,[6] have occupied a basic position in medicinal chemistry for almost a century now. With the microbe's basic position in medicinal chemistry for almost a century now. With the microbes responding to the traditional antibiotics through *β*-lactamases, the need for novel antibiotics prevails, making the synthesis of newer *β*-lactams ever more important. In addition to their use as antibiotics, *β*-lactams are increasingly being used as synthons for other biologically important molecules.[7-10] *β*-Lactams have been found to act as cholesterol acyl transferase inhibitors, thrombin inhibitors, human cytomegalovirus protease inhibitors, matrix metalloprotease inhibitors, cysteine protease, and apoptosis inductors.[6] The biological activity is usually associated with the nature of the groups linked to N-1, C-3 and C-4 of the *β*-lactam molecules.[11] 2-Azetidinone derivatives containing *β*-lactam nucleus have a wide range of pharmaceutical activity and become an integral part of the chemotherapeutic arsenal available to today's medical practitioners.[12]

Thiazolidin-4-ones are thiazolidine derivatives and have an atom of sulfur at position 1, an atom of nitrogen at position 3 and a carbonyl group at position 4.[13] However, thiazolidinone derivatives belong to the most frequently studied moieties and its presence in penicillin was the first recognition of its occurrence in nature.[14] Thiazolidin-4-ones and their derivatives are an important class of compounds in organic and medicinal chemistry.[15] The thiazolidin-4-one ring system is a core structure in various synthetic pharmaceutical agents, displaying a broad spectrum of biological activities such as antitubercular, antibacterial, anti-inflammatory, antioxidant agents, antiviral agents, especially as anti-HIV agents, and their use as anticancer drugs.[6, 13, 16] They received considerable attention during the last two decades as they are gifted with a variety of activities and have a wide range of therapeutic properties.[15]

In the present work, a new series of *β*-lactam and thiazolidin-4-one derivatives have been synthesized by cycloaddition reaction of Schiff's bases with ketene and 2-mercaptobutanoic acid, respectively. The compounds were studied in vivo acute toxicity, antioxidant, antibacterial, and anticancer activity.

2. MATERIALS AND METHODS

Materials and reagents: Allthechemicals and solvents used were of analytical grade supplied from BDH, Fluka, USP, Merck, GCC, PubChem, MOLBASE and Aldrich. 4-hydroxy-3-methoxybenzaldehyde, 2-hydroxybenzaldehyde,2-amino-4-chlorobenzenesulfonamide, sulfamerazine, sulfanilamide, sulfamethazine, sulfathiazole, sulfadiazine, glacial acetic acid, thioglycolic acid, phosphorus oxychloride ($POCl_3$) and zinc chloride ($ZnCl_2$) as well as butylated hydroxyl toluene (BHT) were obtained from sigma-Aldrich. 2-seleno-glycolic acid and *β*-carotene were supplied from MOLBASE and USP respectively. Tween-20 (Polyoxyethylene (20) sorbitan monolaurate), linoleic acid and dimethylformamide was obtained from Fluka. Triethylamine, Na_2SO_4, NaCl and $NaHCO_3$ from Merck product. Dichloromethane, hexane, acetone, methanol and ethyl acetate were obtained from BDH. Hydrochloric acid and 2-mercaptobutanoic acidwere also purchased from GCC and PubChem respectively. Thin-layer chromatography (TLC) was carried out by using aluminium sheet coated with silica gel $60F_{254}$ (Merck), iodine and ultraviolet (UV) light was used for visualized TLC plates.

Physical Measurements: The FT-IR spectra as KBr discs were recorded in the range 4000-400 cm^{-1} using Shimadzu FT-IR model 8400s instrument. The experimental values of ^1H and ^{13}C NMR spectra for the studied compounds were done in a Brucker spectrophotometer (500 MHZ) and using DMSO-d_6 as a solvent and TMS as internal standard (Central Laboratory, University of Tehran, Iran). The mass spectra were measured by the EI technique at 70 eV using Agilent Technologies 5975C spectrometer. Elemental analysis (C,H,N,S) was measured by using CHNS-932 LECO Apparatus. Melting points were measured with a Bauchi 510 melting point apparatus and are uncorrected.

General procedure for the synthesis of Sulfonamide Schiff bases ($5A_1$-$5A_6$, $5A_9$-$5A_{12}$)

The following general method was used to prepare compounds $5A_1$, $5A_2$ and $5A_6$ according to the method ofHassan and Abdullah.[17] An equimolar quantity of sulfonamide derivatives (2-amino-4-chlorobenzenesulfonamide, sulfamerazine, sulfanilamide, sulfamethazine and sulfathiazole, sulfadiazine) (10 mmol) and 4-hydroxy-3-methoxybenzaldehyde (10 mmol) or 2-hydroxybenzaldehyde (10 mmol) were dissolved in a 30 mL of ethanol, then a catalytic amount of glacial acetic acid (2-3 drops) was added and the reaction mixture refluxed for about 5-10 hrs, the progress of the reaction was monitored by TLC using ethyl acetate/ benzene (v/v 2:8) as eluent and ultraviolet (UV) light as appearance, the resulted compoundswas obtained by cooling the reaction mixture to freezingtemperature. The precipitated solids were filtered off from the reaction mixture and washed with cold absolute ethanol, dried, followed by recrystallized in methanol to get the target compounds, as illustrated in Scheme 1.

Compounds **$5A_3$-$5A_5$, $5A_9$-$5A_{12}$** were prepared as previously described in literature.[18-21]

4-chloro-2-((4-hydroxy-3-methoxybenzylidene)amino)benzenesulfonamide (**$5A_1$**)

White solid; yield: 94%; R_f : 0.91; m.p: 197-199 ^oC; Elemental Analysis for $C_{14}H_{13}ClN_2O_4S$ (340.78 g/mol); Calcd: C, 49.34; H, 3.85; N, 8.22; S, 9.41. Found: C, 49.37; H, 3.88; N, 8.22; S, 9.43. IR (KBr) cm^{-1}: 3500 ν(OH), 3385 $\nu_{str.}(NH_2$, Asymmetrical), 3226 $\nu_{str.}(NH_2$, Symmetrical), 2980 ν(CH, Asymmetrical, aliph.),2877 ν(CH, Symmetrical, aliph.),1597 ν(CH=N), 1519 – 1494 ν(C=C), 1332 $\nu_{str.}(SO_2$, Asymmetrical), 1151 $\nu_{str.}(SO_2$, Symmetrical), 912 ν(S-N), 856 ν(C-Cl), 650 $\nu_{str.}$(C-S).

4-((4-hydroxy-3-methoxybenzylidene)amino)-N-(4-methylpyrimidin-2-yl)benzenesulfonamide (**$5A_2$**)

Light yellow solid; yield: 96%; R_f : 0.86; m.p: 251-253 ^oC; Elemental Analysis for $C_{19}H_{18}N_4O_4S$ (398.44 g/mol); Calcd: C, 57.27; H, 4.55; N, 14.06; S, 8.05. Found: C, 57.29; H, 4.51; N, 14.06; S, 8.05. IR (KBr) cm^{-1}: 3483 ν(OH), 3385 ν(N-H), 2943 ν(CH, Asymmetrical, aliph.), 1631 ν(C=N, sulfa ring), 1593 ν(CH=N), 1512 – 1431 ν(C=C), 1330 $\nu_{str.}(SO_2$, Asymmetrical), 1153 $\nu_{str.}(SO_2$, Symmetrical), 1269 ν(C-N), 964 ν(S-N), 678 $\nu_{str.}$(C-S).

4-((4-hydroxy-3-methoxybenzylidene)amino)-N-(pyrimidin-2-yl)benzenesulfonamide (**$5A_6$**)

Light yellow solid; yield: 92%; R_f : 0.79; m.p: 263-265 ^oC; Elemental Analysis for $C_{18}H_{16}N_4O_4S$ (384.41 g/mol); Calcd: C, 56.24; H, 4.20; N, 14.57; S, 8.34. Found: C, 56.27; H, 4.23; N, 14.56; S, 8.31. IR (KBr) cm^{-1}: 3448 ν(OH), 3147 ν(N-H), 2870 ν(CH, symmetrical, aliph.), 1620 ν(CH=N), 1481 – 1454 ν(C=C), 1311 $\nu_{str.}(SO_2$, Asymmetrical), 1145 $\nu_{str.}(SO_2$, Symmetrical), 1276 ν(C-N), 937 ν(S-N), 644 $\nu_{str.}$(C-S).

General procedure for the synthesis of *β*-lactams derivatives ($Z5A_1$-$Z5A_6$, $Z5A_9$-$Z5A_{12}$, $Z5A_{2'}$, $Z5A_{9'}$-$Z5A_{11'}$)

To a stirred solution of imine $5A_1$-$5A_6$, $5A_9$-$5A_{12}$(3.0 mmol),thioglycolic acid (4.5 mmol, 0.42 g) or 2-seleno-glycolic acid (4.5 mmol, 0.63 g) and triethylamine (12.0 mmol, 1.2 gm) in dry dichloromethane (40 mL) maintained at 0 °C under Argon atmosphere, a solution of phosphorous oxychloride (3.3 mmol, 0.51 g) in dry dichloromethane (20 mL) was added dropwise, at 0 °C with constant stirring. The reaction mixture was stirred overnight at room temperature. Thereafter, the mixture was extracted with ethyl acetate, washed successively with 1N HCl (20 mL), water (2 × 20 mL), 5% $NaHCO_3$ (20 mL) and brine (20 mL), then dried (Na_2SO_4) and concentrated. The progress of the reaction was monitored by TLC. The crude product was purified by silica gel column chromatography using 3:7 ethyl acetate / hexane as eluent to afford pure products.[11] The R_f values of all the compounds were determined by using Ethyl acetate: n-Hexane (2:8) as solvent system. The synthetic procedures for the preparation of compounds ($Z5A_1$-$Z5A_6$, $Z5A_9$-$Z5A_{12}$, $Z5A_{2'}$, $Z5A_{9'}$-$Z5A_{11'}$)are presented in Scheme 1.

4-chloro-2-(2-(4-hydroxy-3-methoxyphenyl)-3-mercapto-4-oxoazetidin-1-yl)benzenesulfonamide (**$Z5A_1$**)

Greenish yellow solid, yield: 53%; R_f: 0.87; m.p: 203-204 ^oC; Elemental Analysis for $C_{16}H_{15}ClN_2O_5S_2$ (414.88g/mol); Calcd: C, 46.32; H, 3.64; N, 6.75; S, 15.46. Found: C, 46.39; H, 3.58; N, 6.70; S, 15.40. IR (KBr) cm^{-1}: 3466 ν(OH), 3379 $\nu_{str.}(NH_2$, Asymmetrical), 3248 $\nu_{str.}(NH_2$, Symmetrical), 2960 ν(CH, Asymmetrical, aliph.), 2846 ν(CH, Symmetrical, aliph.), 2492 ν(S-H), 1716 ν(C=O, azetidin-2-one ring), 1521 ν(C-N, azetidin-2-one ring), 1471 ν(C=C), 1396 $\nu_{str.}(SO_2$, Asymmetrical), 1165 $\nu_{str.}(SO_2$, Symmetrical), 885 ν(S-N), 846 ν(C-Cl), 665 $\nu_{str.}$(C-S); ^{1}HNMR (500 MHz, DMSO-d_6)

(δ/ppm): 10.38 (s, 1H, OH), 7.51 (d, 2H, *J* = *10* Hz, Ar-H), 7.37 (s, 1H, Ar-H), 6.89 (d, 1H, *J* = *15* Hz, Ar-H), 6.77 (s, 2H, NH_2), 6.62 (dd, 2H, *J* = *10* Hz, Ar-H), 3.745 (d, 1H, *J* = *5* Hz, C<u>H</u>-N, 2-azetidinone ring), 3.65 (s, 3H, O<u>C</u>H_3), 3.045 (t, 1H, $J_1 = J_2$= 7.5 Hz, C<u>H</u>-S, 2-azetidinone ring), 1.20 (s,1H, SH); ^{13}CNMR (500 MHz, DMSO-d_6) (δ/ppm): 170.92, 153.83, 147.16, 144.55, 136.22, 130.30, 124.69,123.57, 122.36, 121.46, 120.78, 115.63, 111.81, 61.72, 55.99, 45.79; The EI-MS m/s (%): 416.9 $[M]^+$ (1), 396 $[C_{16}H_{13}ClN_2O_4S_2]^{\bullet+}$ (2.2), 367 $C_{15}H_{12}ClN_2O_3S_2^+$ (1), 302 $[C_{15}H_{11}ClN_2OS]^{\bullet+}$ (1.5), 189 $C_6H_4ClNO_2S^+$ (6.1), 86 $C_4H_8NO^+$ (100).

4-(2-(4-hydroxy-3-methoxyphenyl)-3-mercapto-4-oxoazetidin-1-yl)-N-(4-methylpyrimidin-2-yl)benzenesulfonamide (**Z5A$_2$**)

Yellowish brown oil, yield: 57%; R_f: 0.72; Elemental Analysis for $C_{21}H_{20}N_4O_5S_2$ (472.54g/mol); Calcd: C, 53.38; H, 4.27; N, 11.86; S, 13.57. Found: C, 53.44; H, 4.23; N, 11.79; S, 13.52. IR (KBr) cm^{-1}: 3421 ν(OH), 3421 ν(N-H), 2989 ν(CH, Asymmetrical, aliph.), 2499 ν(S-H), 1693 ν(C=O, azetidin-2-one ring), 1560 ν(C-N, azetidin-2-one ring), 1600 ν(C=N, pyrimidine ring), 1473 ν(C=C), 1396 $\nu_{str.}(SO_2$, Asymmetrical), 1165 $\nu_{str.}(SO_2$, Symmetrical), 895 ν(S-N), 642 $\nu_{str.}$(C-S); ^{1}HNMR (500 MHz, DMSO-d_6) (δ/ppm): 10.75 (s, 1H, NH), 8.245 (d, 1H, *J* = 5 Hz, C<u>H</u>=N, pyrimidine ring), 7.85 (d, 2H, *J* = 10 Hz, Ar-H), 7.325 (d, 2H, *J* = 10 Hz, Ar-H), 6.90 (d, 1H, *J* = 10 Hz, Ar-H), 6.84 (s, 1H, Ar-H), 6.815 (d, 1H, *J* = 5 Hz, 5-H, pyrimidine ring), 6.78 (d, 1H, *J* = 10 Hz, Ar-H), 3.87 (d, 1H, *J* = 5 Hz, C<u>H</u>-N, 2-azetidinone ring), 3.77 (t, 1H, $J_1 = J_2$= 5 Hz, C<u>H</u>-S, 2-azetidinone ring), 3.65 (s, 3H, O<u>C</u>H_3), 3.10 (s, 1H, OH), 1.22 (d, 1H, *J* = 15 Hz, SH), 1.16 (s, 3H, CH_3); ^{13}CNMR (500 MHz, DMSO-d_6) (δ/ppm): 171.48, 163.45, 159.98, 152.36, 150.10, 146.75, 142.86, 136.34, 128.48, 127.86, 121.42, 120.13, 114.81, 113.26, 106.26, 69.33, 59.91, 52.35, 25.95; The EI-MS m/s (%): 472.5 $[M]^+$ (1.2), 435 $C_{21}H_{15}N_4O_5S^+$ (1), 362 $C_{16}H_{12}NO_5S_2^+$ (1), 287 $C_{16}H_{16}NO_2S^+$ (1.2), 86 $C_4H_8NO^+$ (100).

4-(3-hydroseleno-2-(4-hydroxy-3-methoxyphenyl)-4-oxoazetidin-1-yl)-N-(4-methylpyrimidin-2-yl) benzenesulfonamide (**Z5A$_{2'}$**)

Dark orange oil, yield: 48%; R_f: 0.65; Elemental Analysis for $C_{21}H_{20}N_4O_5SSe$ (519.43 g/mol); Calcd: C, 48.56; H, 3.88; N, 10.79; S, 6.17. Found: C, 48.63; H, 3.81; N, 10.68; S, 6.21. IR (KBr) cm^{-1}: 3456 ν(OH), 3221 ν(N-H), 2939 ν(CH, Asymmetrical, aliph.), 2872 ν(CH, Symmetrical, aliph.), 2366 ν(Se-H), 1693 ν(C=O, azetidin-2-one ring), 1516 ν(C-N,azetidin-2-one ring), 1593 ν(C=N, pyrimidine ring), 1269 ν(C-N, pyrimidine ring), 1435-1404 ν(C=C), 1315 $\nu_{str.}(SO_2$, Asymmetrical), 1157 $\nu_{str.}(SO_2$, Symmetrical), 985 ν(S-N), 572 $\nu_{str.}$(C-Se); ^{1}HNMR (500 MHz, DMSO-d_6) (δ/ppm): 10.54 (s, 1H, NH), 10.12 (s, 1H, OH), 8.26 (d, 1H, *J* = 25 Hz, C<u>H</u>=N, pyrimidine ring), 7.88 (d, 2H, *J* = 20 Hz, Ar-H), 7.64 (d, 2H, *J* = 15 Hz, Ar-H), 7.24 (d, 1H, *J* = 10 Hz, Ar-H), 6.97 (s, 1H, Ar-H), 6.86 (d, 1H, *J* = 15 Hz, 5-H, pyrimidine ring), 6.58 (d, 1H, *J* = 10 Hz, Ar-H), 4.30 (d, 1H, *J* = 5 Hz, C<u>H</u>-N, 2-azetidinone ring), 3.91 (t, 1H, J_1 = 5 Hz, J_2= 20 Hz, C<u>H</u>-Se, 2-azetidinone ring), 3.04 (s, 3H, O<u>C</u>H_3), 2.29 (s, 1H, SeH), 1.17 (s, 3H, CH_3); ^{13}CNMR (500 MHz, DMSO-d_6) (δ/ppm): 169.70, 159.71, 153.81, 148.77, 144.94, 142.83, 138.91, 130.33, 128.71, 126.86, 121.92, 121.13, 117.01, 115.91, 111.09, 65.16, 58.94, 56.65, 25.83; The EI-MS m/s (%): 519.6 $[M]^+$ (1.0), 435 $C_{21}H_{15}N_4O_5S^+$ (1.2), 407 $C_{16}H_{12}N_2O_4SSe^+$ (1.2), 373 $C_{18}H_{19}N_4O_3S^+$ (1.0), 337 $C_{17}H_{13}N_4O_2S^+$ (1.4), 165 $C_4H_8NOSe^+$ (5.8), 134 $C_8H_8NO^+$ (100).

