Synthesis, Characterization of Cd(II) Complex Using [N{3-(5-amino-2-thione-1,3,4 thiadiazole)methyl imidazole}] and study their anti-microbial, anti-inflammatory property

Santosh W. Kulkarni[1], Suhas P. Janwadkar[2], Kaustubh S. Ghude[3]*, Pooja V. Shinde, Sunil G.Gadakh, Dilip K. Yadav, Shraddha S. Parab, Rohit J. Gawad, Hardik T. Deshmukh

Abstract- Metal complexes with Novel [N{3-(5-amino-2-thione-1,3,4 thiadiazole) methyl imidazole] have been synthesized by the reaction of Azole compound methyl imidazole with [N{3-(5-amino-2-thione-1,3,4 thiadiazole)]. Their reaction with Imidazole led to the formation of the respective Novel reagent [N{3-(5-amino-2-thione-1,3,4 thiadiazole) methyl imidazole]. The formation of metal complexes with [N{3-(5-amino-2-thione-1,3,4 thiadiazole) methyl imidazole] by different stoichiometry of metals like cadmium. The structure of the above compound and complex was confirmed by its spectral characteristics like UV, IR and NMR. This compound shows good biological activities like anti-microbial, (fungal/bacterial) and anti-inflammatory.

Keywords: Azole-(methyl imidazole), thiadiazole -[N{3-(5-amino-2-thione-1,3,4 thiadiazole)], metal -(cadmium), biological activities- (anti-microbial and anti-inflammatory)

INTRODUCTION

Metal complexes with Amino-1,3,4- thiadiazole derivatives are well known as compounds of a wide range of anticancer activity and antibacterial, antiinflammatory property that have been prepared from their azoles and binding of metals and also transition metal complexes surely giving activities like anti-inflammatory and anticancer[1,14,23,26]. The most interesting examples are constituded by 5-amino-134 thiadiazole-derivatives such as thiols, a compound used as radio protective agent, as well as an investigational antitumor and gastroprotective drug acetazolamide and anti depressant agent[2,15]

Complexes of metal ions such as cadmium (II) are of great interests in the aspect of metal based chemotherapeutic drugs, since they are involved in many biological processes. Cadmium (II) complexes have attracted more attention due to their widely reported bioactivities. [3,16] The synthesis of a new complex 2-acetyl-2-thiazoline-thiosemicarbazone with cadmium showed in studies of minimum inhibitory concentration (MIC) great activity against Staphylococcus epidermidis (12.4 mg/mL), Staphylococcus aureus (25 mg/mL), Enterococcus faecalis (50 mg/mL) and Escherichia coli (25 mg/mL) compared to complexes with cadmium. [4,16,17]

1,3,4-Thiadiazole nucleus exhibited remarkable pharmacological activities. Literature indicates that compounds having 1,3,4-Thiadiazole nucleus have wide range of pharmacological activities that include antibacterial, antifungal, antitubercular, A antiviral, antileishmanial, anti-inflammatory, analgesic, CNS depressant, anticonvulsant, anticancer, antioxidant, antidiabetic, molluscicidal, antihypertensive, diuretic.[5,15,16,17,18,25].

The 1,3,4-thiadiazole nucleus is one of the most important and well known heterocyclic, which is a common and integral feature of a variety of natural products and medicinal agents. Thiadiazole nucleus is present as a core structural component in an array of drug categories such as antimicrobial, anti-inflammatory, analgesic, antiepileptic, antiviral, antineoplastic and antitubercular agents etc.[6,18,24]

Imidazoles play an important role in medicinal chemistry, because many of its derivatives have demonstrated significant biological activity.[7,19] with all this in mind, the present paper focused on the synthesis of some fused with novel [N{3-(5-amino-2-thione-1,3,4 thiadiazole) methyl imidazole] and novel

[N{3-(5-amino-2-thione-1,3,4 thiadiazole) methyl imidazole] Cd(II) and study their applications.

Denaturation of proteins is one of the phenomenon's that results in the disturbance of stability andstructure of the protein. The chemistry of proteins has always been important owing to the abundance of these biomolecules in the living system.

with all this in mind, the present paper focused on the synthesis of some fused with novel [N{3-(5-amino-2-thione-1,3,4 thiadiazole) methyl imidazole] and novel [N{3-(5-amino-2-thione-1,3,4 thiadiazole) methyl imidazole] Cd(II) and study their applications.

