Synthesis, Characterization and Antimicrobial Activities of Silver Nanoparticles coated [1,3] Thiazin-4-One derivatives

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Abstract. Heterocyclic compounds have been used extensively used in pharmaceutical research area due to their effective biological activities. Thiazine, a heterocyclic compound with four carbon atoms, one nitrogen and one sulfur atoms which have promising pharmacological activities that have attracted the attention of scientists and researchers. In this research [3-(4-Amino-phenyl)-2-(4-nitro-phenyl)-2,3-dihydro-benzo[e][1,3]thiazin-4-one [comp. (2)] was used as a synthone for the preparation of Schiff bases [comp.(3)-(7)]. The synthesis compounds were characterized by FT-IR, ¹H NMR and spectrophotometric methods. Furthermore, silver nanoparticles was prepared by chemical reduction and characterized by X-Ray diffraction (XRD), infra-red spectrum (FTIR), atomic force microscope (AFM) and scanning electron microscope (SEM). The Ag NPs was used as a base metal for preparing Schiff bases-Ag NPs derivatives. The pure Ag NPs and Ag NPs functionalized Schiff bases nano compounds were used to check their effectiveness against four different types of bacterial (Staphylococcus aurous, Staphylococcus epidermidis, Escherichia coli and Klebsiella sp.) and one type of fungal (Candida albicans) by a serial dilution method.

Keywords: Thiazine, Schiff bases, biological activities, heterocyclic compounds, Silver nanoparticles.

1. Introduction
Thiazines are organic heterocyclic compounds with C₄H₅NS molecular formula. Thiazine is a six member ring system containing two endo cyclic hetero atoms (N and S). Thiazine derivatives may be 1, 2-thiazine, 1, 3-thiazine or 1, 4-thiazines[1]. In recent year, thiazine has been particularly interested in the formulation of synthetic drugs in vowing their proven biological activities[2]. The chemicals that include thiazines in their structures have been also used for dyes and insecticides industries[3]. The interesting pharmacological properties have been associated with thiazine derivatives[4] and this is because of these derivatives have shown valuable antimicrobial[5], analgesic[6], anti-inflammatory[7] and anti-tuberculosis[8] features. Thiazine derivatives are inhibitors for both the myocardial calcium channel[9] and matrix-inhibitors of metalloproteinase[10]. The bio-activities of these types of compounds are come from the presence of the reactive isomethane C= N group in substituted thiazine. Besides, the Schiff bases derived from thiazine have shown antitumor activity[11], antibacterial activity and antifungal activity [12].
Nano materials, that also known nanoparticles (NPs), are very nano sized materials with at least one dimension ranging from 1 to 100 nanometers [13]. In this range, nanoparticles have unique biological activity and other properties compared to substances in their larger form (micro and macro) [14]. Silver nanoparticles have significant physical and chemical properties, e.g. high thermal and electrical conductivity, chemical stability, good optical properties, and large catalytic activity [15,16]. These features have made Ag NPs able to use in the production of drug delivery system, microelectronics, and medical devices [17,18], in addition to showing extensive biological activity due to the toxicity of this compound for viruses, bacteria and fungi [19,20]. The citrate method for the preparation of Ag NPs was plenty used because of its easy preparation method, have a short time as well as giving regular silver nanoparticles[21]. Many researchers around the world have recently improved the biologic and fungal properties of organic compounds by combining them with silver nanoparticles. This study showed remarkable properties in the killing of bacteria and fungi. These study shows a major role in reducing the amount of organic compounds used in the preparation of these antibiotics[22-23].

2. Experimental

2.1. Materials and physical measurements
All chemicals and solvents used in this study were reagents grade and they are available from Sigma-Aldrich and Fluka companies. Melting points were determined on Electro- thermal capillary apparatus and are uncorrected. Purity of the compounds was checked on silica coated Merck-TLC plates using water, chloroform , benzene and acetone as mobile phase. FTIR measurements were recorded on Shimadzu model FT-IR-8400S. Proton NMR spectra 1H-NMR spectra were obtained with a Bruker GmbH 400 MHZ spectrophotometer model Ultra Shield at 400 MHz in DMSO-d6 solution with the TMS as internal standard. X-ray diffraction (XRD) measurements ranging (10-80) were recorded for nano materials by using (Shimadzu– XR–6000 ) device with nickel- copper filter for the x-ray radiation (Cu Kα, λ = 1.5406 Å). Atomic Force Microscope (AFM) images were recorded by using PHYW type. Microscopic image of synthesized Ag NPs and its derivatives were taken with a Zeiss Libra scanning electron microscope (SEM).