4-(2-(4-hydroxy-3-methoxyphenyl)-3-mercapto-4-oxoazetidin-1-yl)benzenesulfonamide (**Z5A$_3$**)

Reddish orange oil, yield: 51%; R_f: 0.88; Elemental Analysis for $C_{16}H_{16}N_2O_5S_2$ (380.44g/mol); Calcd: C, 50.51; H, 4.24; N, 7.36; S, 16.86. Found: C, 50.62; H, 4.21; N, 7.28; S, 16.91. IR (KBr) cm^{-1}: 3500 ν(OH), 3385 ν(NH_2), 2937 ν(CH, Asymmetrical, aliph.), 2490 ν(S-H), 1716 ν(C=O, azetidin-2-one ring), 1591 ν(C-N, azetidin-2-one ring), 1519-1473 ν(C=C), 1325 $\nu_{str.}(SO_2$, Asymmetrical), 1163 $\nu_{str.}(SO_2$, Symmetrical), 997 ν(S-N), 667 $\nu_{str.}$(C-S); ^{1}HNMR (500 MHz, DMSO-d_6) (δ/ppm): 9.83 (s, 1H, OH), 7.28 (d, 2H, *J* = 10 Hz, Ar-H), 7.165 (d, 2H, *J* = 10 Hz, Ar-H), 7.08 (s, 2H,NH_2), 6.81 (d, 1H, *J* = 5 Hz, Ar-H), 6.72 (s, 1H,Ar-H), 6.63 (d, 1H, *J* = 10 Hz, Ar-H), 4.41 (d, 1H, *J* = 5 Hz, C<u>H</u>-N, 2-azetidinone ring), 3.54 (t, 1H, $J_1 = J_2$ = 10 Hz,C<u>H</u>-S, 2-azetidinone ring), 3.75 (s, 3H, O<u>C</u>H_3), 1.22 (d, 1H, *J* =10, SH); ^{13}CNMR (500 MHz, DMSO-d_6) (δ/ppm): 170.90, 150.85, 145.96, 142.00, 137.96, 129.77, 129.05, 120.84, 120.37, 118.82, 113.51, 62.15, 56.44, 45.80; The EI-MS m/s

(%): 381 $[M]^+$ (4.1), 351 $C_{15}H_{15}N_2O_4S_2^+$ (2.5), 279 $C_{13}H_{15}N_2O_3S^+$ (34), 272 $C_{16}H_{18}NO_3^+$ (5.8), 255 $[C_{16}H_{17}NO_2]^{\bullet+}$ (1.2), 194 $[C_9H_{10}N_2OS]^{\bullet+}$ (51.3), 93 $[C_6H_7N]^{\bullet+}$ (65.1), 86 $C_4H_8NO^+$ (100).

N-(4,6-dimethylpyrimidin-2-yl)-4-(2-(4-hydroxy-3-methoxyphenyl)-3-mercapto-4-oxoazetidin-1-yl)benzenesulfonamide (**Z5A$_4$**)

Dark brown oil, yield: 63%; R_f: 0.93; Elemental Analysis for $C_{22}H_{22}N_4O_5S_2$ (486.56g/mol); Calcd: C, 54.31; H, 4.56; N, 11.51; S, 13.18. Found: C, 54.38; H, 4.59; N, 11.47; S, 13.09. IR (KBr) cm^{-1}: 3421 ν(OH), 3200 ν(N-H), 2985 ν(CH, Asymmetrical, aliph.), 2495 ν(S-H), 1712 ν(C=O, azetidin-2-one ring), 1519 ν(C-N, azetidin-2-one ring), 1624, 1597 ν(C=N, pyrimidine ring), 1469 ν(C=C), 1396 $\nu_{str.}(SO_2$, Asymmetrical), 1161 $\nu_{str.}(SO_2$, Symmetrical), 840 ν(S-N), 663 $\nu_{str.}$(C-S); ^{1}HNMR (500 MHz, DMSO-d_6) (δ/ppm): 12.88 (s, 1H, OH), 10.31 (s, 1H, NH), 7.77 (d, 2H, *J* = 10 Hz, Ar-H), 6.91 (dd, 2H, *J* = 10 Hz, Ar-H), 6.77 (s, 1H, 5-H, pyrimidine ring), 6.74 (s, 1H,Ar-H), 6.635 (d, 1H, *J* = 5 Hz, Ar-H), 6.58 (d, 1H, *J* = 5 Hz, Ar-H), 4.38 (t, 1H, $J_1 = J_2$= 10 Hz, C<u>H</u>-N, 2-azetidinone ring), 3.65 (s, 3H, O<u>CH$_3$</u>), 3.04 (t, 1H, $J_1 = J_2$= 10 Hz, C<u>H</u>-S, 2-azetidinone ring), 2.24 (s, 6H, CH_3-n, $2CH_3$), 1.195 (s,1H, SH); ^{13}CNMR (500 MHz, DMSO-d_6) (δ/ppm): 170.93, 163.83, 156.55, 150.93, 147.65, 146.04, 130.44, 129.87, 126.48, 121.54, 120.58, 115.75, 113.83, 106.63, 64.19, 55.97, 45.82, 23.33; The EI-MS m/s (%): 487 $[M]^+$ (1), 368 $C_{19}H_{18}N_3O_3S^+$ (1), 264 $C_{12}H_{14}N_3O_2S^+$ (1.1), 123 $C_7H_7O_2^+$ (5.7), 86 $C_4H_8NO^+$ (100).

4-(2-(4-hydroxy-3-methoxyphenyl)-3-mercapto-4-oxoazetidin-1-yl)-N-(thiazol-2-yl)benzenesulfonamide (**Z5A$_5$**)

Dark brown oil, yield: 54%; R_f: 0.84; Elemental Analysis for $C_{19}H_{17}N_3O_5S_3$ (463.55g/mol); Calcd: C, 49.23; H, 3.70; N, 9.06; S, 20.75. Found: C, 49.34; H, 3.62; N, 8.97; S, 20.81. IR (KBr) cm^{-1}: 3379 ν(OH), 3259 ν(N-H), 2885 ν(CH, symmetrical, aliph.), 2600 ν(S-H), 1739 ν(C=O, azetidin-2-one ring), 1562 ν(C-N, azetidin-2-one ring), 1647 ν(C=N, thiazole ring), 1496 ν(C=C), 1330 $\nu_{str.}(SO_2$, Asymmetrical), 1157 $\nu_{str.}(SO_2$, Symmetrical), 918 ν(S-N), 671 $\nu_{str.}$(C-S); ^{1}HNMR (500 MHz, DMSO-d_6) (δ/ppm): 11.76 (s, 1H, NH), 10.15 (s, 1H, OH), 7.745 (d, 2H, *J* = 10 Hz, Ar-H), 7.53 (d, 2H, *J* = 10 Hz, Ar-H), 7.23 (d, 1H, *J* = 5 Hz, 4-H, thiazole ring), 6.91 (d, 1H, *J* = 10 Hz, Ar-H), 6.82 (s, 1H,Ar-H), 6.685 (d, 1H, *J* = 5 Hz, Ar-H), 6.47 (d, 1H, *J* = 10 Hz, 5-H, thiazole ring), 4.35 (d, 1H, *J* = 5 Hz, C<u>H</u>-N, 2-azetidinone ring), 3.82 (t, 1H, J_1 = 10 Hz, J_2= 5 Hz, C<u>H</u>-S, 2-azetidinone ring), 3.65 (s, 3H,O<u>CH$_3$</u>), 1.23 (d, 1H, *J* = 5 Hz, SH); ^{13}CNMR (500 MHz, DMSO-d_6) (δ/ppm): 171.17, 163.37, 147.14, 145.85, 141.05, 133.58, 131.35, 127.40, 126.77, 121.01, 119.49, 116.15, 111.49, 108.76, 65.10, 59.92, 45.65.

4-(2-(4-hydroxy-3-methoxyphenyl)-3-mercapto-4-oxoazetidin-1-yl)-N-(pyrimidin-2-yl)benzenesulfonamide (**Z5A$_6$**)

Off white solid, yield: 72%; R_f: 0.67; m.p: 179-181 ^oC; Elemental Analysis for $C_{20}H_{18}N_4O_5S_2$ (458.51g/mol); Calcd: C, 52.39; H, 3.96; N, 12.22; S, 13.99. Found: C, 52.32; H, 3.99; N, 12.14; S, 13.93. IR (KBr) cm^{-1}: 3425 ν(OH), 3356 ν(N-H), 2931 ν(CH, Asymmetrical, aliph.), 2870 ν(CH, symmetrical, aliph.), 2420 ν(S-H), 1693 ν(C=O, azetidin-2-one ring), 1531 ν(C-N, azetidin-2-one ring), 1647,1585 ν(2C=N, pyrimidine ring), 1496, 1438 ν(C=C), 1327 $\nu_{str.}(SO_2$, Asymmetrical), 1157 $\nu_{str.}(SO_2$, Symmetrical), 941 ν(S-N), 678 $\nu_{str.}$(C-S); ^{1}HNMR (500 MHz, DMSO-d_6) (δ/ppm): 11.25 (s, 1H, OH), 10.53 (s, 1H, NH), 8.49 (d, 2H, *J* = 15 Hz, 2C<u>H</u>=N, pyrimidine ring), 7.94 (d, 2H, *J* = 10 Hz, Ar-H), 7.76 (d, 2H, *J* = 10 Hz, Ar-H), 7.62 (d, 1H, *J* = 10 Hz, Ar-H), 7.02 (t, 1H, $J_1 = J_2$= 5 Hz, 5-H, pyrimidine ring), 6.57 (d, 2H, *J* = 5 Hz, Ar-H), 4.39 (s, 3H, O<u>CH$_3$</u>), 3.33 (d, 1H, *J* = 10 Hz, C<u>H</u>-N, 2-azetidinone ring), 3.09 (t, 1H, $J_1 = J_2$= 5 Hz, C<u>H</u>-S, 2-azetidinone ring), 2.09 (s,1H, SH); ^{13}CNMR (500 MHz, DMSO-d_6) (δ/ppm): 172.18, 158.73, 157.69, 153.36, 148.71, 141.37, 137.85, 130.29, 123.49, 120.15, 119.04, 115.99, 112.91, 108.39, 69.26, 59.23, 54.02; The EI-MS m/s (%): 458 $[M]^+$ (2.5), 361 $C_{19}H_{13}N_4O_2S^+$ (1.0), 341 $[C_{17}H_{15}N_3O_3S]^{\bullet+}$ (1.0), 236 $C_{10}H_{10}N_3O_2S^+$ (1.0), 80 $[C_4H_4N_2]^{\bullet+}$ (100).

4-(2-(2-hydroxyphenyl)-3-mercapto-4-oxoazetidin-1-yl)-N-(4-methylpyrimidin-2-yl)benzenesulfonamide (**Z5A$_9$**)

Dark orange oil, yield: 66%; R_f: 0.70; Elemental Analysis for $C_{20}H_{18}N_4O_4S_2$ (442.51g/mol); Calcd: C, 54.28; H, 4.10; N, 12.66; S, 14.49. Found: C, 54.37; H, 4.05; N, 12.58; S, 14.51. IR (KBr) cm^{-1}: 3441 ν(OH), 3441 ν(N-H), 2989 ν(CH, Asymmetrical, aliph.), 2692 ν(S-H), 1693 ν(C=O, azetidin-2-one ring), 1546 ν(C-N, azetidin-2-one ring), 1647 ν(C=N,

pyrimidine ring), 1465 ν(C=C), 1396 $\nu_{str.}(SO_2$, Asymmetrical), 1161 $\nu_{str.}(SO_2$, Symmetrical), 995 ν(S-N), 679 $\nu_{str.}$(C-S); ^{1}HNMR (500 MHz, DMSO-d_6) (δ/ppm): 12.37 (s, 1H, NH), 10.55 (s, 1H, OH), 8.315 (d, 1H, *J* = 9 Hz, CH=N, pyrimidine ring), 7.71 (d, 2H, *J* = 3 Hz, Ar-H), 7.44 (d, 2H, *J* = 3 Hz, Ar-H), 7.39 (t, 1H, $J_1 = J_2$ = 3 Hz, Ar-H), 7.28 (d, 1H, *J* = 12 Hz, H-d), 7.08 (t, 1H, J_1 = 12 Hz, J_2= 6 Hz, Ar-H), 6.71 (d, 1H, *J* = 6 Hz, 5-H, pyrimidine ring), 6.57 (d, 1H, *J* = 3 Hz, Ar-H), 5.41 (d, 1H, *J* = 3 Hz, CH-N, 2-azetidinone ring), 4.91 (t, 1H, J_1 = 3 Hz, J_2= 6 Hz, CH-S, 2-azetidinone ring), 2.87 (d, 1H, *J* = 12 Hz, SH), 1.15 (s, 3H, CH_3); ^{13}CNMR (500 MHz, DMSO-d_6) (δ/ppm): 168.72, 159.90, 155.48, 150.84, 148.89, 140.11, 132.54, 130.05, 128.03, 126.75, 122.93, 120.88, 120.18, 116.05, 112.73, 56.07, 54.98, 23.68.

4-(3-hydroseleno-2-(2-hydroxyphenyl)-4-oxoazetidin-1-yl)-N-(4-methylpyrimidin-2-yl)benzenesulfonamide (**Z5A$_{9'}$**)

Dark brown oil, yield: 50%; R_f: 0.58; Elemental Analysis for $C_{20}H_{18}N_4O_4SSe$ (489.41g/mol); Calcd: C, 49.08; H, 3.71; N, 11.45; S, 6.55. Found: C, 49.16; H, 3.66; N, 11.38; S, 6.59. IR (KBr) cm^{-1}: 3560 ν(OH), 3390 ν(N-H), 2985 ν(CH, Asymmetrical, aliph.), 2480 ν(Se-H), 1739 ν(C=O, azetidin-2-one ring), 1577 ν(C-N, azetidin-2-one ring), 1643,1620 ν(2C=N, pyrimidine ring), 1496-1450 ν(C=C), 1319 $\nu_{str.}(SO_2$, Asymmetrical), 1172 $\nu_{str.}(SO_2$, Symmetrical), 900 ν(S-N), 667 $\nu_{str.}$(C-Se); ^{13}CNMR (500 MHz, DMSO-d_6) (δ/ppm): 171.09, 160.52, 158.27, 153.46, 150.06, 146.22, 138.48, 132.10, 130.12, 127.98, 124.17, 122.21, 121.48, 118.55, 113.14, 58.64, 52.87, 25.16; The EI-MS m/s (%): 490 $[M]^+$ (1.2), 400 $C_{15}H_{17}N_2O_4SSe^+$ (2.3), 365 $C_{15}H_{12}NO_3SSe^+$ (2.3), 354 $[C_{18}H_{18}N_4O_2S]^{\bullet+}$ (1.0), 172 $[C_6H_8N_2O_2S]^{\bullet+}$ (35), 123 $[C_3H_8Se]^{\bullet+}$ (11.7), 94 $C_4H_4N_3^+$ (71), 86 $C_4H_8NO^+$ (100).

4-(2-(2-hydroxyphenyl)-3-mercapto-4-oxoazetidin-1-yl)benzenesulfonamide (**Z5A$_{10}$**)

Light orange oil, yield: 79%; R_f: 0.62; Elemental Analysis for $C_{15}H_{14}N_2O_4S_2$ (350.41g/mol); Calcd: C, 51.41; H, 4.03; N, 7.99; S, 18.30. Found: C, 51.50; H, 4.09; N, 7.96; S, 18.26. IR (KBr) cm^{-1}: 3417 ν(OH), 2982 ν(CH, Asymmetrical, aliph.), 2492 ν(S-H), 1689 ν(C=O, azetidin-2-one ring), 1597 ν(C-N, azetidin-2-one ring), 1539-1469 ν(C=C), 1330 $\nu_{str.}(SO_2$, Asymmetrical), 1161 $\nu_{str.}(SO_2$, Symmetrical), 891 ν(S-N), 667 $\nu_{str.}$(C-S); ^{1}HNMR (500 MHz, DMSO-d_6) (δ/ppm): 10.49 (s, 1H, OH), 7.78 (d, 2H, *J* = 10 Hz, Ar-H), 7.735 (d, 2H, *J* = 5 Hz, Ar-H), 7.29 (s, 2H,NH_2), 7.11 (t, 1H, J_1 =10 Hz, J_2= 5 Hz, Ar-H), 6.89 (d, 1H, *J* = 10 Hz, Ar-H), 6.80 (t, 1H, $J_1 = J_2$= 5 Hz, Ar-H), 6.73 (d, 1H, *J* = 10 Hz, Ar-H), 3.405 (d, 1H, *J* = 15 Hz, CH-N, 2-azetidinone ring), 3.04 (t, 1H, J_1 = 5 Hz, J_2= 10 Hz, CH-S, 2-azetidinone ring), 1.20 (d, 1H, *J* =10, SH);^{13}CNMR (500 MHz, DMSO-d_6) (δ/ppm): 170.90, 150.85, 145.96, 137.96, 129.77, 127.83, 125.96, 125.12, 124.34, 123.62, 113.51, 56.44, 45.80; The EI-MS m/s (%): 350 $[M]^+$ (1.0), 300 $[C_{15}H_{12}N_2O_3S]^{\bullet+}$ (1.2), 276 $C_9H_{11}N_2O_4S_2^+$ (6.5), 156 $C_6H_6NO_2S^+$ (4.3), 121 $[C_7H_7NO]^{\bullet+}$ (21.6), 86 $C_4H_8NO^+$ (100).

4-(3-hydroseleno-2-(2-hydroxyphenyl)-4-oxoazetidin-1-yl)benzenesulfonamide (**Z5A$_{10'}$**)

Yellowish brown oil, yield: 65%; R_f: 0.53; Elemental Analysis for $C_{15}H_{14}N_2O_4SSe$ (397.31 g/mol); Calcd: C, 45.35; H, 3.55; N, 7.05; S, 8.07. Found: C, 45.47; H, 3.51; N, 6.98; S, 8.15. IR (KBr) cm^{-1}: 3560 ν(OH), 3379 $\nu_{str.}(NH_2$, Asymmetrical), 3263 $\nu_{str.}(NH_2$, Symmetrical), 2885 ν(CH, symmetrical, aliph.), 2580 ν(Se-H), 1739 ν(C=O, azetidin-2-one ring), 1562 ν(C-N, azetidin-2-one ring), 1496 ν(C=C), 1330 $\nu_{str.}(SO_2$, Asymmetrical), 1157 $\nu_{str.}(SO_2$, Symmetrical), 918 ν(S-N), 536 $\nu_{str.}$(C-Se); ^{1}HNMR (500 MHz, DMSO-d_6) (δ/ppm): 9.57 (s, 1H, OH), 7.92 (d, 2H, *J* = 15 Hz, Ar-H), 7.65 (d, 2H, *J* = 15 Hz, Ar-H), 7.50 (s, 3H, Ar-H,NH_2), 7.08 (d, 1H, *J* = 15 Hz, Ar-H), 7.02 (t, 1H, J_1 =15 Hz, J_2= 10 Hz, Ar-H), 6.94 (t, 1H, J_1 = 15 Hz, J_2= 10 Hz, Ar-H), 4.36 (d, 1H, *J* = 15 Hz, CH-N, 2-azetidinone ring), 3.04 (t, 1H, $J_1 = J_2$= 10 Hz, CH-Se, 2-azetidinone ring), 1.20 (d, 1H, *J* =10, SeH); ^{13}CNMR (500 MHz, DMSO-d_6) (δ/ppm): 174.58, 143.68, 137.92, 135.92, 130.42, 127.85, 127.55, 124.45, 122.11, 121.53, 113.15, 59.94, 45.75; The EI-MS m/s (%): 398 $[M]^+$ (1.1), 287 $C_9H_6NO_3SSe^+$ (1.0), 172 $[C_6H_8N_2O_2S]^{\bullet+}$ (1.2), 156 $C_6H_6NO_2S^+$ (1.2), 86 $C_4H_8NO^+$ (100).