EXPERIMENTAL

All reagents and solvents were used as purchased from commercial suppliers. The ligand TDIZ was synthesized according to the literature method[8] and ligand TDIZ R1 and TDIZC1confirmed by IR spectroscopy by bruker and also the ligand and complexes studied.[20]1H NMR, UV spectra . The IR spectra were recorded in the range of 4000–400 cm^{-1}, NMR spectra were recorded by also bruker and uv recorded by shimadazu 1800.

Methods

Synthesis of Reagent

Preparation of 5 amino –1,3,4- thiadiazole-2- thiol

The above compound was synthesized by Schiff base formation, thiosemicarbazone with carbon disulfide and ethanolic solution to reflux the material for 3-4 hrs. After reflux, the material was neutralized by 0.1 N HCl, and we get the white precipitate. [8]

*[N{3-(5-amino-2-thione-1,3,4 thiadiazole) methyl imidazol*e]

A mixture of equimolar quantities of thiadiazole compound and azole compound (imidazole) was refluxed in dry ethanol for 24 hrs.[9] The excess solvent was distilled off and solid material that separated was collected by filtration, suspended in water, and neutralized to get a free base. The product was filtered, washed with water, dried, and crystallized. Their analytical data are observed.

Cd(II) complex with reagent [N{3-(5-amino-2-thione-1,3,4 thiadiazole) methyl imidazole]

An equimolar concentration of reagent and metal led to the formation of metal complex. Mixing of reagent and metal solution to form a colorful precipitate was observed. This precipitate was dried and recrystallized to remove impurities.

Biological acivities

Procedure:

In vitro anti-inflammatory activity by Protein denaturation method

The reaction mixture (1 mL) consisted of 0.1 mL of egg albumin (from fresh hen's egg), 0.5 mL of Phosphate buffered saline (PBS, pH 6.4) and 0.4 mL of Sample A and Sample B at the concentration 1mg/ml. similar volume of double-distilled water served as control. Then the mixtures were incubated at (37 degree Celsius ±2) in an incubator for 15 min and then heated at 70 degree Celsius for 5 min. After cooling, their absorbance was measured at 660 nm by using vehicle as blank. Diclofenac sodium at concentration 1 mg/ml) was used as reference drug and treated similarly for determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula, % inhibition = absorbance of control - absorbance of test / absorbance of control x 100. Anti-inflammatory activity of different formulation by Protein denaturation.

Procedure: Antibacterial activity against E.coli, Bacillus subtilis, S Aurus , Salmonila Typibacteria by well diffusion method

The inoculums of the micro-organism were prepared from the bacterial cultures. 15
ml of nutrient agar (Hi media) medium was poured in clean sterilized Petri plates
and allowed to cool and solidify. 100 µl of broth of bacterial strain was pipette out
and spread over the medium evenly with a spreading rod till it dried properly. Once
the agar was hardened, then Sample Slides was placed on the plate in the manner
and the plates were incubated at 37^0C for 24 h. Antibacterial activity was evaluated by measuring the diameters of the zone of inhibitions (ZI).[11]

Procedure:

Antifungal activity against Candida albicans A.Nigar(Agar well plate diffusion Method)well difuusion method for the determination of zone of inhibition

Antifungal activity

Stock solution for antifungal activity: For antifungal study sample concentration of 5mg and10 mg stored in a refrigerator till further used.

Antifungal activities of the sample were evaluated by means of agar well diffusion assay.The assay was carried out according to the method of (Hufford *et al*., 1975). Sabourauddextrose agar (Hi media) was used for the growth of fungus. Media with acidic pH (pH 5.5to 5.6) containing relatively high concentration of glucose (40%) is prepared by mixing (SDA) Sabouraud dextrose and distilled water and autoclaved at 121^{o}C for 15 minutes.Twenty five ml of molten (45^{0}C) SDA medium was aseptically transferred into each100mm×15mm sterile Petri dish.

