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Synthesis and anti-mycobacterium study of some fluorine containing schiff bases of quinoline and their metal complexes

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ABSTRACT

Five new quinoline Schiff bases were synthesized starting from 6-fluoro-2-hydroxyquinoline-3-carbaldehyde. In the reaction sequence, Fluorine substituted anilines were condensed to 6-fluoro-2-hydroxyquinoline-3-carbaldehyde. Further Zn (II) and Cu (II) complexes of Schiff bases were synthesized. Structure of synthesized compounds has been confirmed by employing various spectroscopic techniques like H^1NMR, FTIR and Mass Spectroscopy. The newly synthesized Schiff bases and their Zn (II) and Cu (II) complexes were evaluated for their anti tuberculosis activity against Mycobacterium tuberculosis. Initial results indicated that most of the Schiff base derivatives and their metal complexes demonstrated very good anti tuberculosis activities. The most effective compounds SB01 to SB05 series and C01-C05 series have exhibited an MIC value in the range of 1.6 to 3.2 µg/ml against M. tuberculosis H37Rv strain that is comparable to first line anti-tuberculosis drug. Fluorescent properties of synthesized Schiff bases as well as metal complexes were studied.

Key words: Quinoline Schiff base ligand, metal complex, fluorescence, anti-tuberculosis.

INTRODUCTION

Tuberculosis (TB) is a lung infection caused mainly by Mycobacterium tuberculosis (M. tuberculosis [MTB]). It is considered to be one of the most contagious and deadly diseases and is a major threat for public health [1]. The TB situation may become even worse with the spread of HIV-1 worldwide, emergence of multi-drug (Isoniazid and Rifampin) resistant (MDR-TB) and the extensively drug resistant (XDR-TB) strains. Tuberculosis, also known as TB and 'white plaque', is caused by infection with members of the MTB complex, which includes Mycobacterium tuberculosis itself, Mycobacterium africanum, Mycobacterium bovis, Mycobacterium caprae, Mycobacterium microti, Mycobacterium pinnipedii and Mycobacterium canettii [2,3]. Robert Koch was the first scientist who isolated the bacteria, MTB in 1882 and got Nobel Prize for this discovery [4]. TB has been one of the deadliest diseases over the past few decades affecting nearly one-third of the world's population [5] with new infection occurring at 1% of population each year [6]

Although the existing method of curing is very effective against TB, the length of treatment, the toxicity and the potential for drug-drug interactions are factors that highlight the need for new anti-TB drugs [7-8]. Among pharmacologically important heterocyclic compounds, quinoline and its derivatives are significant because of their

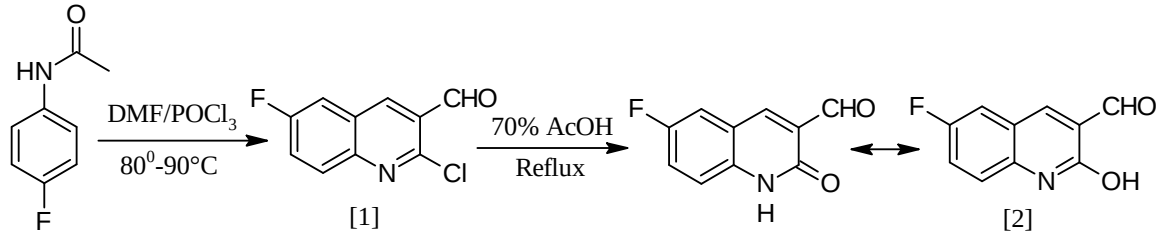
wide spectrum of biological activities and their presence in naturally occurring compounds. Quinoline is a heterocyclic aromatic nitrogen compound characterized by a double-ring structure that contains a benzene ring fused to pyridine at two adjacent carbon atoms [9-10].It can also be named as, benzopyridine, benzo[b]pyridine, 1-azanaphthalene, 1-benzazine and benzazine. In the recent time, quinoline nucleus has gathered an immense attention among chemists as well as biologists as it is one of the key building elements for many naturally occurring compounds. In particular, quinoline alkaloids are found in many different plants including Berberidaceae, Fumariaceae, Papavaraceae and Rutaceae [11–15]. Quinoline and its derivatives are important class of bioactive molecules in the field of drugs and pharmaceuticals. They exhibit significant activity against several viruses including antimalarial [16–18], antibiotic [19-20], anticancer [21], anti-inflammatory [22], antihypertensive [23], tyrokinase PDGF-RTK inhibition [24] and anti-HIV [25-26] properties.

