RESEARCH ARTICLE

Synthesis, Characterization, and Antioxidant, Antimicrobial and Toxic Properties of Novel Δ^2-1,3,4-thiadiazoline and Δ^2-1,3,4-selenadiazoline Derivatives

Zainab K. Al-Khazragie, Bushra K. Al-Salami*, Adnan J. M. Al-Fartosy

Department of Chemistry, College of Science, University of Basrah, Basrah, Iraq

Received: 14th December, 2021; Revised: 10th January, 2022; Accepted: 17th February, 2022; Available Online: 25th March, 2022

ABSTRACT

Two new series of N-(4-acetyl-5-aryl-4,5-dihydro-1,3,4-thiadiazol-2-yl)acetamide and N-(4-acetyl-5-aryl-4,5-dihydro-1,3,4-selenadiazol-2-yl)acetamide compounds (where aryl = 4-nitrophenyl, 4-hydroxy-3-methoxyphenyl, 3-ethoxy-4-hydroxyphenyl, 2-chloroquinolinyl, and 6-chloro-4-oxo-4H-chromenyl) were synthesized in good yields by heterocyclization of thiosemicarbazones (TSCs) and selenosemicarbazones (SSCs) with acetic anhydride, respectively. The new TSCs and SSCs compounds was prepared by condensation reaction of thiosemicarbazide and Selenosemicarbazide with aromatic aldehydes in acidic medium. The structures of newly synthesized 1,3,4-thiadiazoline (TDZs) and 1,3,4-selenadiazoline (SDZs) derivatives were characterized by the analytical and spectroscopic method such as IR, ^{1}H NMR, ^{13}C NMR, mass spectra and elemental analysis. The purity of compound and evaluation of R_f value were determined by TLC. 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 1,3,4-thiadiazoline and 1,3,4-selenadiazoline compounds were tested *in vitro* against *Staphylococcus aureus*, *Bacillus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Finally, antioxidant efficiency of all compounds were detected according to β-carotene bleaching method.

Keywords: Acute toxicity, Antibacterial, Antioxidant activity, Selenadiazoline, Selenosemicarbazone, Thiadiazoline, Thiosemicarbazone.

International Journal of Drug Delivery Technology (2022); DOI: 10.25258/ijddt.12.1.22

How to cite this article: Al-Khazragie ZK, Al-Salami BK, Al-Fartosy AJM. Synthesis, Characterization, and Antioxidant, Antimicrobial and Toxic Properties of Novel Δ^2-1,3,4-thiadiazoline and Δ^2-1,3,4-selenadiazoline Derivatives. International Journal of Drug Delivery Technology. 2022;12(1):113-126.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Recently, there are a considerable interest in the synthesis of sulfur and selenium containing compounds due to their remarkable using in organic synthesis and biological processes.[1] Thus, several heterocycles containing sulfur, selenium, and nitrogen atoms were prepared which are useful substrates for the preparation of various heterocyclic systems.[2] There is a rapidly growing interest for creating several chemotherapeutic agents due to the limitations of the existences drugs to cure and improve survival in many diseases.[3]

Sulphur or selenium is a key component of several major metabolic pathways in human, including thyroid hormone metabolism, antioxidant defence system, and immune function.[4] Also, Sulphur or Selenium compounds can efficiently induce cell death in various cancer cells.[5,6] For example, metal complexes of selenosemicarbazones induced apoptosis through the mitochondria pathway in cancer cells.[7] Additionally, thiosemicarbazone and selenosemicarbazone derivatives efficiently inhibited metastasis and angiogenesis in breast cancer cells.[6,8] It is worth noting that thiadiazoline and their selenium analog were prepared from the reaction of aldehydes or ketones with semicarbazones or hydrazones.[9,10]

Various thiosemicarbazones, selenosemicarbazones and their heterocyclic derivatives (*i.e*. thiadiazoline and selenadiazoline) were used as antimicrobial, anti-HIV-1, anticancer, antioxidant, anti-convulsant and anti-inflammatory agents.[6,11-15] Furthermore, thiadiazole and selenadiazole nucleus such as a toxophoric (-N=C-S- or -N=C-Se-) group are present as a core structural component in drug categories.[16,17] It is well known that thiadiazole and selenadiazole moieties act as hydrogen binding domains and two-electron donor systems as well as constrained pharmacophores.[18,19] The interest in preparation of 1,3,4-thiadiazole and 1,3,4-selenadiazole derivatives, as they are employed in the synthesis of heterocycles due to their wide range of their potentially activity in pharmaceutical, agricultural, and materials chemistry.[16,20,21]

*Author for Correspondence: bkalsalami62@gmail.com

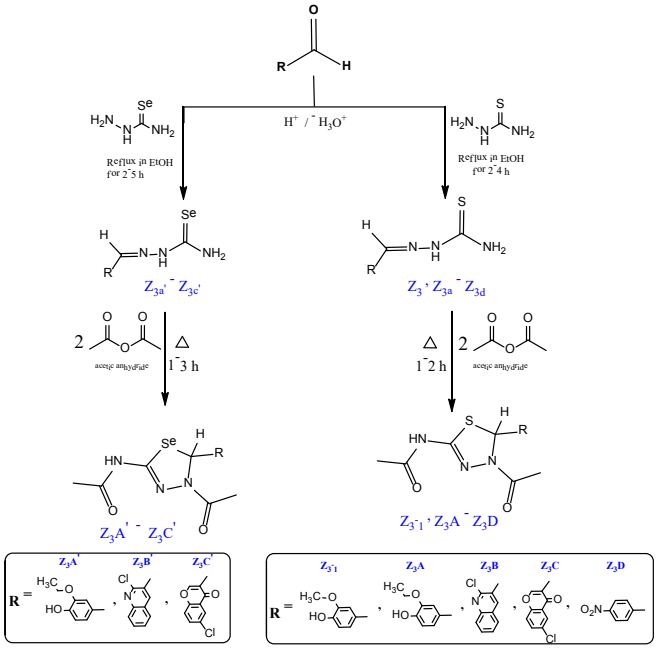
The disubstituted 1,3,4-thiadiazoline have pharmacological and are multifunctional characteristics, for instance ligands containing atoms that donating electrons such as N, S can form complexes with a wide variety of transition metals.[22] 1,3,4-Selenadiazoline and 1,3,4-thiadiazoline derivatives undergo a variety of reactions. They act as 1,3-dipoles or as a source of selenium or sulfur and hence they have attracted much attention for the synthesis of different organoselenium and organosulfur compounds.[19,23]

In view of remarkable pharmacological efficiency of selenadiazoline and thiadiazoline derivatives.[24] herein, we have synthesized and characterized a new series of (TSCs and SSCs) as well as their corresponding 1,3,4-thiadiazolines and 1,3,4-selenadiazolines respectively obtained by cyclization under acetylating condition. The synthesized compounds have been characterized based on spectroscopic methods including elemental analysis (CHNS), IR, ^{1}H and ^{13}C NMR, as well as mass spectroscopy. We also studied *in vivo* acute toxicity, antioxidant and antibacterial of thia- or selenadiazoline derivatives.

MATERIALS AND METHODS

Materials and Reagents

All the chemicals and solvents used were of analytical grade supplied from BDH, Fluka, USP, Merck, PubChem and Aldrich. Thiosemicarbazide, 4-hydroxy-3-methoxybenzaldehyde (vanillin), 3-ethoxy-4-hydroxybenzaldehyde (ethylvanillin), 2-chloroquinoline-3-carbaldehyde, 6-chloro-3-formylchromone, 4-nitronenzaldehyde and glacial acetic acid as well as butylated hydroxyl toluene (BHT) were obtained from sigma-Aldrich. Acetic anhydride and β-carotene were supplied from BDH and USP respectively. Tween-20 (Polyoxyethylene (20) sorbitan monolaurate) and linoleic acid was obtained from Fluka. Selenosemicarbazide were also purchased from PubChem. 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.



Scheme 1: Synthesis of Δ^2-1,3,4-thiadiazolines (**$Z_{3\text{-}1}$, Z_3A-Z_3D**) and Δ^2-1,3,4-selenadiazolines (**Z_3A'-Z_3C'**)

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 ^{1}H 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.

Synthesis

General Procedure for the Synthesis of Thiosemicarbazones (Z_3, Z_3a-Z_3d) and Selenosemicarbazones (Z_3a'-Z_3d'):

The general method of condensation reactions was used to prepare these compounds as follows.[16]

Ethanolic solution (50 mL) of thiosemicarbazide (10 mmol, 0.91 g) or Selenosemicarbazide (10 mmol, 1.37 g) were added with stirring to 50 mL of warm ethanolic solution of selected aldehyde vanillin (10 mmol, 1.52 g), ethylvanillin (10 mmol, 1.66 g), 2-chloroquinoline-3-carbaldehyde (10 mmol, 1.92 g), 6-chloro-3-formylchromone (10 mmol, 2.08 g), and 4-nitronenzaldehyde (10 mmol, 1.51 g), respectively. A few drops of glacial acetic acid were added to mixture and refluxed for 1–3 hours, then cooled the resulting mixture to room temperature. The precipitated solids were filtered off from the reaction mixture, dried, followed by recrystallized in methanol. The completion of the reaction and the purity of the products were confirmed by the TLC using ethyl acetate:n-hexane (2:8). The synthetic procedures for the preparation of compounds (Z_3, Z_3a-Z_3d, Z_3a'-Z_3d') are presented in Scheme 1.

2-(4-hydroxy-3-methoxybenzylidene) hydrazinecarbothioamide (Z_3)

White powder, yield: 88%; R_f: 0.81; m.p: 206–207ºC; Elemental Analysis for $C_9H_{11}N_3O_2S$ (225.27 g/mol); Calcd: C, 47.99; H, 4.92; N, 18.65; S, 14.23. Found: C, 47.97; H, 4.88; N, 18.61; S, 14.20. IR (KBr) cm^{-1}: 3525 ***υ***(OH), 3437 ***υ***(NH_2, asym), 3279 ***υ***(NH_2, sym), 3155 ***υ***(NH), 1593 ***υ***(CH=N), 1512 – 1462 ***υ***(C=C), 1280 ***υ***(C-N), 1111 ***υ***(C=S), 613 ***υ***(C-S).