4-(2-(2-hydroxyphenyl)-3-mercapto-4-oxoazetidin-1-yl)-N-(thiazol-2-yl)benzenesulfonamide (**Z5A$_{11}$**)

Yellowish brown oil, yield: 69%; R_f: 0.91; Elemental Analysis for $C_{18}H_{15}N_3O_4S_3$ (433.52 g/mol); Calcd: C, 49.87; H, 3.49; N, 9.69; S, 22.19. Found: C, 49.80; H, 3.53; N, 9.61; S, 22.14. IR (KBr)

cm^{-1}: 3444 ν(OH), 3444 ν(N-H), 2985 ν(CH, Asymmetrical, aliph.), 2492 ν(S-H), 1689 ν(C=O, azetidin-2-one ring), 1527 ν(C-N, azetidin-2-one ring), 1593 ν(C=N, thiazole ring), 1469 ν(C=C), 1327 $\nu_{str.}(SO_2$, Asymmetrical), 1145 $\nu_{str.}(SO_2$, Symmetrical), 933 ν(S-N), 671 $\nu_{str.}$(C-S); ^{13}CNMR (500 MHz, DMSO-d_6) (δ/ppm): 168.12, 158.54, 148.82, 140.64, 138.75, 131.98, 131.54, 130.97, 128.48, 123.55, 121.93, 121.01, 116.28, 108.43, 50.96, 45.65; The EI-MS m/s (%): 434 $[M]^+$ (2.6), 352 $[C_{15}H_{16}N_2O_4S_2]^{\bullet+}$ (1.2), 320 $C_{15}H_{14}NO_3S_2^+$ (3.9), 200 $C_{13}H_{14}NO^+$ (4.5), 172 $[C_6H_8N_2O_2S]^{\bullet+}$ (35.5), 156 $C_6H_6NO_2S^+$ (47.1), 93 $[C_6H_7N]^{\bullet+}$ (76.8), 76 $C_6H_4^+$ (100).

4-(3-hydroseleno-2-(2-hydroxyphenyl)-4-oxoazetidin-1-yl)-N-(thiazol-2-yl)benzenesulfonamide (**Z5A$_{11'}$**)

Yellow crystalline solid, yield: 73%; R_f: 0.71; m.p: 237-239 ^oC; Elemental Analysis for $C_{18}H_{15}N_3O_4S_2Se$ (480.42g/mol); Calcd: C, 45.00; H, 3.15; N, 8.75; S, 13.35. Found: C, 45.11; H, 3.20; N, 8.79; S, 13.33. IR (KBr) cm^{-1}: 3417 ν(OH), 3417 ν(N-H), 2974 ν(CH, Asymmetrical, aliph.), 2804 ν(CH, symmetrical, aliph.), 2492 ν(Se-H), 1739 ν(C=O, azetidin-2-one ring), 1531 ν(C-N, azetidin-2-one ring), 1647 ν(C=N, thiazole ring), 1519, 1473 ν(C=C), 1361 $\nu_{str.}(SO_2$, Asymmetrical), 1172 $\nu_{str.}(SO_2$, Symmetrical), 941 ν(S-N), 509 $\nu_{str.}$(C-Se); ^{1}HNMR (500 MHz, DMSO-d_6) (δ/ppm): 10.67 (s, 1H, NH), 7.85 (d, 2H, *J* = 15 Hz, Ar-H), 7.71 (d, 2H, *J* = 10 Hz, Ar-H), 7.405 (d, 1H, *J* = 15 Hz, Ar-H), 7.23 (d, 1H, *J* = 5 Hz, 4-H, thiazole ring), 7.18 (t, 1H, $J_1 = J_2$= 5 Hz, Ar-H), 6.82 (d, 1H, *J* = 5 Hz, Ar-H), 6.75 (t, 1H, $J_1 = J_2$= 5 Hz, Ar-H), 6.56 (d, 1H, *J* = 15 Hz, 5-H, thiazole ring), 4.27 (d, 1H, *J* = 20 Hz, C<u>H</u>-N, 2-azetidinone ring), 3.90 (s, 1H, OH), 3.03 (t, 1H, J_1 = 15 Hz, J_2= 5 Hz, C<u>H</u>-Se, 2-azetidinone ring), 1.205 (d, 1H, *J* = 15 Hz, SeH); ^{13}CNMR (500 MHz, DMSO-d_6) (δ/ppm): 174.58, 160.99, 158.44, 147.14, 142.75, 140.04, 134.13, 131.05, 126.11, 123.95, 122.06, 121.01, 118.13, 103.58, 59.92, 45.65; The EI-MS m/s (%): 480 $[M]^+$ (1.1), 335 $[C_{15}H_{16}N_2O_2Se]^{\bullet+}$ (6.4), 303 $[C_9H_8N_2O_3SSe]^{\bullet+}$ (1.0), 185 $[C_8H_{10}Se]^{\bullet+}$ (100), 171 $[C_7H_8Se]^{\bullet+}$ (56.4), 92 $C_4H_8NO^+$ (62.8).

4-(2-(2-hydroxyphenyl)-3-mercapto-4-oxoazetidin-1-yl)-N-(pyrimidin-2-yl)benzenesulfonamide (**Z5A$_{12}$**)

Yellowish brown oil, yield: 62%; R_f: 0.85; Elemental Analysis for $C_{19}H_{16}N_4O_4S_2$ (428.48g/mol); Calcd: C, 53.26; H, 3.76; N, 13.08; S, 14.97. Found: C, 53.35; H, 3.72; N, 13.12; S, 14.96. IR (KBr) cm^{-1}: 3417 ν(OH), 3417 ν(N-H), 2989 ν(CH, Asymmetrical, aliph.), 2492 ν(S-H), 1720 ν(C=O, azetidin-2-one ring), 1597 ν(C-N, azetidin-2-one ring), 1647, 1597 ν(2C=N, pyrimidine ring), 1462 ν(C=C), 1357 $\nu_{str.}(SO_2$, Asymmetrical), 1165 $\nu_{str.}(SO_2$, Symmetrical), 891 ν(S-N), 667 $\nu_{str.}$(C-S); ^{1}HNMR (500 MHz, DMSO-d_6) (δ/ppm): 10.43 (s, 1H, NH), 9.14 (s, 1H, OH), 8.40 (s, 2H, 2C<u>H</u>=N, pyrimidine ring), 8.34 (s, 2H, Ar-H), 7.69 (d, 2H, *J* = 5 Hz, Ar-H), 7.29 (d, 1H, *J* = 10 Hz, Ar-H), 7.16 (t, 1H, $J_1 = J_2$= 10 Hz, Ar-H), 7.10 (t, 1H, J_1 = 5 Hz, J_2 = 10 Hz, 5-H, pyrimidine ring), 6.89 (d, 1H, *J* = 10 Hz, Ar-H), 6.80 (t, 1H, J_1= 10 Hz, J_2=5 Hz, Ar-H), 3.26 (d, 1H, *J* = 15 Hz, C<u>H</u>-N, 2-azetidinone ring), 3.05 (t, 1H, J_1 =10 Hz, J_2= 15 Hz, C<u>H</u>-S, 2-azetidinone ring), 1.2 (d,1H, *J* = 5 Hz, SH); ^{13}CNMR (500 MHz, DMSO-d_6) (δ/ppm): 171.30, 159.76, 158.99, 154.40, 143.62, 138.40, 129.33, 128.20, 127.69, 125.70, 121.71, 119.49, 115.86, 111.25, 46.89, 45.77; The EI-MS m/s (%): 429 $[M]^+$ (1.0), 380 $[C_{19}H_{16}N_4O_3S]^{\bullet+}$ (1.0), 272 $C_{15}H_{14}NO_2S^+$ (1.0), 255 $[C_{15}H_{13}NOS]^{\bullet+}$ (1.1), 138 $[C_8H_{10}S]^{\bullet+}$ (6.4), 86 $C_4H_8NO^+$ (100).

General procedure for preparation of Thiazolidin-4-ones (ZZ5A$_2$-ZZ5A$_6$, ZZ5A$_9$-ZZ5A$_{12}$)

A mixture of Schiff base (**5A$_2$-5A$_6$, 5A$_9$-5A$_{12}$**) (10 mmol) and catalytic amount of zinc chloride (0.05 gm) in DMF (10 mL) was taken and to it 2-mercaptobutanoic acid (20 mmol, 2.4 g) in DMF (10 mL) was added slowly. the reaction mixture was refluxed for 12-16 hrs. The reaction mixture was then poured into crushed ice. The separated solid was neutralized by sodium bicarbonate to remove excess of 2-mercaptobutanoic acid. Solid compounds obtained was filtered, washed several times with water and recrystallized from acetone. The completion of the reaction and the purity of the products were confirmed by the TLC using ethanol: chloroform (3:7).[22] The synthetic procedures for the preparation of compounds (ZZ5A$_2$-ZZ5A$_6$, ZZ5A$_9$-ZZ5A$_{12}$) are presented in Scheme 1.

4-(5-ethyl-2-(4-hydroxy-3-methoxyphenyl)-4-oxothiazolidin-3-yl)-N-(4-methylpyrimidin-2-yl)benzenesulfonamide (**ZZ5A$_2$**)

Dark yellow solid, yield: 81%; R_f: 0.56; m.p: 198-200 °C; Elemental Analysis for $C_{23}H_{24}N_4O_5S_2$ (500.59g/mol); Calcd: C, 55.18; H, 4.83; N, 11.19; S, 12.81. Found: C, 55.26; H, 4.77; N, 11.12; S, 12.90. IR (KBr) cm^{-1}: 3444 ν(OH), 3363 ν(N-H), 2924 ν(CH, Asymmetrical, aliph.), 2854 ν(CH, Symmetrical, aliph.), 1662 ν(C=O, thiazolidinone ring), 1570 ν(C-N, thiazolidinone ring), 1635 ν(C=N, pyrimidine ring), 1504, 1427 ν(C=C), 1269 $\nu_{str.}(SO_2$, Asymmetrical), 1134 $\nu_{str.}(SO_2$, Symmetrical), 972 ν(S-N), 740 $\nu_{str.}$(C-S-C, Asymmetrical), 675 $\nu_{str.}$(C-S-C, Symmetrical); ^{1}HNMR (500 MHz, DMSO-d_6) (δ/ppm): 9.57 (s, 1H, NH), 8.28 (s, 1H, OH), 7.94 (d, 1H, *J* = 10 Hz, C<u>H</u>=N, pyrimidine ring), 7.67 (d, 2H, *J* = 15 Hz, Ar-H), 7.23 (d, 2H, *J* = 15 Hz, Ar-H), 7.06 (d, 1H, *J* = 15 Hz, Ar-H), 6.84 (d, 1H, *J* = 15 Hz, 5-H, pyrimidine ring), 6.70 (s, 1H,Ar-H), 6.55 (d, 1H, *J* = 15 Hz, Ar-H), 5.91 (s, 1H, C<u>H</u>-N, thiazolidinone ring), 4.83 (t, 1H, J_1 = 5 Hz, J_2= 10 Hz, C<u>H</u>-CO, thiazolidinone ring), 3.86 (s, 3H, O<u>CH</u>$_3$), 2.74 (m, 2H, CH_2), 2.61 (t, 3H, J_1 = 15 Hz, J_2= 10 Hz, CH_3), 2.16 (s, 3H, CH_3-pyrimidine ring); ^{13}CNMR (500 MHz, DMSO-d_6) (δ/ppm): 168.25, 162.81, 160.35, 150.72, 150.12, 147.33, 142.90, 138.02, 130.08, 129.64, 122.89, 121.34, 116.17, 112.47, 111.28, 100.31, 59.14, 56.05, 31.85, 21.01, 13.08; The EI-MS m/s (%): 500 $[M]^+$ (1.2), 326 $C_{18}H_{16}NO_3S^+$ (3.8), 302 $[C_{12}H_{18}N_2O_3S_2]^{\bullet+}$ (3.7), 268 $C_{11}H_{10}NO_3S_2^+$ (67.3), 133 $[C_5H_{11}NOS]^{\bullet+}$ (100), 77 $C_6H_5^+$ (88.3).

4-(5-ethyl-2-(4-hydroxy-3-methoxyphenyl)-4-oxothiazolidin-3-yl)benzenesulfonamide (**ZZ5A$_3$**)

Dark brown solid, yield: 77%; R_f: 0.94; m.p: 221-222 °C; Elemental Analysis for $C_{18}H_{20}N_2O_5S_2$ (408.49g/mol); Calcd: C, 52.92; H, 4.93; N, 6.86; S, 15.70. Found: C, 53.02; H, 4.87; N, 6.82; S, 15.73. IR (KBr) cm^{-1}: 3455 ν(OH), 3414 $\nu_{str.}(NH_2$, Asymmetrical), 3383 $\nu_{str.}(NH_2$, Symmetrical), 2924 ν(CH, Asymmetrical, aliph.), 2854 ν(CH, Symmetrical, aliph.), 1672 ν(C=O, thiazolidinone ring), 1593 ν(C-N, thiazolidinone ring), 1512, 1462 ν(C=C), 1342 $\nu_{str.}(SO_2$, Asymmetrical), 1145 $\nu_{str.}(SO_2$, Symmetrical), 940 ν(S-N), 763 $\nu_{str.}$(C-S-C, Asymmetrical), 675 $\nu_{str.}$(C-S-C, Symmetrical); ^{1}HNMR (500 MHz, DMSO-d_6) (δ/ppm): 8.92 (s, 1H, OH), 7.53 (d, 2H, *J* = 10 Hz, Ar-H), 7.49 (d, 2H, *J* = 10 Hz, Ar-H), 6.96 (d, 1H, *J* = 15 Hz, Ar-H), 6.87 (s, 1H, Ar-H), 6.69 (s, 2H,NH_2), 6.63 (d, 1H, *J*=10 Hz, Ar-H), 4.81 (s, 1H, C<u>H</u>-N, thiazolidinone ring), 3.74 (s, 3H, O<u>CH</u>$_3$), 3.52 (t, 1H, J_1 = J_2= 10 Hz, C<u>H</u>-CO, thiazolidinone ring), 2.85 (m, 2H, CH_2), 2.60 (t, 3H, J_1 = J_2= 10 Hz, CH_3); ^{13}CNMR (500 MHz, DMSO-d_6) (δ/ppm): 168.51, 148.97, 148.13, 143.65, 139.91, 136.33, 128.12, 122.91, 121.53, 116.43, 112.59, 61.21, 56.08, 50.05, 30.41, 18.15.

N-(4,6-dimethylpyrimidin-2-yl)-4-(5-ethyl-2-(4-hydroxy-3-methoxyphenyl)-4-oxothiazolidin-3-yl)benzenesulfonamide (**ZZ5A$_4$**)

Light orange solid, yield: 86%; R_f: 0.55; m.p: 272-275 °C; Elemental Analysis for $C_{24}H_{26}N_4O_5S_2$ (514.62g/mol); Calcd: C, 56.01; H, 5.09; N, 10.89; S, 12.46. Found: C, 56.04; H, 5.12; N, 10.84; S, 12.41. IR (KBr) cm^{-1}: 3464 ν(OH), 3252 ν(N-H), 2920 ν(CH, Asymmetrical, aliph.), 2847 ν(CH, Symmetrical, aliph.), 1643 ν(C=O, thiazolidinone ring), 1512 ν(C-N, thiazolidinone ring), 1581 ν(C=N, pyrimidine ring), 1427 ν(C=C), 1350 $\nu_{str.}(SO_2$, Asymmetrical), 1149 $\nu_{str.}(SO_2$, Symmetrical), 968 ν(S-N), 725 $\nu_{str.}$(C-S-C, Asymmetrical), 678 $\nu_{str.}$(C-S-C, Symmetrical); ^{1}HNMR (500 MHz, DMSO-d_6) (δ/ppm): 11.01 (s, 1H, NH), 9.54 (s, 1H, OH), 7.68 (d, 2H, *J* = 15 Hz, Ar-H), 7.24 (d, 2H, *J* = 20 Hz, Ar-H), 7.08 (d, 1H, *J* = 15 Hz, Ar-H), 6.92 (s, 1H,5-H, pyrimidine ring), 6.86 (s, 1H,Ar-H), 6.57 (d, 1H, *J* = 15 Hz, Ar-H), 5.98 (s, 1H, C<u>H</u>-N, thiazolidinone ring), 3.87 (s, 3H, O<u>CH</u>$_3$), 3.54 (t, 1H, J_1 = 10 Hz, J_2= 15 Hz, C<u>H</u>-CO, thiazolidinone ring), 2.76 (m, 2H, CH_2), 2.61 (t, 3H, J_1 = 10 Hz, J_2= 15 Hz, CH_3), 2.24, 2.29 (s, 6H, 2CH_3-pyrimidine ring); ^{13}CNMR (500 MHz, DMSO-d_6) (δ/ppm): 167.68, 163.43, 157.21, 153.31, 148.89, 148.44, 137.58, 130.46, 127.46, 125.77, 122.57, 116.18, 112.43, 110.81, 61.52, 56.07, 34.32, 31.86, 30.98, 23.70; The EI-MS m/s (%): 515 $[M]^+$ (1.1), 449 $C_{24}H_{25}N_4O_3S^+$ (1.0), 407 $C_{18}H_{19}N_2O_5S_2^+$ (1.1), 300 $[C_{12}H_{16}N_2O_3S_2]^{\bullet+}$ (2.1), 105 $[C_3H_7NOS]^{\bullet+}$ (100).

