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Synthesis and Bio-evaluation of 2-Imino-4-amino thiazole Capped Silver Nanoparticles

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Silver nanoparticles capped with *N*-(4-amino-3,5-diphenyl-3H-thiazol-2-ylidine)benzamide is synthesized by simple chemical reduction method, prepared by treatment of aqueous silver ions with hot ethanolic solution of *N*-(4-amino-3,5-diphenyl-3H-thiazol-2-ylidine)benzamide. The size and shape of obtained silver nanoparticles are spherical and uniform with average size of 34 nm and are characterized by using UV-visible, FTIR, XRD, SEM and TEM studies. The capped silver nanoparticle showed significant inhibitory activity against various human pathogenic bacteria with different doses of 25, 50, 75 and 100 µg. The synthesized silver nanoparticle capped compound was potent inhibitory agent when compared with standard antibiotic ciprofloxacin.

Keywords: Antimicrobial, Capping agent, Silver nanoparticles, Triazole derivatives.

INTRODUCTION

2-Iminothiazoline derivatives are important 5-membered heterocyclic compounds have proven to be a structural feature providing a broad spectrum of biological activity. These are playing key role in the development of drugs for hypertension [1], inflammation [2], cancer therapies [3], *etc*. Due to their inherent low toxicities and good pharmacokinetic profile, these compounds have been recognized as privileged structural motif in medicinal chemistry. Recently, 2-imino-thiazolines were found to have antifungal activity [4] and skin whitening properties [5]. The pifithrin (Pft-α) was isolated by screen of chemical libraries having 2-iminothiazoline skeleton is the lead compound of p53 in activators and have received increasing attention due to their possible applications in several major neuro-degenerative disorders such as Alzheimer's disease, Parkinson's disease, stroke, cancers therapy and other pathologies related to various signaling pathways [6-9]. In this present work the derivative of 2-imino thiozoline *i.e.*, N-(4-amino-3,5-diphenyl-3H-thiazol-2-ylidine)benzamide is used as a capping agent to control the size of silver nanoparticles and stabilize them. The capping agent also enhances the biological activity of silver nanoparticles

Synthesis and characterization of *N*-(4-amino-3,5-diphenyl-3H-thiazol-2-ylidine)benzamide: 2-Iminothiazoline derivatives are important class of 5-membered heterocyclic compounds, because of their wide applications [10]. They are not only seen as building blocks in natural products, but are also considered to be extraordinarily useful scaffolds, especially in combinatorial and medicinal chemistry [11-15]. Furthermore, thiazolines have interesting applications in agriculture as acaricides, insecticides and plant growth regulators [16,17].

In general the synthesis of 2-iminothiazoline derivatives was achieved by the reaction of thiourea derivatives with various substituted α-bromoketone in the presence of suitable base [18,19]. Here also the similar reaction procedure was adopted for the synthesis of 4-amino-2-iminothiazole from the reaction of aroylthiourea with bromo benzyl cyanide.

As it was known from the literature that triethylamine works as best base for this kind of transformation [20], the same base was used in this method. The thiourea derivatives were synthesized using the previous standard procedure. The product was fully characterized using all spectroscopic analysis (IR, ^{1}H NMR, ^{13}C NMR and mass). In the IR spectrum, the characteristic peak for NH_2 was observed at 3340-3222 cm^{-1} and C=N was observed at 1590-1550 cm^{-1}. In ^{1}H NMR, NH_2 appeared as broad singlet at δ 3.5 ppm.

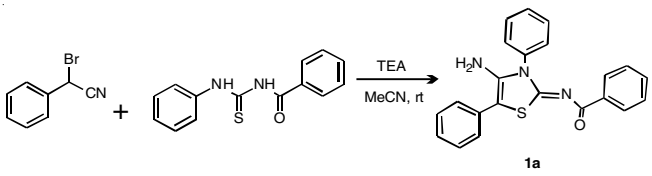
EXPERIMENTAL

All the reagents used were of AR grade. Silver nitrate was obtained from National Refinery Pvt. Ltd. and a 0.1M aqueous solution was used as stock solution. Sodium boro-hydroxide was obtained from Merck, India. Organic-free water was used throughout the experiment.