Schiff bases play an important role as ligand in inorganic chemistry as they easily form stable co-ordinate complexes with most transition metal ions through azomethine group and phenolic group. The development in the field of bioinorganic chemistry has increased the interest in Schiff base complexes, since it has been recognized that many of these complexes may act as models for biologically important species [27–31]. Copper and Zinc are essential trace elements present in living organisms in the form of co-ordinate complexes of macromolecules such as Cytochrome C oxidase, Superoxidase dismutase, Dopamine-B-hydroxylase , Peptidylglycine monooxygenase and many more. Schiff base complexes of transition series metals also show luminescent properties with high quantum yield.

MATERIALS AND METHODS

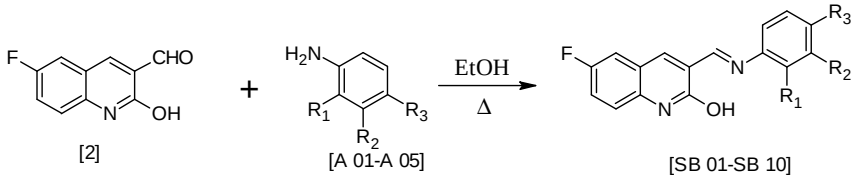
General Information: All chemicals and solvents used in this work were of analytical grade and purchased from Merck Chemicals and SD Fine chemicals. Melting points were determined by using open capillary and are uncorrected. Infrared spectra were recorded on FTIR-7600 Lambda Scientific Pty. Ltd. using KBr pellets. 1H-NMR spectra were recorded on Varian-NMR-Mercury 300 MHz instruments using DMSO-d6 as a solvent and TMS as an internal standard; chemical shifts are expressed as δ values (ppm). Mass spectra (MS) were taken in Mass spectra were recorded on BRUKER ESQUIRE HCT spectrometer. Analytical thin-layer chromatography (TLC) was performed on pre-coated TLC sheets of silica gel 60 F254 (Merck, Darmstadt, Germany), visualized by long- and short-wavelength UV lamps. Chromatographic purifications were performed on Merck silica gel (60−120 mesh).

1.1 SYNTHESIS OF SYNTHESIS OF 6-FLUORO-2-HYDROXYQUINOLINE-3-CARBALDEHYDE. [2]



Titled compound was prepared according to the literature procedure [**32**]. Required Acetanilide was readily prepared from reaction of 4-Fluoroaniline with acetic anhydride in aqueous medium. To a solution of Acetanilide (5 mmol) in dry DMF (15 mmol) at 0^0-5^0 C $POCl_3$ (60 mmol) was added drop wise with constant stirring and the mixture stirred at 80^0-90^0 C for 16 hrs. The mixture was poured into crushed ice, stirred for 5 min. and the resulting solid [1] filtered washed well with water and dried. The compound purified by recrystallization from Ethyl acetate. The suspension of [1] in 70% Acetic acid (10 mmol) was heated under reflux for 5 hrs. Upon cooling the reaction mixture a solid product [2] precipitated out which was filtered. The precipitate was washed well with water, dried and purified by recrystallization from DMF. **M.P.:** 309^0C **Color:** Greenish yellow **^{1}H NMR (300 MHz, DMSO-d_6) δ ppm:** 7.38(m, 1H, H-7), 7.57(m, 1H, H-5), 7.9(m, 1H, H-8), 8.48(d, 1H, H-4), 10.31(s, 1H, CHO), 12.31(s, 1H, OH). **FTIR:** 3153, 3023, 2927,2854, 1685, 1558, 1502, 1434, 1232, 1108, 597 **MASS SPECTRA: [M+H]** 192.41

1.2 SYNTHESIS OF SCHIFF BASES [SB01-05]



A/SB	R_1	R_2	R_3
01	H	H	F
02	F	Cl	H
03	CF_3	H	H
04	H	CF_3	H
05	H	CF_3	CN

6-Fluoro-2-hydroxyquinoline-3-carbaldehyde (0.01mol) and substituted aniline [A01-A05] (0.01mol) taken in round bottom flax containing 10 cm^3 of ethanol. Reaction mixture was refluxed for 30 min. Completion of reaction is checked with TLC. Upon cooling the reaction mixture a solid product precipitated out which was filtered, dried and purified by recrystallization from Ethanol.

