SYNTHESIS, CHARACTERIZATION, IN VITRO ANTIMICROBIAL, ANTHELMINTIC AND DOCKING STUDIES OF NEW 2-[(E)-[(4-1H,1,2,4-TRIAZOL-1-YLMETHYL)PHENYL]IMINO] METHYLPHENOL, AND THEIR COMPLEXES WITH 3D METAL IONS

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ABSTRACT

Objective: The main objectives of this research work is the synthesis and characterization of biologically potential triazole ring containing the Schiff base ligand and their transition metal complexes, followed by screenings of their antimicrobial and anthelmintic activity the results of antimicrobial activity were compared with docking scores.

Methods: The coordination complexes of Co(II), Cu(II), Fe(III) and Zn(II) with Schiff base derived ligand 4-(1H,1,2,4-triazol-1-ylmethyl) aniline and substituted aldehydes have been synthesized. The complexes are characterized by elemental analysis, conductivity measurements, electronic, IR, and 1H NMR spectral data. The synthesized compounds were also screened in vitro antimicrobial activity was carried out according to diffusion method using agar and potato dextrose agar at 100, 500 and 700 mg/ml concentrations in DMF. HEX 8.0 programers were used to perform the docking experiments on nucleotides of S. pyphi at as ligand [PDB: 5860].

Results: Schiff base ligand and their transition metal complexes were studied for antimicrobial activity as well as docking. The results of both studies concluded that 4a, 4c and 4d compounds are more active in minimum inhibition concentration (30 μg/ml) against Staphylococcus aureus (S. aureus), Salmonella typhi (S. typhi) bacteria and Penicillium chrysogenum (P. chrysogenum) fungi. The compounds showed highest docking score (-257.47, -275.61 and -280.17 respectively) with the secondary structure of the alpha-amylase with a nucleotide from S. pyphi in the solid model. In the study of anthelmintic activity among these three compounds, 4d compound exhibits more activity compared with the standard.

Conclusion: The compounds 4a, 4c and 4d were found to be more promising pharmacological activity this observation may promote a further development of this triazole group of compounds which may lead to better pharmacological profile than standard drugs.

Keywords: Schiff bases, Triazoles, Metal complexes, Antimicrobial activity, Docking study

INTRODUCTION

Inorganic elements play a crucial role in biological, medical processes, and it is evident that many organic compounds used in medicine are not purely organic; most of them are activated or biotransformed by the metal ions. [1-3]. The first Schiff base compounds were reported by Hugo Schiff in 1864. In recent years, the chemistry of Schiff bases contains a donor atom and their transition metal complexes, followed by screenings of their antimicrobial and anthelmintic activity the results of antimicrobial activity were compared with docking scores.

Among this pharmacologically important heterocyclic compounds, 1,2,4-triazole with azomethine linkage and derivatives have been known to possess a wide range of biological properties, in our community and have become a threat to human life worldwide.

MATERIALS AND METHODS

Triazol amine, aldehyde and all other chemicals were procured from the Sigma-Aldrich (INDIA), Himedia (INDIA), Lobo Chemicals (INDIA) (Commercially available from local sources) and were used as received without further purification. Freshly distilled solvents were employed for all synthetic purposes. Spectroscopic grade solvents were employed in spectral works.

All other chemicals were of AR grade. The progress of every reaction was monitored by TLC. Yield refers to the isolated yields after crystallization of the compounds that have a purity of ≥95%. 1H NMR spectra were recorded on a JNM-ECS-400 NMR spectrometer at 399.78 and Bruker avance III, 400 MHz, 9.4-tesla magnet with chemical shifts reported in ppm relative to the residual deuterated solvent or the internal standard tetramethylsilane. Elemental analyses were carried out with a Perkin-Elmer 2400 Series II H, C and N analyzer. UV-Vis spectra recorded on varian, Cary 5000. Melting points were determined in an electrically heated apparatus by taking the sample in a glass capillary sealed at one end.

Preparation of the Schiff base ligand

The Schiff base ligand was prepared by condensation of 4-(1H,1,2,4-triazol-1-ylmethyl)aniline (0.01 mol) with 2-hydroxybenzaldehyde (0.01 mol) in a 1:5 ml ethanol solvent the resulting mixtures was refluxed on a water bath for about 3-4 h; reaction was monitored by TLC. After completion of reaction reacting mixture was cooled to room temperature, the yellow color crystals were separated out, which was filtered and washed with cold ethanol, dried and recrystallized in ethanol. M. P. 108±1°C (Yield 75%), IR (KBr, cm⁻¹) 3407 (br), 3093.6 (w), 1620.9 (s), 1571.88 (s), 1512.09 (s), 1456.16 (m), 1269 (s), 1174.5 (s), 1018.3 (s). 1H NMR (DMSO-d6, 400 MHz) 11.61 (1H, s, OH), 8.95 (1H, s, CH-Triazole), 8.74 (1H, s, CH-Triazole).
Preparation of the complexes

