BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, June 2023. Vol. **20**(2), p. 681-689

Diamagnetic Zn(II) and Hg(II) Complexes with Fluconazole: Synthesis, Spectral Characterization and Biological Investigation

P. Keerthika[1]*, S. Balasubramaniyan[1] and R. Govindharaju[2]

[1]Department of Chemistry, Government Arts College (Affiliated to Bharathidasan University, Tiruchirappalli), Ariyalur-621713, Tamil Nadu, India.
[2]Department of Chemistry, Thanthai Hans Roever College (Autonomous), (Affiliated to Bharathidasan University, Tiruchirappalli), Perambalur - 621 220, Tamil Nadu, India.

http://dx.doi.org/10.13005/bbra/3122

(Received: 26 January 2023; accepted: 11 May 2023)

In order to create new mononuclear diamagnetic complexes (M = Zn (II) and Hg (II)), fluconazole and thiocyanate ion ligands were utilized. Physicochemical and spectroscopic approaches were used to describe the synthesized metal complexes. The spectral data show that the fluconazole served as a bidentate ligand and linked to metal ions via the nitrogen of the imine group, the thiocyanate ion connected to metal ions through nitrogen. The antibacterial capacities of the strains of Candida albicans (MTCC 183) for fungi and Escherichia coli (MTCC 732) for bacteria were assessed using the disc diffusion method. The results showed that Zn (II) and Hg (II) complexes have much stronger antibacterial activity than pure ligands because of metal chelation. The complexes interactions with the stable free radical DPPH are measured. The free radical scavenging activities of the complexes and the ligand have been determined by measuring their interaction with the stable free radical DPPH. The complexes have larger antioxidant activity as compared to the ligands.

Keywords: Antimicrobial; Diamagnetic; Fluconazole; Thiocyanate ion.

Fungal strains that cause invasive infections have been posing a serious threat not only to human health all over the world but also to agriculture and the environment[1-3]. The Aspergillus, Candida, Cryptococcus, and Pneumocystis species are primarily to blame for the annual mortality of 1.5 to 2 million people from fungal infections[4]. Patients with compromised immune systems, such as those who have received hematopoietic stem cells or solid organ transplants, and HIV-infected individuals are particularly vulnerable to these pathogens[5]. Currently, fungal infections are treated with four classes of organic compounds categorized according to their mode of action: polyenes like amphotericin B and nystatin, azoles like caspofungin and micafungin, echinocandins like micafungin, and antimetabolites like 5-fluorocytosine[6]. Due to their broad-spectrum antifungal activity, superior efficacy, and acceptable toxicity profile, azoles are the most commonly used antifungal agents[7]. The enzymes (cytochrome P450) involved in the synthesis of ergosterol, a component of the fungal cell membrane, are inhibited by this class of antifungal agents[8,9]. Fluconazole (fcz), a triazole that belongs to the first generation of azoles, was residential to treat infections caused by Candida, such as C. albicans, C. tropicalis, C. parapsilosis, and dermatophytes. In contrast, Aspergillus

*Corresponding author E-mail: lathika.ttp@gmail.com

species or other hyphomycetes, C. krusei, and other hyphomycetes are naturally resistant to fluconazole, whereas C. glabrata occasionally exhibits reduced sensitivity. Additionally, the overuse of this antifungal agent has resulted in a rapid rise in the number of fluconazole-resistant strains[10]. Due to fluconazole's limited antifungal spectrum, development of resistance, and other drawbacks, it is urgent to discover novel compounds that can address these issues.

Diamagnetic Zn(II) and Hg(II) metal complexes containing fluconazole (Fig.1) and thiocyanate ion as ligands will be synthesized and characterized spectrally using microwave assisted synthesis as the primary objectives of this investigation. The biological research is also the focus of the ligands and their complexes.

Experimental Method

Materials

Fluconazole, metal nitrate, and potassium thiocyanate (KSCN) were obtained from the Alfa Aaser Company and used as such. The utilized organic solvents, namely, The AnalaR-grade dimethyl sulfoxide (DMSO), dimethylformamide (DMF), methanol, and ethanol were utilized as-is without further purification.

Synthesis of Zn(II) complex

1g (3.33 mmol) of fluconazole in methanol and 1.00g (3.40 mmol) of KSCN in ethanol were added to the methanolic solution of zinc nitrate (1.00g (3.33 mmol), and this was followed by microwave irradiation for few minutes after each addition by using IFB 25 BG-1S model microwave oven. The resulting precipitate was filtered off, washed with 1:1 ethanol: water mixture and dried under vacuum. A colorless complex was obtained with the yield of 42.44%.

