Journal of Taibah University Medical Sciences (2017) 12(2), 115–124

Taibah University

Journal of Taibah University Medical Sciences

www.sciencedirect.com

Original Article

Antimicrobial activity of novel synthesized coumarin based transitional metal complexes

Jyotirmaya Sahoo, PhD.[a,*] and Sudhir K. Paidesetty, PhD.[b]

[a] *Department of Pharmaceutics, Sri Jayadev College of Pharmaceutical Sciences, Bhubaneswar, India*
[b] *Department of Pharmaceutical Chemistry, School of Pharmaceutical Sciences, Siksha 'O' Anusandhan University, Bhubaneswar, Odisha, India*

Received 6 June 2016; revised 2 October 2016; accepted 9 October 2016; Available online 24 November 2016

الملخص

أهداف البحث: تهدف هذه الدراسة لتوليف بعض المركبات المعدنية الانتقالية الجديدة المستمدة من ٣-اريل آزو ٤-نظائر هيدروكسي الكومارين وتقييم أنشطتها المضادة للجراثيم.

طرق البحث: تم الحصول على توليف مركبات لنظائر الكومارين بواسطة مزج محلول هيدرو-الكحول من ٣-اريل آزو ٤-نظائر هيدروكسي الكومارين مع الكلوريدات الانتقالية. وتم تمييز البيئة الهيكلية للجزيئات التي تم توليفها بطرق مفيدة مختلفة. كما سبق تحديد نشاط مضادات الجراثيم بواسطة طريقة نشر آجار الجيدة.

النتائج: أظهرت مركبات كوبالت أنشطة مضادة للجراثيم ممتازة مقارنة بروابطها.

الاستنتاجات: أظهرت تقارير التحقق من مضادات الجراثيم أن مركبات الكوبالت من ٣-اريل آزو ٤-نظائر هيدروكسي الكومارين أبدت إمكانية نشاط مضاد للجراثيم أقوى من روابطها.

الكلمات المفتاحية: نشاط مضاد للجراثيم؛ نظائر الكومارين؛ مركبات الكوبالت؛ المركبات المعدنية الانتقالية

Abstract

Objectives: To synthesize new transitional metal complexes derived from 3-aryl-azo-4-hydroxy coumarin analogues and to evaluate their antimicrobial activities.

Methods: The syntheses of complexes of coumarin analogues were accomplished by mixing a hydro-alcoholic solution of 3-aryl-azo-4-hydroxy coumarin analogues with transition metal chlorides. The structural environment of the synthesized molecules was characterized using different instrumental methods. The antimicrobial activity of the compounds was determined by the agar well diffusion method.

Results: The cobalt complexes of (*E*)-3-((4-chlorophenyl) diazenyl)-4-hydroxy-2H-chromen-2-one (HL_1): (**4a**) and (*E*)-3-((4-methoxyphenyl) diazenyl)-4-hydroxy-2H-chromen-2-one (HL_2): (**4e**) showed excellent antimicrobial activities compared with their ligands.

Conclusion: The reports of the antimicrobial investigation showed that the cobalt complexes of 3-aryl-azo-4-hydroxy coumarin analogues exhibited potential antimicrobial activity that was stronger than that of their ligands.

Keywords: Antimicrobial activity; Cobalt complexes; Coumarin analogues; Transitional metal complexes

* Corresponding address: Department of Pharmaceutics, Sri Jayadev College of Pharmaceutical Sciences, Bhubaneswar, Odisha, India.
E-mail: jjyotisahoo@rediffmail.com (J. Sahoo)

Peer review under responsibility of Taibah University.

Production and hosting by Elsevier

Introduction

Due to the increasing number of multi-drug-resistant microbial pathogens and the inclusion of emerging infectious diseases such as severe acute respiratory syndrome and avian influenza, the treatment of microbial infections still

1658-3612 http://dx.doi.org/10.1016/j.jtumed.2016.10.004

remains a challenging job with the available antimicrobials and remains a worldwide problem for clinical management.[1] Development of newer molecules with less expense and minimum toxicity for the management of infections due to multi-drug-resistant (MDR) microbial pathogens should represent the vital sphere of antimicrobial research today. A literature survey revealed that azo molecules are popular for versatile uses such as antiseptics,[2] antimicrobial,[3] antidiabetics,[4] antineoplastics,[5] transmissible spongiform encephalopathy,[6] antiulcerative,[7] antoxidant,[8] analgesic,[9] antiinflammatory,[10] antiviral,[11] antitubercular[10] and antitumour[12] activities.