The UV-visible spectra were recorded on a Shimadzu UV-visible spectrophotometer and the solutions were taken in a 1 cm well stoppered quartz cuvette. Fourier transform infrared (FTIR) spectral characteristics of the samples were collected on a Shimadzu FTIR spectrometer with the samples as KBr pellets. The FTIR spectrum was recorded over 45 scans of each sample and the background spectrum was automatically subtracted. The formation of single-phase compound was checked by X-ray diffraction (XRD) technique. The XRD pattern was taken with X-ray diffractometer (XPERT-PRO) at room temperature, using CuK_α radiation $\lambda = 1.5406$ Å over a wide range of Bragg angles ($30° \leq 2\theta \leq 85°$). SEM micrograph of *N*-(4-amino-3,5-diphenyl-3H-thiazol-2-ylidine)benzamide capped silver nanoparticles was obtained on a NOVA-230 with an operating voltage of 10 KV. For SEM analysis, the specimen was suspended in distilled water, dispersed ultrasonically to separate individual particles and one or two drop of the suspension deposited onto holey-carbon coated copper grids and dried under infrared lamp.

Synthesis: To 10 mmol of thiourea derivative in acetonitrile, triethyl amine (10.50 eqiv.) was added and stirred at room temperature (25-30 °C) for 10 min until the solution turns to pale yellow. To that 10.10 mmol of bromobenzylcyanide (which was dissolved in 1 mL acetonitrile) was added drop wise for 10 min, then a yellow precipitate was obtained after 15 min. The solvent was concentrated under reduced pressure to separate solid material; it was filtered and washed with hexane to get rid of the excess triethyl amine. The obtained yellow coloured crystalline compound was purified and characterized (**Scheme-I**).

Characterization of *N*-(4-Amino-3,5-diphenyl-3H-thiazol-2-ylidine)benzamide (1a): m.p.: 78-80 °C; IR (KBr, ν_{max}, cm^{-1}): 3220, 1670,1598,1560,1219; ^1H NMR ($CDCl_3$ 400 MHz): δ 6.0 (S, 2H, NH_2), 6.98.0 (m, 15H, aromat); ^{13}C NMR ($CDCl_3$ 100 MHz): δ 95.3, 124.1, 126.7, 127.2, 129.8, 131.2, 133.7, 135.5, 136.8, 137.6, 167.0, 174.4, 178.5.



1a

Scheme-I: Synthetic approach

Synthesis of silver nanoparticle assembly

N-(4-amino-3,5-diphenyl-3H-thiazol-2-ylidine)-benzamide capped silver nanoparticles: A total of 2.5 mL of 10^{-2} M $AgNO_3$ was added to 75 mL of triply distilled organic-free water, to this 5 mL of 10^{-2} M *N*-(4-amino-3,5-diphenyl-3H-thiazol-2-ylidine)benzamide (dissolved in hot EtOH) was added as stabilizer with stirring. After 10 min of mixing, 2.5 mL of 10^{-2} M KI was added drop wise into the solution slowly, until a green yellow AgI colloid was obtained. The silver colloid was finally obtained when 20 mg of $NaBH_4$ was added to the AgI colloidal solution and the reaction mixture was continually stirred for about 20 min. During the whole reaction, the colour of the colloidal solution changed from green-yellow to nut-brown at the beginning, then to brown and finally to black.

The formation, morphology, photo physical and biological activity of the *N*-(4-amino-3,5-diphenyl-3H-thiazol-2-ylidine)-benzamide capped silver nanoparticles are reported. The silver nanoparticles with uniform shapes and sizes can be obtained by a simple chemical reaction of silver idodide (AgI) and sodium borohydride. The advantage of the method is ease of preparation, convenience in use and especially the obtained silver nanoparticles are uniform in their shapes and sizes. The shapes and sizes of the metal nanoparticles are significant parameters for their bio-evaluation measurements. Furthermore, UV-visible spectroscopy was employed to determine the optimum conditions for the preparation of stable silver colloids. Time-dependent UV-visible spectroscopy and Scanning electron microscopy (SEM) are employed to monitor the process of silver nanoparticles formation. There is a change in the shape of the silver nanoparticles during the formation of the nanoparticles was observed. This may be helpful in understanding the growth of the nanoparticles and creates a new dimension in controlling the shapes of nanoparticles.