4-(5-ethyl-2-(4-hydroxy-3-methoxyphenyl)-4-oxothiazolidin-3-yl)-N-(thiazol-2-yl)benzenesulfonamide (**ZZ5A$_5$**)

Light brown solid, yield: 59%; R_f: 0.51; m.p: 208-210 °C; Elemental Analysis for $C_{21}H_{21}N_3O_5S_3$ (491.60g/mol); Calcd: C, 51.31; H, 4.31; N, 8.55; S, 19.57. Found: C, 51.23; H, 4.33; N, 8.59; S, 19.48. IR (KBr) cm^{-1}: 3452 ν(OH), 3225 ν(N-H), 2924 ν(CH, Asymmetrical, aliph.), 2854 ν(CH, Symmetrical, aliph.), 1716 ν(C=O, thiazolidinone ring), 1539 ν(C-

N, thiazolidinone ring), 1627 ν(C=N, thiazole ring), 1508, 1458 ν(C=C), 1373 $\nu_{str.}(SO_2$, Asymmetrical), 1130 $\nu_{str.}(SO_2$, Symmetrical), 941 ν(S-N), 763 $\nu_{str.}$(C-S-C, Asymmetrical), 686 $\nu_{str.}$(C-S-C, Symmetrical); ^{1}HNMR (500 MHz, DMSO-d_6) (δ/ppm): 9.04 (s, 1H, NH), 8.28 (s, 1H, OH), 7.43 (d, 2H, *J* = 10 Hz, Ar-H), 7.39 (d, 2H, *J* = 5 Hz, Ar-H), 6.88 (d, 1H, *J* = 5 Hz, 4-H, Thiazole ring), 6.82 (s, 1H, Ar-H), 6.715 (d, 1H, *J* = 15 Hz, Ar-H), 6.66 (d, 1H, *J* = 10 Hz, Ar-H), 6.51 (d, 1H, *J* = 10 Hz, 5-H, Thiazole ring), 5.70 (s, 1H, C<u>H</u>-N, thiazolidinone ring), 4.82 (t, 1H, J_1 = 15 Hz, J_2= 10 Hz, C<u>H</u>-CO, thiazolidinone ring), 3.68 (s, 3H, OC<u>H</u>$_3$), 2.86 (m, 2H, CH_2), 2.61 (t, 3H, J_1 =10 Hz, J_2= 15 Hz, CH_3); ^{13}CNMR (500 MHz, DMSO-d_6) (δ/ppm): 169.57, 158.12, 148.89, 148.03, 142.16, 138.53, 135.15, 130.81, 130.03, 122.98, 122.18, 116.07, 112.13, 108.16, 61.50, 56.65, 51.31, 30.89, 20.15; The EI-MS m/s (%): 491 $[M]^+$ (1.0), 447 $[C_{20}H_{21}N_3O_3S_3]^{\bullet+}$ (1.4), 363 $C_{17}H_{19}N_2O_3S_2^+$ (1.2), 261 $C_{13}H_{13}N_2O_2S^+$ (2.2), 172 $[C_6H_8N_2O_2S]^{\bullet+}$ (27), 105 $[C_3H_7NOS]^{\bullet+}$ (100), 92 $C_6H_6N^+$ (69.2).

4-(5-ethyl-2-(4-hydroxy-3-methoxyphenyl)-4-oxothiazolidin-3-yl)-N-(pyrimidin-2-yl)benzenesulfonamide (**ZZ5A$_6$**)

White crystalline solid, yield: 68%; R_f: 0.77; m.p: 230-232 ^{o}C; Elemental Analysis for $C_{22}H_{22}N_4O_5S_2$ (486.56g/mol); Calcd: C, 54.31; H, 4.56; N, 11.51; S, 13.18. Found: C, 54.36; H, 4.50; N, 11.48; S, 13.22. IR (KBr) cm^{-1}: 3425 ν(OH), 3255 ν(N-H), 2924 ν(CH, Asymmetrical, aliph.), 2854 ν(CH, Symmetrical, aliph.), 1716 ν(C=O, thiazolidinone ring), 1585 ν(C-N, thiazolidinone ring), 1651 ν(C=N, pyrimidine ring), 1492, 1438 ν(C=C), 1323 $\nu_{str.}(SO_2$, Asymmetrical), 1153 $\nu_{str.}(SO_2$, Symmetrical), 941 ν(S-N), 725 $\nu_{str.}$(C-S-C, Asymmetrical), 682 $\nu_{str.}$(C-S-C, Symmetrical); ^{1}HNMR (500 MHz, DMSO-d_6) (δ/ppm): 11.27 (s, 1H, NH), 10.60 (s, 1H, OH), 8.46 (d, 2H, *J* = 10 Hz, 2 C<u>H</u>=N, pyrimidine ring), 7.94 (d, 2H, *J* = 15 Hz, Ar-H), 7.61 (d, 2H, *J* = 15 Hz, Ar-H), 7.35 (d, 1H, *J* = 15 Hz, Ar-H), 7.01 (t, 1H, J_1 = 10 Hz, J_2= 5 Hz, 5-H, pyrimidine ring), 6.57 (d, 2H, *J* = 15 Hz, H-f,Ar-H), 6.03 (s, 1H, C<u>H</u>-N, thiazolidinone ring), 3.54 (t, 1H, J_1 = 10 Hz, J_2= 15 Hz, C<u>H</u>-CO, thiazolidinone ring), 3.05 (s, 3H, OC<u>H</u>$_3$), 2.76 (m, 2H, CH_2), 2.61 (t, 3H, J_1 = 15 Hz, J_2= 10 Hz, CH_3); ^{13}CNMR (500 MHz, DMSO-d_6) (δ/ppm): 166.65, 158.72, 157.67, 153.50, 152.22, 139.33, 135.06, 130.29, 125.24, 123.43, 121.46, 115.98, 112.57, 111.57, 70.07, 67.25, 56.40, 24.30, 14.00; The EI-MS m/s (%): 491 $[M]^+$ (1.2), 407 $C_{18}H_{19}N_2O_5S_2^+$ (1.5), 379 $C_{17}H_{19}N_2O_4S_2^+$ (1.5), 267 $C_{12}H_{15}N_2O_3S^+$ (1.6), 185 $C_{10}H_9N_4^+$ (100), 95 $[C_4H_5N_3]^{\bullet+}$ (69.4).

4-(5-ethyl-2-(2-hydroxyphenyl)-4-oxothiazolidin-3-yl)-N-(4-methylpyrimidin-2-yl)benzenesulfonamide (**ZZ5A$_9$**)

Dark yellow solid, yield: 78%; R_f: 0.84; m.p: 155-158 ^{o}C; Elemental Analysis for $C_{22}H_{22}N_4O_4S_2$ (470.56g/mol); Calcd: C, 56.15; H, 4.71; N, 11.91; S, 13.63. Found: C, 56.09; H, 4.74; N, 11.97; S, 13.54. IR (KBr) cm^{-1}: 3471 ν(OH), 3375 ν(N-H), 2924 ν(CH, Asymmetrical, aliph.), 2854 ν(CH, Symmetrical, aliph.), 1716 ν(C=O, thiazolidinone ring), 1589 ν(C-N, thiazolidinone ring), 1620 ν(C=N, pyrimidine ring), 1496, 1435 ν(C=C), 1330 $\nu_{str.}(SO_2$, Asymmetrical), 1149 $\nu_{str.}(SO_2$, Symmetrical), 972 ν(S-N), 756 $\nu_{str.}$(C-S-C, Asymmetrical), 675 $\nu_{str.}$(C-S-C, Symmetrical); ^{1}HNMR (500 MHz, DMSO-d_6) (δ/ppm): 11.19 (s, 1H, NH), 10.21 (s, 1H, OH), 8.27 (d, 1H, *J* = 12 Hz, C<u>H</u>=N, pyrimidine ring), 7.97 (d, 2H, *J* = 18 Hz, Ar-H), 7.73 (d, 1H, *J* = 6 Hz, Ar-H), 7.62 (d, 2H, *J* = 10 Hz, Ar-H), 7.13 (t, 1H, J_1 = 15 Hz, J_2 = 18 Hz, Ar-H), 6.97 (t, 1H, J_1 = 6 Hz, J_2 = 3 Hz,Ar-H), 6.86 (d, 1H, *J* = 3 Hz, Ar-H), 6.55 (d, 1H, *J* = 9 Hz, 5-H, pyrimidine ring), 5.99 (s, 1H, C<u>H</u>-N, thiazolidinone ring), 4.21 (t, 1H, J_1 = J_2= 6 Hz, C<u>H</u>-CO, thiazolidinone ring), 2.81 (m, 2H, CH_2), 2.30 (s, 3H, C<u>H</u>$_3$-pyrimidine ring), 1.15 (t, 3H, J_1 = 21 Hz, J_2= 30 Hz, CH_3); ^{13}CNMR (500 MHz, DMSO-d_6) (δ/ppm): 167.96, 158.18, 154.67, 152.83, 148.93, 142.17, 134.64, 132.32, 130.08, 128.53, 125.92, 122.78, 122.12, 118.34, 115.82, 60.18, 45.79, 28.02, 22.51, 18.22.

4-(5-ethyl-2-(2-hydroxyphenyl)-4-oxothiazolidin-3-yl)benzenesulfonamide (**ZZ5A$_{10}$**)

Dark gray solid, yield: 87%; R_f: 0.79; m.p: 124-125 ^{o}C; Elemental Analysis for $C_{17}H_{18}N_2O_4S_2$ (378.47g/mol); Calcd: C, 53.95; H, 4.79; N, 7.40; S, 16.94. Found: C, 54.01; H, 4.82; N, 7.36; S, 16.86. IR (KBr) cm^{-1}: 3455 ν(OH), 3236 $\nu_{str.}(NH_2$, Asymmetrical), 3171 $\nu_{str.}(NH_2$, Symmetrical), 2989 ν(CH, Asymmetrical, aliph.), 1735 ν(C=O, thiazolidinone ring), 1562 ν(C-N, thiazolidinone ring), 1492, 1465 ν(C=C), 1319 $\nu_{str.}(SO_2$, Asymmetrical), 1157 $\nu_{str.}(SO_2$, Symmetrical), 868 ν(S-N), 756 $\nu_{str.}$(C-S-C, Asymmetrical), 624 $\nu_{str.}$(C-S-C, Symmetrical); ^{1}HNMR (500 MHz, DMSO-d_6) (δ/ppm): 8.87 (s, 1H, OH), 7.92 (d, 2H, *J* = 10 Hz,

Ar-H), 7.55 (d, 2H, *J* = 10 Hz, Ar-H), 7.13 (d, 1H, *J* = 5 Hz, Ar-H), 7.02 (s, 2H,NH_2), 6.935 (t, 1H, J_1 =10 Hz, J_2= 5 Hz, Ar-H), 6.69 (t, 1H, J_1 = 5 Hz, J_2= 10 Hz, Ar-H), 6.48 (d, 1H, *J* = 10 Hz, Ar-H), 5.97 (s, 1H, CH-N, thiazolidinone ring), 3.68 (t, 1H, J_1 = 5 Hz, J_2= 10 Hz, CH-CO, thiazolidinone ring), 2.76 (m, 2H, CH_2), 2.38 (t, 3H, $J_1 = J_2$= 10 Hz, CH_3); ^{13}CNMR (500 MHz, DMSO-d_6) (δ/ppm): 170.36, 152.88, 146.57, 135.17, 130.83, 128.90, 128.19, 125.94, 123.59, 122.72, 112.77, 61.50, 55.02, 31.85, 18.26; The EI-MS m/s (%): 379 $[M]^+$ (5.2), 302 $[C_{12}H_{18}N_2O_3S_2]^{\bullet+}$ (2.5), 277 $C_{13}H_{13}N_2O_3S^+$ (10.1), 222 $[C_{11}H_{14}N_2OS]^{\bullet+}$ (30.7), 171 $C_6H_7N_2O_2S^+$ (56.5), 106 $C_3H_8NOS^+$ (21.3), 77 $C_6H_5^+$ (100).

4-(5-ethyl-2-(2-hydroxyphenyl)-4-oxothiazolidin-3-yl)-N-(thiazol-2-yl)benzenesulfonamide (**ZZ5A$_{11}$**)

Dark brown solid, yield: 91%; R_f: 0.68; m.p: 131-132 °C; Elemental Analysis for $C_{20}H_{19}N_3O_4S_3$ (461.58g/mol); Calcd: C, 52.04; H, 4.15; N, 9.10; S, 20.84. Found: C, 52.11; H, 4.11; N, 9.01; S, 20.91. IR (KBr) cm^{-1}: 3425 ν(OH), 3259 ν(N-H), 2924 ν(CH, Asymmetrical, aliph.), 2854 ν(CH, Symmetrical, aliph.), 1716 ν(C=O, thiazolidinone ring), 1585 ν(C-N, thiazolidinone ring), 1651 ν(C=N, thiazole ring), 1492, 1438 ν(C=C), 1323 $\nu_{str.}$(SO_2, Asymmetrical), 1153 $\nu_{str.}$(SO_2, Symmetrical), 941 ν(S-N), 725 $\nu_{str.}$(C-S-C, Asymmetrical), 682 $\nu_{str.}$(C-S-C, Symmetrical); ^{1}HNMR (500 MHz, DMSO-d_6) (δ/ppm): 9.55 (s, 1H, NH), 7.39 (d, 2H, *J* = 10 Hz, Ar-H), 7.16 (d, 3H, *J* = 15 Hz, Ar-H), 7.07 (t, 1H, J_1 = 15 Hz, J_2= 10 Hz, Ar-H), 6.81 (d, 1H, *J* = 10 Hz, H-j, 4-H, Thiazole ring), 6.75 (t, 1H, $J_1 = J_2$= 10 Hz, Ar-H), 6.56 (d, 1H, *J* = 10 Hz, Ar-H), 6.49 (d, 1H, *J* = 15 Hz, 5-H, Thiazole ring), 5.68 (s, 1H, CH-N, thiazolidinone ring), 4.83 (s, 1H, OH), 3.55 (t, 1H, J_1 = 15 Hz, J_2= 10 Hz, CH-CO, thiazolidinone ring), 2.76 (m, 2H, CH_2), 2.39 (t, 3H, $J_1 = J_2$= 10 Hz, CH_3); ^{13}CNMR (500 MHz, DMSO-d_6) (δ/ppm): 166.74, 161.51, 149.30, 136.99, 130.83, 130.08, 128.92, 128.19, 127.64, 125.16, 122.72, 119.30, 115.62, 112.77, 61.50, 34.32, 31.85, 30.96; The EI-MS m/s (%): 462 $[M]^+$ (1.0), 255 $[C_9H_9N_3O_2S_2]^{\bullet+}$ (0.5), 182 $C_9H_{12}NOS^+$ (4.0), 164 $[C_3H_4N_2O_2S_2]^{\bullet+}$ (3.3), 101 $C_3H_5N_2S^+$ (60.5), 86 $C_4H_8NO^+$ (100).

4-(5-ethyl-2-(2-hydroxyphenyl)-4-oxothiazolidin-3-yl)-N-(pyrimidin-2-yl)benzenesulfonamide (**ZZ5A$_{12}$**)

Yellowish brown solid, yield: 76%; R_f: 0.74; m.p: 148-150 °C; Elemental Analysis for $C_{21}H_{20}N_4O_4S_2$ (456.54 g/mol); Calcd: C, 55.25; H, 4.42; N, 12.27; S, 14.05. Found: C, 55.28; H, 4.39; N, 12.29; S, 14.01. IR (KBr) cm^{-1}: 3452 ν(OH), 3375 ν(N-H), 2924 ν(CH, Asymmetrical, aliph.), 2854 ν(CH, Symmetrical, aliph.), 1716 ν(C=O, thiazolidinone ring), 1585 ν(C-N, thiazolidinone ring), 1635 ν(C=N, pyrimidine ring), 1492, 1438 ν(C=C), 1327 $\nu_{str.}$(SO_2, Asymmetrical), 1149 $\nu_{str.}$(SO_2, Symmetrical), 941 ν(S-N), 756 $\nu_{str.}$(C-S-C, Asymmetrical), 675 $\nu_{str.}$(C-S-C, Symmetrical); ^{1}HNMR (500 MHz, DMSO-d_6) (δ/ppm): 11.32 (s, 1H, NH), 9.60 (s, 1H, OH), 8.48 (d, 2H, *J* = 6 Hz, 2 CH=N, pyrimidine ring), 8.03 (d, 2H, *J* = 6 Hz, Ar-H), 7.60 (d, 2H, *J* = 9 Hz, Ar-H), 7.09 (t, 1H, J_1 = 6 Hz, J_2= 9 Hz, Ar-H), 6.99 (t, 1H, J_1 = 3 Hz, J_2= 6 Hz, 5-H, pyrimidine ring), 6.82 (d, 1H, *J* = 9 Hz, Ar-H), 6.71 (t, 1H, J_1 = 9 Hz, J_2= 6 Hz, Ar-H), 6.55 (d, 1H, *J* = 9 Hz, Ar-H), 6.01 (s, 1H, CH-N, thiazolidinone ring), 4.21 (t, 1H, J_1 = 6 Hz, J_2= 12 Hz, CH-CO, thiazolidinone ring), 2.83 (m, 2H, CH_2), 2.60 (t, 3H, $J_1 = J_2$= 6 Hz, CH_3); ^{13}CNMR (500 MHz, DMSO-d_6) (δ/ppm): 170.13, 156.34, 154.11, 148.16, 138.42, 132.51, 130.06, 128.89, 128.34, 125.09, 120.62, 119.98, 116.73, 113.01, 56.81, 45.97, 24.35, 13.08; The EI-MS m/s (%): 458 $[M]^+$ (1.2), 428 $[C_{19}H_{16}N_4O_4S_2]^{\bullet+}$ (1.2), 363 $C_{15}H_{15}N_4O_3S_2^+$ (1.5), 274 $C_{15}H_{16}NO_2S^+$ (32.3), 251 $C_{10}H_{11}N_4O_2S^+$ (2.3), 185 $C_{10}H_9N_4^+$ (37.7), 121 $[C_8H_{11}N]^{\bullet+}$ (50.2), 105 $[C_3H_7NOS]^{\bullet+}$ (100).

Acute toxicity (LD_{50})

Healthy albino mice of either sex (male and female), age from 7-9 weeks and their body weight ranged between 23-33 g, were used for study acute toxicity of 2-azetidinone (**Z5A$_{11}$**) and 2-azetidinone (**Z5A$_{11'}$**) derivatives. The animals were injected intraperitonially with the first dose 500 mg/kg. The result was read death X or life O after 24 hour, and increases or decreases the amount of dose was constant 50 mg/kg and repeat dosing up or down for 4 mice after changing the result death to life and versa. LD_{50} were calculated based on the diagram and equation of Dixon LD_{50} = Xf +Kd, where Xf: the last dose, K: the interval between dose levels, d: the tabulated value, Table 1.[23]

Table 1: The tabulated Dixon values

	K represented serial tests started with :-				
	O	OO	OOO	OOOO	
XOOO	0.157-	0.154-	0.154-	0.154-	OXXX
XOOX	0.878-	0.861-	0.860-	0.860-	OXXO
XOXO	0.701	0.747	0.741	0.741	OXOX
XOXX	0.084	0.169	0.181	0.182	OXOO
XXOO	0.305	0.372	0.380	0.381	OOXX
XXOX	0.305-	0.169	0.144-	0.142-	OOXO
XXXO	1.288	1.500	1.544	1.549-	OOOX
XXXX	0.555	0.0897	0.985	1.000	OOOO
	X	XX	XXX	XXXX	
	K represented serial tests started with :-				

Antibacterial Activity

The compounds (**Z5A$_2$, Z5A$_9$-Z5A$_{11}$, Z5A$_{2'}$**and **Z5A$_{9'}$-Z5A$_{11'}$**) were screened in vitro for antibacterial properties. The panel of pathogens involved *Staphylococcus aureus* and *Bacillus* as a Gram-positive bacterium, *Escherichia coli* and *Pseudomonas aeruginosa* as a Gram-negative bacterium, by using agar diffusion method. The antibiotic tetracycline was use to calibrate and to comparison with the antibacterial stuff. 0.2 mL of bacterial inoculums were uniformly spread using sterile cotton swab on a sterile Petri dish Mueller Hinton Agar (MHA). The tested compounds and tetracycline drug were dissolved in DMSO with concentrations include (1, 5, 25,125, 250 and 500) mg /mL for each compound. 50 µl from 1-500 mg/mL concentrations of tested compounds and tetracycline were added to every well (7 mm diameter holes cut within the agar gel, 20 mm aside from one another). The plates were incubated for twenty-four h at 36ºC ± 1ºC, under aerobic conditions. After incubation, confluent bacterial growth was observed. Inhibition of the bacterial growth was measured in mm.[24] Furthermore, values of minimum inhibitory concentration (MIC) of those compounds.[25] The MIC was recorded because the lowest concentration at which no visible growth was observed.