2.2.1 6-FLUORO-3-{(*E*)-[(4-FLUOROPHENYL)IMINO]METHYL}QUINOLIN-2-OL [SB01]
Yield: 88% M.P.: 297^{0}C **Color:** Bright Yellow **^{1}H NMR (300 MHz, DMSO-d_6) δ ppm:** 7.34-7.36 (m, 5H), 7.45-7.47 (m, 1H), 7.76-7.79 (m, 1H), 8.64 (m, 1H), 8.75 (s, 1H), 12.21 (s, 1H). **FTIR (KBr) (cm^{-1}):** 3205, 3154, 3004, 2937, 2894, 2844, 1681, 1631, 1504, 1427, 1232, 1126, 977, 831, 775, 599. **MASS SPECTRA:** [M+H] 285.53

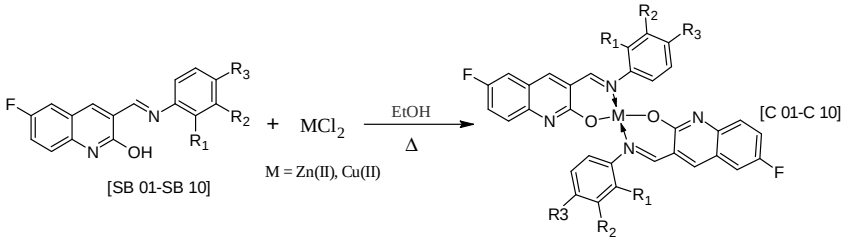
2.2.2 3-{(*E*)-[(3-CHLORO-2-FLUOROPHENYL)IMINO]METHYL}-6-FLUOROQUINO LIN-2-OL [SB02].
Yield: 87% M.P.:248 ^{0}C **Color:** Yellow **^{1}H NMR (300 MHz, DMSO-d_6) δ ppm:** 6.70-6.82 (m, 2H), 7.00 (m, 1H), 7.38 (m, 1H), 7.55-7.65 (m, 2H), 7.79-7.82 (s, 1H), 8.48 (s, 1H), 12.31 (s, 1H). **FTIR (KBr) (cm^{-1}):** 3153, 3095, 3073, 3006, 2937, 2890, 2852, 1660, 1629, 1562, 1500, 1430, 1232, 1108, 902, 819, 599. **MASS SPECTRA:** [M+] 318.88

2.2.3 6-CHLORO-3-[(*E*)-{[2-(TRIFLUOROMETHYL)PHENYL]IMINO}METHYL] QUI NOLIN-2-OL [SB03].
Yield: 92% M.P.: 266^{0}C **Color:** Pale Yellow **^{1}H NMR (300 MHz, DMSO-d_6) δ ppm:** 7.35-7.38 (m, 1H), 7.46-7.65 (m, 5H), 7.75-7.78 (m, 1H), 8.68 (s, 1H), 8.78 (s, 1H), 12.23(s, 1H). **FTIR (KBr) (cm^{-1}):** 3154, 3073, 2927, 2900, 2863, 2821, 1685, 1629, 1560, 1502, 1432, 1232, 1108, 917, 827, 597. **MASS SPECTRA:** [M+H] 335.56

2.2.4 6-FLUORO-3-[(*E*)-{[3-(TRIFLUOROMETHYL)PHENYL]IMINO}METHYL] QUIN OLIN-2-OL [SB04].
Yield: 96% M.P.: 240^{0}C **Color:** Dark Yellow **^{1}H NMR (300 MHz, DMSO-d_6) δ ppm:** 7.26-7.29 (m, 1H), 7.40-7.52 (m, 3H), 7.77-7.89 (m, 2H), 7.84-7.87(m, 1H), 8.61 (s, 1H), 8.69 (s, 1H), 12.27(s, 1H). **FTIR (KBr) (cm^{-1}):** 3320, 3270, 3166, 3097, 3056, 2931, 2886, 2838, 1673, 1627, 1562, 1502, 1430, 1334, 1322, 1230, 1184, 1120, 798, 696, 601. **MASS SPECTRA:** [M+H] 335.81