2-(3-ethoxy-4-hydroxybenzylidene) hydrazinecarbothioamide (Z_{3a})

White powder, yield: 79%; R_f: 0.80; m.p: 202–204ºC; Elemental Analysis for $C_{10}H_{13}N_3O_2S$ (239.29 g/mol); Calcd: C, 50.19; H, 5.48; N, 17.56; S, 13.40. Found: C, 50.18; H, 5.42; N, 17.54; S, 13.41. IR (KBr) cm^{-1}: 3525 ***υ***(OH), 3425 ***υ***(NH_2,

asym), 3190 υ(NH_2, sym), 3151 υ(NH), 1597 υ(CH=N), 1535 - 1504 υ(C=C), 1276 υ(C-N), 1111 υ(C=S), 624 υ(C-S).

2-(3-ethoxy-4-hydroxybenzylidene) hydrazinecarboselenoamide ($Z_{3a'}$)

Brown powder, yield: 44%; R_f: 0.58; m.p: 143–145°C; Elemental Analysis for $C_{10}H_{13}N_3O_2Se$ (286.19 g/mol); Calcd: C, 41.97; H, 4.58; N, 14.68. Found: C, 42.01; H, 4.55; N, 14.68. IR (KBr) cm^{-1}: 3525 υ(OH), 3405 υ(NH_2, asym), 3274 υ(NH_2, sym), 3160 υ(NH), 1589 υ(CH=N), 1516 - 1438 υ(C=C), 1274 υ(C-N), 1065 υ(C=Se), 620 υ(C-Se).

2-((2-chloroquinolin-3-yl)methylene) hydrazinecarbothioamide (Z_{3b})

Yellow powder, yield: 91%; R_f: 0.92; m.p: 190–192°C; Elemental Analysis for $C_{11}H_9ClN_4S$ (264.73 g/mol); Calcd: C, 49.91; H, 3.43; N, 21.16; S, 12.11. Found: C, 49.96; H, 3.39; N, 21.13; S, 12.10. IR (KBr) cm^{-1}: 3410 υ(NH_2, asym), 3282 υ(NH_2, sym), 3151 υ(NH), 1651 υ(C=N, quinoline ring), 1604 υ(CH=N), 1539 – 1473 υ(C=C), 1276 υ(C-N), 1057 υ(C=S), 833 υ(C-Cl), 601 υ(C-S).

2-((2-chloroquinolin-3-yl)methylene) hydrazinecarboselenoamide ($Z_{3b'}$)

White powder, yield: 87%; R_f: 0.61; m.p: 268–269°C; Elemental Analysis for $C_{11}H_9ClN_4Se$ (311.63 g/mol); Calcd: C, 42.40; H, 2.91; N, 17.98. Found: C, 42.44; H, 2.89; N, 17.97. IR (KBr) cm^{-1}: 3448 υ(NH_2, asym), 3280 υ(NH_2, sym), 3100 υ(NH), 1651 υ(C=N, quinoline ring), 1589 υ(CH=N), 1516 – 1485 υ(C=C), 1215, 1118 υ(C-N), 1049 υ(C=Se), 817 υ(C-Cl), 632 υ(C-Se).

2-((6-chloro-4-oxo-4H-chromen-3-yl)methylene) hydrazinecarbothioamide (Z_{3c})

Light yellow crystals, yield: 93%; R_f: 0.72; m.p: 148–150°C; Elemental Analysis for $C_{11}H_8ClN_3O_2S$ (281.72 g/mol); Calcd: C, 46.90; H, 2.86; N,14.92; S, 11.38. Found: C, 46.90; H, 2.88; N, 14.92; S, 11.38. IR (KBr) cm^{-1}: 3421 υ(NH_2, asym), 3236 υ(NH_2, sym), 3174 υ(NH), 1627 υ(CH=N), 1570 υ(C=C, Chromone ring), 1523 – 1458 υ(C=C), 1315, 1261 υ(C-N), 1215 υ(C-O, Chromone ring), 1095 υ(C=S), 875 υ(C-Cl), 655 υ(C-S).

2-((6-chloro-4-oxo-4H-chromen-3-yl)methylene) hydrazinecarboselenoamide ($Z_{3c'}$)

White powder, yield: 76%; R_f: 0.63; m.p: 271-272 °C; Elemental Analysis for $C_{11}H_8ClN_3O_2Se$ (328.61 g/mol); Calcd: C, 40.20; H, 2.45; N, 12.79. Found: C, 40.27; H, 2.47; N, 12.82. IR (KBr) cm^{-1}: 3356 υ(NH_2, asym), 3217 υ(NH_2, sym), 3151 υ(NH), 1697 υ(C=O, Chromone ring), 1639 υ(CH=N), 1581 υ(C=C, Chromone ring), 1519 – 1462 υ(C=C), 1300, 1253 υ(C-N), 1184 υ(C-O, Chromone ring), 1095 υ(C=Se), 895 υ(C-Cl), 636 υ(C-Se).

2-(4-nitrobenzylidene) hydrazinecarbothioamide (Z_{3d})

Dark yellow, yield: 92%; R_f: 0.71; m.p: 252–254°C; Elemental Analysis for $C_8H_8N_4O_2S$ (224.24 g/mol); Calcd: C, 42.85; H, 3.60; N, 24.99; S, 14.30. Found: C, 42.85; H, 3.58; N, 24.98; S, 14.30. IR (KBr) cm^{-1}: 3487 υ(NH_2, asym), 3363 υ(NH_2, sym), 3144 υ(NH), 1581 υ(CH=N), 1523 υ(NO_2, asym), 1338 υ(NO_2, sym), 1446 υ(C=C), 1280 υ(C-N), 1095 υ(C=S), 686 υ(C-S).

Synthesis of N-(4-acetyl-5-aryl-4,5-dihydro-1,3,4-thiadiazol-2-yl)acetamide (Z_{3-1},Z_3A-Z_3D)and N-(4-acetyl-5-aryl-4,5-dihydro-1,3,4-selenadiazol-2-yl)acetamide (Z_3A'-Z_3C') :

The TDZs (Z_{3-1},Z_3A-Z_3D) and SDZs (Z_3A'-Z_3C') were prepared in the following general procedure by reacting the obtained TSCs (Z_3, Z_3a-Z_3d) and SSCs (Z_3A'-Z_3C') with the cyclization agent acetic anhydride. A mixture of (Z_3, Z_3a-Z_3d) (or Z_3A'-Z_3C') (2 mmol) in acetic anhydride (32 mL) was refluxed on oil bath (120°C) for 1–3 h The reaction mixture were monitored by TLC using ethyl acetate:benzene (1:9). After completion of the reaction, the reaction mixture was poured onto crushed ice with vigorous stirring. The formed precipitate was filtered off, washed with water, dried, and recrystallized from ethyl acetate. The various synthetic preparation of 4-acetamide-1,3,4-thia- or selenadiazoles compounds are summarized in Scheme 1.

N-(4-acetyl-5-(4-hydroxy-3-methoxyphenyl)-4,5-dihydro-1,3,4-thiadiazol-2-yl)acetamide (Z_{3-1})

White powder, yield: 63%; R_f: 0.69; m.p: 273–276°C; Elemental Analysis for $C_{13}H_{15}N_3O_4S$ (309.34 g/mol); Calcd: C, 50.47; H, 4.89; N, 13.58; S, 10.37. Found: C, 50.46; H, 4.91; N, 13.57; S, 10.37. IR (KBr) cm^{-1}: 3448 υ(OH), 3167 υ(NH), 2931 υ(CH, aliph.), 1759, 1697 υ(CH_3CON, two groups), 1570 υ(C=N, thiadiazoline ring), 1516 – 1454 υ(C=C), 1230 υ(C-N), 1126 υ(N-N), 709 υ(C-S-C, asym), 659 υ(C-S-C, sym); ^{1}HNMR (DMSO-d_6, 500 MHz): δ/ppm = 12.68 (s, 1H, NH), 11.94 (s,1H, OH), 7.66 (s, 1H, Ar-H), 7.525 (d, 1H, J = 10 Hz, Ar-H), 7.25 (d, 1H, J = 10 Hz, Ar-H), 3.33 (s, 1H, 5-H, thiadiazoline ring), 3.90 (s, 3H, CH_3), 2.30 (s, 3H, CH_3), 2.23 (s, 3H, CH_3); ^{13}CNMR (DMSO-d_6, 500 MHz): δ/ppm = 169.18, 168.86, 161.49, 159.06, 151.88, 141.51, 129.45, 124.30, 120.15, 111.06, 56.53, 22.90, 20.88; The EI-MS (m/s): 309 $[m]^+$, 265 $[C_{11}H_{11}N_3O_3S]^+$, 223 $[C_9H_9N_3O_2S]^+$.

N-(4-acetyl-5-(3-ethoxy-4-hydroxyphenyl)-4,5-dihydro-1,3,4-thiadiazol-2-yl)acetamide (Z_3A)

Off white powder, yield: 64%; R_f: 0.83; m.p: 238–240°C; Elemental Analysis for $C_{14}H_{17}N_3O_4S$ (323.37 g/mol); Calcd: C, 52.00; H, 5.30; N, 12.99; S, 9.92. Found: C, 51.98; H, 5.26; N, 12.97; S, 9.75. IR (KBr) cm^{-1}: 3515 υ(OH), 3155 υ(NH), 2982-2901 υ(CH, aliph.), 1770, 1697 υ(CH_3CON, two groups), 1562 υ(C=N, thiadiazoline ring), 1516 – 1431 υ(C=C), 1207, 1180 υ(C-N), 1118 υ(N-N), 705 υ(C-S-C, asym), 659 υ(C-S-C, sym); ^{1}HNMR (DMSO-d_6, 500 MHz): δ/ppm = 12.67 (s, 1H, NH), 11.77 (s,1H, OH), 7.635 (d, 1H, J = 5 Hz, Ar-H), 7.505 (d, 1H, J = 5 Hz, Ar-H), 7.24 (d, 1H, J = 10 Hz, Ar-H), 6.82 (s, 1H, 5-H, thiadiazoline ring), 4.17 (q, 2H, J_1 = 15 Hz, J_2 = 5, CH_2), 2.29 (s, 3H, CH_3), 2.23 (s, 3H, CH_3), 1.33 (t, 3H, J_1 = 10 Hz, J_2 = 5 Hz, CH_3); ^{13}CNMR (DMSO-d_6, 500 MHz): δ/ppm = 169.17, 168.83, 161.51, 159.02, 151.12, 141.85, 129.38, 124.23, 120.10, 111.99, 64.74, 22.90, 20.81, 14.94; The EI-MS

(m/s): 323 $[m]^+$, 281 $[C_{12}H_{15}N_3O_3S]^+$, 279 $[C_{12}H_{13}N_3O_3S]^+$, 237 $[C_{10}H_{11}N_3O_2S]^+$.