Antioxidant Activity

The antioxidant activity of the Azetidin-2-one, (**Z5A$_2$, Z5A$_9$-Z5A$_{11}$, Z5A$_{2'}$ and Z5A$_{9'}$-Z5A$_{11'}$**) and Thiazolidin-4-one (**ZZ5A$_2$-ZZ5A$_6$, ZZ5A$_9$-ZZ5A$_{12}$**) was determined according to the *β*-carotene bleaching method.[26] The *β*-carotene bleaching method is based on the loss of the yellow color of *β*-carotene because of its reaction with radicals formed by linoleic acid oxidation in an emulsion and according to previous methods.[27] A solution of *β*-carotene was prepared by dissolving 0.01 gm of *β*-carotene in 50 ml of chloroform, 1 ml of this solution was then pipetted into round-bottom rotary flask containing (0.02 ml) of linoleic acid and (0.2 ml) of Tween-20. After removing the chloroform by vacuum evaporation using a rotary evaporator at room temperature, 50ml of distilled water were added to the flask with manual shaking as first stage. The emulsion (3.8 mL) was added to tubes containing 0.2 mL of the prepared compounds and reference (BHT) compound (which prepared by dissolving 0.01 gm of these compounds in 0.2 ml of DMSO) The absorbance was read at 470 nm, the samples were then subjected to thermal autoxidation at 45°C in a water bath for 2 h. Absorbance was measured every 15 min.[26] Antioxidant activity (AA) was calculated as percent of inhibition relative to the control using the following equation :

$$\%AA = 1 - [(Ai - At) / (Ai^* - At^*)] \times 100$$

Where, Ai : is the measured absorbance value of sample at zero time. At : is the measured absorbance value of sample after incubation (105) min at 45°C. Ai* : is the measured absorbance value of control at zero time, At* : is the measured absorbance value of control after incubation (105)min at 45°C.

Anti-Breast Cancer Activity

A) *In vitro*MTT cellular viability assay

The Cytotoxicity of samples on MCF-7 cell line were determined by the MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazoliumbromide) cell viability assay.[28] Cells at a density of 1×10^4 cells/mL (100 µL/well) were seeded in 96-well plates and incubated overnight under 5% CO_2 at 37 °C, followed by exposure to a series of concentrations (6.25, 12.5, 25, 50, 75 and 100 µg/mL) of the tested compounds (Z5A$_{11}$ and Z5A$_{11'}$) and 5-Fluorouracil as reference drug. At the same time, a group only containing culture medium was set as blank control. Each group had three biological repeats. After dosing for 72 h, the cells were washed and then fresh medium (100 µL) supplemented with 28 µL of 2 mg/mL solution of MTT was added to each well. After incubated in the dark for 2 h at 37 °C, removing the MTT solution and the crystals remaining in the wells were solubilized by the addition of 100 µL of DMSO followed by 37 °C incubation for 15 min with shaking.[29] The optical density at 620 (OD620) of each well were measured by plate reader (Synergy H4: Bio-Tek, Winooski, VT, USA). The results are presented as mean ± standard deviation (SD). The survival rate of control cells treated with 0 M the tested compounds was set as 100%. Cell viability was calculated using the following Equation :

Cell viability (%) = [(dosing cell OD – blank OD) / (control cell OD – blank OD)] × 100

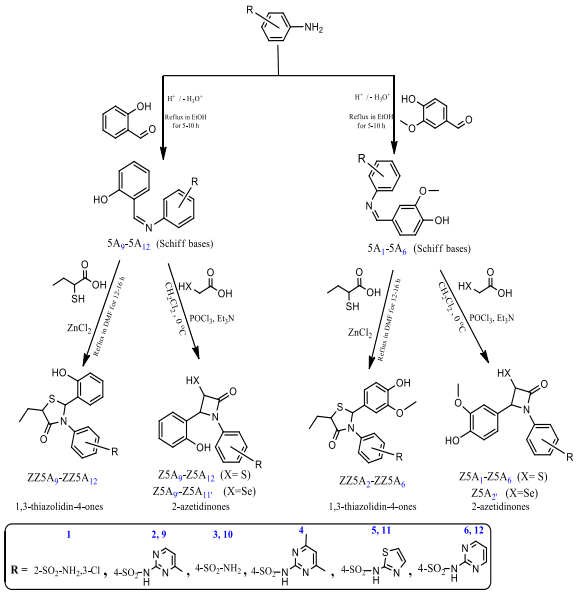
B) Acridine Orange/Ethidium Bromide Staining

Morphological apoptosis of MCF-7 cells treated with different concentrations of the new

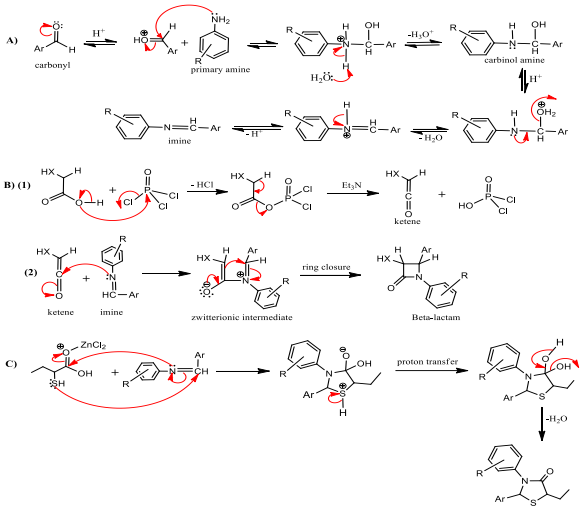
prepared compounds ($Z5A_{11}$ and $Z5A_{11'}$) and standard (5-Fluorouracil) were assessed using an acridine orange/ethidium bromide (AO/EB) staining kit (Solarbio, Beijing, China, Cat No. CA1140). The density of 1×10^4 MCF-7 cells/mL was plated in 6-well plates (1 mL/well) and incubated overnight. The medium was replaced with the tested compounds-containing (6.25, 12.5, 25, 50, 75 and 100 µg/mL) medium and incubated for 48 h under the same conditions mentioned before. Cells were washed with PBS and stained with AO/EB solution (20 µL AO/EB freshly mixed solution of equal volume in 1 mL PBS) for 2–3 min in the dark. After the successive washes, the fluorescent images were taken with an inverted fluorescence microscope (Olympus Corporation, Beijing, China).[30]

3. Results and Discussion

The 2-azetidinone$Z5A_1$-$Z5A_6$, $Z5A_9$-$Z5A_{12}$, $Z5A_{2'}$, $Z5A_9$-$Z5A_{11'}$ and 1,3-thiazolidin-4-one $ZZ5A_2$-$ZZ5A_6$, $ZZ5A_9$-$ZZ5A_{12}$ compounds were prepared via reaction of Schiff's bases with ketene and 2-mercaptobutanoic acid, respectively. The prepared thiazolidin-4-ones are solid Compounds, often melting with decomposition but the attachment of an alkyl group to the nitrogen lowered its melting point compared to the *β*-Lactam compounds. 2-azetidinones and 1,3-thiazolidin-4-ones are stable in air and they are soluble in most non-polar solvents, the suggested mechanism for preparing a 2-azetidinone and thiazolidin-4-one ring are shown in scheme 2. Also, the existence of interactive unsaturated ketone group in 2-azetidinones and thiazolidin-4-ones are accountable for their biological activities.[21] The elemental analysis results C, H, N, S of the studied compounds are in agreement with the theoretical values.



Scheme 1: Synthesis of 2-azetidinones and 1,3-thiazolidin-4-ones



Scheme 2: A- The suggested mechanism of Schiff bases, B- The suggested mechanism of *β*-Lactams Compounds and C- The suggested mechanism of Thiazolidin-4-one Compounds

Spectroscopic analysis

Spectral studies including the observed spectroscopic results for the title compounds are discussed. All the synthesized compounds gave a spectroscopic analysis consistent with the empirical structures. A complete set of spectral data of studied compounds is given in Supplementary data.

Infrared spectra (FT-IR): The infrared spectra show the position and the intensities of the peaks which corresponds to various groups present in each compound. The infrared of prepared compounds

($5A_1$-$5A_6$, $5A_9$-$5A_{12}$) shows characteristic bonds at 1593-1620 cm^{-1}that be attributed to the azomethine ν(CH=N) stretching vibration.[18] All the infrared spectra of the compounds were characterized by a broad band at 3417–3560 cm^{-1}which corresponds to the ν(O-H) stretching vibration.[4] IR spectra of the compounds ($5A_1$, $Z5A_1$, $Z5A_3$, $Z5A_{10}$, $Z5A_{10'}$, $ZZ5A_3$, $ZZ5A_{10}$) show two bands within the range 3171–3414 cm^{-1}which attributed to asymmetric and symmetric stretching of$\nu(NH_2)$ groups. In addition, the medium to weak bands at 3147-3444 cm^{-1}can correspond to the ν(N-H) stretching vibration. Ring closure in 2-azetidinones and 1,3-thiazolidin-4-ones can be observed by the appearance of strong bands at 1643-1739 cm^{-1} and at 1512-1597 cm^{-1} which attributed to the stretching vibration of the carbonyl group ν(C=O) and ν(C-N) respectively.[12,22]

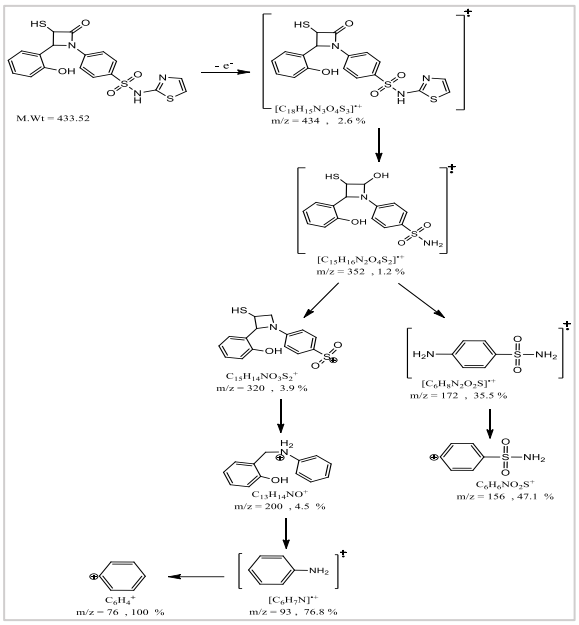
The medium to weak bands at the range 2420-2692 cm^{-1} and at 2366-2580 cm^{-1}can be assigned to the ν(S-H) and ν(Se-H) absorption frequencies respectively.[31] Furthermore, the medium to weak bands which appeared in the range 642-679 cm^{-1} and at 509-667 cm^{-1} are attributed to the ν(C-S) and ν(C-Se) stretching respectively for the 2-azetidinone compounds.[32,33] The spectrum was distinguished by the appearance of distinct absorption bands for ν(C-S-C) at the range 725-763 cm^{-1} and in 624-686 cm^{-1}, which assigned to asymmetrical and symmetrical stretching vibration respectively for the 1,3-thiazolidin-4-ones ($ZZ5A_2$-$ZZ5A_6$, $ZZ5A_9$-$ZZ5A_{12}$).[12,34] All the prepared compounds show featured bands at the range 1269-1396 cm^{-1} and in 1130-1172 cm^{-1}, which assigned to asymmetrical and symmetrical stretching vibration respectively of (SO_2) group.[34] In addition, the strong band at 1006–1161 cm^{-1}can correspond to the phenolic (C-O) stretching vibration. Appearance of strong to medium bands at the range 840-997 cm^{-1} in IR spectrum can be related to stretching of ν(S-N) for the prepared compounds.[4]

^{1}HNMR and ^{13}CNMRSpectra: The structures of all new compounds were confirmed and the formation of five- or four-membered ring by ^{1}HNMR spectra. The ^{1}HNMR spectra of all compounds show a singlet signal at the range δ 8.28-10.60 ppm, which attributed to phenolic group (OH).[4] The β-lactam compounds ($Z5A_1$-$Z5A_6$, $Z5A_9$-$Z5A_{12}$, $Z5A_{2'}$, $Z5A_{9'}$-$Z5A_{11'}$) are characterized by showing triplet signal at δ 3.04-3.82 ppm and which can be assigned to the 3-H proton of 2-azetidinone ring. They also display a doublet signal at δ 3.26-4.41 ppm which is attributed to the 4-H proton of azetidine-2-one ring.[11,35]Furthermore, all β-lactam compounds have doublet signal at δ 1.19-2.09 ppm and δ 1.20-2.29 ppm, which can be assigned to the (SH) and (SeH) protonsrespectively.[36] All the 1,3-thiazolidin-4-onecompounds are characterized by showing triplet signal at δ 3.52-4.83 ppm, which attributed to the (CH-S) proton of thiazolidinone ring. The proton of (CH-N) group of thiazolidinone rings appear at δ 4.81-6.03 ppm.[22]The two signals at δ 2.74-2.86 ppm and at δ 1.15-2.61 ppm are assigned to the CH_2 and CH_3protons of ethyl group respectively for 1,3-thiazolidin-4-one compounds. Also, multiple signals that appear at δ 6.48-8.46 ppm can be attribute to aromatic rings of the studied compounds.[22] In addition, the studied compounds ($Z5A_1$, $Z5A_3$, $Z5A_{10}$, $Z5A_{10'}$, $ZZ5A_3$ and $ZZ5A_{10}$) have singlet signal at δ6.69-7.29 ppm that due to the presence of two protons of (NH_2) group of sulfonamide which innervate the desired results.[4]The proton of (NH) group of compounds ($Z5A_2$, $Z5A_{2'}$, $Z4A_4$-$Z5A_6$, $Z5A_9$, $Z5A_{9'}$, $Z5A_{11}$, $Z5A_{11'}$, $Z5A_{12}$, $ZZ5A_2$, $ZZ5A_4$-$ZZ5A_6$, $ZZ5A_9$, $ZZ5A_{11}$ and $ZZ5A_{12}$) appear at δ 9.04 -12.88 ppm. Therefore, the ^{1}HNMR result supports the formation of four- or five-membered ring.

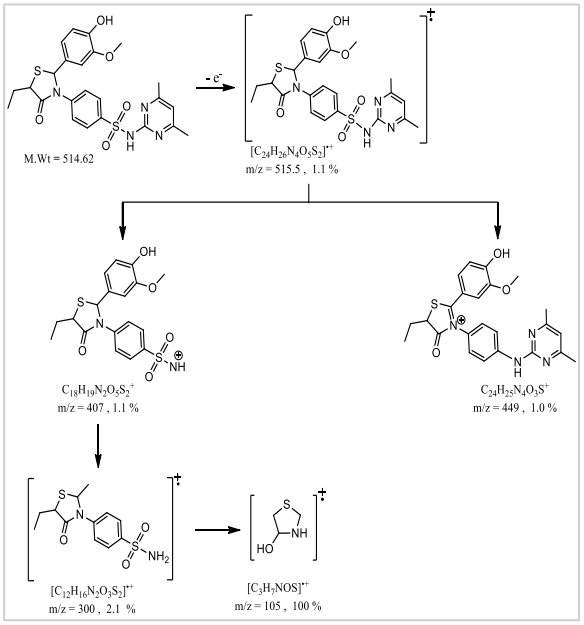
The ^{13}C-NMR spectra of all studied compounds show signal at the range δ (168.12 - 174.58) ppm and signal at δ (166.65 - 170.36) ppm which attribute to carbonyl carbon of the azetidine-2-one and 1,3-thiazolidin-4-one compoundsrespectively.[22] The β-lactam compounds are characterized by showing two signals at δ (50.96-69.33) ppm and δ (45.65-58.94) ppm and which can be assigned to the 4-C and 3-C of 2-azetidinone ring respectively.[11]Also, the spectra of the thiazolidinone derivatives exhibited two signals at δ (56.81-100.31) ppm and δ (34.32-56.05) ppm which can be assigned to the 2-C and 5-C of 1,3-thiazolidin-4-one ring respectively.[22]Furthermore, the two signals of the ethyl group observed at the range δ (21.01-31.85) ppm and at δ (13.08-30.96) ppm for 1,3-thiazolidin-4-one ring. Additionally, the signals of aromatic carbons of these synthesized compounds represented at δ (106.26-163.83) ppm.[4]The ^{13}CNMR spectral data of the 2-azetidinones and Thiazolidin-4-

onesare in accord with suggested structures. Some spectra of compounds showed in Figures 1,2.

EI-mass: Mass spectrometry as a powerful structural characterization technique in coordination chemistry has been successfully used to confirm the molecular ion peaks of the 2-azetidinoneand Thiazolidin-4-one compounds. The peaks intensity brings out an idea about the stability of fragments principally the base peak. The electron impact spectrum of the synthesized compounds is differentiating by high relative intensity molecular ion peaks.[37] The mass spectrum of all studied compounds detects the molecular ion peaks $[M]^+$ are in excellent acceptance with the suggested structures. The potential suggested ion fragments with the appearance of the result of fragmentation of these synthesized compounds are shown in Schemes (3 and 4)and Figure 3, furthermore the peaks intensity gives an idea about the stability of fragments primarily with the base peaks. The mass spectrum of the compound $Z5A_1$shows several fragmentation peaks at m/z 396, m/z 367, m/z 302, and m/z 189, these peaks can be assigned to $[C_{16}H_{13}ClN_2O_4S_2]^{\bullet+}$, $C_{15}H_{12}ClN_2O_3S_2^+$, $[C_{15}H_{11}ClN_2OS]^{\bullet+}$ and $C_6H_4ClNO_2S^+$ ions, respectively. The mass spectrum of the compound $Z5A_{10'}$ shows three fragmentation peaks at m/z 287, m/z 172 and m/z 156, these peaks can be attributed to $C_9H_6NO_3SSe^+$, $[C_6H_8N_2O_2S]^{\bullet+}$ and $C_6H_6NO_2S^+$ ions, respectively. On other hand the mass spectrum of compound $ZZ5A_4$ characterized by the appearance of three fragmentation peaks at m/z 449, m/z 407 and m/z 300which can be attributed to $C_{24}H_{25}N_4O_3S^+$, $C_{18}H_{19}N_2O_5S_2^+$ and $[C_{12}H_{16}N_2O_3S_2]^{\bullet+}$ ions respectively. The base peaks at m/z 86 can be assigned to $C_4H_8NO^+$ ion for most 2-azetidinone compounds. Furthermore, the base peaks of Thiazolidin-4-one compounds shows at m/z 105 which can be assigned to $[C_3H_7NOS]^{\bullet+}$ ion.