2.2.5 4-{[(*E*)-(6-FLUORO-2-HYDROXYQUINOLIN-3-YL)METHY LIDENE]AMINO}-2-(TRIFLUORO METHYL)BENZONITRILE [SB05].
Yield: 80% M.P.: 235 ^{0}C **Color:** Pale Yellow **^{1}H NMR (300 MHz, DMSO-d_6) δ ppm:** 7.28(m, 2H), 7.40-7.52 (m, 3H), 7.82-7.85 (m, 1H), 8.72 (s, 1H), 8.81 (s, 1H), 12.28(s, 1H). **FTIR (KBr) (cm^{-1}):** 3156, 3073, 2904, 2821, 2219, 1685, 1629, 1560, 1502, 1436, 1232, 1108, 906, 827, 682, 597. **MASS SPECTRA:** [M+H] 360.23

1.3 SYNTHESIS OF METAL COMPLEXES: [C01-10]



A solution of metal salt dissolved in ethanol was added gradually to a stirred ethanolic solution of the ligand in the molar ratio 1:2. The reaction mixture was further stirred for 2–4 h at 80^0 C. Then it was cooled in ice bath to ensure the complete precipitation of the formed complexes. The precipitated solid complexes were filtered, washed several times with water. Finally, the complexes were washed with diethyl ether and dried in vacuum desiccators over anhydrous $CaCl_2$.

Schiff Base	Zn (II) Complex	Cu(II) Complex
SB01	C01	C06
SB02	C02	C07
SB03	C03	C08
SB04	C04	C09
SB05	C05	C10

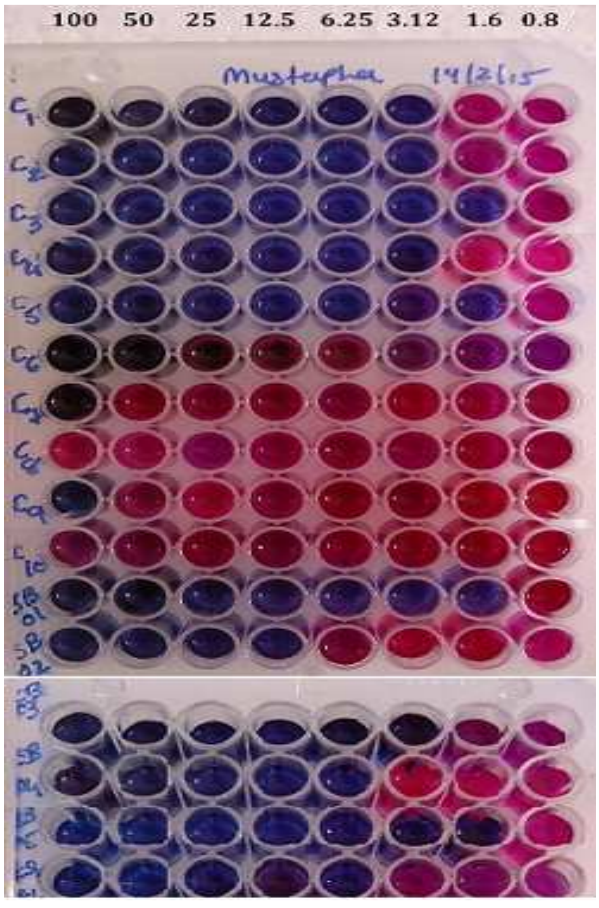


Fig. 1: Anti Mycobacterial activity by Alamar Blue assay (MABA)

2. ANTIMICROBIAL ACTIVITY

The anti Mycobacterial activity of compounds were assessed against M. tuberculosis using micro plate Alamar Blue assay (MABA) Fig. 01. This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method. Briefly, 200µl of sterile de-ionized water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation. The 96 wells plate received 100 µl of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate. The final drug concentrations tested were 100 to 0.2 µg/ml. Plates were covered and sealed with parafilm and incubated at 37ºC for five days. After this time, 25µl of freshly prepared 1:1 mixture of Almar Blue reagent and 10% between 80% was added to the plate and incubated for 24 hrs. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink.