N-(4-acetyl-5-(3-ethoxy-4-hydroxyphenyl)-4,5-dihydro-1,3,4-selenadiazol-2-yl)acetamide (Z_3A'*)*

Light yellow powder, yield: 41%; R_f: 0.7; m.p: 90–92°C; Elemental Analysis for $C_{14}H_{17}N_3O_4Se$ (370.26 g/mol); Calcd: C, 45.41; H, 4.63; N, 11.35. Found: C, 45.47; H, 4.69; N, 11.40. IR (KBr) cm^{-1}: 3352 ***υ***(OH), 3221 ***υ***(NH), 2827 ***υ***(CH, aliph.), 1770, 1697 ***υ***(CH_3CON, two groups), 1658 ***υ***(C=N, thiadiazoline ring), 1519 – 1435 ***υ***(C=C), 1195, 1168 ***υ***(C-N), 1118 ***υ***(N-N), 578 ***υ***(C-Se); ^{1}HNMR (DMSO-d_6, 500 MHz): δ/ppm = 10.43 (s, 1H, NH), 9.97 (s,1H, OH), 7.07 (d, 1H, *J* = 10 Hz, Ar-H), 6.915 (d, 1H, *J* = 5 Hz, Ar-H), 6.79 (d, 1H, *J* = 10 Hz, Ar-H), 5.81 (s, 1H, 5-H, selenadiazoline ring), 4.09 (q, 2H, J_1 = 15 Hz, J_2 = 5, CH_2), 2.22 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 1.30 (t, 3H, J_1 = 5 Hz, J_2 = 10 Hz, CH_3); ^{13}CNMR (DMSO-d_6, 500 MHz): δ/ppm = 169.91, 169.07, 159.43, 157.63, 147.38, 138.15, 130.09, 119.88, 116.54, 113.53, 64.86, 22.23, 21.03, 14.87.

N-(4-acetyl-5-(2-chloroquinolin-3-yl)-4,5-dihydro-1,3,4-thiadiazol-2-yl)acetamide (Z_3B*)*

Dark brown powder, yield: 54%; R_f: 0.51; m.p: 216–218°C; Elemental Analysis for $C_{15}H_{13}ClN_4O_2S$ (348.81 g/mol); Calcd: C, 51.65; H, 3.76; N, 16.06; S, 9.19. Found: C, 51.61; H, 3.78; N, 16.11; S, 9.21. IR (KBr) cm^{-1}: 3163 ***υ***(NH), 2928 ***υ***(CH, aliph.), 1693, 1658 ***υ***(CH_3CON, two groups), 1608 ***υ***(C=N, thiadiazoline ring), 1500 – 1415 ***υ***(C=C), 1238 ***υ***(C-N), 1134 ***υ***(N-N), 860 ***υ***(C-Cl), 717 ***υ***(C-S-C, asym), 620 ***υ***(C-S-C, sym); ^{1}HNMR (DMSO-d_6, 500 MHz): δ/ppm = 12.18 (s,1H, NH), 8.08 (d, 1H, *J* = 10 Hz, Ar-H), 8.755 (d, 1H, *J* = 5 Hz, Ar-H), 7.625 (t, 1H, J_1 = 10 Hz, J_2 = 8 Hz, Ar-H), 7.49 (s, 1H, Ar-H), 7.22 (t, 1H, J_1 = 5 Hz, J_2 = 10 Hz, Ar-H), 6.60 (s, 1H, 5-H, thiadiazoline ring), 2.33 (s, 3H, CH_3), 2.02 (s, 3H, CH_3); ^{13}CNMR (DMSO-d_6, 500 MHz): δ/ppm = 171.36, 169.92, 149.50, 148.72, 147.11, 137.90, 133.77, 132.65, 129.57, 128.91, 123.25, 122.53, 65.06, 23.46, 21.97; The EI-MS (m/s): 348 $[m]^+$, 334 $[C_{14}H_{11}ClN_4O_2S]^+$, 313 $[C_{15}H_{13}N_4O_2S]^+$, 306 $[C_{13}H_{11}ClN_4OS]^+$.

N-(4-acetyl-5-(2-chloroquinolin-3-yl)-4,5-dihydro-1,3,4-selenadiazol-2-yl)acetamide (Z_3B'*)*

Light yellow powder, yield: 77%; R_f: 0.71; m.p: 292–293°C; Elemental Analysis for $C_{15}H_{13}ClN_4O_2Se$ (395.70 g/mol); Calcd: C, 45.53; H, 3.31; N, 14.16. Found: C, 45.59; H, 3.33; N, 14.17. IR (KBr) cm^{-1}: 3205 ***υ***(NH), 2862 ***υ***(CH, aliph.), 1743, 1707 ***υ***(CH_3CON, two groups), 1651 ***υ***(C=N, quinoline ring), 1589 ***υ***(C=N, selenadiazoline ring), 1435 ***υ***(C=C), 1215 ***υ***(C-N), 1118 ***υ***(N-N), 898 ***υ***(C-Cl), 524 ***υ***(C-Se); ^{1}HNMR (DMSO-d_6, 500 MHz): δ/ppm = 12.24 (s,1H, NH), 8.51 (s, 1H, Ar-H), 7.93 (d, 1H, *J* = 10 Hz, Ar-H), 7.665 (t, 1H, J_1 = 10 Hz, J_2 = 5 Hz, Ar-H), 7.37 (d, 1H, *J* = 10 Hz, Ar-H), 7.26 (t, 1H, J_1 = 10 Hz, J_2 = 8 Hz, Ar-H), 6.86 (s, 1H, 5-H, selenadiazoline ring), 2.30 (s, 3H, CH_3), 1.84 (s, 3H, CH_3); ^{13}CNMR (DMSO-d_6, 500 MHz): δ/ppm = 168.43, 161.91, 142.90, 141.63, 138.00, 134.16, 131.40, 130.70, 128.75, 128.26, 126.08, 123.13, 115.89, 23.42, 20.92; The EI-MS (m/s): 396 $[m]^+$, 360 $[C_{15}H_{13}N_4O_2Se]^+$, 307 $[C_{11}H_7ClN_4Se]^+$, 206 $[C_{10}H_9ClN_3]^+$.

N-(4-acetyl-5-(6-chloro-4-oxo-4H-chromen-3-yl)-4,5-dihydro-1,3,4-thiadiazol-2-yl)acetamide (Z_3C*)*

White crystals, yield: 83%; R_f: 0.86; m.p: 177–179°C; Elemental Analysis for $C_{15}H_{12}ClN_3O_4S$ (365.79 g/mol); Calcd: C, 49.25; H, 3.31; N, 11.49; S, 8.77. Found: C, 49.28; H, 3.36; N, 11.50; S, 8.79. IR (KBr) cm^{-1}: 3151 ***υ***(NH), 2947 ***υ***(CH, aliph.), 1693, 1643 ***υ***(CH_3CON, two groups), 1604 ***υ***(C=N, thiadiazoline ring), 1570 ***υ***(C=C, Chromone ring), 1462 – 1415 ***υ***(C=C), 1238 ***υ***(C-N), 1168 ***υ***(C-O, Chromone ring), 1138 ***υ***(N-N), 856 ***υ***(C-Cl), 705 ***υ***(C-S-C, asym), 640 ***υ***(C-S-C, sym); ^{1}HNMR (DMSO-d_6, 500 MHz): δ/ppm = 11.78 (s,1H, NH), 8.025 (d, 1H, *J* = 5 Hz, Ar-H), 7.90 (d, 2H, *J* = 10 Hz, Ar-H), 7.77 (d, 1H, *J* = 10 Hz, Ar-H), 6.64 (s, 1H, 5-H, thiadiazoline ring), 2.24 (s, 3H, CH_3), 2.04 (s, 3H, CH_3); ^{13}CNMR (DMSO-d_6, 500 MHz): δ/ppm = 174.67, 169.97, 168.49, 154.97, 154.07, 147.81, 135.09, 130.81, 124.58, 124.28, 122.10, 121.64, 60.25, 22.98, 22.40; The EI-MS (m/s): 367 $[m]^+$, 325 $[C_{13}H_{10}ClN_3O_3S]^+$, 279 $[C_{11}H_6ClN_3O_2S]^+$, 213 $[C_8H_{11}N_3O_2S]^+$.

N-(4-acetyl-5-(6-chloro-4-oxo-4H-chromen-3-yl)-4,5-dihydro-1,3,4-selenadiazol-2-yl)acetamide (Z_3C'*)*

Very light gray powder, yield: 76%; R_f: 0.9; m.p: 224–226°C; Elemental Analysis for $C_{15}H_{12}ClN_3O_4Se$ (412.69 g/mol); Calcd: C, 43.66; H, 2.93; N, 10.18. Found: C, 43.68; H, 2.98; N, 10.18. IR (KBr) cm^{-1}: 3279 ***υ***(NH), 2931 ***υ***(CH, aliph.), 1743, 1654 ***υ***(CH_3CON, two groups),1697 ***υ***(C=O, Chromone ring), 1604 ***υ***(C=N, selenadiazoline ring), 1570 ***υ***(C=C, Chromone ring), 1462 ***υ***(C=C), 1238 ***υ***(C-N), 1149 ***υ***(N-N), 1114 ***υ***(C-O, Chromone ring), 868 ***υ***(C-Cl), 582 ***υ***(C-Se); ^{1}HNMR (DMSO-d_6, 500 MHz): δ/ppm = 10.38 (s,1H, NH), 8.015 (d, 1H, *J* = 5 Hz, Ar-H), 7.915 (d, 1H, *J* = 15 Hz, Ar-H), 7.80 (s, 1H, Ar-H), 7.79 (s, 1H, Ar-H), 6.83 (s, 1H, 5-H, selenadiazoline ring), 2.11 (s, 3H, CH_3), 2.06 (s, 3H, CH_3); ^{13}CNMR (DMSO-d_6, 500 MHz): δ/ppm = 175.28, 170.21, 168.07, 159.20, 158.43, 137.95, 135.19, 129.93, 129.48, 127.48, 122.72, 121.66, 65.64, 23.63, 20.62; The EI-MS (m/s): 413 $[m]^+$, 260 $[C_8H_{11}N_3O_2Se]^+$, 234 $[C_6H_9N_3O_2Se]^+$, 180 $[C_9H_5ClO_2]^+$.