Synthesis of Hg(II) complex

1.11g (3.60 mmol) of fluconazole in methanol and 0.54g (8.40 mmol) of 0.75g (7.50 mmol) of KSCN in ethanol were added to the methanolic solution of mercury chloride contained 1.00g (3.42 mmol) and this was followed by microwave irradiation for few minutes after each addition by using IFB 25 BG-1S model microwave oven. The resulting precipitate was filtered off, washed with 1:1 ethanol: water mixture and dried under vacuum. A colorless complex was obtained with the yield of 47.02%.

Instrumentations

Thermo Finnegan's Flash EA1112 Series CHNS(O) analyzer was used for the C,H,N elemental analyses. At 30°C, 10-3 M solutions of the metal complex in acetonitrile were used for the molar conductivity measurements with a Systronic Conductivity Bridge (model 304). On a Varian, Cary 5000 model UV-Vis Spectrophotometer, the complexes' UV-Visible spectra were taken. In KBr discs at room temperature, infrared spectra of the complexes and ligands were recorded using a Perkin Elmer Spectrum RX-I FT-IR Spectrometer. The JES FA 200 EPR Spectrometer was used to take measurements at room temperature of the copper complex's electron paramagnetic resonance spectra.

Antimicrobial activity

The free fluconazole and its complexes were tested for in vitro antimicrobial activity by the well diffusion method using the agar nutrient as the medium. The antibacterial and the antifungal activities of the ligands and their complexes were evaluated by the well diffusion method against the strains, cultured on potato dextrose agar as medium. In this typical procedure[11], a well was made on the agar medium inoculated with the microorganisms. The well was filled with the test solution using a micropipette and the plate was incubated for 24 hours for bacteria and 72 hours for fungi at 35oC. At the end of the period, the inhibition zones formed on the medium were evaluated as millimeters (mm) diameter12. The microbial strains employed in the biological assays were Escherichia coli (MTCC 732) for bacteria and Candida albicans (MTCC 183) for fungal strains, obtained from Microbial type culture collection (MTCC) at the institute of Microbial Technology (IMTECH), Chandigarh, India.

Antioxidant activity

Antioxidant testing DPPH (1,1-diphenyl-2-picryl-hydrazyl) was created as a set assay for in vitro radical scavenging evaluation[13] because it exhibits immediate and constant activity for the examined substances. Ascorbic acid (Vitamin C) was used as the standard medication, and the DPPH radical scavenging activity was determined by measuring the change in DPPH's molar absorbance value at 517 nm at various doses (100, 200, 300, 400, and 500 g/mL) in DMSO[14]. One mL of the

aforementioned solutions should be added to one mL of DPPH solution (5 mg per 100 mL) produced in DMSO. The volume of the resulting solution should then be increased with more DMSO. The prepared mixture was shaken briskly and kept at room temperature for about 30 minutes in complete darkness. DPPH and DMSO were used as a reference or blank to determine the compounds' molar absorbance at 517 nm. The colour change of the solution and the DPPH values at 517 nm, which both help measure the antioxidant's radical-scavenging ability, are proof that the antioxidant is actively scavenging DPPH radicals by donating a hydrogen radical or electron to create a stable DPPH-H molecule. The proportion of DPPH's free radical generation that was prevented by radical scavenging activity was calculated using the formula below:

Where Abscontrol stands for the absorbance of the blank and Abssample for the absorbance of the sample. Each test experiment was carried out in triplicate, the data were averaged, and the mean and standard deviation were calculated. A graph is drawn between the concentration (g/mL) on the X-axis and the % scavenging effect on the Y-axis[15,16].

RESULTS AND DISCUSSION

Elemental analysis

The molecular formulas for the metal complexes were derived from the elemental analytical data. It matched the theoretical values perfectly. Based on each complex's molecular formula[17] (Table 1), the theoretical values in parentheses are calculated.

Molar conductance

Molar conductance tests on the complexes with acetonitrile as the solvent revealed that the complexes did not behave like electrolytes at a concentration of 10-3M[18,19]. Complexes can be formulated as non-electrolyte nature (1:0).

