Utility of 2, 4-Dihydroxybenzylidene Thiosemicarbazones in the Synthesis of Some Bioactive and Anti-Corrosive Heterocyclic Compounds

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Abstract:
The important organic compound 3-((2,4-dihydroxybenzylidene) amino)-2-thioximidazolidin-4-one (3) has been prepared by the reaction of 2,4- dihydroxybenzaldehyde with thiosemicarbazide to give 2,4 dihydroxybenzylidene thiosemicarbazone (1), followed by cyclization of compound 1 with ethyl chloroacetate in the presence of fused sodium acetate. The synthesized compound 3 was considered as the started material for synthesis some novel thiohydantoin derivatives (4-13). Also, 2, 4-dihydroxybenzylidene thiosemicarbazone(1) was used for the preparation of 2 and 5. All the synthesized compounds have been characterized on the bases of IR, 1H-NMR, 13C-NMR, mass spectrometer and elemental analyses. Some of 2-thiohydantoin derivatives (5, 6, 11) were tested for in-vitro antibacterial activity against Gram-positive bacteria (Staphylococcus aureus, Bacillus subtilis), Gram-negative bacteria (Salmonella typhimurium, Escherichia coli) and fungi (Candida albicans and Aspergillusfumigatus). This paper also interested with flame retardants and anticorrosive additives based on some previously prepared thiohydantoin derivatives 4- (2-(5-[(4-methoxyphenyl)thiazol-2-yl] hydrazono)methyl) benzene-1, 3-diol (5), 5-(2-chlorobenzylidene-3-(2, 4-dihydroxybenzylidene) amino)-2-thioximidazolidin-4-one (6), and 4-(2-chlorophenyl)-1-(2, 4-dihydroxybenzylidene)-6-oxo-2-thioxo-2,3,5,6- tetrahydro-1H-imidazo [4,5] pyridine-7-carbonitrile (11), which were physically added to epoxy organic coating. The flame retardants technique was observed via a limited oxygen index (LOI) method, and anticorrosive was evaluated with salt spray (or salt fog) test. The coating evaluation showed that, the incorporation of thiohydantoin derivatives into varnish improved the flame residency, corrosion resistance, and some physical properties of mild steel perversely coated with modified epoxy varnish.

Keywords: 2, 4-dihydroxybenzylidene thiosemicarbazone, Multi-component reactions, Anti-microbial and anti -corrosion Activity

1. Introduction
Thiohydantoins are sulfur analogs of hydantoins with one or both carbonyl groups replaced by thiocarbonyl groups [1]. Among the known thiohydantoins, 2-thiohydantoins are most notably known due to their wide applications as hypolipidemic [2], antitumorigenic [3], antimutagenic [4], antithyroidal [5] antiviral (e.g., against herpes simplex virus, HSV) [6], human immunodeficiency virus (HIV) [7] and tuberculosis [8], antimicrobial (antifungal and antibacterial) [9], anti-ulcer and anti-inflammatory agents [10], as well as pesticides [11]. In addition, 2-thiohydantoins are useful intermediates in the synthesis of natural products [12,13]. Also, 2-thiohydantoins have been used as reference standards for the development of C-terminal protein sequencing [14], as reagents for the development of dyes [15] and in textile printing, metal cation complexation and polymerization catalysis [16].

It has been found that some hydantoins derivatives have anti-corrosion inhibitors and flame retardant properties, whereas 2-thiohydantoin [17], 1-(2-ethylamino)-2-methylimidazoline (imidazoline), N-[3(2-amino-ethylamino-ethyl)]-acetamide (amide) and 1-(2-ethylamino)-2-methylimidazolidine (imidazolidine) were used as corrosion inhibitors for steel in a 0.5 M HCl solution [18].

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The inhibition of corrosion of iron in 2M nitric acid and 2M sulfuric acid solutions by substituted phenyl hydantoin as 1-phenylhydantoin (1), 3-hydroxymethyl-1-phenylhydantoin (2), 3-carboxy-1-phenylhydantoin (3), 5-benzylideneydantion (4), 5-methyl-2-thiodydantoin (5), 1,3-dimethyl-5-phenylazo-2-thiodydantoin (6), and 5,5-dimethyl-2,4-dithiodydantoin (7), compounds was measured using weight loss, thermometric, and polarization methods. The three methods gave consistent results. The efficiency of hydanto in derivatives inhibitors under investigation increases, according to 3 > 2 > 4 > 1 > 6 > 5 > 7. The corrosion inhibition may be due to the adsorption of the hydantoin molecules (with a high negative charge density at the hetero atom) on the metal surface (19).

Corrosion and fire have much serious economic, environmental, human health, technological and cultural safety consequences for different societies, so fighting and resisting of corrosion and fire have recently captured the high attention of scientists and scientific researches.

In our daily life some conventional materials such as metals, wood, glass, and ceramic have been progressively replaced with synthetic polymers, due to their versatility, good mechanical and physical properties, low density, and easy molding. Although these advantages of polymeric materials, there are serious drawbacks, such as poor fire resistance, but there are attempts to overcome this problem by inserting a fire retardant into the polymer backbone (20-22).

The flame retardants, which added to the polymer are one of the two types, reactive additives and normal additives: Reactive additives are those added to the polymer during polymerization or a post-processing step that chemically bond to the polymer chain. Either they copolymerize as a new polymer monomer or they graft onto the polymer via a post-polymerization reaction.

Normal additives are those which do not chemically bond to the polymer and are mixed into the polymer either during polymer formulation or during melt compounding of the thermoplastic polymer. Normal additives are part of polymer formulations and tend to be the most common type of flame-retardant additive.

All types of flame-retardant chemistries fall into one (or more) of three mechanisms of flame-retardant action (23).

1.1. Gas Phase Flame Retardants (Ex. Halogen, Phosphorus)

These materials reduce the heat released in the gas phase from combustion by scavenging reactive free radicals.

1.2. Endothermic Flame Retardants (Ex. Metal Hydroxides, Carbons)

These materials function in the gas phase and condensed phase by releasing non-flammable gases (H₂O, CO₂), which dilute the fuel and cool the polymer through endothermic decomposition of the flame-retardant additive. The lower polymer temperature slows the pyrolysis rate. These materials also leave behind a ceramic-like residue, which protects the underlying polymer.

1.3. Char-Forming Flame Retardants (Intumescents)

Exposure to heat initiates a series of chemical and physical processes, leading to a tunescent condition. This state is characterized by fire-resistant insulating foam. The foam serves to isolate heat and oxygen from the fuel source, extinguishing the fire.