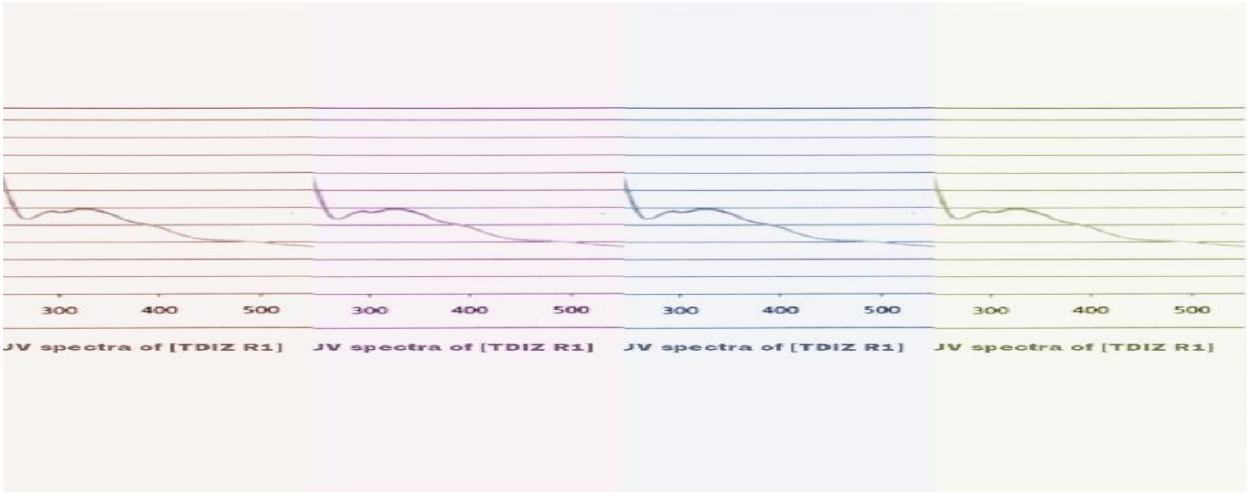
For counting of spore (fungi) were suspended in normal saline to make volume up to1ml and then counted with help of heamocytometer (neubar chamber). Once the agar was hardened,6mm wells were bored using a sterile cork borer. Then 0.1ml (100µl) from each stock solution of the sample having final concentration of 5 mg and 10mg was placed in each the well and the plates were incubated for 72 hour at 29^{o}C. The antifungal activity was measured as the diameter (mm) of clear zone of growth inhibition. (Umadevi *et al.,* 2003). [10,12,13]

Results

Ultraviolet visible spectroscopy [UV], Infrared radiation [IR] and protonated nuclear magnetic resonance[^{1}H NMR] spectral data, biological activities like Anti- Microbial (fungal/bacterial), Anti–Inflammatory of [N{3-(5-amino-2-thione-1,3,4 thiadiazole) methyl imidazole], [N{3-(5-amino-2-thione-1,3,4 thiadiazole) methyl imidazole] - Cd(II).