Table 01: MIC for Schiff Bases and their Zn(II) and Cu(II) complexes

Sample	**MIC µg/ml**	Sample	**MIC µg/ml**
Pyrazinamide	**3.125**	C 01	**3.120**
Ciprofloxacin	**3.125**	C02	**3.120**
Streptomycin	**6.250**	C03	**1.600**
6-F-2-HQL	**6.250**	C04	**3.120**
SB 01	**1.600**	C05	**1.600**
SB 02	**12.500**	C06	**50.00**
SB 03	**3.120**	C07	**100.00**
SB 04	**6.250**	C08	**100.00**
SB 05	**1.600**	C09	**100.00**
		C10	**100.00**

Table 02: ANTI-TUBERCULOSIS RESULTS

Sr.No.	**Samples**	**100 µg/ml**	**50 µg/ml**	**25 µg/ml**	**12.5 µg/ml**	**6.25 µg/ml**	**3.12 µg/ml**	**1.60 µg/ml**	**0.8 µg/ml**
1	**QAL**	**S**	**S**	**S**	**S**	**S**	R	R	R
2	**SB01**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	R
3	**SB02**	**S**	**S**	**S**	**S**	R	R	R	R
4	**SB03**	**S**	**S**	**S**	**S**	**S**	**S**	R	R
5	**SB04**	**S**	**S**	**S**	**S**	**S**	R	R	R
6	**SB05**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	R
7	**C01**	**S**	**S**	**S**	**S**	**S**	**S**	R	R
8	**C02**	**S**	**S**	**S**	**S**	**S**	**S**	R	R
9	**C03**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	R
10	**C04**	**S**	**S**	**S**	**S**	**S**	**S**	R	R
11	**C05**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	R
12	**C06**	**S**	**S**	R	R	R	R	R	R
13	**C07**	**S**	R	R	R	R	R	R	R
14	**C08**	R	R	R	R	R	R	R	R
15	**C09**	**S**	R	R	R	R	R	R	R
16	**C10**	R	R	R	R	R	R	R	R
STD	**Pyrazinamide**	**S**	**S**	**S**	**S**	**S**	**S**	R	R
	Ciprofloxacin	**S**	**S**	**S**	**S**	**S**	**S**	R	R
	Streptomycin	**S**	**S**	**S**	**S**	**S**	R	R	R

S-Sensitive **R- Resistant**

Strain used: M. tuberculosis (H37 RV strain)

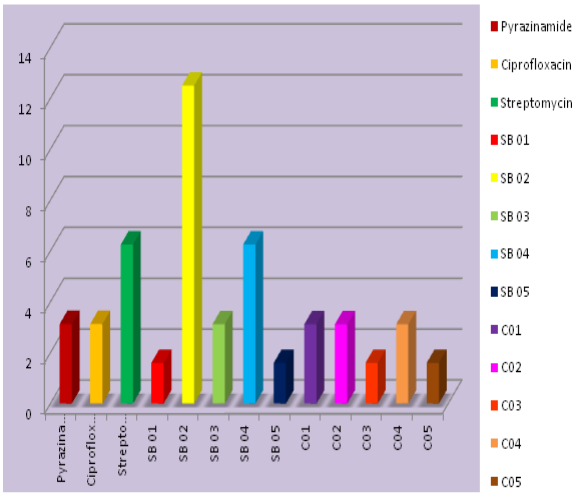


Fig. 02: Graphical representation of MIC for Schiff bases and Zn (II) Complexes

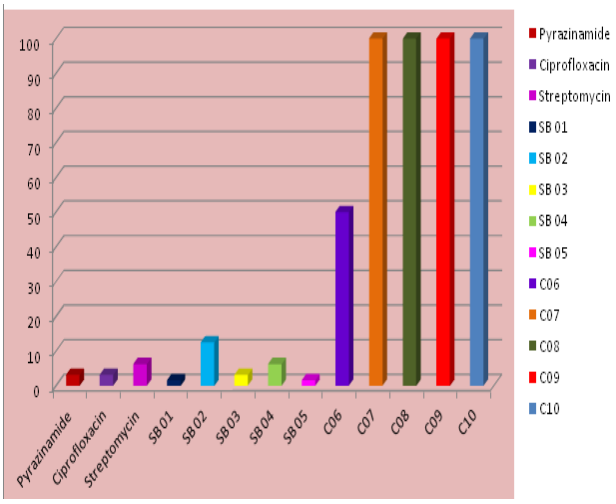


Fig. 03: Graphical representation of MIC for Schiff bases and Cu (II) Complexes

3. FLUORESCENCE STUDY

UV–Visible absorption spectra were obtained with UV spectrophotometer Shimadzu UV-1800 and recorded in quartz cells with 1 cm optical path length. Fluorescence spectra were acquired on a Spectrofluorophotometer Shimadzu RF-5301pc and equipped with quartz cuvette of 1 cm path length using DMF as a solvent.