N-(4-acetyl-5-(4-nitrophenyl)-4,5-dihydro-1,3,4-thiadiazol-2-yl)acetamide (Z_3D*)*

Off white powder, yield: 81%; R_f: 0.74; m.p: 230–232°C; Elemental Analysis for $C_{12}H_{12}N_4O_4S$ (308.31 g/mol); Calcd: C, 46.75; H, 3.92; N, 18.17; S, 10.40. Found: C, 46.71; H, 3.96; N, 18.17; S, 10.40. IR (KBr) cm^{-1}: 3167 ***υ***(NH), 2970 ***υ***(CH, aliph.), 1701, 1647 ***υ***(CH_3CON, two groups), 1608 ***υ***(C=N, thiadiazoline ring), 1519 ***υ***(NO_2, asym), 1350 ***υ***(NO_2, sym), 1446 – 1404 ***υ***(C=C), 1238 ***υ***(C-N), 1138 ***υ***(N-N), 713 ***υ***(C-S-C, asym), 609 ***υ***(C-S-C, sym); ^{1}HNMR (DMSO-d_6, 500 MHz): δ/ppm = 11.86 (s,1H, NH), 8.23 (d, 2H, *J* = 10 Hz, Ar-H), 7.54 (d, 2H, *J* = 10 Hz, Ar-H), 6.97 (s, 1H, 5-H, thiadiazoline ring), 2.24 (s, 3H, CH_3), 2.05 (s, 3H, CH_3); ^{13}CNMR (DMSO-d_6, 500 MHz): δ/ppm = 170.08, 168.14, 148.71, 147.58, 146.27, 127.00 (2C), 124.60 (2C), 65.50, 22.98, 22.28; The EI-MS (m/s): 308 $[m]^+$, 266 $[C_{10}H_{10}N_4O_3S]^+$, 222 $[C_8H_6N_4O_2S]^+$, 163 $[C_8H_7N_2S]^+$.

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 thiadiazoline (Z_3C) and selenadiazoline (Z_3C') 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[25] LD_{50} = Xf +Kd, where Xf: the last dose, K: the interval between dose levels, d: the tabulated value, Table 1.[26]

Antibacterial Activity

The compounds (Z_3A-Z_3C, Z_3A'-Z_3C') 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 used 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 24 hours at 36 ± 1ºC, under aerobic conditions. After incubation, confluent bacterial growth was observed. Inhibition of the bacterial growth was measured in mm.[27] Furthermore, values of minimum inhibitory concentration (MIC) of those compounds.[28] The MIC was recorded because the lowest concentration at which no visible growth was observed.

Antioxidant Activity

The antioxidant activity of the 1,3,4-thiadiazoline and 1,3,4-selenadiazoline (Z_{3-1}, Z_3A-Z_3D, Z_3A'-Z_3C') was determined according to the β-carotene bleaching method.[29] 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.[30,31] 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, 50 ml 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 compounds Z_{3-1}, Z_3A-Z_3D , Z_3A'-Z_3C' 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 hours. Absorbance was measured every 15 minutes.[29] Antioxidant activity (AA) was calculated as percent of inhibition relative to the control using the following equation:

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

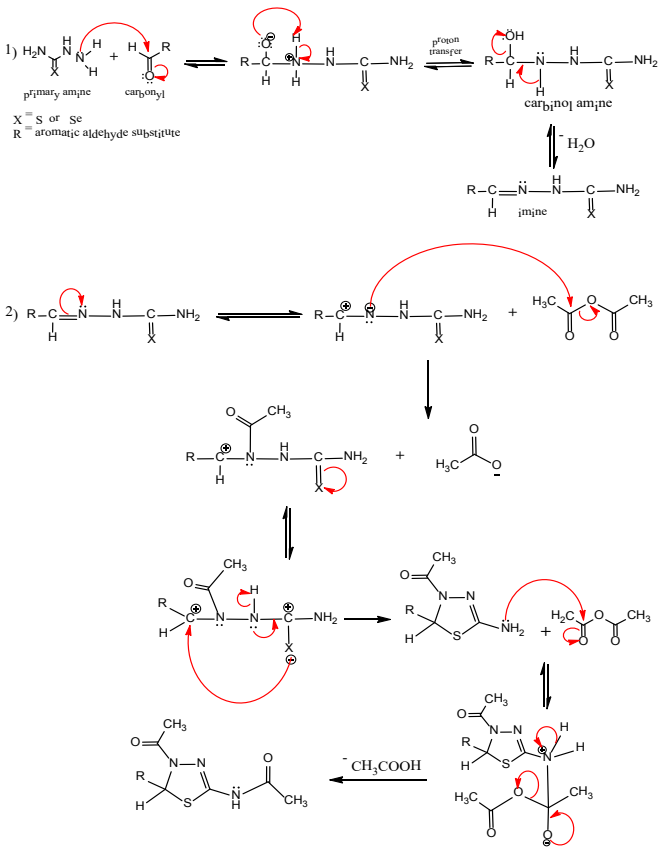
Where, Ai: is the measured absorbance value of sample at zero time. At: is the measured absorbance value of sample after incubation for 105 minutes at 45°C. Ai*: is the measured absorbance value of control at zero time, At*: is the measured absorbance value of control after incubation 105 minutes at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

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 :-				

RESULTS AND DISCUSSION

The Δ^2-1,3,4-thiadiazoline and Δ^2-1,3,4-selenadiazoline compounds (Z_{3-1}, Z_3A-Z_3D and Z_3A'-Z_3C') were prepared via reaction of thiosemicarbazone and selenosemicarbazone derivatives with acetic anhydride. The prepared compounds are solid Compound, stable in air, and they are partially soluble in most polar solvents, the suggested mechanism for preparing a thiadiazoline or selenadiazoline ring is shown in Scheme 2.



Scheme 2: The suggested mechanism for preparing compounds

The elemental analysis results C, H, N, S of the compounds agree with the theoretical values.

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. On comparing the IR spectral data of the TSCs (Z_3, Z_3a–Z_3d) and SSCs (Z_3a'–Z_3c') with the IR spectra of TDZs ($Z_{3\text{-}1}$, Z_3A–Z_3D) and SDZs (Z_3A'–Z_3C') respectively the following can be pointed out: The infrared of prepared compounds (Z_3, Z_3a–Z_3d and Z_3a'–Z_3c') show two bands within the range 3487–3217 cm^{-1} which attributed to asymmetric and symmetric stretching of $\boldsymbol{\upsilon}$ (NH_2) groups.[6] All the infrared spectra of the compounds TSCs and SSCs were characterized by a strong band at 1581–1639 cm^{-1} which corresponds to the azomethine $\boldsymbol{\upsilon}$(CH=N) stretching vibration.[6,16] The strong bands at 1057–1111 cm^{-1} and the medium bands that observed at 1049–1095 cm^{-1} can be assigned to the $\boldsymbol{\upsilon}$(C=S) and $\boldsymbol{\upsilon}$(C=Se) absorption frequencies respectively.[16,32] Furthermore, the strong to medium bands which appeared in the range 601–686 cm^{-1} and at 620–636 cm^{-1} are attributed to the $\boldsymbol{\upsilon}$(C-S) and $\boldsymbol{\upsilon}$(C-Se) stretching respectively.[33-35]

The infrared of the TDZ and SDZ compounds shows characteristic bonds at 1770-1693 cm^{-1} that be attributed to the stretching vibration of the carbonyl group (C=O).[36,37] IR spectra of the TDZs and SDZs showed the absence of $\boldsymbol{\upsilon}$ (NH_2), $\boldsymbol{\upsilon}$(C=S) and $\boldsymbol{\upsilon}$(C=Se) of acetamide thia- and selena- diazole compounds and the appearance of new bands at 1707–1643 cm^{-1} assigned to amide carbonyls $\boldsymbol{\upsilon}$ $(C=O)_{amide}$, confirms evidently five-membered ring formation from TSCs and SSCs compounds.[37] Ring closure in TDZs and SDZs can be observed by the appearance of strong bands at ~1608 cm^{-1} which attributed to the azomethine $\boldsymbol{\upsilon}$(C=N) groups, where it is expected to increase the absorption frequency of the azomethine group in the five-membered ring. In addition, the strong to medium bands at 1238–1180 cm^{-1} can correspond to the $\boldsymbol{\upsilon}$(C-N) stretching vibration.[37] Furthermore, the spectrum was distinguished by the appearance of distinct absorption bands for $\boldsymbol{\upsilon}$ (C-S-C) at the range 717–705 cm^{-1} and in 659–609 cm^{-1}, which assigned to asymmetrical and symmetrical stretching vibration respectively for the compounds ($Z_{3\text{-}1}$, Z_3A-Z_3D).[38] Appearance of medium to weak bands at the range 582–524 cm^{-1} in IR spectrum can be related to stretching of $\boldsymbol{\upsilon}$(C-Se) for the compounds (Z_3A'-Z_3C').[39]

^{1}HNMR and ^{13}CNMR Spectra

The structures of all new compounds were confirmed and the formation of five-membered ring by ^{1}HNMR spectra. The ^{1}HNMR spectra of all compounds show a singlet signal at the range δ (3.33-6.97) ppm, which attributed to the 5-H proton of thia- or selenadiazoline ring.[37] They also display a singlet signal at δ (10.38–12.68) ppm which is attributed to the NH proton.[16] All the compounds are characterized by showing two singlet signals at δ (2.11–2.33) ppm and δ (1.84–2.23) ppm, which can be assigned to methyl group of the acetyl ($COCH_3$) and acetamide ($NHCOCH_3$) groups respectively.[6] Also multiple signals that appear at δ (6.7–8.7) ppm can be attribute to aromatic rings of the studied compounds. The compounds $Z_{3\text{-}1}$, Z_3A and Z_3A' are characterized by showing singlet signal at δ (9.97-11.94) ppm and which can be assigned to phenolic group (OH).[40] Furthermore, the protons of (OCH_2) group of compounds Z_3A and Z_3A' were observed as a quartet signal at δ (4.17 and 4.09) ppm, respectively. The signal at δ (3.90) ppm is assigned to the OCH_3 group for compound $Z_{3\text{-}1}$. Therefore, the ^{1}HNMR result supports the formation of five-membered ring.[41]

The ^{13}CNMR spectra of the studied compounds ($Z_{3\text{-}1}$,Z_3A-Z_3D and Z_3A'-Z_3C') show signal at the range δ (137.95–151.88) ppm of the (C=N) carbon, and signal appear at δ (60.25-111.99) ppm and δ (65.64-115.89) ppm which attribute to C-S and C-Se carbon signal, respectively. These signals confirm the formation of the thia- or selenadiazoline ring from new (thio- or selenosemicarbazones) with acetic anhydride.[6,37] Also, all spectra exhibited two signals at δ (161.91-169.92) ppm and δ (168.43-171.36) ppm which can be assigned to carbonyl carbon of the acetyl ($COCH_3$) and acetamide ($NHCOCH_3$) groups respectively.[42] Furthermore, the signals of aromatic carbons of these synthesized compounds represented at δ (120.10–159.43) ppm. The ^{13}CNMR spectral data of the 4-acetamide-1,3,4-thiadiazoles and 4-acetamide-1,3,4-selenadiazoles are in accord with suggested structures. Some spectra of compounds showed in Figures 1 to 3.