2.2. Preparation of Materials

2.2.1. Preparation of 2-(4-nitrophenyl)-4H-benzo[d][1,3]oxathin-4-one [comp. (1)]: This compound has been synthesized by following the reported procedures [24]. (3.85g, 0.025mole) of 2-mercapto benzoic acid was mixed with (8.75g , 0.05mole ) 4-Nitrobenzaldehyde in 100 ml of absolute ethanol. The mixture was magnetically stirred and (2.70g, 20 mmole) of aluminum chloride was slowly added, then the mixture was stirred again for 8 hr at 25 °C, then 200 ml of distilled water was gradually added until precipitate was formed, which re-crystallized from absolute ethanol, dried at 80 °C for 24 hr. Yield: 58%. M.p143-144°C. FTIR (KBr, ν, cm⁻¹):1703 (C=O); 1521,1344 (NO2); 2906,2845 (Aliphatic C-H); 7.78 (d d,2H, Ar -H), 7.70 (d d,2H, Ar -H), 7.29-7.30 (d d, 2H, Ar -H), 6.1 (s, 1H, Aliphatic C-H) 6.1(s,1H) C-H of thiazine ring .

2.2.2. Preparation of 3-(4-aminophenyl) -2-(4-nitrophenyl) -2,3-dihydro -4H-benzo[e] [1,3] thiazin-4-one [comp. 2]: A mixture of (0.01mole, 2.90g) of substrate comp. (1) in 50 ml of absolute ethanol and (0.01mole, 2.87g) of p-phenylene diamine in 50 ml of absolute ethanol. This mixture was stirred at room temperature and refluxed for 6 hr. The crystals were collected after evaporate the solvent, then filtrated, washed with petroleum ether, and dried at 80 °C for 24 hr [25]. Yield: 70% ; M.p:164 -166 °C, FTIR (KBr, ν,cm⁻¹):1680 (C=O); 3444 ,3354 (NH2); 2953, 2891 (Aliphatic C-H). 1H -NMR (400 MHz, DMSO-d6, δ, ppm): 5.3 (s,2H, NH2), 8.3-6.4 (m,12H , Ar –H,C-H of thiazine ring).
2.2.3. Preparation of [comp. (3-7)]: These compounds were synthesized according to a modified procedure described in the reported reference [25]. Substituted benzaldehyde (1mmol) was dissolved in absolute ethanol with a few drops of glacial acetic acid and then (1mmol) of comp. [2] derivatives was added respectively. The mixture was refluxed for 8 hr. The solid was filtered and recrystallized from dioxane and dried at 80 °C for 24 hr.

The prepared Schiff base compounds are:

Comp.3= 3-(4-((4-hydroxybenzylidene)amino)phenyl)-2-(4-nitrophenyl)-2,3-dihydro-4H-benzo[e][1,3]thiazin 4-one. Yield: 64% ; M.p: 275-277 °C. FTIR (KBr, ν, cm⁻¹):1676 (C=O), 1583 (C=N), 3354 (O-H). ¹H-NMR (400 MHz, DMSO-d6, δ, ppm): 9.7 (s, 1H, O-H), 8.89 (s,1H, CH=N), 8.5-6.4 (m, 17H, Ar-H,C-H of thiazine ring).

Comp.4 = 3-(4-((4-chlorobenzylidene)amino)phenyl)-2-(4-nitrophenyl)-2,3-dihydro-4H-benzo[e][1,3]thiazin-4-one. Yield: 65% ; M.p: 281-284 °C. FTIR (KBr, ν, cm⁻¹):1678 (C=O), 1587 (C=N), 1096(C-Cl). ¹H-NMR (400 MHz, DMSO-d6, δ, ppm): 8.8 (s, 1H, CH=N), 8.7-6.55 (m, 17H, Ar-H, C-H of thiazine ring).

Comp.5 = 3-(4-((4-bromobenzylidene)amino)phenyl)-2-(4-nitrophenyl)-2,3-dihydro-4H-benzo[e][1,3]thiazin-4-one. Yield: 71% ; M.p: 286-288 °C. FTIR (KBr, ν, cm⁻¹):1678 (C=O), 1587 (C=N), 1075 (C-Br). ¹H-NMR (400 MHz, DMSO-d6, δ, ppm): 8.6 (s, 1H, CH=N),8.03-6.4 (m, 17H, Ar-H, C-H thiazine ring).

Comp.6 = 3-(4-((4-nitrobenzylidene)amino)phenyl)-2-(4-nitrophenyl)-2,3-dihydro-4H-benzo[e][1,3]thiazin-4-one. Yield: 75%. M.p: 260-262 °C. FTIR (KBr, ν, cm⁻¹):1680 (C=O), 1585 (C=N), 1512,1344 (NO2). ¹H-NMR (400 MHz, DMSO-d6, δ, ppm): 8.8 (s, 1H, CH=N),8.02-6.2 (m, 17H, Ar-H,C-H thiazine ring).