TABLE 03: THE EXCITATION AND EMISSION WAVELENGTH WITH INTENSITY

Compound	Color of Compound	λ_{max} Absorption (intensity)	λ_{max} Emission (intensity)
6-FQAL	Yellowish green	298 (2.2)	465 (010.24)
SB01	Bright Yellow	395 (2.3)	309 (003.50)
SB02	Yellow	305 (1.3)	473 (028.75)
SB03	Pale Yellow	306 (2.0)	310 (003.40)
SB04	Dark Yellow	307 (3.3)	310 (009.80)
SB05	Pale Yellow	286 (3.9)	455 (067.21)
C01	Yellow	307 (3.7)	507 (053.09)
C02	Yellow	299 (2.0)	**472 (139.59)**
C03	Bright Yellow	302 (1.0)	**483 (518.02)**
C04	Bright Yellow	311 (3.0)	491 (047.35)
C05	Yellow	286 (3.3)	**447 (185.15)**
C06	Dark green	306 (1.8)	508 (021.63)
C07	Yellowish green	306 (1.9)	471 (040.57)
C08	Bright green	307 (2.2)	467 (064.00)
C09	Dark green	303 (1.4)	476 (005.70)
C10	Green	286 (1.6)	**398 (558.86)**

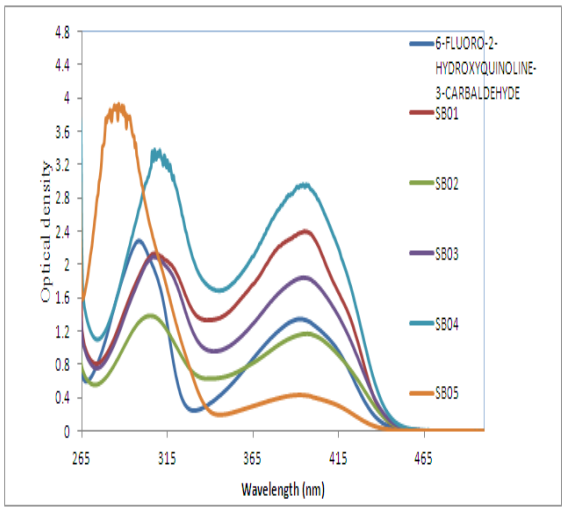


Fig. 04: UV-VISIBLE ABSORPTION SPECTRA OF SCHIFF BASES

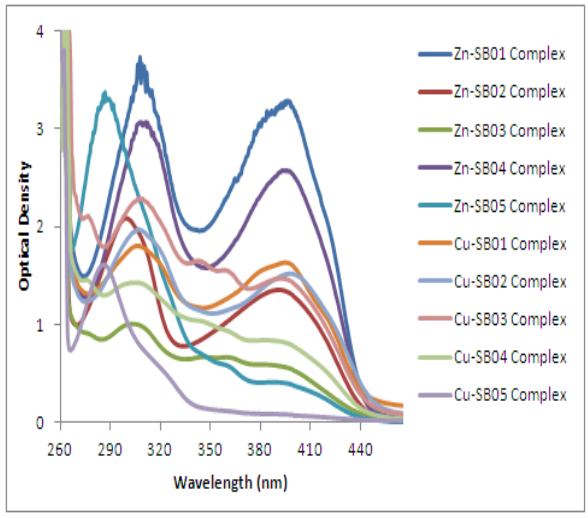


Fig. 05: UV-Visible Absorption Spectra of Zn(II) and Cu(II) complexes

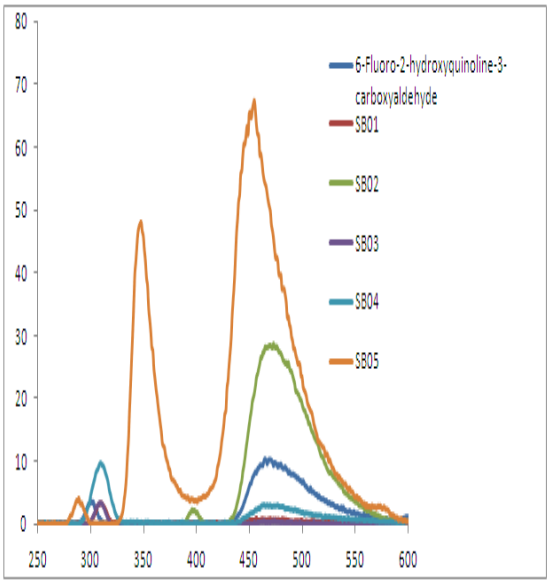


Fig. 06: FLUORESCENCE SPECTRA OF SCHIFF BASES

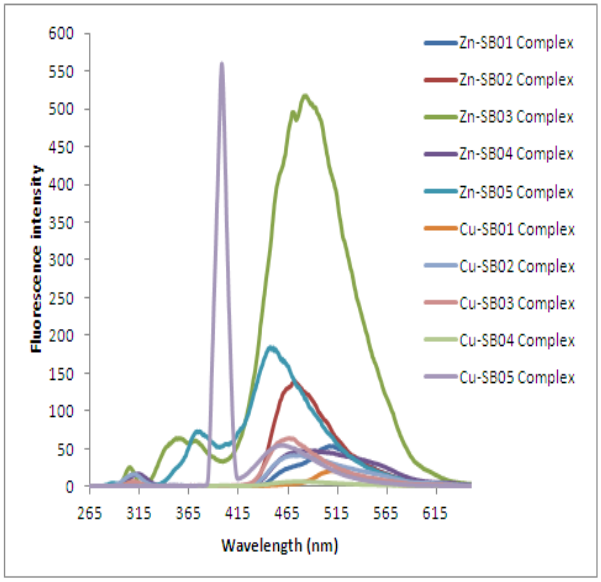


Fig. 07: FLUORESCENCE SPECTRA OF ZN(II) AND CU(II) COMPLEXES

RESULTS AND DISCUSSION

Spectral studies of synthesized compounds support the suggested structures of the Schiff bases as well as their metal complexes. The strong peak in the region 1660-1680 cm^{-1} in the Schiff base assigned to azomethine (HC=N). Formation of Schiff base is confirmed by disappearance of NMR signal at 10.31 which is responsble for aldehydic proton and appearance of new signal in the range of 8.48-8.81 which attributed to the azomethine proton (HC=N). Mass Spectral evaluation predicts the molecular weights of the desired Schiff base compounds.