EI-mass

Mass spectrometry employs documentation obtained when a molecule is ionized by electron impact and several ions consist of and examined on the basis of their mass to charge ratio. 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. The mass spectrum of the compounds ($Z_{3\text{-}1}$, Z_3A-Z_3D, Z_3B' and Z_3C') 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. Furthermore, the peaks intensity gives an idea about the stability of fragments primarily with the base peaks. The mass spectrum of the compound Z_3A shows three fragmentation peaks at m/z 281, m/z 279 and m/z 237, these peaks can be attributed to $[C_{13}H_{15}N_3O_3S]^+$, $[C_{12}H_{13}N_3O_3S]^+$ and $[C_{10}H_{11}N_3O_2S]^+$ ions, respectively. Also, a peak at m/z 209 can be assigned to $[C_9H_9N_2O_2S]^+$ ion. The mass spectrum of the compound Z_3B shows several fragmentation peaks at m/z 334, m/z 313, m/z 306, m/z 237 and m/z 207, these peaks can be assigned to $[C_{14}H_{11}N_4O_2SCl]^+$, $[C_{15}H_{14}N_4O_2S]^+$,

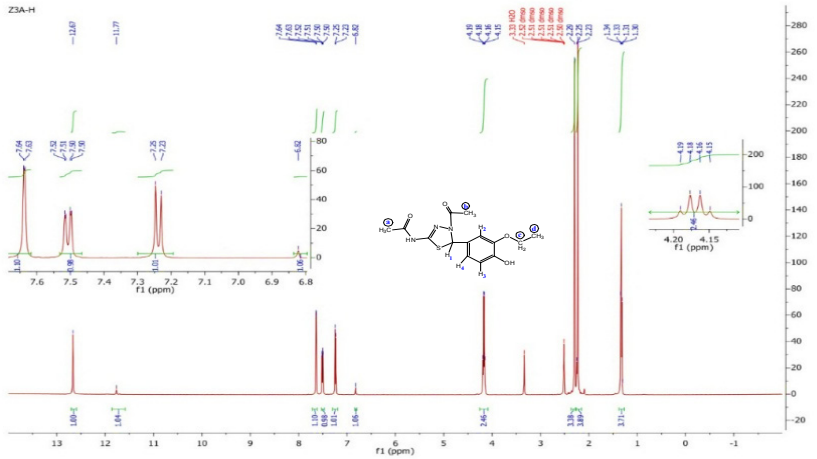


Figure 1: ^{1}HNMR spectrum of compound Z_3A

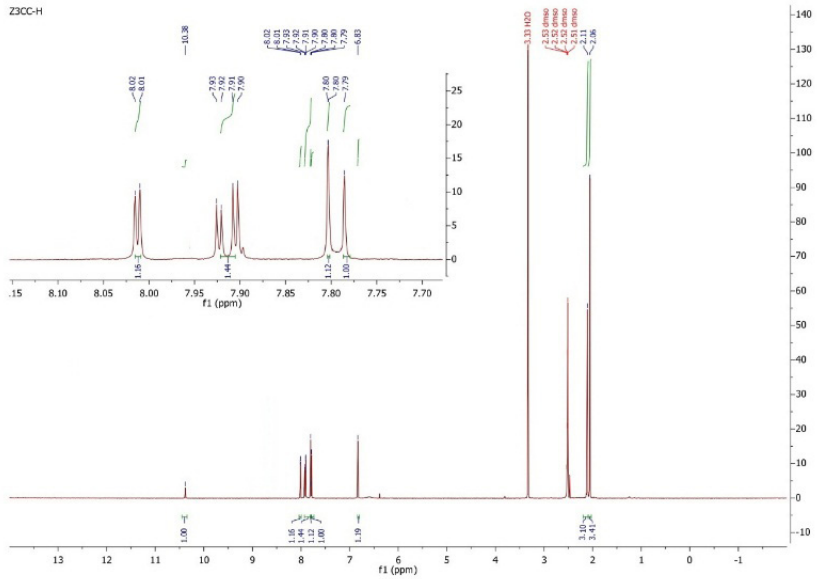


Figure 2: ^{1}HNMR spectrum of compound Z_3C'

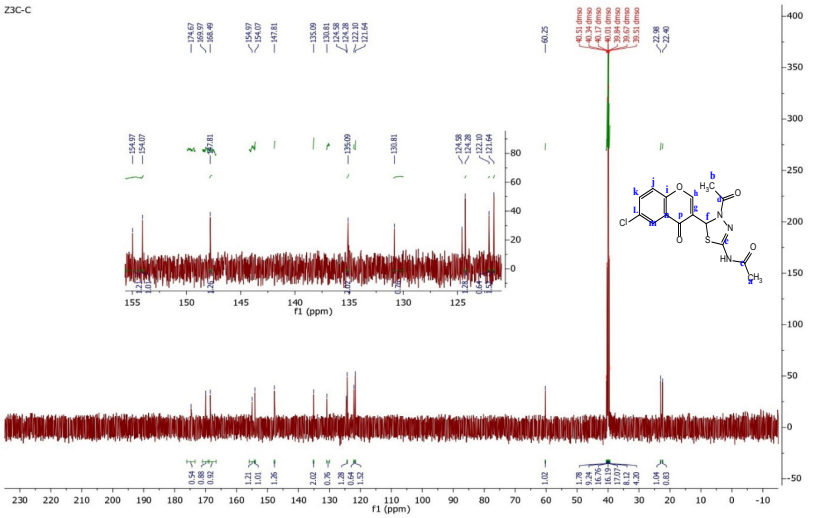
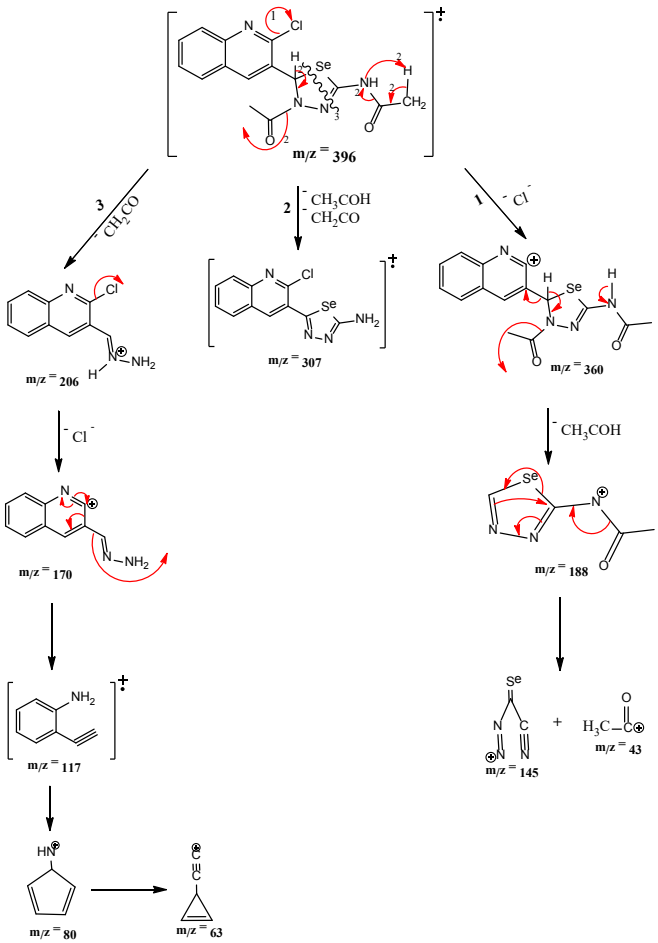
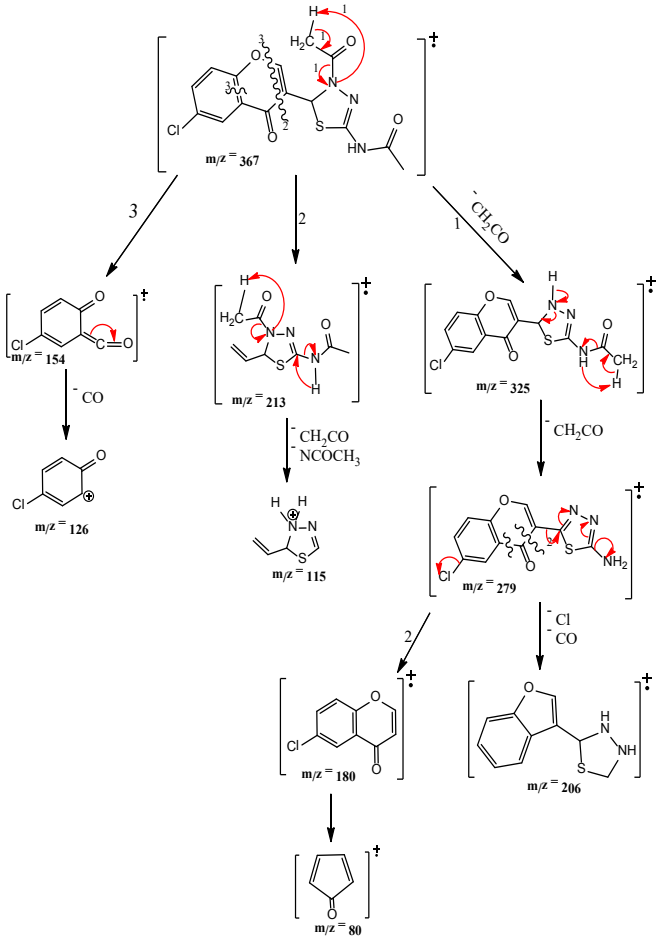


Figure 3: ^{13}C NMR spectrum of compound Z_3C



Scheme 3: The fragmentation pattern proposed for compound (Z_3B')



Scheme 4: The fragmentation pattern proposed for compound (Z_3C)