A literature survey also revealed that azo-bearing ligands have a modified therapeutic effect when combined with transition metal ions.[13,14] The complexes of transition metals have significant biological functions including antibacterial, antifungal and anticancer activities.[15] Cobalt, copper, nickel and zinc are the potentially used metal ions that form low-molecular-weight complexes, which are found to be effective against various diseases. Literature support even suggests that metal complexes are more active than their ligands because the metal complexes serve as a vehicle for activation of the ligands as principal cytotoxic species.[16,17]

The biological aspects of metallic ligands depend upon the ease of cleaving the bond between the metal ion and the ligand. It is well known that the metals present in complexes generally accelerate the drug action and the efficacy of therapeutic agents and that the pharmacological efficiencies of drug-based metal complexes depend upon the nature of the metal ion and the ligands.[18] The present work is the continuation of our previously reported work to obtain some novel complexes where the 4-chlorophenyl and 4-methoxyphenyl substituted 4-hydroxycumarin azo-analogues are conjugated with different transitional metals with an intention to produce target molecules possessing good antimicrobial properties.[3,19]

Materials and Methods

All the chemicals used in the present studies were of synthetic grade and were obtained from Merck specialties Ltd. and HiMedia laboratories Pvt. Ltd, (Mumbai, India). The prepared products were analysed by FT/IR (JASCO FT/IR 4100 Spectrophotometer) using KBr pellets. An LC-MS column C_6 (150 mm × 4.6 mm) with a 5 µm particle size (Shimadzu-Mass spectrophotometer) was used. The ^{1}H NMR spectra were recorded on a Bruker ^{1}H NMR 400 *MHz* using tetramethylsilane as an internal standard, and the chemical shifts were reported in δ ppm. The UV (Jasco V-630 Spectrophotometer) and elemental analyses for C, H, N and S were performed on a Perkin Elmer model 2400 CHNS/O analyser. A Shimadzu XRD 7000 was used for the study of the structural environment of the synthesized ligands and their metal complexes. The Faraday balance technique was employed for the measurement of magnetic susceptibility of the metal complexes. The *in vitro* antimicrobial activities against different bacterial and fungal pathogens were determined by the agar well diffusion method, sourced from *IMTECH*, India, Chandigarh.

*Synthesis of ligands (**3a–3b**)*

The synthetic procedures yielding the ligands *(E)-3-((4-chlorophenyl) diazenyl)-4-hydroxy-2H-chromen-2-one (HL_1)*: (**3a**) and *(E)-3-((4-methoxyphenyl) diazenyl)-4-hydroxy-2H-chromen-2-one (HL_2)*: (**3b**) were carried out as described previously.[3]

*Synthesis procedure of metal complexes (**4a–4h**)*[20]

A mixture of 25 mL was prepared with the appropriate metal chloride (Cu(II), Ni(II), Co(II) and Zn(II) of 10 mmol in ethanol and water, 1:1). The above mixture was added to the solution of azo compound **3a–3b** (0.40 g, 10 mmol) in ethanol and water in equal proportions to obtain 50 mL of solution. The resulting solution was refluxed for 30 min at a controlled temperature not more than 78 °C. The precipitated complexes were separated by filtration using Whatman filter paper. Separated precipitates were washed with 1:1 ethanol-water. Finally, obtained products were recrystallized from diethyl ether and air dried.

Spectral characterization

*(E)-3-((4-chlorophenyl)diazenyl)-4-hydroxy-2H-chromen-2-one (HL_1), (**3a**)*

Off yellow-coloured powder; yield 91%; Rf: 0.7; m.p.: 235–40 °C; UV–Vis (λ max, DMSO): 409 nm; IR (KBr, ν, cm^{-1}): 3445(O–H str.), 1619 (C=C str.), 1500 (–N=N–), 1726 (C=O str.), 1298 (=C–O str.), 828 (1, 4 disubst. Ar.); ^{1}H NMR (DMSO-d_6, δ ppm, 400 *MHz*): 16.86 (s, 1H, 4-enolic OH), 7.65 (m, coumarin H-7), 7.45 (m, coumarin H-6), 7.47 (d, coumarin H-8), 7.90 (d, coumarin H-5), 7.21–7.39 (m, 4H, Ar–H); LC-MS (RT, % area); 2.291, 60.04 *m/z*; 301.2 (M+1); Analysis for $C_{15}H_9ClN_2O_3$: Calcd: C, 59.91; H, 3.02; N, 9.32; Found: C, 59.93; H, 3.04; N, 9.36%.