An alcoholic solution of Schiff base (45 ml, 0.002 mol) was added to the alcoholic solution of CoCl$_2 \cdot 6$H$_2$O, CuCl$_2 \cdot 2$H$_2$O, FeCl$_3$ and ZnCl$_2$ (5 ml of 0.001 mol) metal salt, one at a time, the resultant mixture colour change instantly and it was refluxed on a water bath for about 3 h. Then, the reaction mixture was cooled to room temperature; the solid separated was collected by filtration, washed thoroughly with water, then ethanol followed by either, finally dried in vacuum over fused CaCl$_2$.

Scheme 1: Synthesis of 2-[(E)-[[4-(1H-1,2,4-triazol-1-ylmethyl)phenyl]imino]methyl]phenol

Scheme 2: synthesis of metal complexes with ligand, M=Co, Cu, Zn and Fe

In vitro antibacterial and antifungal assay

The antibacterial and antifungal activities at different concentration of newly synthesized trazole Schiff base ligand and their Fe(III), Co(II), Cu(II) and Zn(II) complexes have been studied by using agar and potato dextrose agar on the basis of the previously reported diffusion method [16-18]. The antibacterial and antifungal activities were done at 100, 500 and 700 mg/ml concentrations in DMF using three bacteria (S. aureus, Bacillus subtilis (B. subtilis), s. typi) and two fungi (Aspergillus niger (A. niger) P. Cryosugenum). The bacterial strains were incubated for 24 h at 37 °C and fungal stains were incubated for 48 h at 37 °C. Standard for antibacterial (Chloramphenicol, Fluconazole) and antifungal drugs (Kanamycin) were used for comparison under similar condition.

Anthelmintic activity (in vitro)

The anthelmintic assay was evaluated by the method given in the literature [18] with minor modifications. The assay was performed on adult Indian earthworms, Peretima posthuma, due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings [18]. Earthworms (P. posthuma) collected from moist soil and washed with normal saline to remove all fecal matter were used for the anthelmintic study. The earthworms of 3 to 4 cm in length and 0.1 to 0.2 cm in width were used for the experimental protocol. The compounds were subjected to anthelmintic studies against earthworms at 2 and 10 mg /ml by using Albendazole as a standard drug. The paralyzing and death time was noted, and their mean was calculated for triplicate sets. The death time was ascertained by placing the earthworms in warm water (50 °C) which stimulated the movement if the worm was alive.

The results of anthelmintic studies are tabulated in table 5.

RESULTS AND DISCUSSION

All the metal complexes were stable at room temperature and non-hygrosopic in nature. On heating, they decompose at high temperatures. The complexes were insoluble in water but are soluble in organic solvent. The elemental analysis, physical properties, and analytical data of the ligand and complexes were summarized below.

IR spectra

The electronic spectra of the ligand and all the complexes were recorded in DMF at room temperature. The absorption band of the
The electronic spectra of the Cu(II) complex showed the broad band at 12820 cm⁻¹ due to 4T₂ (F) → 4T₁g (P) transition for Cu(II) complex. On the basis of electronic spectra tetrahedron geometry around Cu(II) ion is suggested. The conductivity measurement showed that the complex was non-electrolytic in nature.

The electronic spectra of the Fe(III) complex showed a strong absorption band at 22471 cm⁻¹ and it was not possible to identify the type of the d-d transition, this is due to a strong charge-transfer (CT) band appeared from the UV-region to the visible region. Generally, a tentative interpretation expects the structure of Fe(III) complex to be tetrahedron geometry; the conductivity measurement showed that the complex was non-ionic, Table 2.



In Zn(II) complex do not possess any d-d transitions due to d[10] configuration, the conductivity measurement showed that the complex was non-ionic, and possible geometry of the complex was predicted as a square planar.

### Antimicrobial activity

Antibacterial activity carried out by a well diffusion method using the nutrient agar medium, DMSO as control and chloramphenicol is used as a standard bactericide. Antifungal activity was carried out by a well diffusion method using potato dextrose agar (PDA) medium, DMSO as control and fluconazole is used as a standard fungicide [20, 21]. The results of the biological properties of the synthesized compounds are screened for bacterial and fungal strains (Tables 3 and 4 respectively).