In this paper, we have described the synthesis and Antimycobacterial properties of series of Schiff bases SB01-SB05 derived from 6-fluoro-2-hydroxyquinoline-3-carbaldehyde. Of all these target compounds SB01, SB03, SB05, C01, C02, C03, C04 and C05 showed promising activity against M. tuberculosis (H37 RV strain). Zn (II) complexes showed better antimycobacterial properties as compared to Cu (II) complexes.

The spectral characteristics such as absorption (λ-abs) and emission (λ-em) of the all new compounds (SB01-SB05 and C01-C10) were measured in DMF as a solvent. The absorption spectra of compounds SB01-SB05 showed absorption band in the range of 286-395 nm and compounds C01-C10 showed absorption band in the range of 286-307 nm. The emission spectra of compounds SB01-SB05 showed emission band in the range of 309-473 nm and compounds C01-C10 showed emission band in the range of 398-508 nm. Compounds C02, C03, C05 and C10 showed higher emission intensity. Complex formation of Schiff bases induces a marked hyperchromic and bathochromic shifts. Zn (II) showed strong fluorescent properties as compared to Cu (II) complexes.

CONCLUSION

In summary, we have designed and synthesized the Zn (II) and Cu (II) complexes of Schiff bases containing quinoline core. Zn (II) complexes show better fluorescence as well as anritubercular activities than Cu (II) complexes. Schiff bases containing quinoline core serve as potential antimycobacterial agents, as compared to standard drugs in market. This work highlights the applications of quinoline Schiff bases and their metal complexes in the field of pharmaceutical as well as LED developments.