Antimicrobial studies of 2-imino-4-amino thiazole and their silver nanoparticles

Human pathogenic bacteria: Human pathogenic bacteria species *Salmonella typhi, Vibrio cholera, Shigella dysenteriae, Staphylococcus aureus* are used in this study. These were collected from Department of Microbiology, Andhra Medical College, Visakhapatnam, India.

Preparation of bacterial inoculums: The microorganisms were inoculated into Muller Hinton broth and incubated at 35 ± 2 °C for 4 h. The turbidity of the resulting suspensions was diluted with MH broth to obtain a transmittance of 25 % at 580 nm. That percentage was found spectrophotometrically comparable to 1 McFarland turbidity standard. This level of turbidity is equivalent to approximately 3.0×10^8 CFU/mL. The Bausch & Lomb® spectrophotometer, Model Spectronic 20 was used to adjust the transmittance of the working suspensions. This suspension used as inoculums.

Agar-well diffusion assay: The modified agar-well diffusion method of Perez *et al.* [21] was employed. Each selective medium was inoculated with the microorganism suspended in Muller Hinton broth. Once the agar was solidified, it was punched with a 6 mm diameter wells and filled with required concentration of compounds. Ciprofloxacin (antibiotic) used as standard for positive control while pure solvents were used as negative control. Results were determined based on size of the inhibitory zone surrounding the wells containing the extract, on comparing with standard and blank. The diameter of zones of inhibition was measured in mm using Hi-Media zone reader.

Minimum inhibitory concentration: The minimum inhibitory concentration of synthesized silver-nanoparticle was determined using broth dilution assay. The medium containing different concentrations of compounds *viz.*, 100 mg to 100 µg per mL prepared by serial dilution (10^{-1} dilution). After inoculation of culture, the tubes were incubated for 24 h at 37 °C. The MIC of each sample was determined by measuring the optical density in the spectrophotometer (Electronics India) at 580 nm and compared the result with those of the non-inoculated broth used as blank. Control was prepared with media and inoculums only without compounds. The experiment was conducted according to NCCLS standards.

RESULTS AND DISCUSSION

UV-visible spectroscopy: Fig. 1 shows UV-visible spectra of the obtained silver colloids. The surface plasmon resonance (SPR) band is broad indicating poly-dispersed nanoparticles. A smooth and narrow absorption bands at 434 nm is observed for *N*-(4-amino-3,5-diphenyl-3H-thiazol-2-ylidine)benzamide capped silver nanoparticles. The optical absorption spectra of metal nanoparticles are dominated by surface plasmon resonances (SPR), which shift to longer wavelengths with increase of particle size. The position and shape of plasmon absorption of silver nanoclusters are strongly dependent on the particle size, dielectric medium and surface-adsorbed species. The surface plasmon absorption of silver nanoparticles have the short wavelength band in the visible region around 409 nm is due to the transverse electronic oscillation.

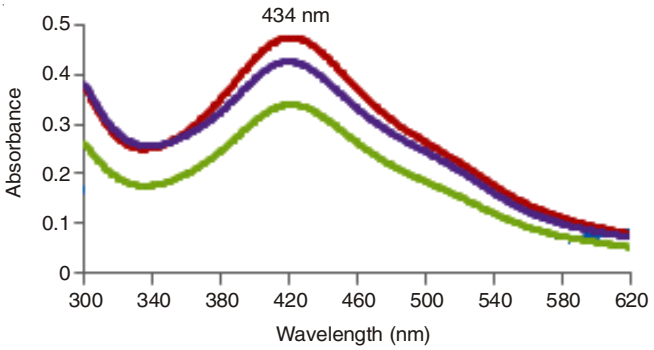


Fig. 1. UV-visible absorbance spectra of 2-imino-4-amino thiazole capped silver nanoparticles in DMSO

Scanning electron microscopy: The SEM images of synthesized 2-imino-4-amino thiazole capped silver nanoparticles shown in Fig. 2, indicates that the particles are nearly crystalline and spherical.