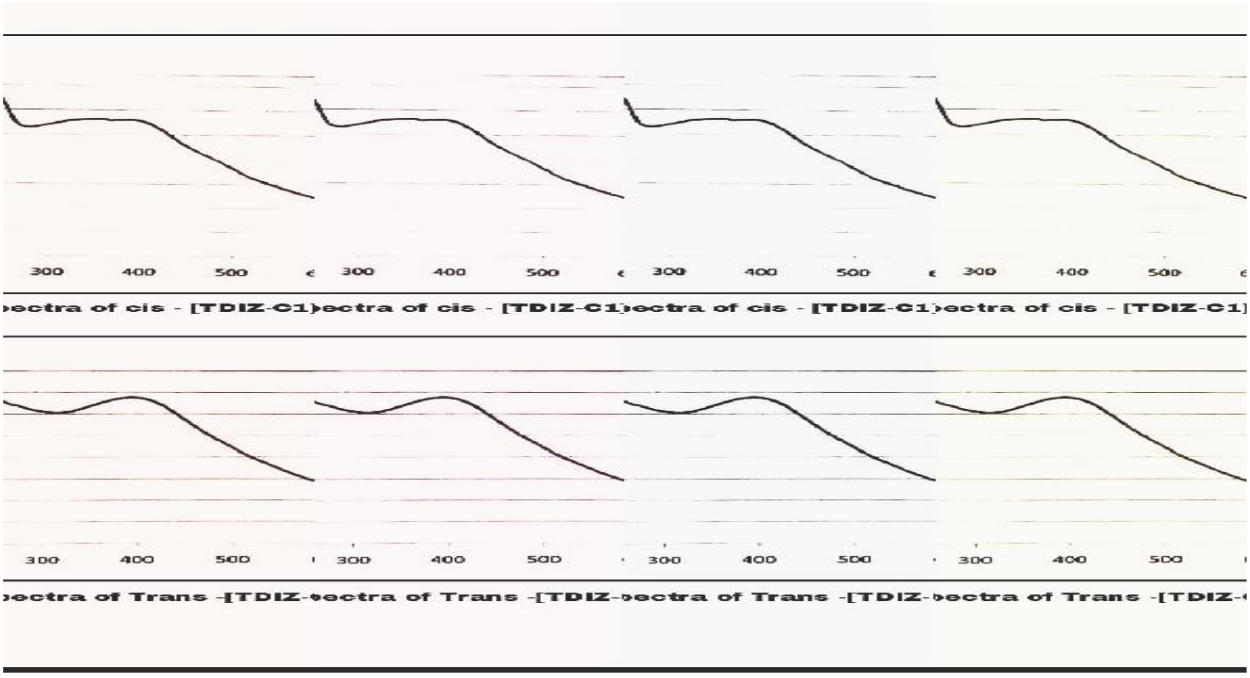
UV Spectra

The UV- Visible data can be used as a supporting evidence for structural Elucidation[20]. The UV and visible spectra of several Schiff base metal complex have been studied and show the graph in this paper. The electronic absorbation spectrum of TDIZ R1 in DMSO solvent in the UV visible region show the peaks at various intensity band 240nm~330 nm ,i.e $\pi - \pi *$ *and* $n - \pi *$ was observed in the graph.



For the cis-Cd(II) complex, three absorbation band were observed, one band observed at 220 nm, 382nm and another will be at 485nm.

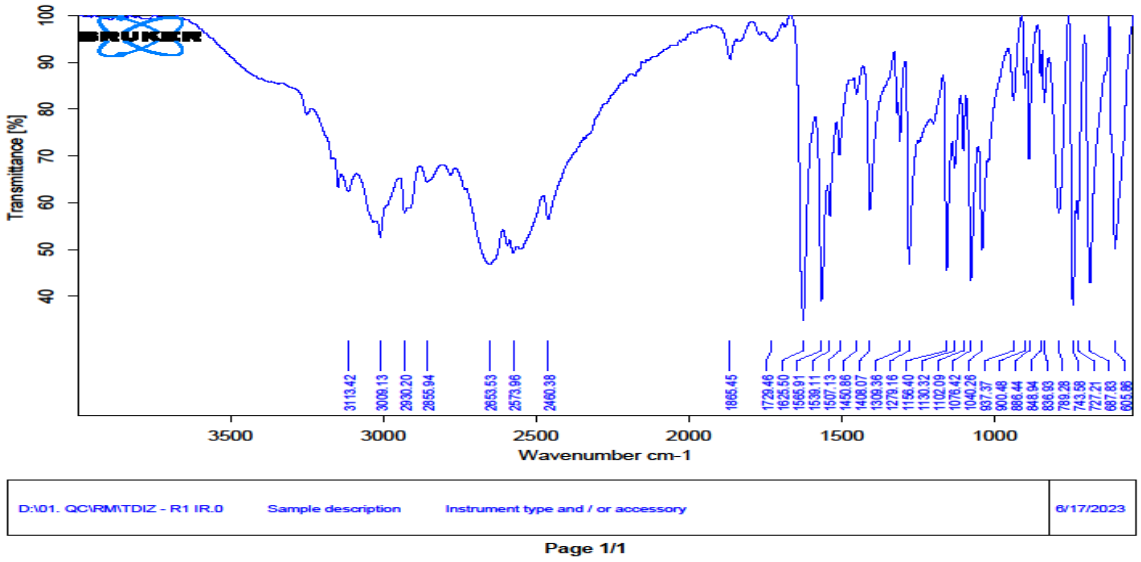
For the trans- For the trans-Cd(II) complex, three absorbation band were observed, one band observed at 225 nm, 382nm and another will be at 495nm.