Scheme 3: The fragmentation pattern proposed for compound ($Z5A_{11}$)



Scheme 4: The fragmentation pattern proposed for compound ($ZZ5A_4$)

Biological activity

Median lethal dose (LD_{50})

The lethal dose (LD_{50}) of the studied compounds ($Z5A_{11}$ and $Z5A_{11'}$) *in-vivo* was determined in mice via intraperitonially injecting dosages ranging from 500-700 mg/kg with equal spacing (concentrations) between doses. Our data revealed that LD_{50} values were 658.45 and 718.6 mg/kg for the compounds $Z5A_{11}$ and $Z5A_{11'}$, respectively. The results may give

an indicated about the moderately toxicity effect of the studied compounds and clinical change that observed in the mice after giving different doses. The toxic signs observed in injected mice may be manifested in some behaviours such as tremors, straight tail, salivation, urination, lacrimation, defecation, shortness of breath, excitation, muscle fasciculations, capillary bulge, convulsions and also the tortuous reflex in some treatments, and finally Death at high toxic doses, Table 2.[38,39]

Table 2: Toxicity results (LD_{50}) of and toxic signs on mice

Test characterization	Results	
	Z5A$_{11}$	**Z5A$_{11'}$**
Doses range	500-650=150 mg/kg	300-700=150 mg/kg
First dose	500 mg/kg	500 mg/kg
Last dose	650 mg/kg	700 mg/kg
Up and down dose	50 mg/kg	50 mg/kg
Median lethal dose (LD_{50}) mg/kg	658.45 mg/kg	718.6 mg/kg
Effective dose (LD_{50} /10) mg/kg	65.845 mg/kg	71.86 mg/kg
No. of mice	8 (XOXXOXOO)	8 (XXOOOOXX)
Onset of toxic signs	5-16 minutes	5-24 minutes
Toxic signs	Rolling convulsions, excitation, salivation, choreoathetosis, tremors, death	Salivation, dyspnoea, convulsions, excitation, tremors, muscle fasciculation, death

Antibacterial activity

The sensitivity of four human pathogenic microbes (two of Gram-positive bacteria: *Staphylococcus aureus, Bacillus* and two of Gram-negative bacteria: *Escherichia coli*, *Pseudomonas aeruginosa*) to the new synthetic heterocyclic compounds (**Z5A$_2$**, **Z5A$_9$-Z5A$_{11}$**, **Z5A$_{2'}$**and **Z5A$_{9'}$-Z5A$_{11'}$**) was tested and compared to that of commercially available antibacterial antibiotic tetracycline. Our study confirmed that the 2-azetidinonecompounds had antibacterial activity (increases as the compound concentration increases) against the studied bacteria, also minimum inhibitory concentration MIC which can define as the lowest concentration of the compound in medium which out visible growth of the test organisms in concentration ranging from 1-500 mg/mL, as shown in Table 3.

All the scientific studies reported that the antibiotics had the ability to introduce the main basis for the therapy of microbes infections. On the other hand, the bacteria had a highly genetic variability which enables them to rapidly evade the effect of antibiotics via developing antibiotic resistance. Furthermore, the development in recent years of the ability of pathogenic bacteria and parasites to resist multi-drugs has resulted in major clinical problems in the treatment of infectious diseases.[40] The toxicity of some antimicrobial drugs on host tissues and other problems have raised the need for attention in the search for new antimicrobial substances. Moreover, *Escherichia coli* is one of the most dangerous microbes that cause many common diseases in humans, frequently associated with urinary tract infections, a common problem in stressed people and office owners who share communal toilets and followed by the risk of *pseudomonas aeruginosa* infection, which is often associated with infant diseases. Also, the main human bacterial agent causing a variety of variety of potentially serious infections and clinical manifestations is *Staphylococcus aureus* if allowed to enter the bloodstream or internal tissues.[41]

In the present work, the antibacterial activity of the new synthetic compounds may be attributed to the fact that these two groups of bacteria differ by its cell wall component and its thickness. The ability of these new compounds to cause the bacterial colonies to disintegrate probably results from their interference with the bacterial cell wall, thereby inhibiting the microbial growth.[41]

Among the new synthetic heterocyclic compounds, Z5A$_{2'}$ was found to be more effective than positive control (tetracycline) against Gram-negative bacteria (E. coli) with an inhibition zone (IZ) of 12, 16, 28 and 31 mm at the concentration of 5, 25,125, and 250 mg/mL, respectively. This result may come from the fact that the membrane of Gram-negative bacteria is surrounded by an outer membrane containing lipopolysaccharides, which makes the compound able to combine with the lipophilic layer in order to enhance the permeability of the membrane to Gram-negative bacteria. In conclusion, the antibacterial activity of any compound may be related to the cell wall structure of bacteria due to the importance of this wall for bacterial survival. Thus the ability of antibiotics to kill or inhibit the growth of bacteria is may be through inhibition of a step in peptidoglycan synthesis by gram positive bacteria.[42,43]

In the case of antibacterial activity against Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus*), all compounds were found to have activity ranged between high and moderate. Our results indicated that the compound Z5A$_{11'}$possessed the highest antibacterial activity against Gm+Ve (*Staphylococcus aureus*) with an IZ of (10, 23, 29, 30, and 31 mm) at concentrations of (1, 5, 25, 125, 250 mg/mL). Also, Z5A$_{11'}$ compound showed more potent compared to the positive control (IZ= 0-25) mm at the same concentration. From the other hand, Our data pointed out that compound Z5A$_{2'}$ showed a good antibacterial activity against Gm+Ve (*Bacillus*) with an IZ ranging from (12-31) mm as compared to

tetracycline (IZ = 11-30 mm) at the concentrations (5-250)mg/mL.

The antimicrobial activity of these new synthetic heterocyclic compounds is may attributed to the basis of their structures, mainly possessing the phenolic-OH group. Also, the presence the hydrogen of the phenolic group can enhance the toxicants to combine with constituents of living tissues. The accumulation of phenolic groups in the lipid bilayer may disrupt lipid–protein interaction and increase membrane permeability, further causing alterations in membrane structure and accelerating the extensive leakage of intracellular constituents, finally destroying membrane integrity to facilitate the entry of more antibacterial agents.[44] Furthermore, the mechanism of action of sulfonamide is inhibition the action of dihydropteroate synthase and blocking the net biosynthesis of folate coenzymes, therefore it represents bacteriostatic compounds.[45]

Finally, all *β*-lactam drugs are selective inhibitors of bacterial cell wall synthesis and therefore active against growing bacteria.[46] The biological activity of β-lactam skeleton is believed to be associated with the chemical reactivity of the ring and on the substituents especially at nitrogen of 2-azetidinone ring.[47]

The MIC of tested compounds in this study against the test organisms ranged between (1-500) mg/mL, Table 3. Antimicrobial agents with low activity against an organism had a high MIC while a highly active antimicrobial agent gave a low MIC. The most resistant microorganisms were *Escherichia coli* and *Pseudomonas aeruginosa*, whereas the most sensitive microorganisms were *Staphylococcus aureus* and *Bacillus*. The lowest MIC value of (1) mg/mL was recorded on *S. aureus* with compound $Z5A_{11'}$, whereas the lowest MIC value of (5) mg/mL was obtained on *Bacillus* with compounds $Z5A_2$, $Z5A_{2'}$, $Z5A_{10}$and $Z5A_{11}$. The compounds $Z5A_{2'}$,$Z5A_{11}$ and $Z5A_{11'}$were more active as compared with its precursors and had the lowest MIC value of (5) mg/mL was obtained on *Escherichia coli* and on *Pseudomonas aeruginosa*. However, the highest MIC value of 250 mg/mL was recorded on *E. coli* and on *Pseudomonas aeruginosa* with compounds ($Z5A_2$ and $Z5A_{10'}$), whereas the highest MIC value of (250) mg/mL was obtained on *Staphylococcus aureus* and on *Bacillus* with compound $Z5A_9$. The results of the present study suggest that the 2-azetidinone compounds possess remarkable toxic activity against bacteria and may assume pharmacological importance.[48]

Antioxidant Activity

Reactive oxygen species (ROS) such as superoxide anions, hydrogen peroxide, hydroxyl and nitric oxide radicals are being generated during bioorganic redox process and normal cellular metabolism, play a significant role in oxidative stress related to the development and pathogenesis of life-limiting various diseases such as cancer, diabetes mellitus, arteriosclerosis, rheumatoid arthritis, and others.[27] It is scientifically known that exposure of a normal cells to free radical lead to damage structures via interfering with functions of enzymes and critical macromolecules within cell such as lipids, proteins and nucleic acids. Conversely, antioxidants are man-made or natural substances which possess the ability to prevent or delay some types of cell damage caused by free radical-induced oxidative stress. In the past decade, the scientists of medical chemists, food chemists, and biologists have focused their attention largely on the research and testing of a variety of new and effective natural or synthetic antioxidants as a preventive strategy against human diseases in order to reduce and/or inhibit oxidative damage related to free radical reactions.[27]

In the present study, antioxidant activity of the new synthetic compounds was quantified by the *β*-carotene bleaching method. In this method, linoleic acid undergoes an oxidation reaction to form unstable hydroperoxides which easily attack and oxidize the *β*-carotene molecules rich in double bonds, causing the beta-carotene molecule to lose its colour and double bond rapidly. In this method, linoleic acid undergoes oxidation reaction to unstable hydroperoxides which easily attack and oxidation of the double bonding rich *β*-carotene molecules making it a rapid decolorization and lose their double bonds. Hence, presence of antioxidant compound can hinder the extent of *β*-carotene bleaching by neutralizing the linoleate-free radical and other free radicals formed in the system.[27] Accordingly, the absorbance values were decreased rapidly in the samples devoid of antioxidants, while in the presence of one of the antioxidants it was observed that they retained their colour and therefore their absorbance was high for a longer period.[49]

The results in Table 4 and Figures 4-5 were indicated an increase in the antioxidant activity of the synthetic compounds and standard in the order of$Z5A_9$< $Z5A_{10'}$< $ZZ5A_2$ <$ZZ5A_{11}$<$Z5A_{2'}$<$Z5A_{11'}$< $ZZ5A_9$<$Z5A_{11}$<$ZZ5A_4$< BHT with corresponding

percentages values of (52.1, 54.0, 55.5, 56.4, 56.9, 57.3, 60.2, 61.1, 75.8 and 84.8) %, respectively. On the other hand, the lowest activity was observed for compounds $ZZ5A_6$, $ZZ5A_5$, $ZZ5A_{12}$, $Z5A_{10}$, $ZZ5A_{10}$, $Z5A_2$, $ZZ5A_3$ and $Z5A_{9'}$ with corresponding inhibition ratio (48.3, 47.4, 46.9, 44.5, 41.7, 41.2, 35.1 and 26.1) % respectively. A possible explanation for the higher antioxidant activity of these compounds ($ZZ5A_4$, $Z5A_{11}$, $ZZ5A_9$, $Z5A_{11'}$,$Z5A_{2'}$,$ZZ5A_{11}$, $ZZ5A_2$,$Z5A_{10'}$,$Z5A_9$) might be due to the following reasons; first, since compound $ZZ5A_4$have an additional methoxy group which increase the antioxidant activity, this activity may be correlated with the introduction of electron donor substituent which stabilizes the generated radical during oxidation.[50] Second, compounds $Z5A_{11'}$,$Z5A_{2'}$ and $Z5A_{10'}$ have Se-H moieties which increase the antioxidant activity by the interaction with the active site of protein to form a new seleno-protein (Enz-SeH) moiety in the active site.[51]Furthermore, the organoselenium compounds had an ability to catalyzes the reduction of harmful peroxides by glutathione (GSH) and thereby protects the biomolecules against oxidative damage.[51]Third, these compounds contains hydroxyl group, which have ability of scavenging free radical. Furthermore,phenolic compounds, which can represent an inhibitor of the process of oxidation, even at comparatively smallconcentration, usually involve an aromatic ring as part of the molecular structure, with one or more hydroxyl groups. They can act as antioxidants as their broad conjugated π-electron systems allow ready donation of electrons or hydrogen atoms from the hydroxyl moieties to free radicals,[52] where the phenoxide free radical ($ArO^{\cdot}$) is stabilized by resonance.[53]

The finding that compound $ZZ5A_4$possessed a strong protective effect is interesting and points to the potential use of this new compound as an agent to overcome oxidative stress that associated with cellular metabolism and disease conditions.[54] The mechanism by which $ZZ5A_4$ protects the body's cells from oxidative damage may require further study and investigation.

Interestingly, the relative antioxidant effect of some *β*-lactamor thiazolidin-4-one antibiotics such as ampicillin on oxygen-reactive species (ROS) has been reported and a possible therapeutic role for β-lactamagents in protecting host tissues from oxidative damage has been proposed. Actually, keto lactam ring or thiazolidine ring is responsible to initiate the free radical scavenging activity due to its N-H and C=O moieties.[54, 55]

Notably, scientific studies have confirmed that compounds in general, including those that have antioxidant properties, may be subjected to metabolism *in vivo* through specialized enzymatic systems in the body, which often convert lipophilic chemical compounds into polar products that are easily secreted. Moreover, because the metabolism of any compound can result in an increase or a decrease in its toxicity.[27] Therefore, we expect that $ZZ5A_4$ and other new synthetic compounds to enter different metabolic pathways in the body that may differently modify from their structure and/or toxicity and this require further researchs. Again, the possible exact mechanism via which compound $ZZ5A_4$ and the new other synthetic compounds protects against oxidative damage will be the matter of future studies and must be confirmed in a more controlled experimental design.[27]

Cell Cytotoxicity (anticancer) study

The process of carcinogenesis initiates from a set of mutations induced by carcinogens, that affect regulation of proliferation and involves series of molecular events which trigger progressive changes from pre-invasive histological transformation to an invasive neoplastic process.[56] On the other hand, Chemopreventive intervention involves a pharmacological approach that utilizes natural, synthetic or biologic chemical agents with an objective to reverse, suppress or prevent carcinogenic progression. Also, the efficacy of a Chemopreventive agent depends on its ability to inhibit the development of invasive cancer, either by blocking the transformative, hyperproliferative and inflammatory processes that initiate carcinogenesis or by arresting or reversing the progression of premalignant cells to malignant by suppressing angiogenesis and metastasis. Furthermore, the appropriate use of Chemopreventive agent depends on the understanding of its mechanism of action at all levels i.e. at molecular, cellular, tissue and organs levels, as well as in the animal as a whole.[57]

Hence, an interest in the pharmacological effects of bioactive compounds, both of prepared or isolated from natural products, on cancer treatments and prevention has increased dramatically over the past twenty years. It has been shown to possess numerous anti-cancer activities in various cancer cells through

different forms of cytotoxic effects without exhibiting considerable damage to normal cells.[58]

For this, one of the first goals of researchers and scientists is to discover and develop a new anti-cancer drug that has good efficacy and does not cause any of the side effects of current chemotherapy drugs. Therefore, the need for a time-saving, low-cost, high-throughput drug efficacy testing system has led to the emergence of an in vitro Model cytotoxicity testing on human cancer cell lines.[57]

In this work, the cytotoxic effects of the synthesized compounds against breast cancer cell line (MCF-7) were evaluated using 5-fluorouracil (5-FU) as a reference cytotoxic drug. The IC_{50} and cell viability percent of MCF-7 cancerous at different concentrations ranging from 6.25-100 µg/mL are given in Table 5. The results showed that compounds **Z5A$_{11}$** and **Z5A$_{11}$'**were comparable to that of 5-FU (positive control) while compound **Z5A$_{11}$** is a slightly less cytotoxic agent than 5-FU (Table 5). It is evident that, the tested compounds showed anticancer activity in all concentrations and the effects of these compounds were dose dependent, *i.e.* by increasing the concentration in the culture media; the percentage of cells viability is decreased (this means that the percentage of dead cells has increased). IC_{50} values ranged from 94.05 to 96.12 µg/mL. Also, we can note that the cytotoxic activity of compound **Z5A$_{11}$** was higher in cancerous cells when compared with the compound **Z5A$_{11}$'** especially at a concentration 100 µg/mL.

β-lactam compounds revealed their pharmaceutical significance as anticancer agents. Numerous antitumor *β*-lactams that are currently used to treat cancer, such as anthracyclines, bleomycin, mitomycin C, dactinomycin, and mithramycin. The major mechanism of action for these antitumor *β*-lactams is inhibition of cell wall synthesis, DNA intercalation or inhibition of DNA synthesis.[48] The presence of 2-azetidinone ring (C_3H_2NO) in the molecular structure of compounds **Z5A$_{11}$** and **Z5A$_{11}$'** is related to anticancer activity by inhibiting the transpeptidase enzyme, which catalyzes the cross-linking of the peptidoglycan strands in the cell wall phase of the cancer cell wall biosynthesis. The *β*-lactam ring can bind to the active site of the transpeptidase enzyme since its structure resembles that of the substrate, which is the terminal D-ala-D-ala dipeptide of the pentapeptide of each monomer unit. Note that D-ala-D-ala dipeptide of the substrate can exist in multiple conformations formed by rotation around the C–C single bonds but a *β*-lactam molecule has a limited variety of conformation because of the rigidity of the four-membered lactam ring. Of the many conformations possible for the terminal dipeptide the one that binds to the enzyme resembles the structure of the *β*-lactam ring, and thus, the two can compete for binding to the active site of the enzyme. The –C(O)–N bond of the *β*-lactam mimics the –C(O)–N of the peptide bond of the terminal dipeptide. Therefore, inhibition the formation of the cancer cell wall, which leads to cells death.[48] In addition, found that a class of beta-lactams, the *N*-thiolated beta-lactams, induce tumor cell apoptosis by introducing DNA damage in a potent, and more importantly, a tumor cell-specific manner with little or no effect on normal cells.[59, 60] Cainelli *et al*., describe that 4-alkylidene-beta lactams inhibit matrix metalloproteinases-2, and -9 (MMP), essential for the tumor-induced neovascularization.[41] Banik *etal.*, also show that beta-lactams with polyaromatic substituents induce tumor cell death in a variety of breast cancer cell lines.[48] As well, the presence of (-S-C=N-) moieties in the tested compounds is related to anticancer activity by the interaction with the active site of protein through hydrogen bonding bringing about the hindrance development of cells,[61,62] however, several novel classes of beta-lactams have been shown to possess anticancer properties as well.[48]

On the other hand, the present results clearly indicated that the compound **Z5A$_{11}$** had an ability to induced apoptosis of MCF-7 Cells, as illustrated in Figure 8. Acridine orange (AO) is a vital dye and will stain the nuclei of both live and dead cell to green while ethidium bromide (EB) will stain only cells that have lost membrane integrity to red. Thus, live cells will appear uniformly green while early apoptotic cells will have condensed or fragmented nuclei with bright green color. Late apoptotic cells will show condensed and fragmented orange chromatin. The results showed that increased the compound **Z5A$_{11}$** concentration resulted in gradual increases in orange and red staining accompanied by reductions in green staining of nuclei, indicating cell damage and apoptosis (Figure 8).Therefore, high concentration (100 µg/mL) of **Z5A$_{11}$** could cause serious membrane damage in around 85% of cells. Moreover, these results indicate that apoptotic rate gradually increase with the **Z5A$_{11}$** concentration and treatment time. It is verified that at around 100 µg/mL**Z5A$_{11}$** can induce half of the cells to undergo apoptosis at 48 h, which is consistent with the IC_{50} results.