UV-Visible spectrum of complexes

The d shell is complete and unavailable for bonding in the Zn(II) complex. The absence of d electrons in the metallic bond may be the reason why the metal is so soft in comparison to other transition metals. Zn2+ ions lack a ligand field stabilization effect due to their complete d shell. The charge transfer[20] transition CT band is shown as an absorption band at 32258 cm-1 in the electronic spectrum of the Zn(II) complex in Fig.2. The empirical formula suggests that the Zn(II) complex has a tetrahedral geometry with sp3 hybridization and is diamagnetic.

The diamagnetic Hg(II) complex d10 has no bonding potential because its d shell is completely filled. The absence of d electrons in the metallic bond probably accounts for the metal's relatively softness in comparison to other transition metals. Because of its complete d shell, Hg2+ ions have no effect on stabilizing the ligand field. In the electronic spectrum, the Hg(II) complex has only one absorption band at 37735 cm-1, which is known as the charge transfer transition CT band[21] in Fig.2. The Hg(II) complex is said to have a tetrahedral geometry with sp3 hybridization based on analytical, conductance, and spectral data.

FT-IR Spectra of free ligands and their complexes

The FT-IR spectra of the free ligands and their complexes were recorded in the 4000-400 cm-1 range[22]. According to C=N stretching vibrations, the fluconazole FT-IR spectrum contains strong and weak absorption bands at 1612 cm-1 and 1597 cm-1, respectively[23,24]. The stretching vibrations of C-N revealed distinct peaks at 3298 cm-1 (OH stretch), 1582 cm-1 (C=C aromatic symmetric stretch), and 1512 cm-1 (C=C aromatic asymmetric stretch) at 1107 cm-1 strongly and weakly. According to the bands at 1509 cm-1 and 1098 cm-1 that were shifted to

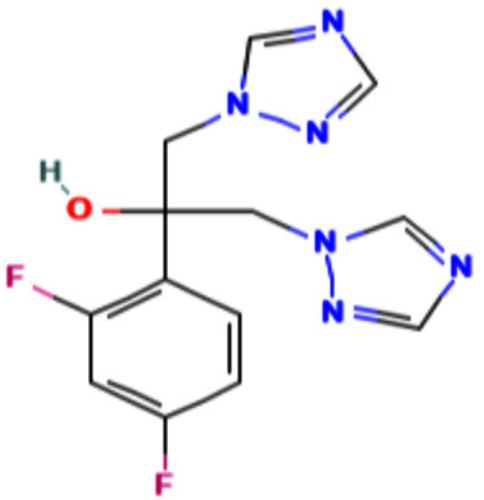

Fig. 1. Structure of fluconazole

lower frequencies, fluconazole was coordinated to metals in a bidentate manner through the imine nitrogen of C=N in metal complexes[25,26].

The IR spectra of the complexes organized have been additionally in comparison with the spectra stated for the thiocyanato complexes. The C-N stretching frequency of N-bonded complicated of thiocyanate ion became almost 2050 cm-1 shown in Fig.3 and Fig.4, It became moved via way of means of 58-23 cm-1 to better frequency variety in all of the complexes[27].

The new bands are found in the spectra of the all synthesized metal complexes, suggesting that the asymmetric and symmetric vibrations of metal-nitrogen ?(M-N) bond as other evidence for binding of ligand to metal center are seen at 460-490 cm–1 as moderate and weak peaks28. This indicates that the ketoconazole coordinates