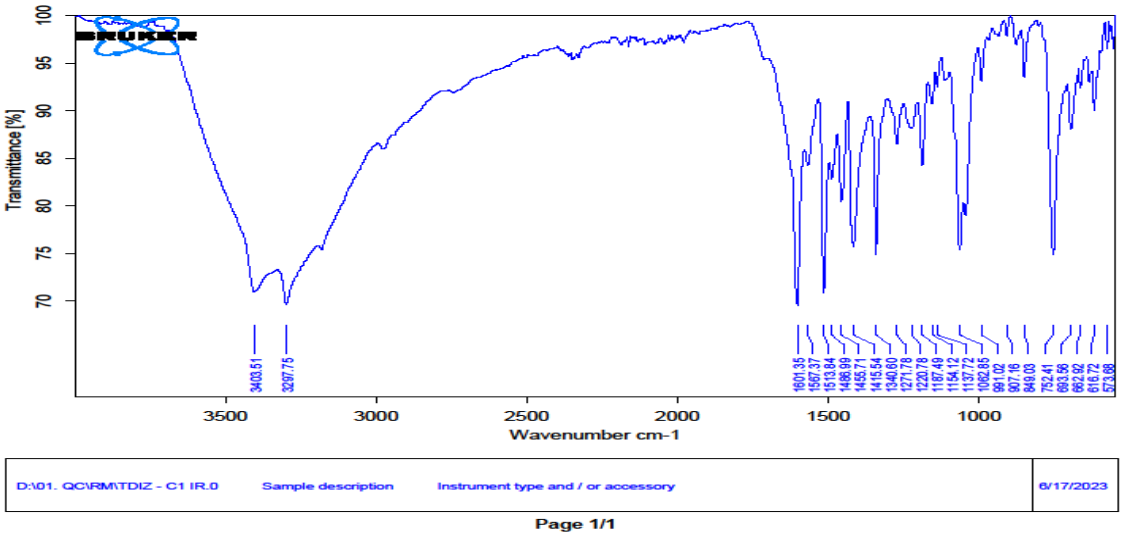


IR Spectra

[N{3-(5-amino-2-thione-1,3,4 thiadiazole) methyl imidazole], [N{3-(5-amino-2-thione-1,3,4 thiadiazole) methyl imidazole] - Cd(II)

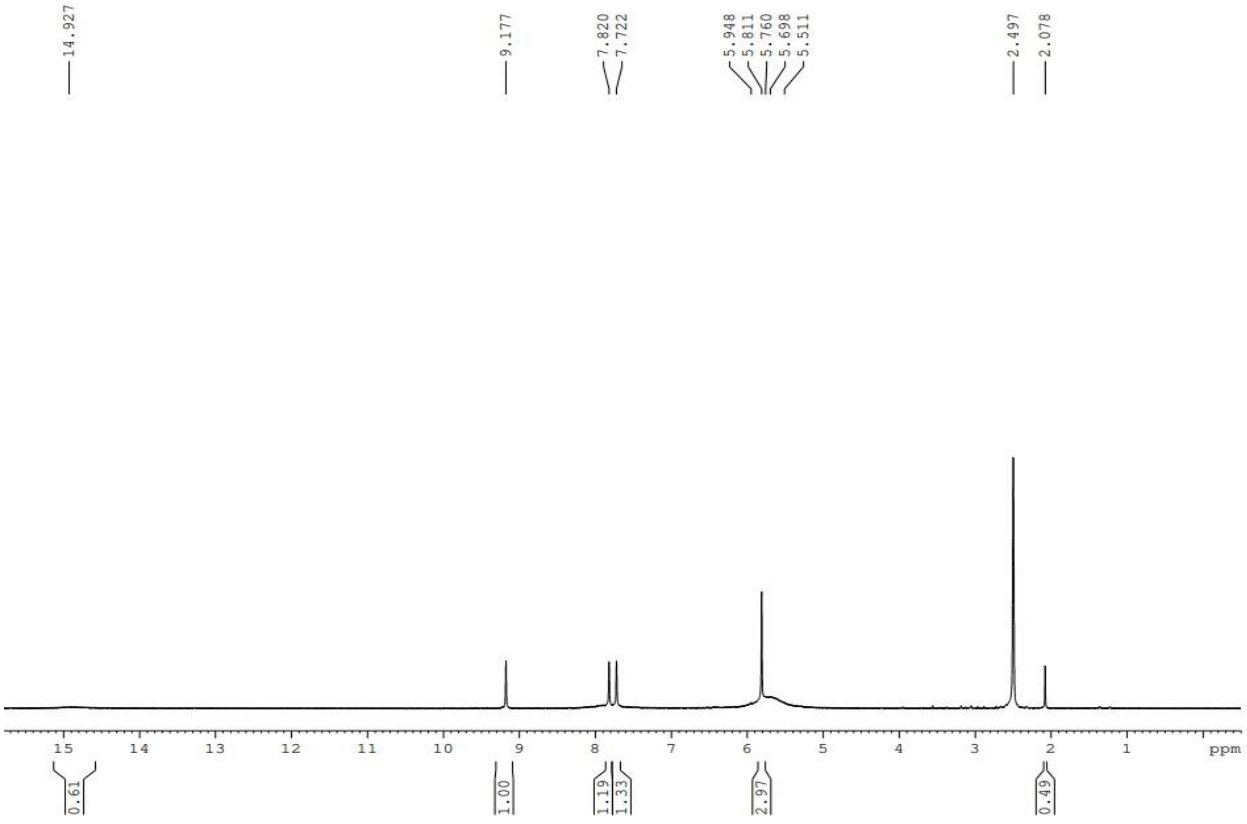
The C=C stretching vibration bands are observed at about show in graph. The presence of N-H and C=C; absorption bands also confirmed that the title compounds. These bands appear at below the graph.