### Table 3: Results of the antibacterial activity–zone of inhibition

| Compounds      | Growth Inhibition against bacteria in mm | Growth inhibition against fungi in mm |
|----------------|-----------------------------------------|--------------------------------------|
|                | S. aureus | B. subtilis | S. typhi | A. niger | P. crysogenum |
| 3a 100 µg/ml   | 4.0±0.1   | 1.0±0.0    | 1.0±0.0 | 7.0±0.0 | 3.0±0.0       |
| 3a 500 µg/ml   | 4.1±0.1   | 1.0±0.0    | 1.0±0.0 | 7.0±0.0 | 3.0±0.0       |
| 3b 700 µg/ml   | 5.0±0.0   | 2.0±0.0    | 1.9±0.0 | 12.9±0.1| 6.0±0.0       |
| 4a 100 µg/ml   | 4.0±0.0   | 8.0±0.0    | 3.1±0.1 | 1.0±0.0 | 7.0±0.0       |
| 4a 500 µg/ml   | 3.9±0.1   | 10.9±0.0   | 7.0±0.0 | 3.9±0.1 | 9.0±0.0       |
| 4b 700 µg/ml   | 4.1±0.1   | 13.0±0.0   | 7.1±0.1 | 4.0±0.0 | 10.0±0.0      |
| 4b 500 µg/ml   | 1.0±0.0   | 8.9±0.0    | 1.0±0.0 | 1.0±0.0 | 8.0±0.0       |
| 4b 700 µg/ml   | 1.9±0.05  | 11.0±0.0   | 1.0±0.0 | 3.0±0.0 | 9.0±0.0       |
| 4c 110 µg/ml   | 3.9±0.07  | 11.9±0.0   | 3.9±0.0 | 4.9±0.05| 10.9±0.0      |
| 4c 500 µg/ml   | 11.9±0.1  | 1.0±0.0    | 13.0±0.0| 2.0±0.0 | 3.0±0.0       |
| 4c 500 µg/ml   | 14.0±0.0  | 3.0±0.0    | 10.9±0.0| 3.0±0.0 | 5.0±0.0       |
| 4c 700 µg/ml   | 11.9±0.0  | 3.0±0.0    | 10.0±0.0| 4.0±0.05| 6.0±0.0       |
| 4d 100 µg/ml   | 3.0±0.0   | 11.0±0.0   | 1.0±0.0 | 6.0±0.05| 5.0±0.0       |
| 4d 500 µg/ml   | 4.9±0.05  | 12.9±0.0   | 2.9±0.0 | 9.0±0.05| 7.0±0.0       |
| 4d 700 µg/ml   | 6.9±0.1   | 13.9±0.0   | 5.0±0.0 | 12.0±0.0| 8.0±0.0       |
| Std 1 100 µg/ml| 4.9±0.05  | 3.9±0.0    | 3.9±0.0 | 6.0±0.0 | 7.0±0.0       |
| Std 1 500 µg/ml| 7.0±0.0   | 7.0±0.0    | 6.9±0.05| 9.0±0.0 | 8.0±0.0       |
| Std 1 700 µg/ml| 8.0±0.0   | 8.0±0.0    | 8.0±0.0 | 10.0±0.0| 9.0±0.0       |
| Std 2 100 µg/ml| 7.0±0.0   | 7.0±0.0    | 7.0±0.0 | 7.0±0.0 | 7.0±0.0       |
| Std 2 500 µg/ml| 8±0.0     | 8±0.0      | 8±0.0  | 8±0.0   | 8±0.0         |
| Std 2 700 µg/ml| 9±0.0     | 9±0.0      | 9±0.0  | 9±0.0   | 9±0.0         |

Less than 8 mm—inactive; 10–12 mm—moderately active; above 12 mm—highly active, *Chloramphenicol; Fluconazole, (sample size n=3)
Most of the synthesized compounds showed inhibition property against the strains used. Among the test samples, Co(II), Zn(II) and Fe(III) complexes showed more activity when compared to the standards used. After comparing the zone of inhibition, the selected compounds were checked for their MIC (minimum inhibition concentration) values. The MIC values of less than 30 μg/ml are shown in table 3. It is observed that 4a, 4c, and 4d compounds showed most promising activity. The compounds Co(II) and Fe(III) complexes have been considered as good inhibitors and also shows over inhibition relative to the previously reported compounds [18].

| Compound | Conc. (μg ml⁻¹) | Growth inhibition against bacteria in mm | Growth inhibition against fungi in mm |
|----------|----------------|----------------------------------------|-------------------------------------|
|          |                | S. aureus | B. subtilis | S. typhi | A. Niger | P. Cryosogenum |
| 4a       | 30 μg/ml       | 1±0.1    | 15±0.2     | 8±0.1   | 12±0.0   | 15±0.1           |
| 4c       | 30 μg/ml       | 14±0.2   | 8±0.2      | 13±0.0  | 8±0.1    | 11±0.0           |
| 4d       | 30 μg/ml       | 13±0.1   | 1±0.2      | 14±0.1  | 13±0.0   | 10±0.1           |