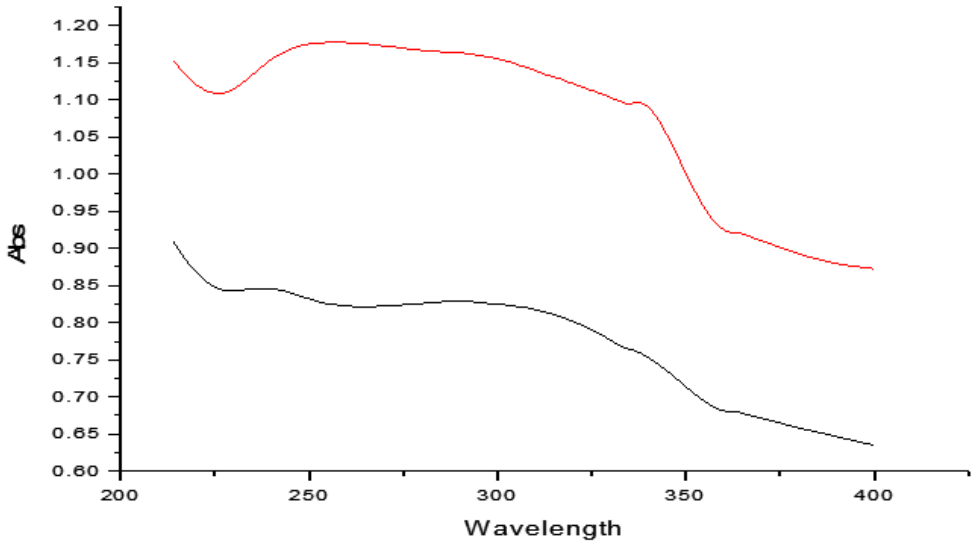

Fig. 2. UV-Visible Spectrum of Zn(II) and Hg(II) complexes

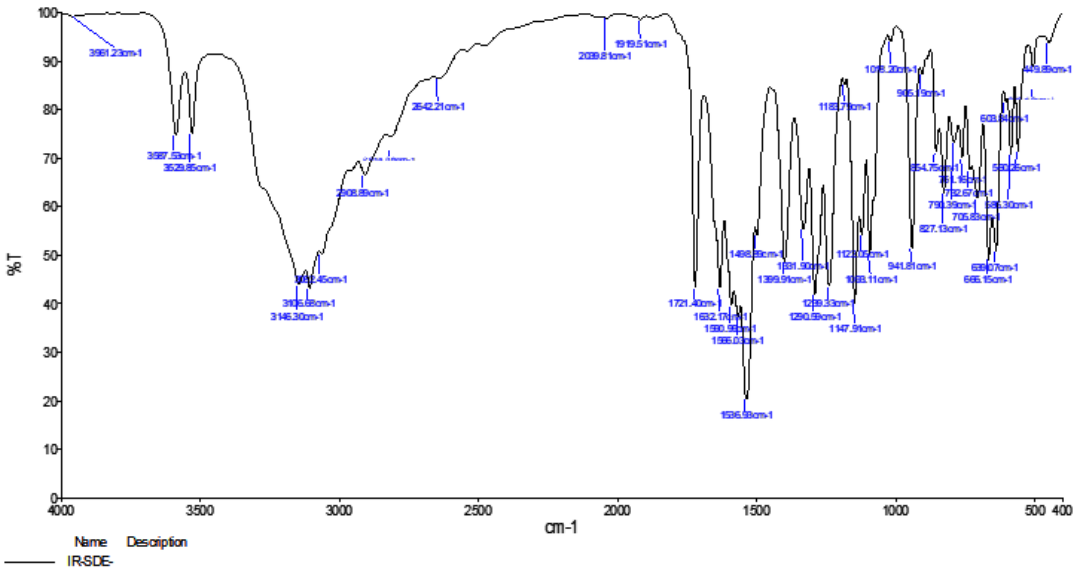

Fig. 3. FT-IR spectrum of Zn(II) complex

to the metal via, nitrogen atom. The bands at 484-535cm-1 have been tentatively assigned to ?(M-N) mode29, which confirm the thiocyanate ion coordinate to the metal via., nitrogen atom.

Biological activity

Antibacterial activity

Using the agar-properly diffusion technique under in vitro conditions, the synthesized Zn(II) and Hg(II) complexes as well as their free ligand fluconazole are examined in relation to the microorganism Escherichia coli (MTCC 732). When compared to the unattached ligand fluconazole, the complexes have the ability to play nice with microorganisms30. Because of increased activity of the metal complexes can be explained on the basis of chelation theory, it tends to make the ligand act as powerful and potent bactericidal agents, thus killing more of the bacteria than the ligand alone.

Antifungal activity

Through the agar-well diffusion method, the antifungal properties of the loose ligand fluconazole and the synthesized Zn(II), Hg(II) complexes are examined against the fungus Candida albicans (MTCC 183)[31]. Compared to fluconazole without a ligand, the complexes are more effective against the fungi shown in Fig.5 because of metal chelating.