NMR Spectra

In the ^{1}H NMR spectra of [N{3-(5-amino-2-thione-1,3,4 thiadiazole) methyl imidazole]- the characteristic downfield signal at δ [show in the graph]. ppm attributed to the -N=C-SH(-NH-C=S of the tautomer) in the compound is present, as is a sharp signal at δ …. [Show in the graph] .ppm attributable to the N-NH_2 group in the parent azoles.

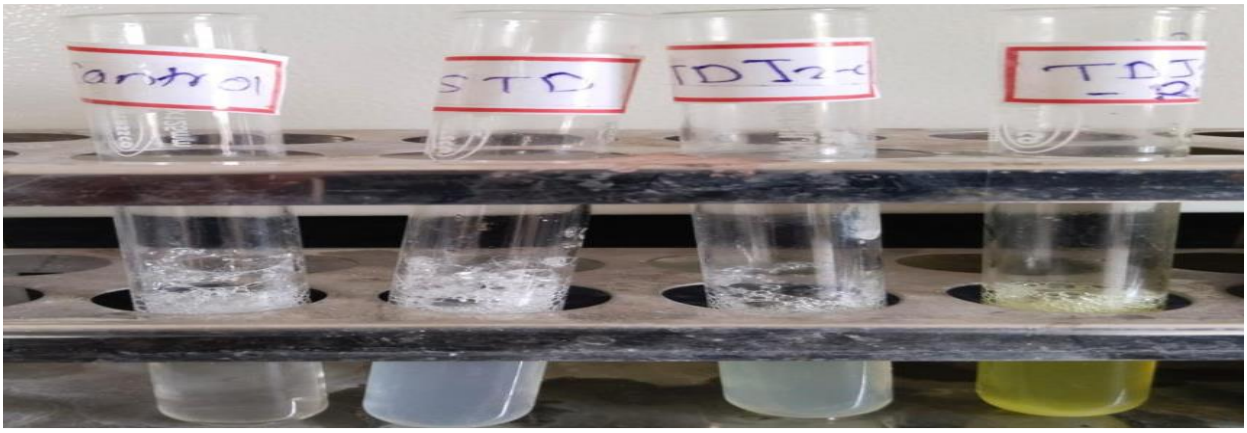


When [N{3-(5-amino-2-thione-1,3,4 thiadiazole) methyl imidazole] react with cadmium chloride to form a complex, the NMR spectrum of the complex may not show signals for the cadmium ion itself due to its paramagnetic nature. [22]However ,the ligand in the complex can still exhibit signals.