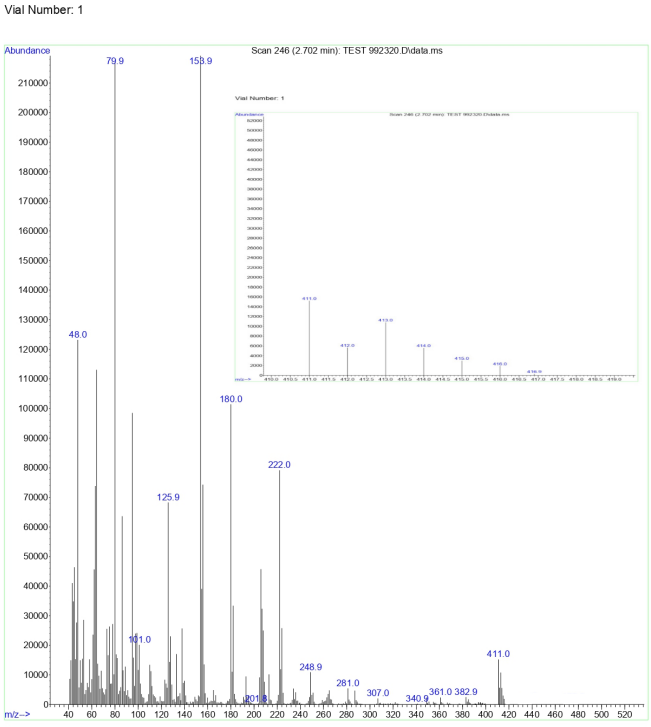


Figure 4: Mass spectrum of the compound Z_3C'

$[C_{13}H_{10}N_4SCl]^+$, $[C_{10}H_8N_3SCl]^+$ and $[C_{10}H_6NSCl]^+$ ions respectively. On other hand the mass spectrum of compound Z_3C' characterized by the appearance of many fragmentation peaks at m/z 260, m/z 234, m/z 180 and m/z 126 which can be attributed to $[C_8H_{11}N_3O_2Se]^+$, $[C_6H_9N_3O_2Se]^+$, $[C_9H_5O_2Cl]^+$ and $[C_6H_3OCl]^+$ ions respectively. The higher mass peak at m/z 80 can be assigned to $[C_5H_4O]^+$ ion.

Biological Activity

Median Lethal Dose (LD_{50})

The lethal dose (LD_{50}) of the studied compounds (Z_3C and Z_3C') *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 Z_3C and Z_3C', 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 the tortuous reflex in some treatments, and finally Death at high toxic doses, Table 2. [43,44]

Antibacterial Activity

The sensitivity of four human pathogenic microbes (two of Gram-positive bacteria: *S. aureus*, *Bacillus* and two of Gram-negative bacteria: *E. coli*, *P. aeruginosa*) to the new synthetic heterocyclic compounds (Z_3A-Z_3C, Z_3A'-Z_3C') was tested and compared to that of commercially available antibacterial antibiotic tetracycline. Our study confirmed that the Δ^2-1,3,4-thiadiazoline and Δ^2-1,3,4-selenadiazoline compounds 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.

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

Test characterization	*Results*	
	Z_3C	*Z_3C'*
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

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.[45] 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, *E. coli* is one of the most dangerous microbes that cause many common diseases in humans, frequently associated with urinary tract infections, a frequent problem in stressed people and office owners who share communal toilets and followed by the risk of *P. 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 *S. aureus* if allowed to enter the bloodstream or internal tissues.[46]

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

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

	Diameter of inhibition zone (mm) Bacillus								*Diameter of inhibition zone (mm) S. sciuri*						
	Concentration (mg/mL)								*Concentration (mg/mL)*						
Compounds	*1*	*5*	*25*	*125*	*250*	*500*	*MIC*	*Compounds*	*1*	*5*	*25*	*125*	*250*	*500*	*MIC*
Z3A	0	0	0	11	11	12	125	Z3A	0	0	0	0	11	13	250
Z3A'	0	0	0	19	19	20	125	Z3A'	0	0	0	12	13	15	125
Z3B	0	0	14	14	14	16	25	Z3B	0	0	11	12	12	20	25
Z3B'	0	0	0	0	13	14	250	Z3B'	0	0	0	0	13	14	250
Z3C	0	0	13	18	20	20	25	Z3C	0	20	22	23	25	33	5
Z3C'	**0**	**0**	18	30	32	40	25	Z3C'	0	21	22	26	30	33	5
tetracycline	5	11	14	22	30	50	1	tetracycline	0	4	10	14	25	48	5

	Diameter of inhibition zone (mm) E. coli								*Diameter of inhibition zone (mm) P. aeruginosa*						
	Concentration (mg/mL)								*Concentration (mg/mL)*						
Compounds	*1*	*5*	*25*	*125*	*250*	*500*	*MIC*	*Compounds*	*1*	*5*	*25*	*125*	*250*	*500*	*MIC*
Z3A	0	0	0	0	0	4	500	Z3A	0	0	0	0	0	11	500
Z3A'	0	0	0	0	0	5	500	Z3A'	0	0	0	13	14	16	125
Z3B	0	0	0	0	0	4	500	Z3B	0	0	0	0	0	4	500
Z3B'	0	0	0	0	0	6	500	Z3B'	0	0	0	0	0	6	500
Z3C	0	0	10	18	35	50	25	Z3C	0	0	0	20	20	25	125
Z3C'	0	0	0	0	18	30	250	Z3C'	0	0	12	18	30	40	25
tetracycline	0	8	11	15	21	44	5	tetracycline	0	6	8	17	30	52	5

interference with the bacterial cell wall, thereby inhibiting the microbial growth.[46]

Among the new synthetic heterocyclic compounds, Z_3C was found to be more effective than positive control (tetracycline) against gram-negative bacteria (*E. coli*) with an inhibition zone (IZ) of 18, 35 and 50 mm at the concentration of 125, 250 and 500 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 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.[47,48]

In the case of antibacterial activity against Gram-positive bacteria (*S. aureus* and *Bacillus*), all compounds were found to have activity ranged between high and moderate. Our results indicated that the compounds Z_3C and Z_3C' possessed the highest antibacterial activity against Gm^{+Ve} (*S. aureus*) with an IZ of (20, 22, 23, 25 and 21, 22, 26, 30 mm) at concentrations of (5, 25, 125, 250 mg/mL), respectively. Also, Z_3C and Z_3C' compounds showed more potent compared to the positive control (IZ= 4-25) mm at the same concentration. From the other hand, our data pointed out that compound Z_3C' showed a good antibacterial activity against Gm^{+Ve} (*Bacillus*) with an IZ ranging from (18–32) mm as compared to tetracycline (IZ = 14–30 mm) at the concentrations (25–250) mg/mL.

The antimicrobial activity of these new synthetic heterocyclic compounds may be attributed to the basis of their structures, mainly possessing electron withdrawing groups like chlorine. Also, the presence of halogen atom in the molecule increases the lipophilicity of the molecule and facilitates hydrophobic interactions of the molecule with specific binding sites on either receptor or enzymes. Furthermore, the Cl ion in the compounds can enhance the antibacterial activity due to the killing microbes or inhibiting their multiplication by blocking their active site. Moreover, the presence of heteroatoms resulted in an increase in the antimicrobial activity.[49,50]

Finally, it is believed that the biological activity of thiadiazoline or selenadiazoline skeletal may relate to the chemical reaction of the ring as well as to substituents especially at (-N=C-S- or -N=C-Se-) of thia-or selenadiazoline ring.[16,19] Thus, thiadiazoline and selenadiazoline can be considered as selective inhibitors of bacterial cell wall synthesis and are therefore effective against bacterial growth.[9,51]

The MIC of tested compounds in this study against the test organisms ranged between (5–500) 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 *E. coli* and *P. aeruginosa*, whereas the most sensitive microorganisms were *S. aureus* and *Bacillus*. The lowest MIC value of 5 mg/mL was recorded on *S. aureus* with compounds Z_3C and Z_3C', whereas the lowest MIC value of 25 mg/mL was obtained on Bacillus with compounds Z_3B, Z_3C and Z_3C'. The compound Z_3C was more active as compared with its precursors and had the lowest MIC value of 5 mg/mL was obtained on *E. coli*, and compound Z_3C' (MIC = 25 mg/mL) was obtained on *P. aeruginosa*. However, the highest MIC value of 500 mg/mL was recorded on *E. coli* with compounds (Z_3A, Z_3A', Z_3B and Z_3B') and on *P. aeruginosa* with compounds (Z_3A, Z_3B and Z_3B'). The results of the present study suggest that the Z_3C and Z_3C' possess remarkable toxic activity against bacteria and may assume pharmacological importance.[52]

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.[30,40] 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.[30,53]

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.[30] 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.[54]

The results in Table 4 and Figures 5 to 7 were indicated an increase in the antioxidant activity of the synthetic compounds and standards in the order of $Z_3A < Z_3C' < Z_3B' < Z_3D <$ BHT with corresponding percentages values of 54.8, 56.2, 66.4, 66.8 and 80.6%, respectively. On the other hand, the lowest activity was observed for compounds $Z_{3\text{-}1}$, Z_3A' and

Table 4: Antioxidant activity of prepared compounds

Comp. symbol	*Aj*	*At*	*Aj**	*At**	*AA%*
BHT	0.523	0.481	0.435	0.218	80.6
$Z_{3\text{-}1}$	0.457	0.327	0.435	0.218	40.09
Z_3A	0.477	0.379	0.435	0.218	54.8
Z_3A'	0.437	0.303	0.435	0.218	38.2
Z_3B	0.503	0.417	0.435	0.218	60.4
Z_3B'	0.450	0.377	0.435	0.218	66.4
Z_3C	0.516	0.375	0.435	0.218	35
Z_3C'	0.443	0.348	0.435	0.218	56.2
Z_3D	0.513	0.441	0.435	0.218	66.8

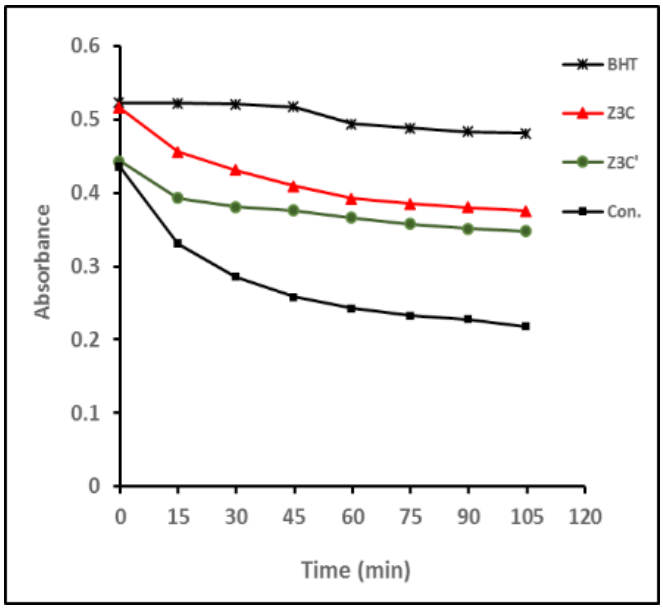


Figure 5: Antioxidant activity of compounds Z_3C and Z_3C'