Unfortunately, epoxy resins tend to burn easily with high smoke and gas release. Therefore, this paper is concerned with enhancing the fire resistance of epoxy resins by adding some thiohydantoin derivatives, which acting as normal flame retardants containing nitrogen, as well as corrosion inhibition.

2. Experimental

2.1. Instruments

All melting points were measured with a Gallenkamp melting point apparatus. IR spectra (KBr) were acquired with a Perkin–Elmer model 157 infrared spectrophotometer. ¹H-NMR spectra were recorded on a Bruker spectrophotometer at 400 MHz using tetramethylsilane (TMS) as internal standard. ¹³C-NMR spectra were recorded on the same spectrometer at 100 MHz in DMSO-d₆ as solvent. Electron Ionization (EI)-MS were measured at a ShimadzuGC-MS-QP-1000 EX mass spectrometer instrument operating at 70 ev.

2.2. Synthesis

2.2.1. Synthesis of 2, 4 dihydroxybenzylidenethiosemicarbazones (1)

A mixture of 2,4-dihydroxybenzaldehyde (0.01 mol) and thiosemicarbazide (0.01 mol) in ethanol (30 mL) was heated under reflux for 4 h, then cooled. The solid formed was filtered off, dried and purified by recrystallization from ethanol to give compound 1 as pale yellow crystals, m.p. 200-202°C, yield 78%, FT-IR (KBr cm⁻¹), 3374(OH), 3270-3125(NH₂), 3233(NH), 1631 (C=O), 1353 (C=S). ¹H NMR (δ ppm) (DMSO-d₆) : δ = 6.11.06 -9.74 (s, 2H, 2xOH), 9.75 (s, 1H, NH), 8.3 (s, 1H, CH=N), 7.71 (s, 2H, NH₂) and 6.24 -7.66 (m, 3H, Ar-H) ppm; Anal Calc for C₅H₅N₂O₃S (M. wt. = 211): C, 45.49; H, 4.26; N, 19.90; S, 15.16; Found: C, 45.46; H, 4.23; N, 19.87; S, 15.13.

2.2.2. Synthesis of N, N-1-triacetoxy-2-(2,4-dihydroxybenzylidene) hydrazinecarbothioamide (2)

A mixture of 1 (0.01 mol) and acetic anhydride (15 mL) was heated under reflux for 3 h, then cooled and poured into ice-diluted hydrochloric acid. The solid obtained was filtered off, washed with water, dried and purified by recrystallization with ethanol to give compound 2 as pale brown crystals, m.p. 127-129°C, yield 50%, FT-IR (KBr cm⁻¹),
2.2.3. Synthesis of 3-[(2, 4-dihydroxybenzylidene) amino]-2-thioximidazolidin-4-one (3)

A mixture of 1 (0.01 mol) and ethyl chlorocacetate (0.01 mol) in the presence of fused sodium acetate in 30 mL was heated under reflux for 6 h, and then cooled. The solid formed was filtered off, dried and purified by recrystallization from ethanol to give 3 as pale yellow crystals, m.p. 260-262°C, yield 70%; FT-IR (KBr cm⁻¹): vmax: 3360(OH), 3244(NH),1697(C=O),1627.9(C=N),1381(C=S). ¹H NMR (δppm) (DMSO-d6): δ 10.88-11.61 (s, 2H, 2×OH), 8.30(s, 1H, CH=N), 6.21-7.53(m, 3H, H-Aromatic ring), 2.18-2.48(s, 9H, 3×COCH₃), ppm; Anal Calcld for C₁₅H₁₃N₅O₆S (M. wt = 385): C, 43.64; H, 3.89; N, 10.90; S, 8.32; Found: C, 43.61; H, 3.86 N, 10.88; S, 8.30.

2.2.4. Synthesis of 4-((3- acetyl-5-oxo-2-thioximidazolidin-1-yl) imino) methyl)-1, 3-phenylene diacetate (4)

A mixture of 3 (0.01 mol) and acetic anhydride (15 mL) was heated under reflux for 3 h, then cooled and poured into ice-diluted hydrochloric acid. The solid obtained was filtered off, washed with water, dried and purified by recrystallization with ethanol to give 4. C₁₇H₁₅N₅O₆S (M. wt. 377), pale brown powder, m.p. 178-180°C, yield 50%; FT-IR (KBr cm⁻¹), umax: 1723(C=O),1649 (C=N), 1367(C=S); MS (m/z %): 377(M⁺, 22) and 251.9, 35); Anal Calcld: C, 50.93; H, 3.98; N, 11.14 ; S, 8.49; Found: C, 50.90; H, 3.95; N, 11.11; S, 8.46.

2.2.5. Synthesis of 4-((2-5-(4-methoxyphenyl) thiazol-2-yl hydrazono) methyl) benzene-1, 3-diol (5)

A mixture of 1 (0.01 mol) and 9-bromomethyl aryl ketones such as (4-methoxy phenacyl bromide) (0.01 mol) in the presence of fused sodium acetate in 30 mL was heated under reflux for 6 h, then cooled and poured into water. The solid formed was filtered off, dried and purified by recrystallization from ethanol to give 5. C₁₇H₁₃N₅O₆S (M. wt. 341), brown color, m.p. 190-192°C, yield 50%; FT-IR (KBr cm⁻¹), umax:3444-3476(OH), 3298 (NH), 1623 (C=N), 1501 (C=C). ¹H NMR (δppm) (DMSO-d6): 6.10.02-11.10 (s,2H,OH), 9.67 (s,1H,NH), 8.22(s,1H, CH=NH),6.23-7.97(m,8H, Ar-H and proton thiazole),3.9( OCH₃) ppm. ¹³C- NMR spectrum (100 MHz, DMSO-d₆, δ ppm) 177(C=S), 168.08 (C=O), 158.42(C=O), 141.15(CH₃); 133.37,130.38, 128.7, 111, 112.7, 108.17,102.7, (phenyl ring), 57.19(OCH₃); MS :m/z 342.2 (14%) of C₁₇H₁₃N₅O₆S and m/z at 325(12%); Anal Calcld: C, 59.82; H, 4.40; N, 12.31; S, 9.38; Found: C, 59.80; H, 4.37, N, 12.28;S, 9.35.