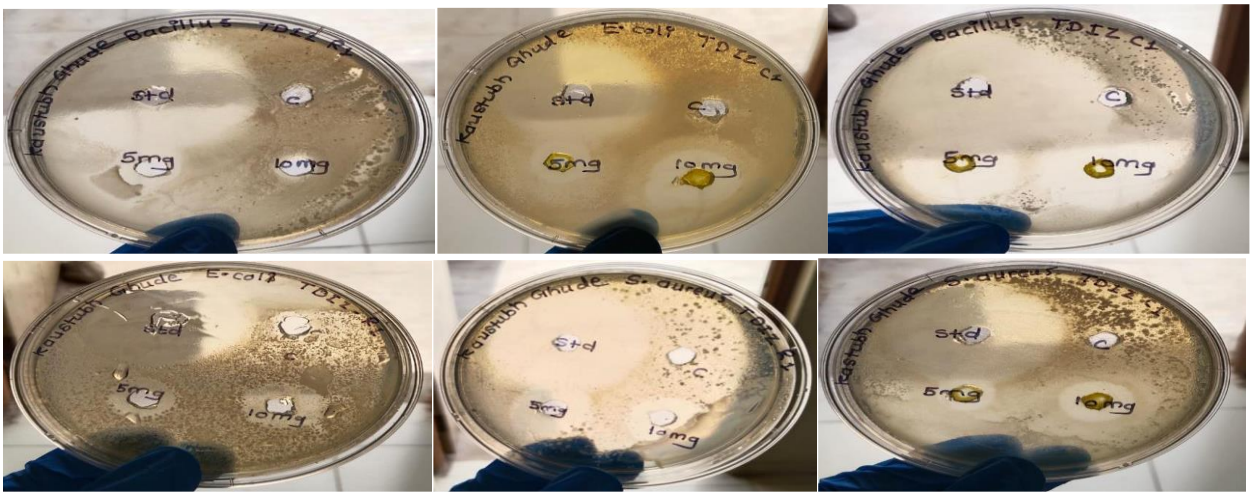
- Aromatic protons from thiadiazole and imidazole rings: 7.0-8.5(multiplets).
- Methyl protons from the methyl group attached to the imidazole ring: around 2.0ppm (singlet)
- Amino protons from the amino group: around 4.0-6.0 (singlet or multiplet).

Biological activities

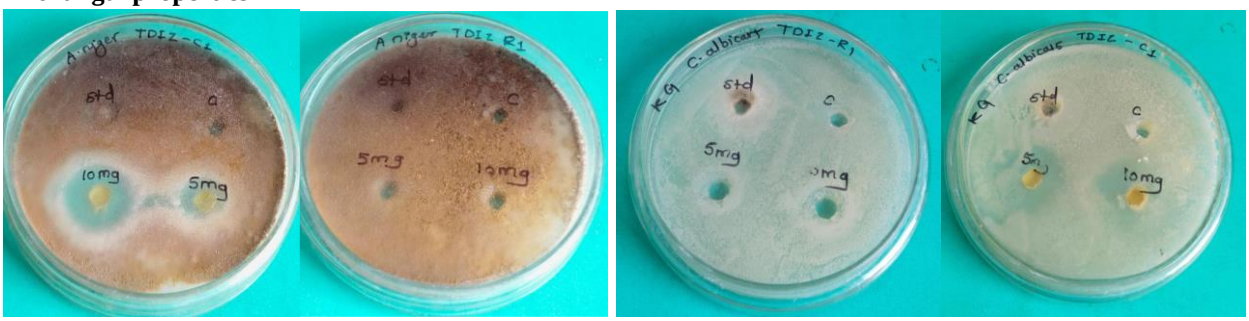
Anti inflammatory properties



Antibacterial properties



Antifungal properties



CONCLUSIONS

In conclusion, the formation of metal complex like Cd(II) using novel [N{3-(5-amino-2-thione-1,3,4 thiadiazole) methyl imidazole] was successfully achieved, followed by their conversion into target molecules. The spectral data and biological activities of the synthesized compound were also examined.

anti-inflammatory activity:
In conclusion All Samples: TDIZ-R1, Samples: TDIZ-C1 were used to carry out in vitro anti-inflammatory activity by using protein denaturation inhibition assay at the concentration 1mg/ml. The all Samples: TDIZ-R1, Samples: TDIZ-C1 showed good anti-inflammatory activity as compared to standard drug(Diclofenacsodium).

anti-bacterial activity:
The given Samples: TDIZ-R1, Samples: TDIZ-C1 used for the
antibacterial activity by using bacterial strain E.coli, Bacillus subtilis, S Aurus, SalmonilaTypi. Which at the concentration 10 mg Showed good activity as compared to standard.

antifungal activity: The given Sample –TDIZ-R1 Sample – TDIZ-C1 showed Good Antifungal activity.

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