Less than 8 mm-inactive; 10-12 mm-moderately active; above 12 mm-highly active, (n-3)

**Anthelmintic activity**

The results of anthelmintic activity of synthesized compounds are collected in table 5. It is clear that all the newly synthesized triazole compounds exhibited more activity than the standard, against the earthworms used. The concentration of test samples and the standard used were 10 mg/ml in DMF. The impact of most of the compounds was more than that of the standard. The results are concluded that the activity of the complex is more potential is may be due to the presence triazole ring.

| Compounds | Time in min |
|-----------|-------------|
|           | Pin pinch  | Paralysis | Death |
| 3a        | 24.07±0.1  | 28.45±0.1 | 37.26±0.1 |
| 4a        | 12.45±0.2  | 15.00±0.1 | 28.34±0.2 |
| 4b        | 17.34±0.0  | 25.49±0.0 | 29.05±0.0 |
| 4c        | 21.50±0.1  | 27.03±0.2 | 36.00±0.1 |
| 4d        | 11.42±0.1  | 16.13±0.1 | 24.05±0.2 |
| Albendazole (Stan.) | 18.0±0.2 | 25.0±0.1 | 28.20±0.0 |
| DMF       | 18.30±0.0  | 32.47±0.0 | 46.08±0.1 |

Less than 18 min-highly active; 18-25 min-moderately active; above 25 mm-inactive, (n-3)

**Molecular docking studies**

Molecular docking studies were conducted in order to validate the obtained pharmacological data and to provide understandable evidence for the observed antimicrobial activity of all synthesized compounds. Molecular docking study is a well-established technique to determine the interaction of two molecules and find the best orientation of ligand would form a complex with minimum overall energy. All the synthesized compounds were docked Structure of TREXI in complex with a nucleotide of S. typhi at ten different orientations. The structure of the protein mentioned above [PDB: 3B60] was retrieved from the Protein Data Bank [www. rcsb. org (DOI: 10.2210/pdb3b60/pdb)] and further modified for docking calculations. The ligand molecules were drawn and analysed using Chem Draw Ultra 8.0. 3D, coordinates were prepared using dock server. It was observed that the most active compound of the series, i.e., Co(II) and Fe(III) was predicted to be a most significant antibacterial activity are also found to have good docking scores as shown in the fig. 1 The acting force of this binding mode mainly depends on hydrogen bonding, electrostatic forces, van der Waals forces and hydrophobic interaction due to non-polar residue interaction and water structure effect alteration. For the receptor, all compounds exhibited more binding interaction energy with a least docking score expect legend compare to the standard and hence complexes have been considered as good inhibitors and also shows potential over inhibition relative to the previously reported compounds [18].

| Correlation type | Shape only |
|------------------|------------|
| Grid Dimension   | 0.6        |
| Receptor range   | 180        |
| Ligand Range     | 180        |
| Twist range      | 360        |
| Distance Range   | 40         |

**Table 4: Results of the antifungal activity–minimum inhibition concentration**

**Table 5: Anthelmintic activity of the legend and their complexes**

**Table 6: Parameters used for docking study**

| Compounds | Tyrosine kinase (RTK) |
|-----------|-----------------------|
| 3a        | -177.58               |
| 4a        | -257.47               |
| 4b        | -250.47               |
| 4c        | -275.61               |
| 4d        | -280.17               |
| Standard  | -205.28               |
CONCLUSION

Based on the reported results, it is concluded that the ligand act as bidentate, coordinating through one of the nitrogen atom and the oxygen atom. In the present investigations, all the complexes are found to be mononuclear, based on spectral evidence possible geometry of the synthesized compounds are predicted. The antimicrobial activity results indicated that some of the tested compounds showed the most promising antibacterial and antifungal activities. These observations may promote a further development of our research in this field. Further development of this group of compounds may lead to better pharmacological profile than standard drugs and serve as templates for the construction of better drugs to combat bacterial and fungal infection. After studying the docking poses and binding modes of the docked compounds, the necessity of hydrogen bond formation for enhancing the activity of this class of compounds can be highly advocated.

ACKNOWLEDGMENT

The authors are thankful to Department of Chemistry Central College Campus, Bangalore University for the Elemental analysis, IR spectral analysis.

CONFLICT OF INTERESTS

Declared none

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How to cite this article
• Jithendra Kumara KS, Krishnamurthy G, Sunil Kumarn. Synthesis, characterization, \textit{in vitro} antimicrobial, anthelmintic and docking studies of NEW 2-[(E)-{[4-(1H-1,2,4-triazol-1ylmethyl) phenylimino]methyl}phenol, and their complexes with 3D metal ions. Int J Pharm Pharm Sci 2016;8(9):134-139.