2.2.6. Synthesis of 5-(2-chlorobenzylidene)-3-((2, 4-dihydroxybenzylidene) amino)-2-thioximidazolidin-4-one (6)

A mixture of 3 (0.01 mol) and o-chlorobenzaldehyde (0.01 mol) in ethanolic solution of NaOH (0.5 g in 30 mL ethanol) was heated under reflux for 8 h, cooled to room-tem, poured onto iced cold water and drops of 10% HCl. The solid obtained was filtered off, washed well with hot water, dried and recrystallized from ethanol to give 6. C₁₇H₁₃ClN₅O₆S (M. wt. 373.5), reddish brown color, m.p. 160-162°C, yield 80%; FT-IR (KBr cm⁻¹), umax : 3444-3437(brOH), 3260(NH), 1705-1695(C=O), 1633 (C=N), 1511 (C=C), 1381(C=S). ¹H NMR (δppm) (DMSO-d6): δ 10.01-10.66(s, 2H, OH), 10.40(s,1, 1NH), 8.58(s, 1H, CH=N),6.33-7.54(m, 8H, Ar-H and olefinic proton) ppm. ¹³C- NMR spectrum (100 MHz, DMSO-d₆, δ ppm) 177(C=S), 160 (C=O), 157.02(C=O), 143.3(NH), 133.8, 129.9, 126.7, 112.5,102-107 (phenyl ring); Anal Calcld: C, 54.62; H, 3.21; Cl, 9.50; N, 11.24; S, 8.57; Found: C, 54.60; H, 3.18; Cl, 9.47; N, 11.21; S, 8.54.

2.2.7. Synthesis of 4-((3-acetyl-4-((2-chlorobenzylidene) -5- oxo-2-thioximidazolidin-1-yl) imino) methyl)-3- hydroxyphenylacetate (7)

A mixture of 6 (0.01 mol) and acetic anhydride (15 mL) was heated under reflux for 3 h, then cooled and poured into ice-diluted hydrochloric acid. The solid obtained was filtered off, washed with water, dried and purified by recrystallization with ethanol to give 7. C₁₇H₁₃ClN₅O₆S (M. wt. 457.5), pale brown color, m.p. 140-142°C, yield 50%; FT-IR (KBr cm⁻¹), umax : 3353 (OH), 1717(C=O), 1651 (C=N), 1368(C=S); MS: m/z 457 (12%) of C₁₃H₁₃ClN₅O₆S and m/z at m/z 408.9(8%); Anal Calcld: C, 55.08; H, 3.50;Cl, 7.76; N, 9.18; S, 6.99; Found: C, 55.05; H, 3.49; Cl, 7.71; N, 9.15; S, 6.96.

2.2.8. Synthesis of 3-(2- chlorophenyl)-6- ((2, 4 -dihydroxybenzylidene) amino) 2, 3a, 4, 6-tetrahydroimidazo[4,5c] pyrazole-5(3H) thione (8)

A mixture of 6 (0.01 mol), and hydrazine hydrate (0.01mol) were added in ethanol (30 mL) was heated under reflux for 8 h, then cooled. The solvent was evaporated to dryness and theresidue was recrystallized from ethanol to give 8. C₁₇H₁₃ClN₅O₆S (M. wt. 387.5), reddish brown color, m.p. 110-112 °C and yield 60%; FT-IR (KBr cm⁻¹), umax : 3380-3310 (OH),3270(NH),1625 (C=N), 1357(C=S) ; Anal Calcld: C, 52.65; H, 3.61; Cl, 9.16; N, 18.06; S, 8.26; Found: C, 52.62; H, 3.58; Cl, 9.13; N, 18.03; S, 8.23.

2.2.9. Synthesis of 10-(2-chlorobenzylidene)-3-((2,4-dihydroxybenzylidene) amino)-3,9,10,10α-tetrahydrobenzo[b]imidazo[4,5-e][1,4] diazepine-2(1H)-thione (9)

A mixture of 6 (0.01 mol) with 1,2-diaminobenzene (0.01 mol) in sodium ethoxide (0.5 gm in 30 mL ethanol). The reaction mixture was refluxed for 8 h, then cooled and poured into ice cold water, drop 10% HCl. The solid obtained was filtered off, washed with hot water, dried and purified by recrystallization with ethanol to give 9. C₁₇H₁₃ClN₅O₆S (M. wt. 463.5), brown color, m.p. 138-140°C, yield 75%; FT-IR (KBr cm⁻¹), umax:3437 (OH), 3210(NH), 1627 (C=N), 1316(C=S). ¹H NMR (δppm) (DMSO-d6): δ 12.85-12.95(s,2H, 2×OH),9.68(S,1H, NH),8.3(s,1H, CH=N),7.36-7.61(m,7H, Ar-H) and 6.92(s,1H,
NH-diazoepine) ppm. $^{13}$C-NMR spectrum (100 MHz, DMSO-d$_6$, $\delta$, ppm)181(C=S), 143.3(CH=N), 134, 143.5, 103-108, 121.9, 128, 114.8, 126.5, 134, 143.5, 128, 126, 128.1, 128.6, 132(two benzene ring), 58.1, 52.6(diazoepine). Anal Calcd: C, 59.55; H, 3.88; Cl, 7.66; N, 15.10; S, 6.90; Found: C, 59.52; H, 3.85; Cl, 7.63; N, 15.07; S, 6.87.

2.2.10. 4-(((1,9-diacetyl-10- (2-chlorophenyl)-2-thiaoxy-1,9,10,10a-tetrahydrobenzo[b]imidazo[4,5-e] [1,4] diazepin-3(2H)-y1) imino)-3)-hydroxyacetophenone (10)

A mixture of 9 (0.01 mol) and acetic anhydride (25 mL) was heated under reflux for 3 h, then cooled and poured into ice-diluted hydrochloric acid. The solid obtained was filtered off, washed with water, dried and purified by recrystallization with ethanol to give 10. C$_{29}$H$_{28}$N$_3$O$_4$S (M.wt.589.5), dark brown color, m.p.108 - 110 ºC, yield 60%; FT-IR (KBr cm$^{-1}$), umax : 3437(OH), 1623 (C=N), 1312 (C=S). $^{1}$H NMR ($\delta$ppm) (DMSOd$_6$): $\delta$ 12.86(s,1H, OH).8.3(s,1H, CH=N).7.36- 7.61(m,7H, Ar-H), 2.20-2.50 (s, 9H,CH$_3$) ppm; MS: m/z 592 (M+2, 9.04%); Anal Calcd: C, 59.03; H, 4.07; Cl, 7.02; N, 11.87; S, 5.43; Found: C, 59.00; H, 4.04; Cl, 6.90; N, 11.84; S, 5.40.