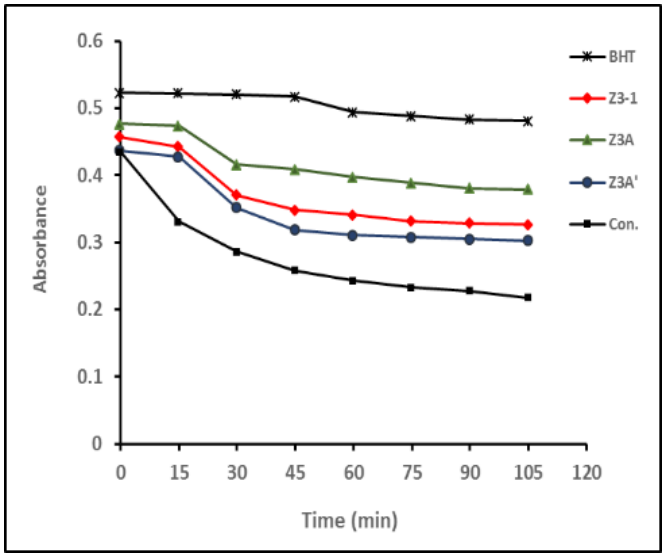


Figure 6: Antioxidant activity of compounds $Z_{3\text{-}1}$, Z_3A and Z_3A'

Z_3C with corresponding inhibition ratio (40.09, 38.2, 35%), respectively. A possible explanation for the higher antioxidant

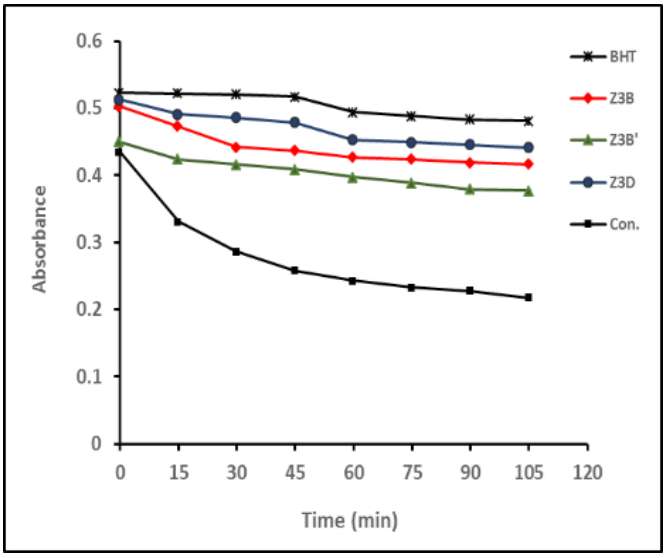


Figure 7: Antioxidant activity of compounds Z_3D, Z_3B and Z_3B'

activity of these compounds (Z_3D, Z_3B', Z3C' and Z_3A) might be due to the following reasons; first, since compound Z_3D has a thiadiazole ring which can act as a scavenger for radicals to prevent oxidative cellular damage and thus enhance antioxidant properties.[55] Second, compounds Z_3B' and Z_3C' have selenadiazole ring which increase the antioxidant activity. Furthermore, the organoselenium compounds had an ability to catalyze the reduction of harmful peroxides by glutathione (GSH) and thereby protects the biomolecules against oxidative damage.[56] Third, compound Z_3A contains phenolic hydroxyl and ethoxy groups that have high scavenging capacity against reactive oxygen species as well as being hydrogen-donating groups, making them very good antioxidants.[57,58]

The finding that compound Z_3D 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.[55] The mechanism by which Z_3D protects the body's cells from oxidative damage may require further study and investigation.

Interestingly, previous studies showed that some of thiazole and selenazole antibiotics such as Febuxostat and ebselen possess a relative antioxidant activity against oxygen-reactive species (ROS).[55,59] Furthermore, the ring of thiadiazole or selenadiazole has an ability to initiate the free radical scavenging activity may be due to its C-S-C or C-Se-C moieties.[16]

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.[53] Therefore, we expect that Z_3D 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 Z_3D 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.[30]

CONCLUSION

Two new series of Δ^2-1,3,4-thiadiazoline and Δ^2-1,3,4-selenadiazoline compounds derived from thiosemicarbazones and selenosemicarbazones respectively were prepared. The compounds show moderate antibacterial activities against *S. aureus*, *Bacillus*, *E. coli* and *P. aeruginosa*. The most elegant result as antibacterial activity was obtained for compounds Z_3C and Z_3C' while the synthesized compound Z_3D showed high activity as an antioxidant agent. The present study reported moderate *in vivo* toxic effects by LD_{50} measurement of new compounds (Z_3C and Z_3C').

REFERENCES

1. Ando W, Tokitoh N, Kabe Y. Synthesis and Reaction of Novel Sulfur Containing Heterocyclic Systems. Phosphorus, Sulfur, and Silicon and the Related Elements. 1991 Apr 1;58(1-4):179-205.
2. Al-Balas QA, Al-Smadi ML, Hassan MA, Al Jabal GA, Almaaytah AM, Alzoubi KH. Multi-Armed 1, 2, 3-Selenadiazole and 1, 2, 3-Thiadiazole Benzene Derivatives as Novel Glyoxalase-I Inhibitors. Molecules. 2019 Jan;24(18):3210. https://doi.org/10.3390/molecules24183210
3. Aziz NM, Rowland JH. Trends and advances in cancer survivorship research: challenge and opportunity. Semin. Radiat. Oncol., 2003;13(3):248–266. doi: 10.1016/S1053-4296(03)00024-9
4. Chen T, Wong YS, Zheng W, Liu J. Caspase-and p53-dependent apoptosis in breast carcinoma cells induced by a synthetic selenadiazole derivative. Chemico-biological interactions. 2009 Jun 15;180(1):54-60. https://doi.org/10.1016/j.cbi.2008.12.010
5. Sanmartín C, Plano D, Sharma AK, Palop JA. Selenium compounds, apoptosis and other types of cell death: an overview for cancer therapy. International journal of molecular sciences. 2012 Aug;13(8):9649-9672. https://doi.org/10.3390/ijms13089649
6. El-Desoky SI, Badria FA, Abozeid MA, Kandeel EA, Abdel-Rahman AH. Synthesis and antitumor studies of novel benzopyrano-1, 2, 3-selenadiazole and spiro [benzopyrano]-1, 3, 4-thiadiazoline derivatives. Medicinal Chemistry Research. 2013 May;22(5):2105-2114. DOI 10.1007/s00044-012-0201-0
7. Srdić-Rajić T, Zec M, Todorović T, Anđelković K, Radulović S. Non-substituted N-heteroaromatic selenosemicarbazone metal complexes induce apoptosis in cancer cells via activation of mitochondrial pathway. European journal of medicinal chemistry. 2011 Sep 1;46(9):3734-3747.
8. Filipovic NR, Bjelogrlic S, Portalone G, Pelliccia S, Silvestri R, Klisuric O, Sencanski M, Stankovic D, Todorovic TR, Muller CD. Pro-apoptotic and pro-differentiation induction by 8-quinolinecarboxaldehyde selenosemicarbazone and its Co(III) complex in human cancer cell lines. Med. Chem. Comm., 2014;1-29. DOI: 10.1039/C6MD00199H
9. Al-Smadi M, Ratrout S. New 1, 2, 3-Selenadiazole and 1, 2, 3-Thiadiazole Derivatives. Molecules. 2004 Nov;9(11):957-967.
10. Al-Rubaie AZ, Al-Jadaan SA, Muslim SK, Saeed EA, Ali ET, Al-Hasani AK, Al-Salman HN, Al-Fadal SA. Synthesis, characterization and antibacterial activity of some new ferrocenyl selenazoles and 3, 5-diferrocenyl-1, 2, 4-selenadiazole. Journal of Organometallic Chemistry. 2014 Dec 15;774:43-47.
11. Geng S, Wu Q, Shi L, Cui F. Spectroscopic study one thiosemicarbazone derivative with ctDNA using ethidium bromide as a fluorescence probe. International journal of biological macromolecules. 2013 Sep 1;60:288-294. http://doi.org/10.1016/j.ijbiomac.2013.06.002
12. Kamal M, Shakya AK, Jawaid T. 1, 3,4-Thiadiazole as antimicrobial agent: a review. Int. J. Biomed. Res., 2011;2(1): 41-61.
13. Hua G, Li Y, Fuller AL, Slawin AMZ, Woollins JD. Facile Synthesis and Structure of Novel 2,5-Disubstituted 1,3,4-Selenadiazoles. Eur. J. of Org. Chem., 2009 (10), 1612-1618 (2009). DOI:10.1002/ejoc.200900013
14. Shen H, Zhu H, Song M, Tian Y, Huang Y, Zheng H, Cao R, Lin J, Bi Z, Zhong W. A selenosemicarbazone complex with copper efficiently down-regulates the 90-kDa heat shock protein HSP90AA1 and its client proteins in cancer cells. BMC cancer. 2014 Dec;14(1):1-10.
15. Mhaidat NM, Al-Smadi M, Al-Momani F, Alzoubi KH, Mansi I, Al-Balas Q. Synthesis, antimicrobial and in vitro antitumor activities of a series of 1, 2, 3-thiadiazole and 1, 2, 3-selenadiazole derivatives. Drug Design, Development and Therapy. 2015;9:3645-3652.
16. Ebrahimi HP, Hadi JS, Alsalim TA, Ghali TS, Bolandnazar Z. A novel series of thiosemicarbazone drugs: from synthesis to structure. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2015 Feb 25;137:1067-1077. doi: http://dx.doi.org/10.1016/j.saa.2014.08.146
17. Lalezari I, Shafiee A, Khorrami J, Soltani A. Selenium Heterocycles XXII: Synthesis and Antibacterial and Antifungal Activities of Arylsulfonyl-1, 2, 3-selenadiazoles. Journal of pharmaceutical sciences. 1978 Sep 1;67(9):1336-1338. doi: 10.1002/jps.2600670945.
18. Shrivastava K, Purohit S, Singhal S. Studies On Nitrogen And Sulphur Containing Heterocyclic Compound: 1,3,4 - thiadiazole. Asian J. Biomed. *Pharm. Sci*., 2013;3(21):6-23.
19. Arsenyan P, Pudova O, Lukevics E. A novel method for the synthesis of 2,5-diarylselenophenes. Tetrahedron Lett. 2002;43(27):4817-4819.
20. Soriano-Garcia M. Organoselenium compounds as potential therapeutic and chemopreventive agents: a review. Current medicinal chemistry. 2004 Jun 1;11(12):1657-1669. DOI: https://doi.org/10.2174/0929867043365053
21. Chen T, Zheng W, Wong YS, Yang F. Mitochondria-mediated apoptosis in human breast carcinoma MCF-7 cells induced by a novel selenadiazole derivative. Biomedicine & pharmacotherapy. 2008 Feb 1;62(2):77-84. DOI: 10.1016/j.biopha.2007.12.002
22. Kornis G. 1,3,4-Thiadiazoles Comprehensive heterocyclic chemistry, A. R. Katritzky, C. W. Rees, Eds., Pergamon Press, Oxford, 1984;**6**(4B):545.
23. Segi M, Tanno K, Kojima M, Honda M, Nakajima T. An efficient 1, 3-dipolar cycloaddition between aromatic selenoaldehydes and nitrile oxides or nitrile imines: an easy access to selenium-containing five-membered heterocyclic ring system. Tetrahedron letters. 2007 Mar 26;48(13):2303-2306. https://doi.org/10.1016/j.tetlet.2007.01.153
24. Atta SM, Farrag DS, Sweed AM, Abdel-Rahman AH. Preparation of new polycyclic compounds derived from benzofurans and furochromones. An approach to novel 1,