Comp.7 = 3-(4-((4-(dimethylamino)benzylidene)amino)phenyl)-2-(4-nitrophenyl)-2,3-dihydro-4H-benzo[e][1,3]thiazin-4-one. Yield: 61% ; M.p: 277-280 °C. FTIR (KBr, ν, cm⁻¹):1683 (C=O), 1589 (C=N), 2931,2858 (Aliphatic C-H). ¹H-NMR (400 MHz, DMSO-d6, δ, ppm): 8.6 (s, 1H, CH=N), 8-6.1 (m, 17H, Ar-H,C-H thiazine ring).
2.2.4. Preparation of silver nanoparticles and functionalized with [compound (1-6)]: AgNO3 (17.0 mg) was dissolved in 100 mL of water in a 250 mL Tri-neck flask. The solution was heated to boiling with strong magnetic stirring. After boiling for 2 min, aqueous solution of sodium citrate (35 mM, 10 ml) was added to the flask in very slow drops. The solution gradually turns greenish yellow in a few minutes, indicating the formation of Ag NPs. The boiling solution is maintained for an additional 6 min. After that, the solution was allowed to cool down to room temperature under magnetic stirring. After cooling, the solution of compounds (I-VII) (17 × 10-3g soluble in 5 mL ethanol) was added and
stirred with Ag NPs solution for 24 hr. The reaction mixture was then evaporated and the solid product was obtained in the nano form [26,27].

2.3. Biological Activities
Thiazine derivatives [2-7] were in vitro screened for antibacterial activity against two Gram positive (Staphylococcus aurous and Staphylococcus epidermidis) and two Gram negative bacteria microorganisms (Escherichia coli and Klebsiella sp.) and fungal strains namely Candida albicans using well diffusion method [21]. Dimethyl sulfoxide DMSO was run as a control and the test was performed at 10mg/mL concentration using DMSO as solvent. The bacteria and fungi were sub refined in agar and potato dextrose agar medium and these plates were incubated for 24 h for bacteria and 48 h for fungi at 37 ºC. The zone inhibition observed around the cups after respective incubation was measured in mm[28].

3. Results and discussions

3.1. Organic compounds:
The new thiazine derivatives were prepared following the reaction sequences depicted in scheme 1. Comp.[1] is the key intermediates for the compounds synthesized later in this work. It has been prepared by the condensation of the thiosalicylic acid with p-nitrobenzaldehyde. Preparation of 3-(4-aminophenyl)-2-(4-nitrophenyl)-2,3-dihydro-4H-benzo[e][1,3]thiazin-4-one [comp. 2] was prepared by reaction of comp.[1] and p-aminoaniline. The structure of all compounds were proven based on the melting point (m.p), thin layer chromatography (TLC) and spectral data. FTIR spectra through the appearance of stretching vibration of NH₂ group, (asymmetrical and symmetrical) at 3444-3354 and C=O stretching vibrations at 1675 cm⁻¹. ¹H-NMR spectrum of comp.[2] exhibited singlet signals 6.7 ppm was assigned to NH₂ proton. The aromatic protons were appeared at (7.1-8.3) ppm. Condensation of the amino group of derivative 2 with Aromatic aldehyde in absolute ethanol gave the Schiff base [comp.93-70]. The formation of these Schiff bases were indicated by the presence in their FTIR spectra of the (CH=N) stretching band at [1583, 1587, 1587, 1585, 1589 cm⁻¹] respectively combined with the disappearance of NH₂ stretching band of comp.[2] and carbonyl group of Aromatic aldehydes.

3.2. Silver Nanoparticles (Ag NPs):

3.2.1. X-Ray Diffraction (XRD) of Silver nanoparticles: Fig. (1) shows the X-ray diffraction pattern of produced crystalline Ag NPs. The diffraction pattern shows four sharp and well-defined diffraction lines at 2θ = 38.24°, 44.42°, 64.59° and 77.51°, can be assigned to the reflections of the face centered cubic (fcc) structure of Ag NPs (111), (200), (220) and (311) respectively, which is well agreement with the literature report JCPDS File No. 04-0783. From the above peaks and by using Deby- Scherrer equation, the average size was found to be 22.07 nm [29,30].
3.2.2. Atomic Force Microscope (AFM) of Ag NPs: It is important to study the topography of the materials in nanoscale and to know the morphology and surfaces for the nanoparticles. AFM investigations of pure silver nanoparticles in Fig. (2). In the three-dimensional and percentage distribution diagram shapes, the highest rise in the measured sample was about 5 nm while the average diameters about 76.33 nm.
3.3. Organic compounds functionalized with Silver nanoparticles

3.3.1. XRD of Compounds [1-6] functionalized with Silver nanoparticles: Fig. (3) shows the X-ray diffraction pattern of prepared compounds which including Ag NPs and thiazine derivatives (Comp. 2-7) functionalized with Ag NPs. Through X-ray diffraction, Silver Nanoparticles peaks (111), (200), (220) and (311) were appeared in the same region (JCPDS File No. 04-0783) but with different intensities, as noted some of the new peaks in different intensities and in different regions. This explains that organic compounds [comp.(2-7)] have react with Ag NPs and mixed with them and New-[1,3]Thiazin-4-One derivatives functionalized with silver nanoparticles were formed[31].