2.2.11. Synthesis of 4 - (2-chlorophenyl)-1- (2,4-dihydroxynbenzylidene)-6- oxo-2-thiaoxy-2,3,5,6- tetrahydro-1H-imidazo [4,5c] pyridine -7-carbonitrile (11)

A mixture of chalcone of thiohydantion(0.01 mol; 2.18gm), (0.02 mol; 2.66gm) ethylcyanoacetate and ammonium acetate (0.01mol; 0.77gm) in ethanol (30mL) was heated under reflux for 12 h. Cooled and poured into ice cold water, drop 10%HCl. The solid obtained was filtered off, washed with hot water, dried and purified by recrystallization with ethanol to give 11. C$_{24}$H$_{24}$N$_3$O$_2$S (M.wt. 437.5), brown color, m.p. 188 -190 ºC and yield 65%; FT-IR (KBr cm$^{-1}$), umax : 3363 (OH), 3317(NH), 2260 (C=O,N), 1630(C=N), 1625 (C=N), 1537(C=S). $^{1}$H NMR ($\delta$ppm) (DMSOd$_6$): 8.11.36-10.10(s,2H, 2×OH),11.19-11.10(s,2H,1NH),8.46(s,1H, CH=N),6.31-7.37 (m,3H, Ar-H) ppm. Anal Calcd: C, 54.86; H, 2.74; Cl, 8.11; N, 16.00; S, 7.31; Found: C, 54.83; H, 2.70; Cl, 8.08; N, 15.97; S, 7.28.

2.2.12. Synthesis of 3 - (chlorophenyl)-1- (2,4-dihydroxynbenzylidene)-2,6- dithiaoxy-2,3,5,6- tetrahydro-1H-imidazo [4,5c] pyridine -7-carbonitrile (12)

A mixture of 11 (0.01mol), and phosphorouspentasulphide (0.01 mol) in the presence of catalytic drop off (pyridine) were heated under reflux for 6 h. Cooled and poured into ice cold water, drop 10% HCl. The solid obtained was filtered off, washed with hot water, dried and purified by recrystallization with toluene to give 12. C$_{26}$H$_{26}$N$_3$O$_2$S$_2$ (M W453.5), brown color, m.p. 300 -302°C and yield 60%; FT-IR (KBr cm$^{-1}$), umax : 3433(OH),3270(NH),1625(C=N), 1304(C=S). Anal Calcd: C, 52.97; H, 2.65; Cl, 7.83; N, 15.43; S, 14.11; S, 14.11. Found: C, 52.94; H, 2.63; Cl, 7.80; N, 15.40; S, 14.08.

2.2.13. Synthesis of 4- (2-chlorophenyl)-1-2,4-dihydroxy benzylidine)- amino)-3,6-dihydroimidazo[4,5-d] pyrazolo[3,4-b] pyridine -2H-thione (13)

A mixture of 12 (0.01mol), and hydrazine hydrate (0.01mol) was added in ethanol (30 mL) was heated under reflux for 8 h, and then cooled. The solvent was evaporated to dryness and the residue was recrystallized from ethanol to give 13. C$_{29}$H$_{28}$N$_4$O$_2$S (M 451.5), pale brown color, m.p. 310-312°C and yield 60%; FT-IR (KBr cm$^{-1}$), umax : 3464 (OH), 3336-3237(NH$_2$),3181(NH),1282 (pyrazole ring).$^{1}$H NMR ($\delta$ppm) (DMSOd$_6$): 6.18.80-10.6(s,2H, 2×OH),10.80-9.5(s,2H, NH),8.3(s,1H, CH=N),7.08-7.34(m,3H, Ar-H) and 4.5(s,2H, NH$_2$) ppm. Anal Calcd: C, 53.16; H, 3.10; Cl, 7.86; N, 21.70; S, 7.08; Found: C, 53.13; H, 3.07; Cl, 7.83; N, 21.67; S, 7.05.

2.3. Coating Composition and Film Preparation

The coating compositions were prepared by means of incorporating thiodyantion derivatives (5, 6, and11), in the ratio of 1.0, 2.0 and 3 %, into epoxy varnish. The coating compositions were applied to steel and wood panels by means of a brush or by spraying. All efforts were made to maintain a uniform film thickness of 50 +/- 5μm for evaluating the properties of modified films.

2.3.1. Drying Time

To determine Set-To-Touch Time-lightly touch the test film at a point not less than 15 mm from the film edges with the tip of a clean finger and immediately places the fingertip against a piece of clean glass. Observe if any of the coating transferred to the glass. The film considered to set to-touch when it still shows a tacky condition, but none of it adheres to the finger (Surface dry).

2.3.2. Hard Dry Time

With the end of the thumb resting on the test film and the forefinger supporting the test panel, exert a maximum downward pressure (without twisting) of the thumb on the film. Lightly polish the contacted area with a soft cloth. The film considered dry-hard when any mark left by the thumb completely removed by the polishing operation (24).

2.4. Anti-Corrosion Testing

The test pieces were 20 x 20 x 3 mm. The paint formulations were evaluated on coated mild steel panels. To ensure that the steel panels were free of all surface contamination before the coating application, mechanically pre-treatment, involving polishing with wire brushing and sand paper for cleaning of the surface to obtain a smooth surface, followed by degreasing with acetone and then rinsed with distilled water, dried between two filter papers. A salt fog chamber was utilized for each of the resin formulations; with a set of three coated panels being placed in the salt spray chamber.
according to ASTM (25). The corrosion resistance was evaluated in terms of blistering, scribe failure and a degree of rusting, in relation to ASTM standards (26–28).

2.5. Flame Retardant Testing Method

The performance of epoxy varnish, with incorporated thiohydantoin derivatives (5, 6 and 11) additives, was evaluated in a limited oxygen index (LOI) chamber. LOI values were determined by standardized test methods (29). The test panels were prepared using a combustible material (wood specimen). It was important that the panels were free of any surface contamination, or imperfections, prior to the coating application. Hand tool cleaning (sand paper) was carefully used to treat the faces and edges of the panels. Final dry film thickness (DFT) was 50 +/- 5µm. In all cases, the film application was applied by means of brushing. Following 10 days of air drying, the panels under study were heated at 50-60°C for 2 h. to eliminate any remaining solvent.

2.6. Screening Antimicrobial Activity

Preliminary screening for the antimicrobial activity of synthesized compounds using standardized disc-agar diffusion method (30) against Staphylococcus aureus, Bacillus subtilis (Gram positive bacteria), and Salmonella, Escherichia coli (Gram-negative bacteria), and Candida albicans (yeast) Aspergillus fumigatus (fungi) compared with standard antimicrobial agents. The diameters of zone of inhibition were measured and compared with that of the standard, and the values were tabulated. Chloramphenicol was used as standard for the reference in the case of Gram – positive bacteria, Cephalothin was used as a standard reference in the case of Gram – negative bacteria and cycloheximide was used as the standard reference in the case of yeasts and fungi) and the observed zone of inhibition is presented in Table 1.