Table 3: Sensitivity of human pathogenic selected microbes to the new synthetic heterocyclic compounds.

Compounds	Diameter of inhibition zone (mm) *Bacillus*						
	Concentration (mg/mL)						
	1	5	25	125	250	500	MIC
$Z5A_2$	0	15	15	17	17	33	5
$Z5A_{2'}$	0	12	17	25	31	35	5
$Z5A_9$	0	0	0	0	14	17	250
$Z5A_{9'}$	0	0	14	14	15	18	25
$Z5A_{10}$	0	12	19	20	22	25	5
$Z5A_{10'}$	0	0	11	21	25	25	25
$Z5A_{11}$	0	10	12	19	20	22	5
$Z5A_{11'}$	0	0	18	20	21	29	25
tetracycline	5	11	14	22	30	50	1

Compounds	Diameter of inhibition zone (mm) *Staphylococcus aureus*						
	Concentration (mg/mL)						
	1	5	25	125	250	500	MIC
$Z5A_2$	0	0	26	27	29	34	25
$Z5A_{2'}$	0	11	20	29	31	30	5
$Z5A_9$	0	0	0	0	11	18	250
$Z5A_{9'}$	0	0	0	13	19	25	125
$Z5A_{10}$	0	12	18	22	24	26	5
$Z5A_{10'}$	0	0	10	21	23	25	25
$Z5A_{11}$	0	16	18	18	19	27	5
$Z5A_{11'}$	10	23	29	30	31	40	1
tetracycline	0	4	10	14	25	48	5

Compounds	Diameter of inhibition zone (mm) *Escherichia coli*						
	Concentration (mg/mL)						
	1	5	25	125	250	500	MIC
$Z5A_2$	0	0	24	27	28	30	25
$Z5A_{2'}$	0	12	16	28	31	34	5
$Z5A_9$	0	0	0	0	11	17	250
$Z5A_{9'}$	0	0	10	10	12	13	125
$Z5A_{10}$	0	0	10	11	14	25	25
$Z5A_{10'}$	0	0	0	0	16	17	250
$Z5A_{11}$	0	11	11	12	13	13	5
$Z5A_{11'}$	0	19	21	22	23	28	5
tetracycline	0	8	11	15	21	44	5

Compounds	Diameter of inhibition zone (mm) *Pseudomonas aeruginosa*						
	Concentration (mg/mL)						
	1	5	25	125	250	500	MIC
$Z5A_2$	0	0	24	25	28	30	25
$Z5A_{2'}$	0	14	18	25	27	33	5
$Z5A_9$	0	0	0	0	13	18	250
$Z5A_{9'}$	0	0	11	13	15	19	25
$Z5A_{10}$	0	0	13	14	17	26	25
$Z5A_{10'}$	0	0	0	0	15	20	250
$Z5A_{11}$	0	10	11	17	17	17	5
$Z5A_{11'}$	0	17	25	25	28	29	5
tetracycline	0	6	8	17	30	52	5

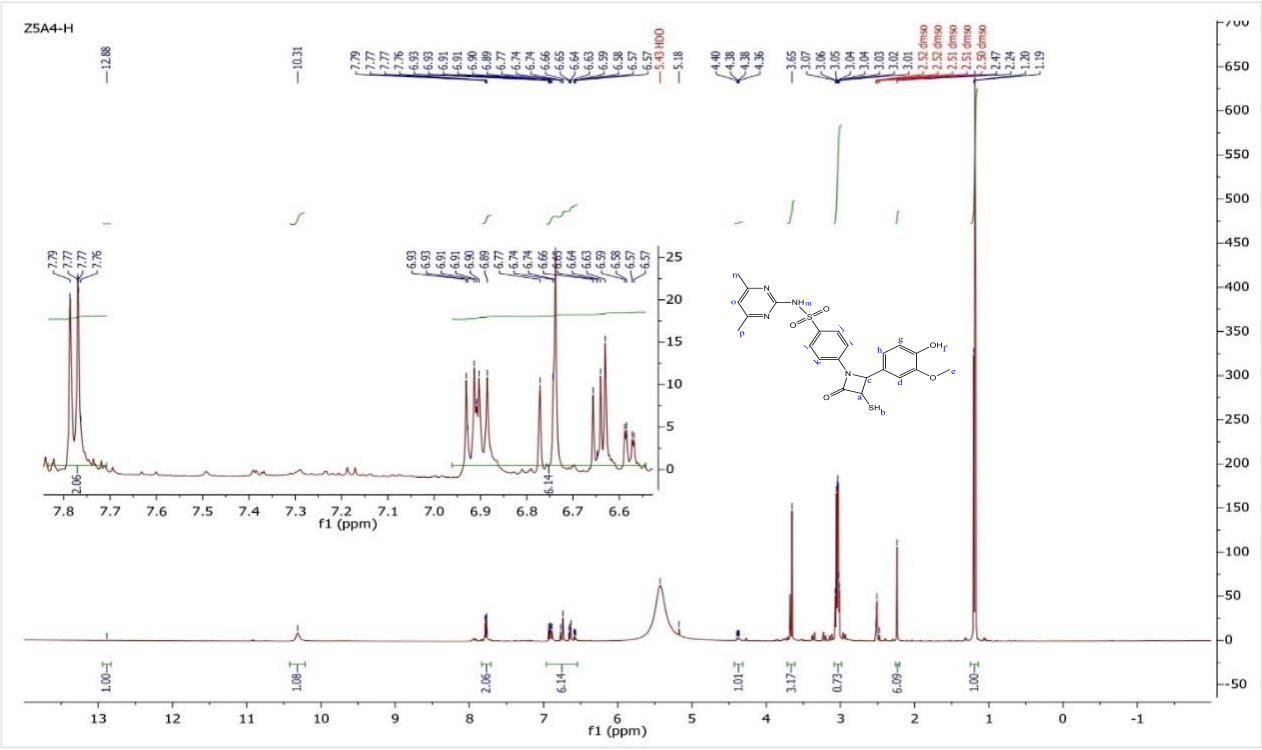


Fig. 1 : ^{1}HNMR spectrum of compound $Z5A_4$

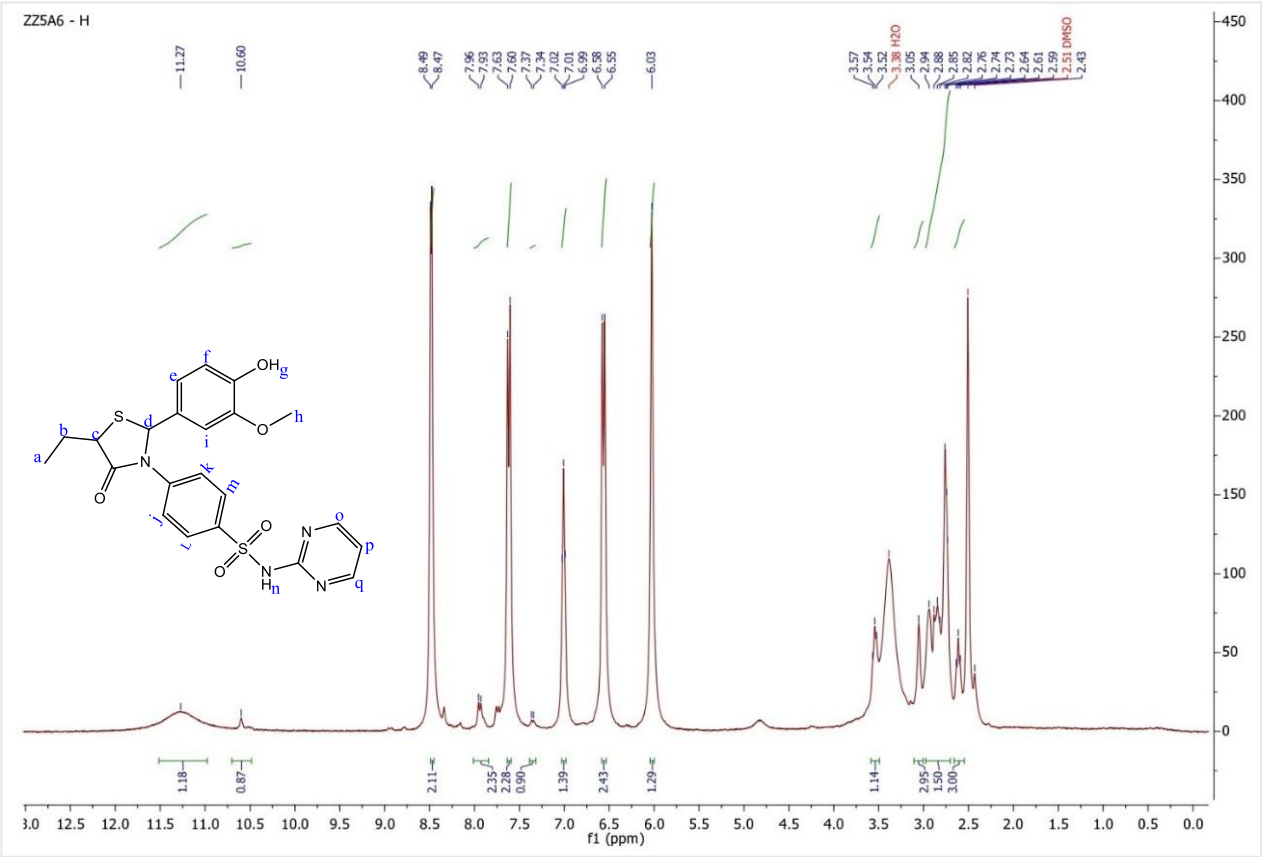


Fig. 2 : ^{1}HNMR spectrum of compound ZZ5A$_6$

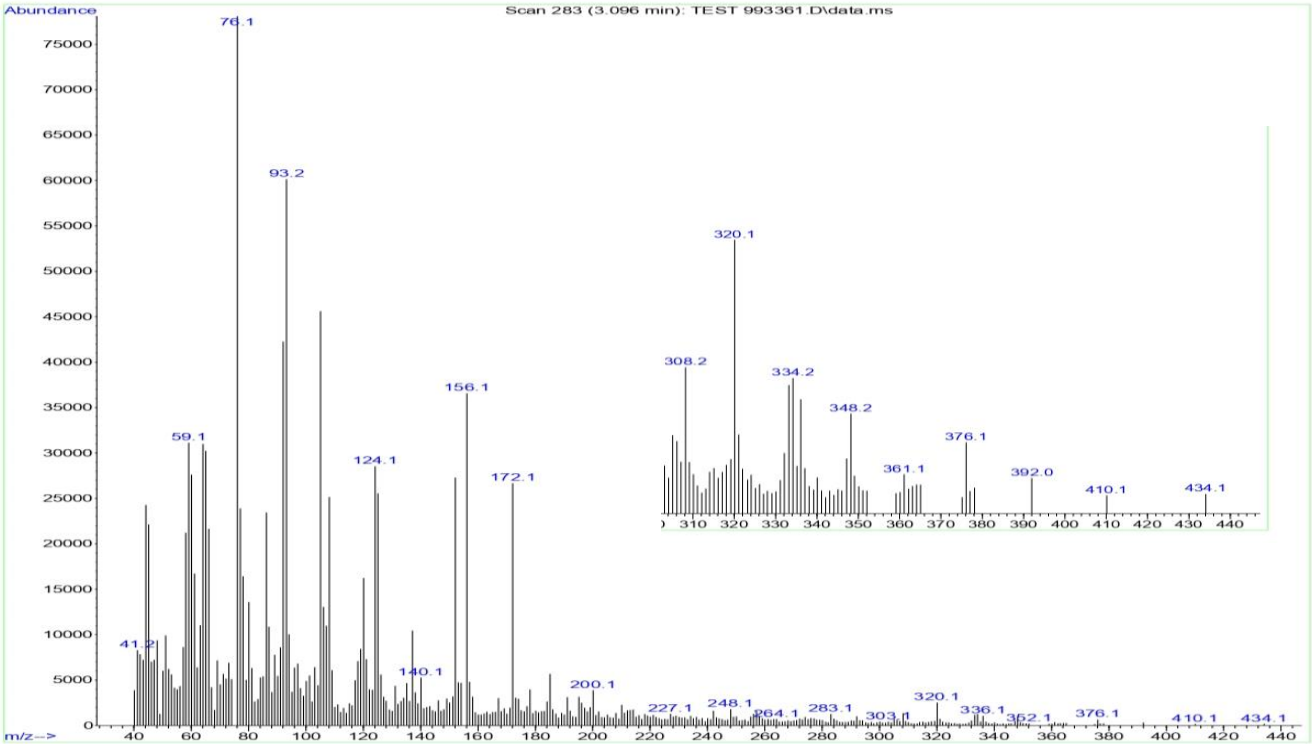


Fig. 3 : Mass Spectrum of the compound **Z5A11**

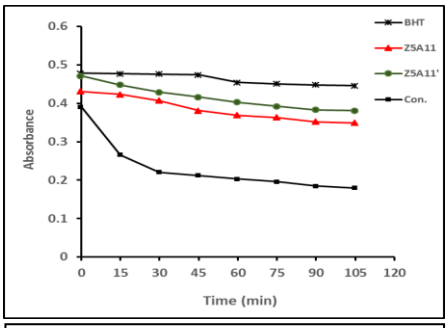


Figure 4: Antioxidant Activity of Compounds **Z5A$_{11}$** and **Z5A$_{11'}$**

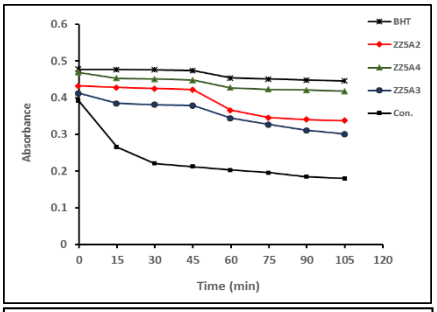


Figure 5: Antioxidant Activity of Compounds ZZ5A$_2$, ZZ5A$_3$and ZZ5A$_4$

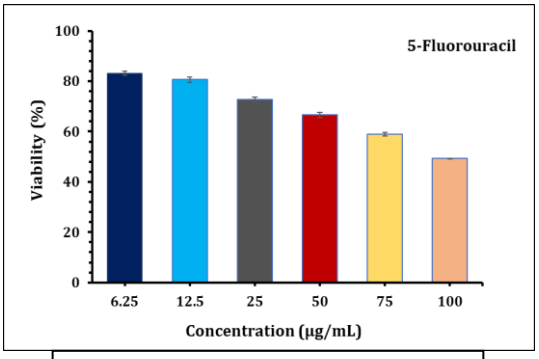


Figure 6: Anticancer Activity of drug **5-Fluorouracil** at (6.25-100) µg/mL

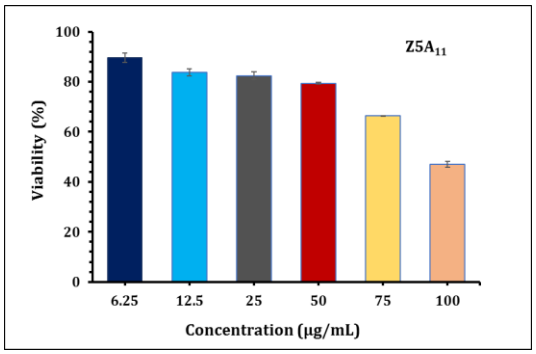


Figure 7: Anticancer Activity of Compound **Z5A$_{11}$** at (6.25-100) µg/mL

Table 4: Antioxidant Activity of Prepared Compounds

Comp. symbol	Aj	At	Aj*	At*	AA%
BHT	0.478	0.446	0.391	0.18	84.8
Z5A$_2$	0.421	0.309	0.391	0.18	46.9
Z5A$_{2'}$	0.453	0.362	0.391	0.18	56.9
Z5A$_9$	0.399	0.298	0.391	0.18	52.1
Z5A$_{9'}$	0.461	0.352	0.391	0.18	48.3
Z5A$_{10}$	0.406	0.283	0.391	0.18	41.7
Z5A$_{10'}$	0.428	0.331	0.391	0.18	54.0
Z5A$_{11}$	0.431	0.349	0.391	0.18	61.1
Z5A$_{11'}$	0.471	0.381	0.391	0.18	57.3
ZZ5A$_2$	0.432	0.338	0.391	0.18	55.5
ZZ5A$_3$	0.412	0.301	0.391	0.18	47.4
ZZ5A$_4$	0.469	0.418	0.391	0.18	75.8
ZZ5A$_5$	0.424	0.287	0.391	0.18	35.1
ZZ5A$_6$	0.410	0.254	0.391	0.18	26.1
ZZ5A$_9$	0.455	0.371	0.391	0.18	60.2
ZZ5A$_{10}$	0.392	0.275	0.391	0.18	44.5
ZZ5A$_{11}$	0.473	0.381	0.391	0.18	56.4
ZZ5A$_{12}$	0.443	0.319	0.391	0.18	41.2

Table 5: The IC_{50} Values and the Percent of Cell Viabilityof the Tested Compounds in Breast Cancer Cell Line MCF-7, the values are the mean ± SD

Compounds	Cell Viability %						IC_{50} µg/mL
	Concentration (µg/mL)						
	6.25	12.5	25	50	75	100	
$Z5A_{11}$	89.54±1.83	83.75±1.52	82.39±1.49	79.42±0.33	66.32±0.05	47.02±1.12	96.12
$Z5A_{11'}$	80.95±1.92	83.67±1.85	85.45±0.93	86.60±0.83	94.64±1.04	99.54±0.63	…..
5-Fluorouracil	83.13±0.86	80.69±1.07	72.76±0.86	66.57±1.06	58.93±0.61	49.29±0.06	94.05

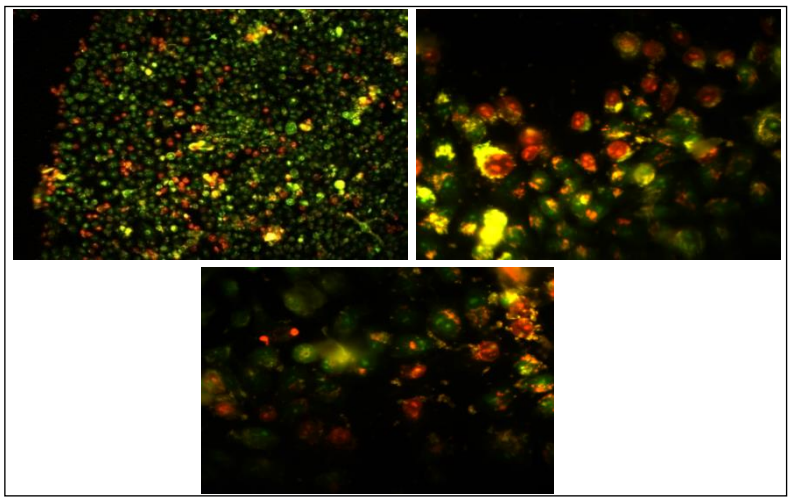


Figure 8: Anticancer Activity of Compound **$Z5A_{11}$** at (100 and 75) µg/mL

4. Conclusion

The present study concluded that the *β*-lactamand thiazolidinone compounds derived from sulfonamide were prepared, characterized and biological evaluated as antibacterial, 2-azetidinone ring in studied compounds likewise assumed a significant job in the restraint of receptor enzyme. Presence of hydroxyl group in the biologically active molecules has appeared to assume a vital job in their antioxidant and anticancer agents. The compounds show moderate antibacterial activities against *Staphylococcus aureus*, *Bacillus*, *Escherichia coli* and *Pseudomonas aeruginosa*. The most elegant result as antibacterial activity was obtained for compounds $Z5A_2$, $Z5A_{2'}$ and $Z5A_{11'}$while the synthesized compound $ZZ5A_4$ showed high activity as an antioxidant agent. Compound $Z5A_{11}$ have greater anticancer activity and the Percentage inhibition of cell viability by compound was 47.02 % at concentration 100 µg/mL. The present study reported moderate *in vivo* toxic effects by LD_{50} measurement of new compounds ($Z5A_{11}$ and $Z5A_{11'}$).