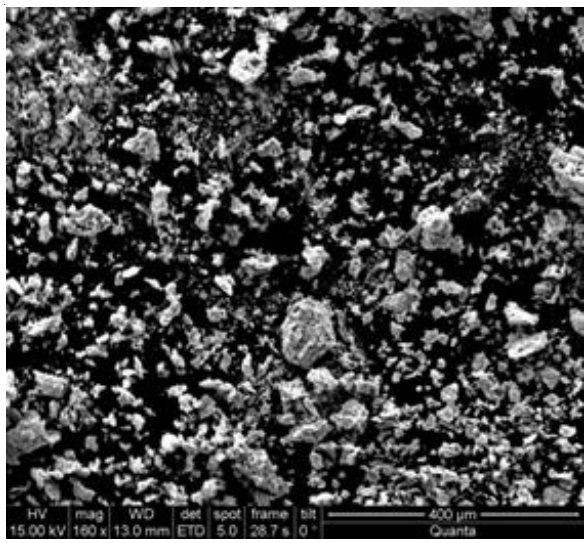


Fig. 2. SEM images of synthesized 2-imino-4-amino thiazole capped silver nanoparticles

The Scherrer rings, characteristic of fcc silver is clearly observed and showing that the structure seen in the SEM image is nanocrystalline in nature. It is also observed that the silver nanoparticles are scattered over the surface and no aggregates are noticed under SEM. The difference in size is possibly due to the fact that the nanoparticles are being formed at different times. Scanning electron microscopic (SEM) measurement of the synthesized nanoparticles shows that they are in spherical shape.

X-ray diffraction: XRD spectra of silver nanoparticles measured in the small and wide angle region are shown in Fig. 3. The XRD spectrum confirms the tendency of nanoparticles to form the organized structures, as seen from the peaks in the small angle XRD spectrum. The peaks are broadened because of the nano crystalline nature of silver nanoparticles. By comparing with standard database values, all the peaks can be indexed to face-centered cubic (fcc) silver crystal structure. Three peaks at 2y values of 38.299, 64.853 and 77.542, correspond to the (1 1 1), (2 0 0) and (2 2 0) planes of silver nanoparticle, respectively.

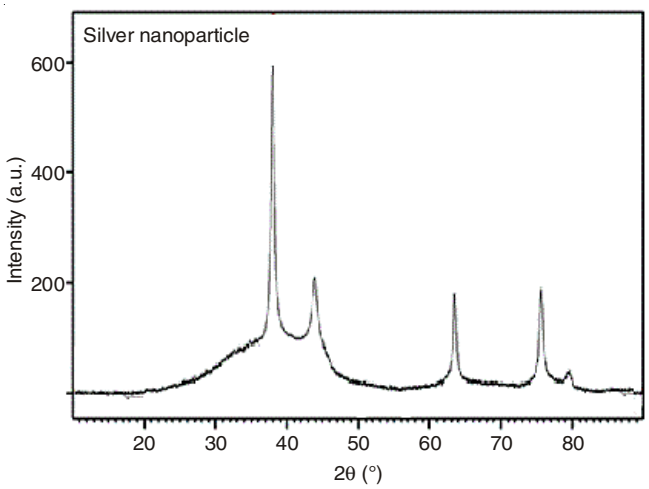


Fig. 3. XRD patterns 2-imino-4-amino thiazole capped silver nanoparticles

From this Fig. 3, it can be noticed that the silver particles capped with 2-imino-4-amino thiazole appeared basically amorphous and abroad. Size-dependent and structure-specific features in diffraction patterns can be quite striking in nano meter-sized particles. Small particles have fairly distinct diffraction patterns, both as a function of size and as a function of structure type. In general, regardless of structure, there is a steady evolution in the aspect of diffraction profiles, as particles become larger no abrupt changes occur but more details will be resolved when features grow continuously from the diffraction profile. These observations form the basis for a direct technique of diffraction pattern analysis that can be used to obtain structural information from experimental diffraction data. The silver particles are almost crystalline with the appearance of diffraction peaks at the scattering angles (2y) at 38-39, which could be indexed to the scattering from the planes (111), (200), (220) and (311), respectively. It is well known that with diminishing crystallite size the measured XRD pattern exhibits broadening and very often overlapping reflections. The broadening of the reflections is inversely proportional to the crystallite size (*i.e.* size of coherently diffracting domains). The relation is known as Scherrer's equation where "y" is the diffraction angle of a particular reflection. The total diffracted intensity for a given Bragg reflection from a crystallite is the