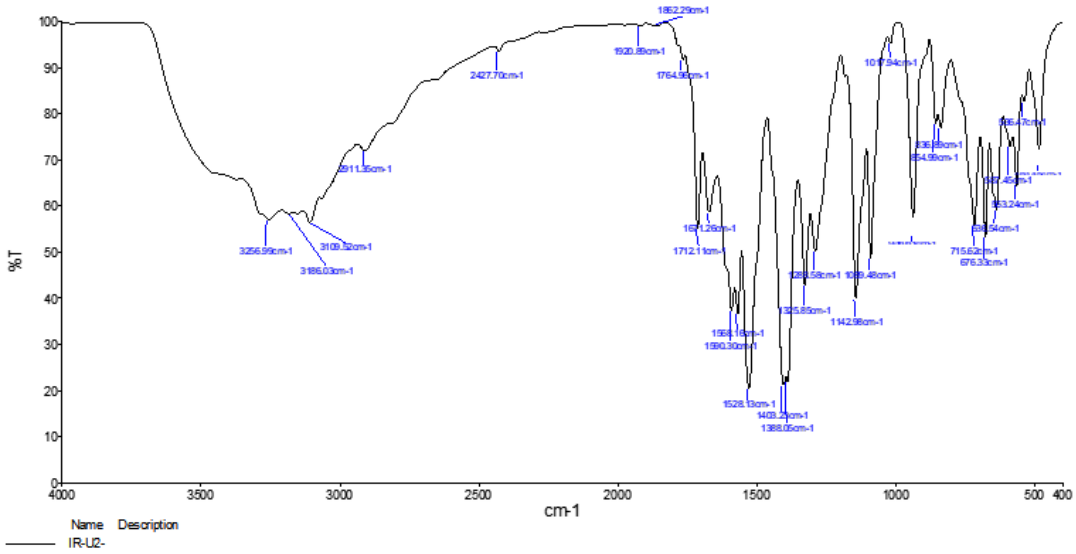

Fig. 4. FT-IR spectrum of Hg(II) complex

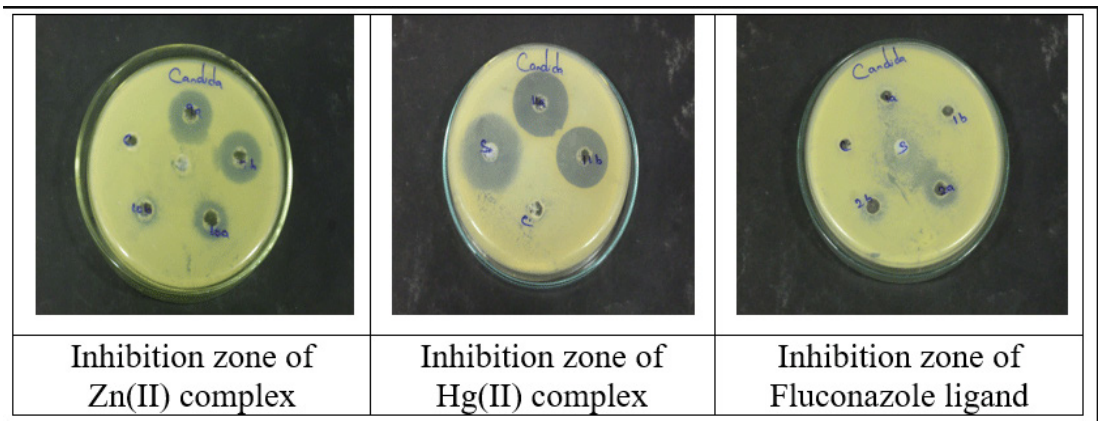

Fig. 5. Antimicrobial activity of Zn(II), Hg(II) complexes and fluconazole ligand against