References

[1] A. M. Mansour, Selective coordination ability of sulfamethazine Schiff-base ligand towards copper (II): Molecular structures, spectral and SAR study. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, **123**, 257–266 (2014).

[2] N. Anand, in Mechanism of Action of Antimicrobial and Antitumor Agents, 3, J. W. Corcoran, F. E. Hahn, J. F. Snell, K. L. Arora Eds., Springer, Berlin, Heidelberg, 668-698 (1975).

[3] A. Tačić, V. Nikolić, L. Nikolic and I. Savić, Antimicrobial Sulfonamide Drugs. *Advanced technologies*, **6** (1), 58-71 (2017).

[4] H. S. Al-Atbi, I. J. Al-Assadi, B. K. Al-Salami and S. Q. Badr, Study of New Azo-Azomethine Derivatives of Sulfanilamide: Synthesis, Characterization, Spectroscopic, Antimicrobial, Antioxidant and Anticancer Activity. *Biochem. Cell. Arch.*, **20** (2), 4161-4174 (2020).

[5] A. Bytyqi-Damoni, H. Genç, M. Zengin, D. Demir, N. Gençer and O. Arslan, Novel β-Lactam Compounds as Activators for Polyphenoloxidase. *Chemistry Select*, **5**,7671 – 7674 (2020).

[6] N. A. A. Elkanzi, Short Review on Synthesis of Thiazolidinone and β-Lactam. *World Journal of Organic Chemistry*, **1** (2), 24 (2013).

[7] F. Broccolo, G. Carnally, G. Caltabiano, C. E. A. Cocuzza, C. Fortuna, G. Galletti, P. D.Giacomini, G. Musumarra, R. Musumeci and A. Quitavalla, Design, Synthesis, and Biological Evaluation of 4-Alkyliden-Beta Lactams: New Products with Promising Antibiotic Activity against resIstant Bacteria.*J. Med. Chem.*, **49**, 2804-2811 (2006).

[8] B. Alcaide, P. Almendros, C. Aragoncillo,β-Lactams: Versatile Building Blocks for the Stereoselective Synthesis of Non-β-Lactam Products. *Chem. Rev*., **107**, 4437-4492 (2007).

[9] M. O'Driscoll, K. Greenhalgh, A. Young, E. Turos, S. Dickey and D. V. Lim, Studies on the Antifungal Properties of N-Thiolated β-Lactams. *Bioorg. Med. Chem.*, **16** (16), 7832-7837 (2008).

[10] E. Turos, G. S. K. Reddy, K. Greenhalgh, P. Ramaraju, S. C. Abeylath, S. Jang, S. Dickey and D. V. Lim,Penicillin-bound polyacrylate nanoparticles: restoring the activity of beta-lactam antibiotics against MRSA. *Bioorg. Med. Chem. Lett.*, **17** (12), 3468-3472 (2007).

[11] A. Bhalla, S. S. Bari, S. Berry, J. Bhalla, S. Vats, S. Mandal and S. Khullar, Facile synthesis of novel monocyclic trans- and cis-3-oxy/thio/seleno-4-pyrazolyl-β-lactams. *ARKIVOC*, **vii**, 10-27 (2015).

[12] J. A. Patel, B. D. Mistry and K. R. Desai,Conventional and microwave induced synthesis of various azetidinone and thiazolidinone derivatives from 3-[(1E)-1-aza-2-(2-chloro-7-methoxy-3-quinolyl)-vinyl]-4-(aryldiazenyl) phenol and their antimicrobial screening. *Indian Journal of Chemistry*, **47B**, 1695-1700 (2008).

[13] A. K. Jain, A. Vaidya, V. Ravichandran, S. K. Kashaw and R. K. Agrawal, Recent developments and biological activities of thiazolidinone derivatives: A review. *Bioorg. Med. Chem.*, **xxx**,1-18 (2012). http://dx.doi.org/10.1016/j.bmc.2012.03.069

[14] S. P. Singh, S. S. Parmar, K. Raman, V. I. Stenberg,Chemistry and biological activity of thiazolidinones. *Chem. Rev.*, **81** (2), 175-203 (1981).

[15] Devprakash and U. A. Bhoi,A complete review of thiazolidine-4-ones. *Journal of Pharmacy Research*, **4** (7), 2436-2440 (2011).

[16] Zainb Mohammed abd al-khaliq, "Synthesis, characterization and antibacterial activity of new series of sulfamethoxazole derivatives", M.Sc. Thesis, University of Al-Mustansiriyah, College of Pharmacy, Iraq (2015).

[17] S. A. Hassan and M. N. Abdullah, Synthesis, Spectroscopic study and Biological activity of some New Heterocyclic compounds derived from Sulfadiazine. *ZANCO Journal of Pure and Applied Sciences* (*ZJPAS*), **31** (6), 92-109 (2019).

[18] A. Gupta and A. K. Halve, Synthesis & Antifungal Screening of Novel Azetidin-2-ones. *Open Chemistry Journal*, **2**, 1-6 (2015).

[19] H. H. Abd-Ali , B. K. Al-Salami and M. A. Abd, Synthesis, Characterization And Antibacterial studies of some Azomethine and Azo Compound Derivatives of selected Sulfa Drugs. *SSRG International Journal of Applied Chemistry* (*SSRG-IJAC*), **7** (2), 32-47 (2020).

[20] S. S. Mohamed, A. R. Tamer, S. M. Bensaber, M. I. Jaeda, N. B. Ermeli, A. A. Allafi, I. A. Mrema, M. Erhuma, A. Hermann, and A. M. Gbaj, Design, synthesis, molecular modeling, and biological evaluation of sulfanilamide-imines derivatives as potential anticancer agents. *NaunynSchmiedebergs Arch Pharmacol.*, **386** (9), 813–822 (2013).

[21] M. Krátký, M. Dzurková, J. Janoušek, K. Koneˇcná, F. Trejtnar, J. Stolaˇríková and J. Vinšová, Sulfadiazine Salicylaldehyde-Based Schiff Bases: Synthesis, Antimicrobial Activity and Cytotoxicity. *Molecules*, **22**, 1573 (2017).

[22] B. Mistry and S. Jauhari, Synthesis and characterization of some quinoline based azetidinones and thiazolidinones as antimicrobial agents. *Arch. Appl. Sci. Res.*, **2** (6), 332-343 (2010).

[23] W. A. Al-Masoudi, M. A. Al-Diwan and I. J. Hassan, Synthesis, acute toxicity and modelling docking studies of azo compound derived from sulphonamide and pyrimidine derivative. *Der Pharma Chemica*, **7** (9), 1-5 (2015).

[24] A. Smânia, F. D. Monache, E. F. A. Smânia, and R. S. Cuneo, Antibacterial activity of steroidal compounds isolated from Ganoderma applanatum (Pers.) Pat. (Aphyllophoro-mycetideae) Fruit body. *Int. J. Med. Mushrooms*, **1**, 325-330 (1999).

[25] B. K. Al-Salami, A. L. Al-Fadhly, A. Adil Al-Fregi, Synthesis, characterization and Biological Activity Study of some New compounds containing Amine and Azomethine Group and their platinum (II) complexes. *Der. Pharma. Chemica.*, **8** (19), 488 (2016).

[26] A. Ahmeda, M. A. Hossain and Z. Ismail, Antioxidant properties of the isolated flavonoids from the medicinal plant. *Phyllanthus niruri*. *As. J. Food Ag-Ind.*, **2** (03), 373-381 (2009).

[27] A. J. M. Al-Fartosy, Antioxidant properties of methanolic extract from *Inula graveolens* L. *Turk J Agric For*, **35** (6), 591-596 (2011).

[28] A. M. Al-Shammari, W. N. Al-Esmaeel, A. A. Al-Ali, A. A. Hassan, and A. A. Ahmed, Enhancement of Oncolytic Activity of Newcastle Disease virus Through Combination with Retinoic Acid Against Digestive System Malignancies. *Molecular Therapy*, **27** (4S1),126-127 (2019).

[29] R. I. Freshney, Culture of animal cells a manual of basic technique and specialized applications, 6th Ed., Wiley-Blackwell, 732 (2010).

[30] K. Liu, P. C. Liu, R. Liu and X. Wu, Dual AO/EB staining to detect apoptosis in osteosarcoma cells compared with flow cytometry. *Medical science*

monitor basic research, **21**, 15–20 (2015). doi:10.12659/MSMBR.893327.

[31] C. E. Mungan, U. Happek, T. Z. Hossain and A. J. Sievers, Infrared Spectroscopy of the Stretching Modes of SeH- and TeH- in KCl and KBr.*Journal of Physics and Chemistry of Solids*, **56** (5), 735-743 (1995). DOI:10.1016/0022-3697(94)00188-X

[32] M. S. AL-Gwady, Synthesis of 2-Amino-5-Substituted-1,3,4-Thiadiazoles (ATDA) and Their Derivatives Using Conventional and Microwave Techniques. *J. Raf. Sci.,***20** (1), 1-7 (2009).

[33] A. Z. Al-Rubaie, W. A. Al-Masoudi, A. J. Hameed, L. Z. Yousif and M. Graia, Synthesis, Reaction and Antiviral Activity of 2,4-Diaryl-1,3-selenazoles. *J. Korean Chem. Soc.*, **52** (1), 36-46 (2008).

[34] P. D. Mehta, N. P. S. Sengar, E. V. S. Subrahmanyam and D. Satyanarayana,Synthesis and Biological Activity Studies of Some Thiazolidinones and Azetidinones. *Indian J. Pharm. Sci.*, **68** (1),103-106 (2006).

[35] S. Alam, P. Hasan, B. Aneja, M. B. Ahmad and M. Abid, $POCl_3$ Mediated Staudinger Reaction of Imines with Ketenes: Synthesis of Monocyclic β-Lactam and 1,3-Oxazinone Derivatives. *Rasayan J. Chem.*(*RJC*), **9** (2), 101-111 (2016).

[36] Reich and U. Wisc., Proton Nuclear Magnetic Resonance Spectroscopy. *Chem.*,**605**, Hans J. Reich, University of Wisconsin, 28-29 (2017).

[37] B. K. AL-Salami, Z. K. AL-Khazragie and A. A. Al-Fregi, Synthesis, Characterization, Antimicrobial Activity and Antioxidant of Azo Schiff Bases Containing Sulfanilamide. *Journal of Global Pharma Technolog*, **10** (03), 952-962 (2018).

[38] D. J. Sharp, G. C. Rogers and J. M. Scholey,Microtubule motors in mitosis. *Nature*, **407** (6800), 41-47 (2000). doi: 10.1038/35024000.

[39] A. Rispin, D. Farrar, E. Margosches, K. Gupta, K. Stitzel, G. Carr, M. Greene, W. Meyer, D. McCall, Alternative methods for the median lethal dose (LD50) test: The up-and-down procedure for acute oral toxicity. *Ilar J.*, **43**, 233–243 (2002).

[40] L. M. Al-Smadi, R. Mansour, A. Mahasneh, O. F, Khabour, M. M. Masadeh and K. H. Alzoubi, Synthesis, Characterization, Antimicrobial Activity, and Genotoxicity Assessment of Two Heterocyclic Compounds Containing 1,2,3-Selena- or 1,2,3-Thiadiazole Rings. *Molecules*, **24**, 1-11 (2019).

[41] P. Piewngam and M. Otto, Probiotics to prevent Staphylococcus aureus disease. *GUT MICROBES*, **11** (1), 94–101 (2020).

[42] D. U. Thomba, S. R. Mirgane, R. U. Ambhure, R. P. Pawar and K. L. Ameta, "Synthesis and Antimicrobial Study of Novel Schiff Bases and Metal Complexes". *Biochemistry and Biophysics* (BAB), **3**, (2017).

[43] A. A. El-Sherif and T. M. Eldebss, "Synthesis, spectral characterization, solution equilibria, in vitro antibacterial and cytotoxic activities of Cu(II), Ni(II), Mn(II), Co(II) and Zn(II) complexes with Schiff base derived from 5-bromosalicylaldehyde and 2-aminomethylthiophene". *Spectrochim Acta*, **79A**, 1803e14 (2011).

[44] Y. Wu , J. Bai, K. Zhong, Y. Huang, Q. Huayi, Y. Jiang and H. Gao, "Antibacterial Activity and Membrane-Disruptive Mechanism of 3-p-trans-Coumaroyl-2-hydroxyquinic Acid, a Novel Phenolic Compound from Pine Needles of Cedrus deodara, against Staphylococcus aureus". *Molecules*, **21**, 1084 (2016).

[45] L. R. AL- Rubaie and R. J. Mhessn, "Synthesis and Characterization of Azo Dye Para Red and New Derivatives". *E-Journal of Chemistry*, **9** (1), 465-470 (2012).

[46] M. Rezaei, M. Komijani and S. M. Javadirad, " Bacteriostatic Agents". *Licensee Intech Open*, (2012).

[47] S. Dhanya and A. Aravind, "Synthesis, characterization and evaluation of antioxidant activities of some new quinazolino-acetidinone derivatives". *Journal of Chemical and Pharmaceutical Research*, **7** (12),849-856 (2015).

[48] D. Kuhn, C. Coates, K. Daniel, D. Chen, M. Bhuiyan, A. Kazi, E. Turos and Q. Ping Dou, Beta-Lactams and Their Potential Use as Novel Anticancer Chemotherapeutics Drugs. *Frontiers in Bioscience*, **9**, 2605-2617 (2004).

[49] S. Miladi and M. Damak, "*In Vitro* Antioxidant A ctivities of Aloe vera Leaf Skin Extracts". *Journal of Society of Chemstry*, Tunisie, **10**, 101-109 (2008).

[50] K. N. Mohana and C. B. Pradeep Kumar, "Synthesis and Antioxidant Activity of 2-Amino-5-methylthiazol Derivatives Containing 1,3,4-Oxadiazole-2-thiol Moiety". International Scholarly Research Notices, Organic Chemistry, (2013).

[51] F. V. Singh and T. Wirth, "Synthesis of Organoselenium Compounds with Potential Biological Activities", V. K. Jain and K. I. Priyadarsini, Tamil Nadu, India, 78 (2018).

[52] J. M. Canadanovic-Brunet, S. M. Djilas, G. S. Cetkovic and V. T. Tumbas, Free-radical scavenging activity of wormwood (*Artemisiaabsinthium*L.) extracts. *J. Sci. Food Agric.*, **85**, 265–272 (2005).

[53] R. Apak, K. Guclu, B. Demirata, M. Ozyurek, S. E. Celik, B. Bektasoglu, K. Berker and D. Ozyurt, Comparative evaluation of various total antioxidant capacity assays applied to Phenolic compounds with the CUPRAC assay. *Molecules*, **12**, 1496-1547 (2007).

[54] M. K. Bhattacharjee, "Chemistry of Antibiotics and Related Drugs",S. Nature and company is Springer International Publishing AG Switzerland, Long Island University Brooklyn, NY, USA, 63-69 (2016).

[55] M. M. Hossain, M. D. Aziz, R. Ahmed, M. Hossain, A. Mahmud, T. Ahmed and E. H. Mazumder, "*In Vitro* Free Radical Scavenging Activity of Some β-Lactams And Phenolics". *International Journal of Pharmacy and Pharmaceutical Sciences*, **2** (2), (2010).

[56] N. V. Zandwijk, Chemoprevention in lung carcinogenesis – An overview. *European Journal of Cancer*, **41**, 1990-2002 (2005).

[57] H. Mukhtar, Chemoprevention: Making it a success story for controlling human cancer. *Cancer Letters*, (2012).doi: 10.1016/j.canlet.2012.05.016.

[58] R. S. Katiyar, N. R. Singhvi, R. V. Kushwaha, Lal. Ramji and N. Suryanarayana, VA mycorrhizal association in arjuna and jamun trees in forest of Bhandara region, Maharashtra, India. *International Journal of Agricultural Sciences*, **4**, 229-232 (2009).

[59] D. M. Smith, A. Kazi, L. Smith, T. E. Long, B. Heldreth, E. Turos and Q.P. Dou, A novel beta-lactam antibiotic activates tumor cell apoptotic program by inducing DNA damage. *Mol Pharmacol*, **61**, 1348-1358 (2002).

[60] Kazi, A., R. Hill, T. E. Long, D. J. Kuhn, E. Turos and Q. P. Dou, Novel N-thiolated beta-lactam antibiotics selectively induce apoptosis in human tumor and transformed, but not normal or non-transformed, cells. *BiochemPharmacol*, **67**, 365-374 (2004).

[61] S. I. El-Desoky, F. A. Badria, M. A. Abozeid, E. A. Kandeel and A. H. Abdel-Rahman, Synthesis and antitumor studies of novel benzopyrano-1,2,3-selenadiazole and spiro[benzopyrano]-1,3,4-thiadiazoline derivatives. *Med. Chem Res*, **22**, 2105–2114 (2013). DOI 10.1007/s00044-012-0201-0

[62] A. J. M. Al-Fartosy and M. H. Ati, A Predictive clinical markers to make prostate cancer and benign prostate hyperplasia easy diagnosis. *Biochem. Cell. Arch.*, **21** (2), 2939-2947 (2021).