sum of independently diffracted intensities by each of the unit-cell columns making-up the crystallite. It means that the calculated size distribution is in fact a distribution of diffraction column lengths in a given crystallographic direction perpendicular to the diffraction planes and not of crystallite (coherently diffracting domains) sizes. Theoretical considerations show that the interference function of a polycrystalline or nano crystalline solid is identical to that of an arrangement of isolated particles with the same size or size distribution as those of the polycrystalline or nano crystalline solid. Thus the values of Scherrer's formula are solely an estimate of a volume-weighted average column length. This explains the difference between the experimental and theoretical values and the values from the Scherrer's formula is termed ''apparent crystallite size''.

FT-IR analysis: The IR spectra shown in Fig. 4. the silver nanoparticles capped by 2-imino-4-amino thiazole and the free 2-imino-4-amino thiazole molecule are similar to one another, indicating that the organic molecules have indeed become a part of the nanoparticles. However, there is remarkable difference in the peak intensity found between the peaks of IR spectra of free 2-imino-4-amino thiazole and 2-imino-4-amino thiazole capped silver nanoparticles. The reason for this intensity difference between the spectra is believed to be the amino molecules on the nano particle, forming a relatively closely packed amino layer and thereby the molecular motion being constrained. Thus, this steric constraining effect on the transverse mode (rocking mode, wagging mode, *etc*.) is stronger than the longitudinal mode (stretching mode, *etc*). Therefore, the change of the peak intensity of the longitudinal modes is smaller than that of the transverse mode. The C–N stretching mode is due to the position of the C–N bond nearest to the surface of the silver particle and a chemical bond can form between N and Ag atoms.

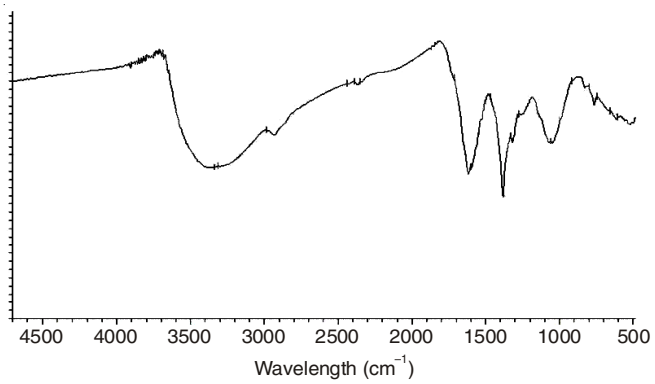


Fig. 4. FT-IR spectra of 2-imino-4-amino thiazole capped silver nanoparticles

From the spectrum of free 2-imino-4-amino thiazole, it can be noticed that the azo group appeared in 1620-1597 cm^{-1} region, respectively. The two bands appeared between 1384 and 1319 cm^{-1} are assigned to the stretching vibration of C-N group. The band appeared at 767 cm^{-1} is assigned to C–S stretching mode. Generally, the vibrational spectrum of silver nanoparticles capped with 2-imino-4-amino thiazole molecule is similar to the spectrum of 2-imino-4-amino thiazole.

Particle size measurement using TEM: Silver nanoparticles that produced were examined using transmission electron microscopy. A sample of silver nanoparticles from a freshly synthesized clear yellow sol was prepared by drying a small drop on a carbon-coated 200-mesh copper grid. The TEM image of one region of the sample is shown in Fig. 5. The TEM image shows the silver particles are spherical with sizes of 34 ± 2 nm.