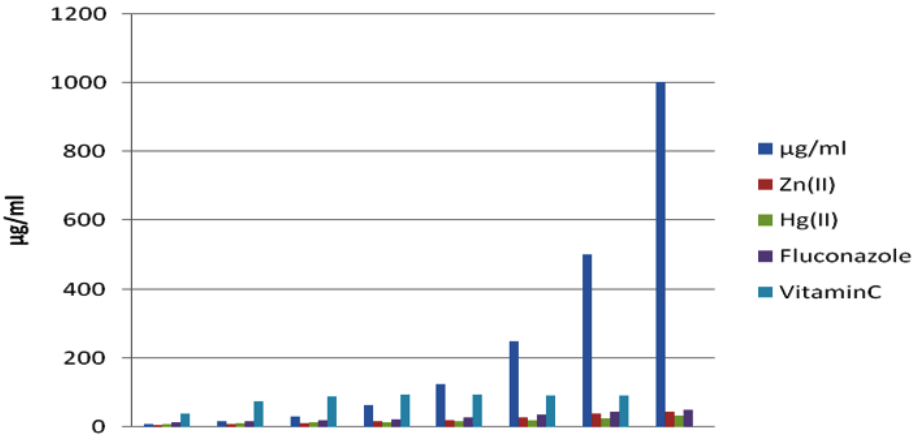

Fig. 6. Antioxidant activity of ligand and its complexes

Table 1. Elemental analysis and molar conductance

S.No	Ligands/Complexes	Elements found (Calculated) %				Λm
		C	H	N	M	
($\Omega^{-1}cm^2$ mol^{-1})						
1	$[Zn(FAZ)_2(SCN)_2]$	41.37(39.53)	7.99(5.82)	26.94(27.73)	21.23(22.44)	94.37
2	$[Hg(FAZ)_2(SCN)_2]$	40.37(35.83)	8.99(7.21)	26.94(25.34)	17.36(19.13)	67.66

(Theoretical values are given in the parentheses)

Candida albicans

Antioxidant activity (Radical Scavenging Activity)

The antioxidant activity of the free ligand fluconazole and the complexes were determined using the DPPH scavenger method and vitamin C as a standard. The reducing ability of DPPH radicals was determined by the antioxidant-induced decrease in absorbance at 517 nm[32]. The graph plots percentage capture efficiency on the y-axis and concentration (ug/ml) on the x-axis. The scavenging capacities of complexes were compared with vitamin C as a standard[33,34]. Metal complexes showed enhanced activity as radical scavengers compared to ascorbic acid. These results are in good agreement with previous metal complex studies where the ligand is expected to have antioxidant activity and the metal moiety enhances that activity[35-41]. The scavenging activity of ligands and their complexes is shown in Fig.6.

CONCLUSION

Using fluconazole and thiocyanate ions as ligands, we recently tried to construct and analyze diamagnetic Zn(II) and Hg(II) complexes. Microwave irradiation was used to create fresh metal complexes. Various chemical and spectral investigations were used to identify the produced complexes. We evaluated the synthetic complexes antimicrobial efficacy. Compared to free ligand, metal complexes have substantial antibacterial and antioxidant action. Because of increased activity of the metal complexes can be explained on the basis of chelating theory, it tends to make the ligand act as powerful and potent bactericidal agents, thus killing more of the bacteria than the ligand alone.

ACKNOWLEDGEMENT

The authors would like to express their gratitude to the principal of the Government Arts

College in Ariyalur, Tamil Nadu, for providing infrastructure facilities for the Department of Chemistry. They also acknowledge the measurement data provided by STIC, Cochin University, Mumbai, SAIF, IIT, Chennai, and collaborators.

Conflict of Interest

I hereby declare that all the authors and corresponding authors do not have any conflict of interest.

Funding Sources

There is not any external source of funding for conducting the research.