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REFERENCES

[1] Brooks JT, Kaplan JE, Holmes KK, Benson C, Pau A, Masur H. *Clin Infect Dis*, **2009**; 48(6), 09–11.
[2] Grange JM. In: Schaaf S, Zumla AL, *Saunders*; **2009**. 44–59.
[3] Dye C, Scheele S, Dolin P, Pathania V, Raviglione MC. *JAMA* **1999**; 282(6), 77–86.
[4] Nobel Foundation. *The Nobel Prize in Physiology or Medicine*; **1905**.
[5] World Health Organization (WHO). *Tuberculosis fact sheet*; **2009**.
[6] World Health Organisation. *Global tuberculosis report*; **2012**.
[7] Dye C, Williams BG. *Science*, **2010**; 328(8), 56–61.
[8] Burman WJ., *Clin Infect Dis*, **2010**; 50(1), 65–72.
[9] Solomon VR, Lee H., Curr Med Chem, **2011**; 18(1), 488–508.
[10] Manske RH., Chem Rev **1942**; 30(1), 13–44.
[11] Prescott TAK, Sadler IH, Kiapranis R, Maciver SK., *J Ethnopharmacol*, **2007**; 109(2), 89–94.
[12] Srivastava V, Negi AS, Kumar JK, Gupta MM, Khanuja SPS., *Bioorg Med Chem* **2005**; 21(5), 892–908.
[13] Canel C, Moraes RM, Dayan FE, Ferreira D., *J Ethnopharmacol*, **2000**; 54(1), 15–20.
[14] Du W., *Tetrahedron*, **2003**; 59(86), 49–87.
[15] Byler KG, Wang C, Setzer WN., *J Mol Model*, **2009**; 15(14), 17–26.
[16] LaMontagne MP, Markovac AMS, Sami Khan M. Antimalarials., *J Med Chem*, **1982**; 25(96), 4–8.
[17] LaMontagne MP, Blumbergs P, Strube RE. Antimalarials., *J Med Chem*,**1982**; 25(109), 4–7.
[18] Nasveld P, Kitchener S., *Trans Royal Soc Trop Med Hyg*, **2005**; 99, 2–5.
[19] Mahamoud A, Chevalier J, Davin-Regli A, Barbe J, Pages JM., *Curr Drug Targets* **2006**; 7(84), 3–7.
[20] Eswaran S, Adhikari AV, Shetty NS., *Eur J Med Chem*, **2009**; 44(46), 37–47.
[21] Denny WA, Wilson WR, Ware DC, Atwell GJ, Milbank JB, Stevenson RJ., **2006,** *US Patent 7064117B2*.
[22] Leatham PA, Bird HA, Wright V, Seymour D, Gordon A., *Eur J Rheumatol Inflamm*, **1983**; 6(2), 09–11.
[23] Muruganantham N, Sivakumar R, Anbalagan N, Gunasekaran V, Leonard JT., *Biol Pharm Bull*, **2004**; 27(168), 3–7.
[24] Maguire MP, Sheets KR, McVety K, Spada AP, Zilberstein A., *J Med Chem*, **1994**; 37(21), 29–37.
[25] Wilson WD, Zhao M, Patterson SE, Wydra RL, Janda L, Strekowski L., *Med Chem Res* **1992**; 2(1), 02–10.
[26] Strekowski L, Mokrosz JL, Honkan VA, Czarny A, Cegla MT, Patterson SE, et al., *J Med Chem*, **1991**; 34(17), 39–46.
[27] Z.H. Chohan, S.K.A. Sheazi,, *Synth. React. Inorg. Met. Org. Chem,*. **1999**, 29, 105.
[28] C. Jayabalakrishnan, K. Natarajan, *Synth. React. Inorg. Met. Org. Chem.*, **2001**, 31, 983.
[29] T. Jeeworth, H.L.K. Wah, M.G. Bhowon, D. Ghoorhoo, K. Babooram, *Synth. React. Inorg. Met. Org. Chem.* **2000,** 30, 1023.
[30] N. Dharmaraj, P. Viswanalhamurthi, K. Natarajan, *Transition Met. Chem.* **2001**, 26, 105.
[31] C.H. Colins, P.M. Lyne, Microhiul Methods, *University Park Press*, Baltimore, **1970**, 422.

[32] Ambika Shrivastav and R M Singh, *Indian Journal of Chemistry*, **2005**, 44B, 1868-1875.