An antibiotic is a growing problem, some of this is due to the overuse of antibiotics in human, but some of it is probably due to the use of antibiotics as growth promoters in food of animals (31). So, there is growing demand for new antibiotics. Thiohydantoin derivatives were reported to exhibit interesting antimicrobial activity (32).

3. Results and Discussion

3.1. Chemistry

The synthetic pathways of preparation of novel heterocyclic compounds containing hetero nitrogen and sulfur atoms are shown in (scheme 1). In the present work, the starting 2,4-dihydroxybenzylidenedithiosemicarbazones (1) was prepared from 2,4-dihydroxy benzaldehyde and thiosemicarbazide in ethanol afforded (1). The structure 1 was confirmed by elemental analysis and spectral data.

Treatment of 2,4-dihydroxybenzylidene dithiosemicarbazones (1) with acetic anhydride under reflux yielded the corresponding N,N,1-triacetoxy-2-(2,4-dihydroxybenzylidene)hydrazine carbethoamide (2). The structure of the compound was confirmed on the basis of their elemental analysis and spectral date such as IR and NMR spectra. The IR and NMR of the compound showed disappearance of NH and CH₂ groups and revealed absorption bands characteristic for C=O and CH₃ groups in IR spectrum and nine protons of 3×COCH₃ at δ = 2.48 ppm in ¹HNMR spectrum.

The reaction of 2,4-dihydroxybenzylidene dithiosemicarbazones (1) with ethyl chloroacetate and ethyl chloroacetate in ethanol (33) containing fused sodium acetate to afford the corresponding 3-{(2,4-dihydroxybenzylidene)amino}-2-thioxoimidazolidin-4-one (3). The structure (3) was elucidated by spectral data and elemental analysis (cf. Exp). The IR spectrum which showed absorption bands at 3360, 3244 cm⁻¹, attributable to νOH, νNH, and the two protons attributable NH, CH=N revealed signals in ¹HNMR spectrum (cf. Exp). Acetylation of compound 3 with acetic anhydride under reflux gave 4-{[(3-acetyl-5-oxo-2-thioxoimidazolidin-1-yl)methy]-1,3-phenylene diacetate (4). The infrared, which showed absorption bands of NH and 2×OH while in ¹HNMR which showed absence absorption bands of NH and two OH groups.

On the other hand, 2, 4-dihydroxybenzylidene dithiosemicarbazones (1) was allowed to react with 9-bromomethylaryl ketone such as (4-methoxy phenyl bromide) in ethanol containing fused sodium acetate afforded the corresponding 4-{[(2-(5-(4-methoxy phenyl)thiazol-2-yl)hydrazono)methyl]benzene-1,3-diol (5). The confirmed structure of compound 5 by elemental analysis and spectral data. The infrared spectra which showed absorption bands at 3476, 3298 cm⁻¹, attributable to νOH, νNH. The ¹HNMR and ¹³C NMR spectra of compound 5 revealed signals at δ=9.67, 3.80 ppm (NH and OCH₃) and δ=141.15, 57.19 for (CH=N and OCH₃).
On the other hand In (scheme2) condensation of 3-((2,4-dihydroxybenzylidene)amino)-2-thioximidazolidin-4-one (3) with aromatic aldehydes such as (2-chlorobenzaldehyde) ethanolic sodium hydroxide solution to formation 5-(2-chlorobenzylidene)-3-(2,4-dihydroxybenzylidene) amino)-2-thioximidazolidin-4-one (6). The structure of 6 was confirmed by analytical and spectral data. The IR spectrum showed absorption bands at 3444, 3260 attributable to (υOH) and (υNH). 1HNMR spectrum of 6 showed signals at δ=6.70, 8.58 ppm due to present of one proton of (C=CH, olefinic) and one proton of (CH=N). The 13C-NMR spectrum of compound 6 revealed the signals characteristic for (C=CH, olefinic) at δ = 120.2, whereas, the (CH=N) at δ = 143.3 ppm.

The structure of 6 was established chemically via reaction with acetic anhydride under reflux to produce 4-((3-acetyl-4-(2-chlorobenzylidene)-5-oxo-2-thioximidazolidin-1-yl)methyl)-3-hydroxyphenyl acetate (7). The IR spectra of compound 7 which showed absence bands of NH and one OH group. Also, MS of compound 7 gave fragments showing the isotopic pattern due to the presence of chlorine atom and appear molecular ion peak like molecular weight (cf. Exp).

Treatment of 6 with hydrazine hydrate in ethanol to give 3-(2-chlorophenyl)-6-((2,4-dihydroxybenzylidene)amino)2,3a,4,6-tetrahydroimidazo[4,5c]pyrazole-5-(3H) thione (8). The IR spectrum which lack absorption band of C=O group.

Also 10-(2-chlorophenyl)-3-(2,4-dihydroxybenzylidene)amino)-3,9, 10, 10a-tetrahydrobenzo[b]imidazo[4,5-e][1,4]diazepin-2-(1H)-thione (9) were prepared from the condensation of chalcone derivative 6 with O-phenylenediamine in sodium ethoxide (NaOH/EOH).

The structure of 9 was confirmed by analytical and spectral data. IR spectrum showed disappearance of C=O of chalcone and appearance of NH bands of diazepine. 1HNMR spectra showed signal at 6.92 ppm attributable to one singlet proton for NH of diazepine. The 13C-NMR spectra revealed the signets.

The structure of compound 9 was established chemically via the reaction with acetic anhydride under boiling to give 4-(((1,9-diacetyl-10-(2-chlorophenyl)-2-thioxo-1,9,10,10a-tetrahydrobenzo[b]imidazo[4,5-e][1,4]diazepin-3(2H)-yl)methyl)-3-hydroxyphenyl acetate (10). IR spectrum which of absorption bands of only one OH and disappeared of two NH and other OH groups. Its 1HNMR spectrum showed lack signals of three protons corresponding to (two NH and one OH). The mass spectrum showed m/z 592(22%) promoting its molecular formula (cf. Exp).
On other hand the reaction of 3-[(2,4-dihydroxybenzylidene)amine]-4-oxo-imidazolidin-2-thiones (6) with ethyl cyanoacetate in the presence of ammonium acetate in ethanol under reflux afforded the corresponding 4-(2-chlorophenyl)-1-(2,4-dihydroxybenzylidene)-6-oxo-2-thioxo-2,3,5,6-tetrahydro-1H-imidazo [4,5c]pyridine-7-carbonitrile (11) in scheme 3.