REFERENCES

1. Kathiravan MK, Salake AB, Chothe AS, Dudhe PB, Watode RP, Mukta MS, Gadhwe S. The biology and chemistry of antifungal agents: a review. Bioorganic & medicinal chemistry. 2012;20(19):5678-98.
2. Fisher MC, Henk DA, Briggs CJ, Brownstein JS, Madoff LC, McCraw SL, Gurr SJ. Emerging fungal threats to animal, plant and ecosystem health. Nature. 2012;484(7393):186-94.
3. Risana MM, Balasubramaniyan S, Govindharaju R, Meenakshi VM, Jayalakshmi B, Ramachandramoorthy T. Synthesis,Spectral Characterization and Biopotential Significance of Co(II) and Ni(II) Complexes With Biologically Active Ligands. Int. J. Pharm. Sci. Drug Res.2020; 12(1): 22-28.
4. Denning DW, Bromley MJ. How to bolster the antifungal pipeline. Science.2015;347(6229):1414-6.
5. Pfaller MA, Diekema D. Epidemiology of invasive candidiasis: a persistent public health problem. Clinical microbiology reviews. 2007;20(1):133-63.
6. Cao X, Sun Z, Cao Y, Wang R, Cai T, Chu W, Hu W, Yang Y. Design, synthesis, and structure–activity relationship studies of novel fused heterocycles-linked triazoles with good activity and water solubility. Journal of medicinal chemistry. 2014;57(9):3687-706.
7. Maertens JA. History of the development of azole derivatives. Clinical Microbiology and Infection. 2004:1-0.
8. Graninger W, Diab-Elschahawi M, Presterl E. Antifungal agents. Clinically Relevant Mycoses: A Practical Approach. 2019:31-42.
9. Nguyen YT, Kim N, Lee HJ. Metal Complexes as Promising Matrix Metalloproteinases Regulators. International Journal of Molecular Sciences. 2023;24(2):1258.
10. Corrêa JC, Salgado HR. Review of fluconazole properties and analytical methods for its determination. Critical reviews in analytical chemistry. 2011;41(2):124-32.
11. Irobi ON, Moo-Young M, Anderson WA. Antimicrobial activity of Annatto (Bixa orellana) extract. International Journal of Pharmacognosy. 1996;34(2):87-90.
12. Risana MM, Balasubramaniyan S, Govindharaju R, Meenakshi VM, Jayalakshmi B, Ramachandramoorthy T. Synthesis,Spectral Characterization and Biopotential Significance of Co(II) and Ni(II) Complexes With Biologically Active Ligands. Int. J. Pharm. Sci. Drug Res.2020; 12(1): 22-28.
13. Chen Y, Wang M, Rosen RT, Ho CT. 2, 2-Diphenyl-1-picrylhydrazyl radical-scavenging active components from Polygonum multiflorum thunb. Journal of agricultural and food chemistry. 1999;47(6):2226-8.
14. Govindharaju R, Muruganantham N, Balasubramaniyan S, Palanivelan L, Jayalakshmi B, Rajalakshmi K, Ramachandramoorthy T. Synthesis, Spectral Characterization and Biological Evaluation of Cr (III) Complex with Mixed N, N and O-donor Ligands. International Journal of Pharmaceutical Investigation. 2019;9(4).
15. Risana MM, Balasubramaniyan S, Govindharaju R, Meenakshi VM, Jayalakshmi B, Ramachandramoorthy T. Synthesis,Spectral Characterization and Biopotential Significance of Co(II) and Ni(II) Complexes With Biologically Active Ligands. Int. J. Pharm. Sci. Drug Res.2020; 12(1): 22-28.
16. Govindharaju R, Muruganantham N, Balasubramaniyan S, Palanivelan L, Jayalakshmi B, Rajalakshmi K, Ramachandramoorthy T. Synthesis, Spectral Characterization and Biological Evaluation of Cr (III) Complex with Mixed N, N and O-donor Ligands. International Journal of Pharmaceutical Investigation. 2019;9(4):158-163.
17. Bukhari SB, Memon S, Tahir MM, Bhanger MI. Synthesis, characterization and investigation of antioxidant activity of cobalt–quercetin complex. Journal of Molecular Structure. 2008;892(1-3):39-46.
18. Abdallah SM, Zayed MA, Mohamed GG. Synthesis and spectroscopic characterization of new tetradentate Schiff base and its coordination compounds of NOON donor atoms and their antibacterial and antifungal activity. Arabian Journal of Chemistry. 2010;3(2):103-13.
19. Raman N, Kulandaisamy A, Jeyasubramanian

K. Synthesis, spectroscopic characterization, redox, and biological screening studies of some Schiff base transition metal (II) complexes derived from salicylidene-4-aminoantipyrine and 2-aminophenol/2-aminothiophenol. Synthesis and Reactivity in Inorganic and Metal-Organic Chemistry. 2001;31(7):1249-70.