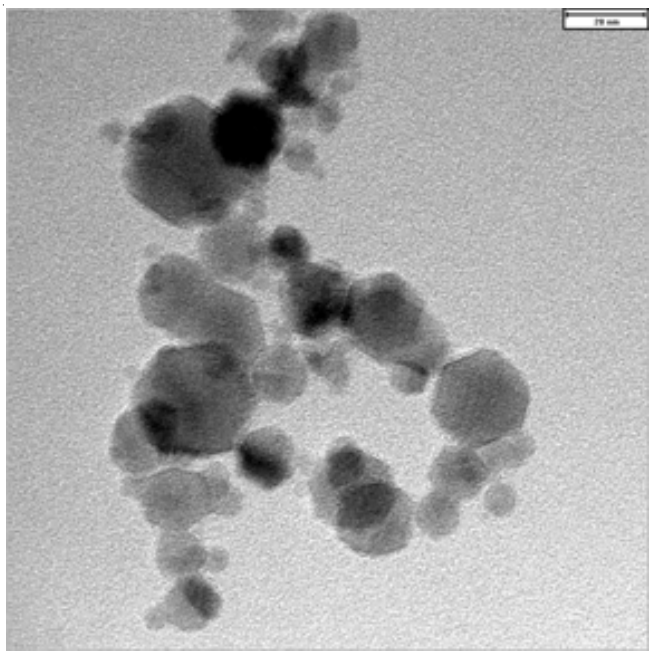


Fig. 5. Transmission electron micrographs of the silver nanoparticles used in this work (a) The bar marker represents 20 nm

Antibacterial activities: The synthesized 2-imino-4-amino thiazole capped silver nanoparticles showed significant inhibitory activity against various human pathogenic bacteria species, like *S. typhi, V. cholera, S. dysenteriae* and *E. faecalis.* It was found that, the synthesized silver nanoparticle compound was potent inhibitory agent when compared with standard antibiotic. 13mm was the highest zone of inhibition showed by compound against *S. typhi* and *S. dysenteriae* (Table-1). *E. faecaulis* and *V. cholerae* showed sensitivity to compounds when compared with compounds inhibitory potential against *S. typhi* and *S. dysenteriae*. From Table-2, synthesized silver nanoparticle compound showed dose dependent inhibitory activity. Zone of inhibition was increases with concentration of compound. 1mg/mL is the lowest MIC of compound against *V. cholera.* The results are comparable with antibiotic ciprofloxacin.

TABLE-1
ANTIBACTERIAL ACTIVITY OF COMPOUNDS AGAINST HUMAN PATHOGENS

	Zone of inhibition (mm)*			
	S. typhi	*V. cholera*	*S. dysenteriae*	*E. faecaulis*
Compound **1a**	6	8	6	7
AgNPs^{+}	13	11	13	12
DMSO	8	7	7	7
Ciprofloxacin	17	17	15	15

$^{+}$50 µg of compound (1 µg/µL concentrated), *6 mm is the well size

TABLE-2
DOSE DEPENDENT INHIBITORY EFFECT OF $AgNPs^+$ ON VARIOUS HUMAN PATHOGENIC BACTERIA SPECIES

Type of human pathogenic bacterial sps.	Causing disease	Zone of inhibition (mm) at different doses			
		25 µg	50 µg	75 µg	100 µg
Salmonella typhi	Typhiod fever	11	14	16	17
Vibrio cholera	Cholera	15	14	16	18
Shigella dysenteriae	Dysentery	12	14	15	13
Enterococcus faecalis	Gastro, intestinal infections	13	12	13	15

Conclusion

N-(4-Amino-3,5-diphenyl-3H-thiazol-2-ylidine)-benzamide capped silver nanoparticles with uniform size and shape are synthesized by easy and convenient method and the morphology, photo physical and biological activities were studied. The synthesized 2-imino-4-amino thiazole capped silver nanoparticles showed significant inhibitory activity against various human pathogenic bacteria species, like *S. typhi, V. cholera, S. dysenteriae* and *E. faecalis.* This compound showed dose dependent inhibitory activity. Zone of inhibition was increases with concentration of compound. It was found that compound was potent inhibitory agent when compared with standard antibiotic.

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