The structure 11 was elucidated on the basis of spectral data and element analysis. The IR spectrum show appearance of absorption band at 2260 cm⁻¹ attributable to $\nu C\equiv N$. HNMR spectrum, which lack of signal of C=CH group and revealed signal at $\delta=11.19$-11.10 ppm attributable of NH of the pyridine ring.

Reaction of compound 11 with phosphorous pentasulfide in dry pyridine (36) to afford 3-(chlorophenyl)-1-(2,4-dihydroxybenzylidene)-2,6-dithioxo-2,3,5,6-tetrahydro-1H-imidazo [4,5c]pyridine-7-carbonitrile (12). The structure of 12 was confirmed by element analysis and spectral data. In the IR spectrum, which disappearance of C=O which replaced by C=S group at 1304 cm⁻¹. The synthesis of 4-(2-chlorophenyl)-1-(2,4-dihydroxybenzylidene) amino-3,6-dihydroimidazo [4,5-d] pyrazolo[3,4-b]pyridine-2H-hione (13) via the reaction of a compound 12 with hydrazine hydrate in ethanol (scheme 3).

The compound 13 was established by spectral data. The infrared spectrum of 13 was identified by absence of $\nu C\equiv N$ of two groups and which revealed an absorption band in 1282 cm⁻¹ of pyrazole ring and absorption band 3336-3237 cm⁻¹ (NH₂). The ¹H NMR spectrum, which showed of the signal of two protons for NH₂ $\delta=4.5$ ppm signal of one proton for NH-pyrazole ring $\delta=10.8$ ppm.

### 3.2. Screening Antimicrobial Activity

The results of this study showed that the compound (5, 6 and 11) was the high potent thiohydantoin derivative against Staphylococcus aureus, Bacillus subtilis (Gram positive bacteria), more than Salmonella, Escherichia coli (gram-negative bacteria). (5, 6 and 11) compounds synthesized have high potentials against Candida albicans (yeast) both (6 and 11) not have any effect at Aspergillusfumigatus (fungi), but compound (5) has low effective at Aspergillusfumigatus (fungi).
Table 1: Anti-Microbial Activity of Thiohydantoin Derivatives

| Organism                        | Gram - positive bacteria | Gram - negative bacteria | Yeasts and Fungi** |
|---------------------------------|--------------------------|--------------------------|--------------------|
|                                 | Staphylococcus aureus (ATCC 25923) | Bacillus subtilis (ATCC 6635) | Salmonella typhimurium (ATCC 14028) | Escherichia coli (ATCC 25922) | Candida albicans (ATCC 10231) | Aspergillus fumigatus |
| Conc. sample                    |                          |                          |                    |                                |                               |                   |
|                                  | 1 mg/ml                  | 0.5 mg/ml                | 1 mg/ml            | 0.5 mg/ml                      | 1 mg/ml                       | 0.5 mg/ml         |
| 5                                | 19I                       | 16I                      | 15I                | 13I                            | 7L                            | 6L                 |
| 6                                | 13I                       | 10I                      | 18I                | 16I                            | 9L                            | 8L                 |
| 11                               | 14I                       | 11I                      | 18I                | 14I                            | 11L                           | 10L                |
| Control                          | 35                        | 26                       | 35                 | 25                             | 36                            | 28                 |

The antimicrobial screening data show that the compounds exhibit antimicrobial properties. The increased activity of the novel thiohydantoin can be explained by the hydroxyl group, which acts as more powerful and potent bactericidal agents.

The structural feature may increase the activity of the tested compound since possess free Cl and hydroxyl groups. π-electron delocalization over the thiohydantoin increases the lipophilic character and favors its permeation through the lipid layer of the bacterial membranes. It was concluded that thiohydantoin acting as electrophilic agents have a positive effect and inhibited bacterial growth.

3.3. Coating Evaluation

After the successful preparation of the Thiohydantoin compounds, some of these compounds (5, 6 and 11) were selected, and physically inserted into a coating formulation as modifiers, after that, the influence of these modifications on some properties of epoxy coating such as the drying time of film formation, as well as the possibility of using these derivatives as anti-corrosive and as flame retardants, were studied. These results are tabulated in table (2) and represented in the figure (1) for drying time, table (3) and figures (2-5) for anti-corrosion, and table (4) and figure (7) for flame retardant.

3.4. Drying Time

From the previous table 2and Figure 7 it is clear that insert thiohydantoin modifiers leads to shortening in both surface and hardener drying times. As the increasing percentage of modifier, the drying times decrease.

Table 2: Surface Dry and Hardener Dry of Epoxy Modifier with Thiohydantoin Derivatives at 25ºC
3.5. Corrosion Resistance

The modified epoxy paint compositions were prepared by means of incorporating thiohydantoin derivatives (5, 6, and 11) by the ratio of 1.0, 2.0 and 3.0% by weight into epoxy paint and adjusted film thickness about 50 ± 5µm were evaluated as the anti-corrosive. After preparing the coated test panels according to experimental section, they were exposed to salt spray tests (salt fog) according to ASTM for 500 h. The blistering size is graded from 10 to 0, where 10 representative of no blistering and 0 representative of the largest blister. Blistering frequency is denoted by F, M, MD and D (few, medium, medium dense and dense). Painted, or coated, specimens subjected to a corrosive environment are also evaluated by recording the average maximum and minimum creep age from the scribe mark. Scribe failure is also rated on a scale from 10 to 0, with 10 being (zero mm) from the scribe mark and 0 is (16 mm) from the scribe mark. Finally, the comparison of the surface appearance, to determine the percentage of the area that has been rusted, is also used. The rust grade is rated on a scale from 10 to 0, where 10 is non-rusting and 0 is severe rusting. The corrosion resistance of the painted films of modified sample is given in Table (3) and Fig (2,3,4, ad5); explains the photo of the painted films after salt test spray (5% of NaCl).