20. Avaji PG, Kumar CV, Patil SA, Shivananda KN, Nagaraju C. Synthesis, spectral characterization, in-vitro microbiological evaluation and cytotoxic activities of novel macrocyclic bis hydrazone. European Journal of medicinal chemistry. 2009;44(9):3552-9.
21. Christofis P, Katsarou M, Papakyriakou A, Sanakis Y, Katsaros N, Psomas G. Mononuclear metal complexes with Piroxicam: Synthesis, structure and biological activity. Journal of inorganic biochemistry. 2005;99(11):2197-210.
22. Chadha R, Saini A, Jain DS, Venugopalan P. Preparation and solid-state characterization of three novel multicomponent solid forms of oxcarbazepine: Improvement in solubility through saccharin cocrystal. Crystal growth & design. 2012;12(8):4211-24.
23. Northcote-Smith J, Suntharalingam K. Targeting chemotherapy-resistant tumour sub-populations using inorganic chemistry: Anti-cancer stem cell metal complexes. Current Opinion in Chemical Biology. 2023;72:102237.
24. Feelders RA, Hofland LJ, De Herder WW. Medical treatment of Cushing's syndrome: adrenal-blocking drugs and ketaconazole. Neuroendocrinology. 2010;92(Suppl. 1):111-5.
25. Rice SA, Givskov M, Steinberg P, Kjelleberg S. Bacterial signals and antagonists: the interaction between bacteria and higher organisms. Journal of molecular microbiology and biotechnology. 1999 Aug 1;1(1):23-31.
26. Bagihalli GB, Avaji PG, Patil SA, Badami PS. Synthesis, spectral characterization, in vitro antibacterial, antifungal and cytotoxic activities of Co (II), Ni (II) and Cu (II) complexes with 1, 2, 4-triazole Schiff bases. European journal of medicinal chemistry. 2008;43(12):2639-49.
27. Wright GD. Resisting resistance: new chemical strategies for battling superbugs. Chemistry & biology. 2000;7(6):R127-32.
28. Indra I, Janah FM, Aryani R. Enhancing the Solubility of Ketoconazole via Pharmaceutical Cocrystal. InJournal of Physics: Conference Series 2019; 1179(1):012134.
29. Ikram M, Rehman S, Faiz A. Synthesis, characterization and antimicrobial studies of transition metal complexes of imidazole derivative. Bulletin of the Chemical Society of Ethiopia. 2010;24(2): , 201-207.
30. Avaji PG, Kumar CV, Patil SA, Shivananda KN, Nagaraju C. Synthesis, spectral characterization, in-vitro microbiological evaluation and cytotoxic activities of novel macrocyclic bis hydrazone. European Journal of medicinal chemistry. 2009;44(9):3552-9.
31. Gautam S, Kumar R, Singh P, Gandhi N, Jain P. Pharmacological aspects of Co (II), Ni (II) and Cu (II) schiff base complexes: An insight. Results in Chemistry. 2023:100849.
32. Govindharaju R, Balasubramaniyan S, Palanivelan L, Risana MM, Meenakshi VM. Synthesis, characterization and binding properties towards CT-DNA of mixed-ligand Cu (II) complex with 2-aminobenzonitrle and octanoate ion. Int J Pharm Sci & Res. 2019;10(11):5137-45.
33. El-Gammal OA, El-Bindary AA, Mohamed FS, Rezk GN, El-Bindary MA. Synthesis, characterization, design, molecular docking, anti COVID-19 activity, DFT calculations of novel Schiff base with some transition metal complexes. Journal of Molecular Liquids. 2022;346:117850.
34. Chioma F, Ezugwu CI, Okpareke O. Synthesis, characterization, DFT and biological studies of Fe (II), Cu (II), and Zn (II) complexes of keto-imine chelators. Inorganica Chimica Acta. 2023;545:121255.
35. Ashry ES. E1, Ramadan E, Kassem E, Kassem AA and Hager M. Adv Heterocycl Chem. 2005;68:1.
36. Singh K, Barwa MS, Tyagi P. Synthesis, characterization and biological studies of Co (II), Ni (II), Cu (II) and Zn (II) complexes with bidentate Schiff bases derived by heterocyclic ketone. European Journal of Medicinal Chemistry. 2006;41(1):147-53.
37. Avaji PG, Kumar CV, Patil SA, Shivananda KN, Nagaraju C. Synthesis, spectral characterization, in-vitro microbiological evaluation and cytotoxic activities of novel macrocyclic bis hydrazone. European Journal of medicinal chemistry. 2009;44(9):3552-9.
38. Shivakumar K, Shashidhar, Vithal Reddy P, Halli MB. Synthesis, spectral characterization and biological activity of benzofuran Schiff bases with Co (II), Ni (II), Cu (II), Zn (II), Cd (II) and Hg (II) complexes. Journal of Coordination Chemistry. 2008;61(14):2274-87.
39. Muruganantham N, Govindharaju R, Anitha P. An investigation of the DNA binding properties of Mn^{2+}, Co^{2+} and Ni^{2+}, complexes with 2-aminobenzonitrile and octanoate ion as ligands. International Journal of Pharmaceutical Sciences and Research. 2019; 10 (12), 5606-5611.
40. Avaji PG, Kumar CV, Patil SA, Shivananda KN,

Nagaraju C. Synthesis, spectral characterization, in-vitro microbiological evaluation and cytotoxic activities of novel macrocyclic bis hydrazone. European Journal of medicinal chemistry. 2009;44(9):3552-9.

41. Avaji PG, Kumar CV, Patil SA, Shivananda KN, Nagaraju C. Synthesis, spectral characterization, in-vitro microbiological evaluation and cytotoxic activities of novel macrocyclic bis hydrazone. European Journal of medicinal chemistry. 2009;44(9):3552-9.