![Fig (3): X-RD of comp. (2-7) Functionalized with Silver nanoparticles](image-url)
3.3.2. Scanning electron Microscope (SEM) of Compounds [2, 3 and 5] functionalized with Ag NPs:
The morphological measurements of the prepared silver capped-organic molecules have given us a
great considerate of the properties of this material. The SEM of comp.[2]-Ag NPs showed the
presence of non-regular nanostructured structures due to the comp.[2] while the silver appears as a
spheres onto the surface. The results show that thickness range of 20-40 nm as shown in Fig. (4) (a).
Whilst the diameter of Ag NPs were found in the range of 15-23 nm. Regarding the comp.[3]-Ag NPs
once again the SEM showed the presence of non-regular granulator nanosheets with thickness of the
sheet 20-35 nm and Ag NPs diameter in the range of 15-29 nm Fig (4) (b). As for the comp.[5] -Ag
NPs compound, the measurements showed that the composition of the resulting material with a sheet
structure is more clear. The silver nanoparticle structures were spherical and a diameter was between
26-42 nm as shown in Fig. (4) (c).

![Fig. 4 SEM image of Compounds [2, 3 and 5] functionalized with Silver NPs.](image)

This resulting structures indicate the roughness of the surface of these materials, providing a good
platform for penetration by providing a greater contact area with bacteria or even in other applications,
which depend on the existence of a large surface area. The presence of granular structures dating back
to nanoparticles explains the interaction between silver and organic matter and demonstrates once
again the bonding on the surface of the material.

4. The Biological Activity

These compounds were dissolved in 5 ml DMSO in order to prepare 0.2 M to study the inhibition
biological activity for antibacterial against (Staphylococcus aurous, Staphylococcus epidermidis,
Escherichia coli and Klebsiella sp.) and anti-fungal against Candida albicans. The biological activities
have been studied using compound [2-7] dissolved in DMSO and its composites with Ag NPs after
dissolved in DMSO too. The functionalized compounds with Ag nanoparticles gave good positive
result against bacteria and fungi than un functionalized compounds as shown in table 1. These results
indicate that the Ag nanoparticles promote the biological activity of organic compounds and this could be attributed to the small size and high surface area and its chemical active to Causes Penetrate wall microbial cell and destroyed[32,33].

Table (1): The results of the biological activity of organic compound and their derivatives with Ag NPs.

| No. | Compounds | Zone of Inhibition in (mm) |
|-----|-----------|---------------------------|
|     |           | S. Aureus | S. Epidermidis | E. Coli | Klebsiellan Sp. | C. Albicans |
| 1   | DMSO      | ----      | ----           | ----    | ----            | ----        |
| 2   | comp. 2   | 11        | 13             | 15      | 8               | 17          |
| 3   | comp. 3   | 18        | 10             | 12      | 11              | 9           |
| 4   | comp. 4   | 12        | 16             | 11      | 9               | 13          |
| 5   | comp. 5   | 10        | 14             | 9       | 18              | 7           |
| 6   | comp. 6   | 17        | 12             | 7       | 4               | 13          |
| 7   | comp. 7   | 12        | 15             | 13      | 7               | 9           |
| 8   | comp. 2-Ag| 22        | 24             | 19      | 14              | 25          |
| 9   | comp. 3-Ag| 29        | 33             | 25      | 27              | 17          |
| 10  | comp. 4-Ag| 24        | 27             | 29      | 30              | 31          |
| 11  | comp. 5-Ag| 22        | 29             | 37      | 34              | 36          |
| 12  | comp. 6-Ag| 19        | 21             | 20      | 22              | 29          |
| 13  | comp. 7-Ag| 19        | 24             | 21      | 19              | 17          |

Fig. 5 Inhibition zone diameter (IZD) with thiazine derivatives without and with Ag NPs.
5. Conclusion

Novel thiazine derivatives are prepared and characterized on the basis of analytical and spectral data. The analysis of these compounds against pathogenic microorganisms shows that these compounds have the potential to inhibit the metabolic growth of some microorganisms to a certain extent. Antimicrobial activity of the compounds depends on the nature of substituent present on the thiazine ring and the functional groups. When these organic compounds are mixed with Ag NPs nanoparticles, antimicrobial activity is highly dependent on Ag NPs because of its small size, large surface area and chemical nature of silver in killing microbes, as well as the presence of organic compounds with Ag NPs.

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7. References

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