| Sample          | Modifier | Blistering Size | Blistering Frequency | Scribe Failure (mm) | Rust Grade |
|-----------------|----------|-----------------|----------------------|---------------------|------------|
| Blank           | 0        | 2               | M                    | 13                  | 2          |
| Epoxy modified  | 1.00     | 3               | M                    | 6                   | 4          |
| with 5          | 2.00     | 4               | M                    | 3                   | 6          |
|                 | 3.00     | 7               | MD                   | 2                   | 6          |
| Epoxy modified  | 1.00     | 5               | MD                   | 10                  | 3          |
| with 6          | 2.00     | 6               | Few                  | 6                   | 7          |
|                 | 3.00     | 8               | M                    | 4                   | 8          |
| Epoxy modified  | 1.00     | 4               | Few                  | 10                  | 6          |
| with 11         | 2.00     | 6               | M                    | 7                   | 8          |
|                 | 3.00     | 6               | MD                   | 6                   | 8          |

Table 3: Evaluation the Corrosion Resistance of the Painted Films

![Figure 4: Plot of Effect Modification and Concentration on Surface Dry & Hard Dry- of Modified Epoxy.](image1)

![Figure 5: Plot of Effect Modification and Concentration on Blistering of Modified Epoxy](image2)
Concerning with the blistering test the dates given in tables (3) show that all modifiers improve the blistering and as the concentration of modifier increase blistering resistance increase. Particularly modified varnish by modifier 5-(2-chlorobenzylidene)-3-(2,4-dihydroxybenzylidene) amino)-2-thioximidazolidin-4-one (6). About the Scribe failure test from the data given in tables (3); it is clear that the adding of modifiers improves the scribe value. It is clear that the compounds4-((2-(5-(4-methoxyphenyl) thiazol-2-yl) hydrazono )methyl) benzene-1,3-diol (5) give the best results which shifts from 13 mm within epoxy varnish along to (2 mm) within epoxy varnish modified 3%. The order of inhibition can be arranged as follows: 5>6>11.

Finally rust grade of epoxy modified films the adding of modifiers in all percentage improves the rust grade inhibition, especially the modifier 4- (2-chlorophenyl)-1- (2,4- dihydroxybenzylidene )-6-oxo-2-thioxo-2,3,5,6- tetrahydro-1H-imidazo [4,5c]pyridine-7-carbonitrile (11). From the previous study it is clear that there are satisfactory differences between the blank and modified samples, the thiohydantoin derivatives (5, 6 and 11) are considered a corrosion inhibitor for mild steel when added to epoxy coating. The improvement of corrosion resistance may be attributed to the introducing of a compound that containing nitrogen, sulfur, and oxygen atoms and aromatic rings into its structure. Corrosion inhibition is caused by the adsorption of thiohydantoin molecules onto the surface of the mild steel as shown in Figure (6).
3.5.1. Evaluation of Thiohydantoin Derivatives as a Flame Retardant

Flame retardancy are materials that are conducted to provide fire protection for flammable consumer goods, as well as to mitigate fire growth in a wide range of fires. This paper is in the field of applied science that is concerned with the study of flame retardants.

The LOI is defined as the minimum concentration of oxygen, expressed as a percentage that will support combustion of a polymer. It is measured by passing a mixture of oxygen and nitrogen over a burning specimen, and reducing the oxygen level until a critical level is reached. The LOI is expressed as:

$$\text{LOI} = 100 \left( \frac{[O_2]}{[O_2] - [N_2]} \right)$$

This method proved suitable as a semi-quantitative indicator of the effectiveness of the flame retardant during the research and development phase of the work. This was due to the fact that the equipment is relatively inexpensive and the size of the test sample that is required is reasonably small. It is clear that the incorporation of thiohydantoin derivatives into epoxy paint, in the ratio’s mentioned in the experimental section, results in enhanced flame retardancy when compared with an epoxy control sample. The results obtained from the LOI test are shown in table (4) and figure (7), which proved that increasing the percentage of modifier of thiohydantoin derivative compound (5, 6, and 11) lead to increasing limiting oxygen index (LOI) (11 > 5 > 6).

The improvement of LOI value may be attributed to the introducing of a compound that containing nitrogen, sulfur, oxygen, and chlorine atoms and aromatic rings within their structure.

| Sample                  | Modifier % | Limiting Oxygen Index (LOI) |
|-------------------------|------------|----------------------------|
| Blank                   | 0.00%      | 20                         |
| Epoxy modified with 5   | 1.00%      | 30                         |
|                         | 2.00%      | 38                         |
|                         | 3.00%      | 40                         |
| Epoxy modified with 6   | 1.00%      | 26                         |
|                         | 2.00%      | 32                         |
|                         | 3.00%      | 34                         |
| Epoxy modified with 11  | 1.00%      | 33                         |
|                         | 2.00%      | 40                         |
|                         | 3.00%      | 45                         |

Table 4: Limiting Oxygen Index (LOI) of Modified Epoxy Thiohydantoin

Figure 8: The Photo of the Painted Samples after Exposed to Salt Spray Test (5%NaCl)

Figure 9: Adsorption of Thiohydantoin on Mild Steel Surface
Figure 10: Limiting Oxygen Index (LOI) – Flame Retardant Additive Curve in Absence and Presence of Different Concentration of Modified Epoxy

4. Conclusion

In the present work, a series of some new heterocyclic compounds (1-13) containing thiohydantoin moiety were synthesized, the structures of the new compounds were confirmed by suitable physical, chemical methods and were tested for their antimicrobial activities. Compounds (5,6, and 11) where the most potent thiohydantoin derivative against Gram-positive and Gram-negative bacteria. Also, they are exhibiting an impressive result as a new green corrosion inhibitor as well as flame retardant additives when physically incorporating into epoxy resin to form a new modified epoxy varnish. Corrosion inhibitor was carried out by using salt spray technique, and flame retardant was evaluated by measuring the limiting oxygen index (LOI) of the coated films.

5. References

i. Johnson, T. B.; Chernoff, L. H. (1913). J. Am. Chem. Soc, 35, 1208.

ii. (a) Tompkins, J. E. (1986). J. Med. Chem., 29, 855; (b) Elwood, J. C.; Richert, D. A.; Westerfeld, W. W. (1972). Biochem. Pharmacol, 21, 1127.

iii. Al-Obaid, A. M.; El-Subbagh, H. I.; Khodair, A. I.; Elmazar, M. M. (1996). Anticancer Drugs, 7873.

iv. 4. (a) Takahashi, A.; Matsuoka, H.; Ozawa, Y.; Uda, Y. (1998). J. Agric. Food Chem., 46, 5037; (b) Froelich, E.; Fruehan, A.; Jackman, M.; Kirchner, F. K.; Alexander, E. J.; Archer, S. (1954). J. Am. Chem.Soc, 76, 3099.