2, 3-thia-, and selena-diazolofurochromones of anticipated antitumor activities. European journal of medicinal chemistry. 2010 Nov 1;45(11):4920-4927. doi:10.1016/j.ejmech.2010.07.065
25. 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. 2015;7(9):1-5.
26. Dixon WJ. Efficient Analysis of Experimental Observations. Ann. Rev. Pharmacol. Toxicol. 1980;20:441-462.
27. Smânia A, Monache FD, Smânia EFA, Cuneo RS. Antibacterial activity of steroidal compounds isolated from Ganoderma applanatum (Pers.) Pat. (Aphyllophoro-mycetideae) Fruit body. Int. J. Med. Mushrooms. 1999;1:325-330.
28. Al-Salami BK, Al-Fadhly AL, Al-Fregi AA. Synthesis, characterization and Biological Activity Study of some New compounds containing Amine and Azomethine Group and their platinum (II) complexes. Der. Pharma. Chemica. 2016;8(19): 488.
29. Ahmeda A, Hossain MA, Ismail Z. Antioxidant properties of the isolated flavonoids from the medicinal plant. Phyllanthus niruri. As. J. Food Ag-Ind. 2009;2(03):373-381.
30. Al-Fartosy AJM, Antioxidant properties of methanolic extract from *Inula graveolens* L. Turk J Agric For. 2011;35(6):591-596.
31. Al-Atbi HS, Al-Salami BK, Al-Assadi IJ. New azo-azomethine derivative of Sulfanilamide: Synthesis, characterization, Spectroscopic, Antimicrobial and Antioxidant activity study. J. Phys. Conf. Ser. 2019;1294(5):052033.
32. Collard-Charon C, Renson M. Étude Infra-Rouge Des Substances Possédant Une Liaison C=Se Adàjacente à un ou Plusieurs Atomes D›azote II. Sélénosemicarbazides. Bull. des Soc. Chim. Belg. 1963;72(5-6):291-303. DOI:10.1002/bscb.19630720501
33. Sharma YR. Elementary Organic Spectroscopy, Principles and Chemical Applications, S. Chand and Company Ltd., New Delhi, India, 132 (2008).
34. Gingras BA, Suprunchuk T, Bayley CH. Localization Of The C= Se Vibration In The Infrared Spectra Of Selenosemicarbazones. Canadian Journal of Chemistry. 1965 Jun 1;43(6):1650-1655.
35. Al-Rubaie AZ, Al-Masoudi WA, Hameed AJ, Yousif LZ, Graia M. Synthesis, reaction and antiviral activity of 2, 4-diaryl-1, 3-selenazoles. Journal of the Korean Chemical Society. 2008;52(1):36-46.
36. Roeges NP, Baas JM. A guide to the complete interpretation of infrared spectra of organic structures. New York: Wiley; 1994.
37. Usova EB, Krapivin GD, Zavodnik VE, Kulnevich VG. Synthesis and Properties of 5-furyl(aryl)-Δ^2- 1,2,4 -triazolines and -Δ^2-I,3,4-thiadiazolines. Molecular and Crystal Structure of 2-Acetylamino-5-phenyl- Δ^2-1,3,4- thiadiazoline. Chemistry of Heterocyclic Compounds. 1994;30(10):1158-1164.
38. Ghasemian M, Kakanejadifard A, Azarbani F, Zabardasti A, Shirali S, Saki Z, Kakanejadifard S. The triazine-based azo-azomethine dyes; synthesis, characterization, spectroscopy, solvatochromism and biological properties of 2,2'-(((6-methoxy-1,3,5-triazine-2,4-diyl)bis(sulfanediyl)bis(2,1-phenylene)) bis(azanylylidene)bis(methanylylidene))bis(4-(phenyldiazenyl) phenol). Spectrochim. Acta A Mol. Biomol. Spectrosc., 2015;138; 643-647.
39. Enchev V, Angelova S. Ab initio study of the tautomerism of 2, 5-substituted diazoles. Structural Chemistry. 2010;21(5):1053-1060.
40. Al–Salami BK, Al–Khazragie ZK, Al-Fregi AA. Synthesis, Characterization, Antimicrobial Activity and Antioxidant of Azo Schiff Bases Containing Sulfanilamide 8. Journal of Global Pharma Technology. 2018;10(3):952-962.
41. Fatondji HR, Kpoviessi S, Gbaguidi F, Bero J, Hannaert V, Quetin-Leclercq J, Poupaert J, Moudachirou M, Accrombessi GC. Structure–activity relationship study of thiosemicarbazones on an African trypanosome: Trypanosoma brucei brucei. Medicinal Chemistry Research. 2013 May;22(5):2151-2162. DOI:10.1007/s00044-012-0208-6
42. Farshori NN, Banday MR, Ahmad A, Khan AU, Rauf A. Synthesis, characterization, and in vitro antimicrobial activities of 5-alkenyl/hydroxyalkenyl-2-phenylamine-1, 3, 4-oxadiazoles and thiadiazoles. Bioorganic & medicinal chemistry letters. 2010 Mar 15;20(6):1933-1938.
43. Sharp DJ, Rogers GC, Scholey JM. Microtubule motors in mitosis. Nature. 2000 Sep;407(6800):41-47. doi: 10.1038/35024000.
44. Rispin A, Farrar D, Margosches E, Gupta K, Stitzel K, Carr G, Greene M, Meyer W, McCall D. Alternative methods for the median lethal dose (LD50) test: the up-and-down procedure for acute oral toxicity. ILAR journal. 2002 Jan 1;43(4):233-243.
45. Al-Smadi LM, Mansour R, Mahasneh A, Khabour O.F., Masadeh MM, Alzoubi KH. Synthesis, Characterization, Antimicrobial Activity, and Genotoxicity Assessment of Two Heterocyclic Compounds Containing 1,2,3-Selena- or 1,2,3-Thiadiazole Rings. Molecules, 2019;24:1-11.
46. Piewngam P, Otto M. Probiotics to prevent Staphylococcus aureus disease. Gut Microbes. 2020;11(1):94-101.
47. Thomba DU, Mirgane SR, Ambhure RU, Pawar RP, Ameta KL. Synthesis and Antimicrobial Study of Novel Schiff Bases and Metal Complexes. Biochemistry and Biophysics (BAB), 2017;3.
48. El-Sherif AA, Eldebss TM. 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. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2011 Sep 1;79(5):1803-1814.
49. Hejchman E, Kruszewska H, Maciejewska D, Sowirka-Taciak B, Tomczyk M, Sztokfisz-Ignasiak A, Jankowski J, Młynarczuk-Biały I. Design, synthesis, and biological activity of Schiff bases bearing salicyl and 7-hydroxycoumarinyl moieties. Monatshefte für Chemie-Chemical Monthly. 2019 Feb;150(2):255-266.
50. Norrihan S, Md Abu A, Md Abdus S, Fasihuddin B A, Mohd Razip A. Synthesis, spectral characterization and biological activities of Organotin (IV) complexes with ortho-vanillin-2-hydrazinopyridine (VHP). Open Journal of Inorganic Chemistry. 2012 Apr 24;2012.
51. Bhattacharjee MK. Chemistry of Antibiotics and Related Drugs, S. Nature and company is Springer International Publishing AG Switzerland, Long Island University Brooklyn, NY, USA, 2016;63-64.
52. Tsemeugne J, Fondjo EF, Tamokou JD, Rohand T, Ngongang AD, Kuiate JR, Sondengam BL. Synthesis, Characterization, and Antimicrobial Activity of a Novel Trisazo Dye from 3-Amino-4H-thieno[3,4-c][1]benzopyran-4-one. Int. J. Med. Chem., 2018, 1-8 (2018).
53. Al-Fartosy AJM. Protective Effect of Galactomannan Extracted from Iraqi *Lycium Barbarum* L. Fruits Against Alloxan-Induced Diabetes in Rats. Amer Biochem Biotech, 2015;11(2):73-83.
54. Miladi S, Damak M. *n Vitro* Antioxidant A ctivities of Aloe vera Leaf Skin Extracts. Journal of Society of Chemstry, Tunisie, 2008;10:101-109.
55. Hasegawa M. A Facile One-Pot Synthesis of 4-Alkoxy-1,3-

benzenedicarbonitrile. Heterocycles, 1998;857-864. DOI: 10.3987/COM-97-S(N)89

56. Singh FV, Wirth T. Synthesis of Organoselenium Compounds with Potential Biological Activities, V. K. Jain and K. I. Priyadarsini, Tamil Nadu, India, 78 (2018).
57. Mohana KN, Kumar CB. Synthesis and antioxidant activity of 2-amino-5-methylthiazol derivatives containing 1, 3, 4-oxadiazole-2-thiol moiety. International Scholarly Research Notices. 2013;2013.
58. Shakir RM, Ali KF, Hussain DF. Synthesis and Antioxidant Ability of Some New 6-amino-7H-[1, 2, 4] triazolo [3, 4-b][1, 3, 4] thiadiazin-3-yl) Derivatives Bearing 2, 6-Dimethoxy-4-(methoxymethyl) Phenol Moiety. Orient. J. Chem. 2017 Oct 1;33(5):2492-2500.
59. Elsherbini M, Hamama WS, Zoorob HH, Bhowmick D, Mugesh G, Wirth T. Synthesis and antioxidant activities of novel chiral ebselen analogues. Heteroatom Chemistry. 2014 Nov;25(5):320-325.