v. (a) Marx, J. V.; Richert, D. A.; Westerfeld, W. W. (1970). J. Med. Chem., 13, 1179; (b) Cheymol, J.; Chabrier, P.; Gay, Y.; Lavedan, J. P. (1951). Arch. Int. Pharmacodyn. Ther., 88, 342; (c) Cheymol, J.; Chabrier, P.; Gay, Y. (1951). Arch. Int. Pharmacodyn. Ther., 87, 321.

vi. El-Barbary, A. A.; Khodair, A. I.; Pedersen, E. B.; Nielsen, C. (1994). J. Med. Chem., 37, 73.

vii. (a) Chérouvrier, J. R.; Carreaux, F.; Bazureau. (2004). J. P. Molecules, 9, 867, and references citedtherein; (b) Khodair, A. I.; El-Subbagh, H. I.; El-Emam, A. A. (1997). Boll. Chim. Farm., 136, 561. (2006). Molecules, 11, 749.

viii. Archer, S.; Unser, M. J.; Froelich, E. (1956). J. Am. Chem. Soc., 78, 6182.

ix. (a) Lacroix, G.; Bascou, J. P.; Perez, J.; Gadras, A. (2000). U. S. Pat. 6,018,052; (b) Lacroix, G.; Bascou, J. P.; Perez, J.; Gadras, A. (1997) U. S. Pat. 5,650,519; (c) Marton, J.; Énisz, J.; Hosztafi, S.; Timar, T. (1993). J. Agric. Food Chem., 41, 148.

x. Curran, A. C. W. (1976). U. S. Pat., 3, 984,430.

xi. Nagpal, K. L. (1984).U. S. Pat. 4; 1984,473,393.

xii. Rou’e, N.; and Bergman, J. (1999). ‘Synthesis of the marine alkaloid leucettamine B,’ Tetrahedron, vol. 55, no. 51, pp. 14729–14738.

xiii. Jakse, R.; Recnik, S.; Svete, J.; Golobic, A.; Golic, L.; and B. Stanovnik (2001,1969). ‘A simple synthesis of alyspinosin analogues by dimethylamine substitution in N,N-

xiv. (dimethylamino)methylidene derivatives of five-membered heterocycles,’ Tetrahedron, vol. 57, no. 39, pp. 8395–8403, 8, 4735.

xv. (a) Mo, B.; Li, J.; Liang, S. (1997). Anal. Biochem., 249, 207; (b) Cromwellt, L. D.; Stark, G. R. (1969). Biochemistry, 8, 4735.

xvi. (a) Nelson, J. V.; Helber, M. J.; Brick, M. C. (1997) U. S. Pat. 5,695,917; (b) Ooi, T.; Fukui, T.; Kobayashi, M.; Ueno, K.; Kagami, K.; Suzuki, M.; Nishino, K. (1996). U. S. Pat. 5,482,814.

xvii. Kandi,S. S.; El-Hefnawy, G. B.; Baker, E. A. (2004) Thermochim. Acta, 414, 105.

xviii. Yuce , A.O.; Kardas , G. (2012) Adsorption and inhibition effect of 2 thiohydantoin on mild steel corrosion in 0.1 M HCl], J. Corrosion Science 58 , 86–94.
xix. Cruz, J.; Martín, R.; Genesca, J.; García, E. (2004). 'Experimental and theoretical study of 1-(2-ethylamino)-2-methylimidazoline as an inhibitor of carbon steel corrosion in acid media', J. Electroanalytical Chemistry 566, 111-121.

xx. Madkour, L. H.; Hassanein, A. M.; Ghoneim, M. M.; and Eid, S. A. (2001) 'Inhibition Effect of Hydantoin Compounds on the Corrosion of Iron in Nitric and Sulfuric Acid Solutions', Monatshefte für Chemie 132, 245-258.

xxi. Abd El-Wahab, H. (2015). 'Synthesis and characterisation of the flame retardant properties and corrosion resistance of Schiff's base compounds incorporated into organic coating', J. Pigment and Resin Technology; 44/2, 101-108.

xxii. Lu, S. and Hamerton, I. (2002). 'Recent developments in the chemistry of halogen-free flame retardant polymers', Progress in Polymer Science, Vol. 27, 1661-1712.

xxiii. Namazi, H. (2017). 'Polymers in our daily life', Biodegradability, 7, 73-74.

xxiv. Alexander B. M.; and Jeffrey W. G. (2013). 'An overview of flame retardancy of polymeric materials, application, technology, and future directions', J. FIRE AND MATERIALS, 37, 259-279.

xxv. ASTM Method, D1640-D1640m. (2018). 06.01.

xxvi. ASTM method, D609. (2017). 06.01.

xxvii. ASTM method, D714. (2017). 06.01.

xxviii. ASTM Method D 1654. (2017). 06.01.

xxix. ASTM Method D 610. (2019). 06.01.

xxx. ISO 4589-1. (2017), and ASTM D 2863. (2017). 06.01.

xxxi. Bauer-Kibry, J. (1966), and CLSI. (2006).

xxxii. Johnson, J.; Kuskowski, M.; Menard, M.; Gajewski, A.; Xercavins, M. and Garau, J. (2006). J. Infect. Dis., 194(1): 71-8.

xxxiii. Roue, N.; Bergman, J. (1999) Tetrahedron, 55, 14729.

xxxiv. Mohamed, S.M.; unis, M.; and Abdel-Hady, H. (2006). 'Synthesis and mass spectra of some New Heterocyclic compounds containing thiophene moiety'. Egypt. J. chem, 49(2), 209-223.

xxxv. El-Hady, H.A. (2012). 'Syntheses and antimicrobial activity of some new thiohydantoin and thiazol derivatives'. Derpharma chemical, 416:2202-2207.

xxxvi. EL-Ossaily, Y.A.; Zaki, R.M; and Metwally, S.A. (2014). 'Investigation and Synthesis of Some Novel Spiro Heterocycles Related to Indoline Moiety'. J. Sci. Res. 6(2), 293-307.

xxxvii. Sayed, H.H.; Flefel, E.M.; AbdEl-Fattah, A.M.; El-Sofany, W.I.; Hassan, A.M. (2010)'Focus on the Synthesis and Reactions of Some New Pyridine Carbonitrile Derivatives as Antimicrobial and Antioxidant Agents'. Egypt. J. Chem. 53, No.1, pp.17-35.

xxxviii. Duncana, S.H.; Flinta, H.J.; Stewarta C.S. (2006)'Inhibitory activity of gut bacteria against Escherichia coli O157 mediated by dietary plant metabolites'. FEMS Microbiol. Lett. 164: 283-288.