*(E)-3-((4-methoxyphenyl)diazenyl)-4-hydroxy-2H-chromen-2-one (HL_2), (**3b**)*

Brick red-coloured powder; yield 93%; Rf: 0.8; m.p.: 205–10 °C; UV–Vis (λ max, DMSO): 413 nm; IR (KBr, ν, cm^{-1}): 3479 (O–H str.), 2929 (CH_2 str.), 1603 (C=C str.), 1745 (–C=O str.), 1505 (–N=N–), 1487, 1113 (=C–O–CH_3 str), 1246 (=C–O str.), 758 (1, 2 disubst. Ar.); ^{1}H NMR (DMSO-d_6, δ ppm, 400 *MHz*): 16.81 (s, 1H, 4-enolic OH), 7.65 (m, coumarin H-7), 7.46 (m, coumarin H-6), 8.09 (d, coumarin H-5), 7.57 (d, coumarin H-8), 6.94–7.34 (d, 4H, Ar–H), 3.83 (s, 3H, Ar–OCH_3); LC-MS (RT, % area); 2.291, 60.04 *m/z*; 297.0 (M+1); Analysis for $C_{16}H_{12}N_2O_4$: Calcd: C, 64.86; H, 4.08; N, 9.46; Found: C, 64.91; H, 4.11; N, 9.43%.

*Nickel complex of (E)-3-((4-chlorophenyl)diazenyl)-4-hydroxy-2H-chromen-2-one (HL_1), (**4b**)*

Light green-coloured powder; yield 37%; Rf: 0.8; m.p.: 245–50 °C; UV–Vis (λ max, DMSO): 440 nm; IR (KBr, ν, cm^{-1}): 1625 (C=C str.), 1529 (–N=N–), 1723 (C=O str.), 1299 (=C–O str.), 829 (1, 4 disubst. Ar.), 446 (Ni–N), 535 (Ni–O); ^{1}H NMR (DMSO-d_6, δ ppm, 400 *MHz*): 7.71 (m, coumarin H-7), 7.36 (m, coumarin H-6), 7.38 (d, coumarin H-8), 8.11 (d, coumarin H-5), 7.26–7.33 {m, 8H, $(C_6H_4)_2$;

LC-MS (RT, % area); 1.147, 63.31 *m/z*; 654.01 (M+1); Analysis for $C_{32}H_{24}ClN_4NiO_6$: Calcd: C, 58.70; H, 3.69; N, 8.56; Ni, 8.56 Found: C, 58.68; H, 3.72; N, 8.58; Ni, 8.54%.

*Cobalt complex of (E)-3-((4-methoxyphenyl)diazenyl)-4-hydroxy-2H-chromen-2-one (HL_2), (**4e**)*

Brown-coloured powder; yield 43%; Rf: 0.7; m.p.: 245–50 °C; UV–Vis (λ max, DMSO): 450 nm; IR (KBr, ν, cm^{-1}): 3012 (Ar H), 2939 (CH_2 str.), 1619 (C=C str.), 1725 (–C=O str.), 1525 (–N=N–), 1488, 1115 (=C–O–CH_3 str), 1248 (=C–O str.), 759 (1, 2 disubst Ar.), 449 (Co–N), 538 (Co–O); ^{1}H NMR (DMSO-d_6, δ ppm, 400 *MHz*): 7.64 (m, coumarin H-7), 7.41 (m, coumarin H-6), 8.07 (d, coumarin H-5), 7.49 (d, coumarin H-8), 6.91–7.23 {m, 8H, $(C_6H_4)_2$}, 3.82 {s, 6H, $(OCH_3)_2$}; LC-MS (RT, % area); 1.871, 51.04 *m/z*; 651.0 (M + 1); Analysis for $C_{33}H_{27}CoN_4O_7$: Calcd: C, 60.93; H, 4.18; N, 8.61; Co, 9.06 Found: C, 60.94; H, 4.15; N, 8.65, Co, 9.09%.

Antimicrobial activity

The above newly synthesized 4-HC azo-analogues and their metal complexes were investigated over different freshly sub cultured microbial strains, viz. *Escherichia coli* (MTCC 614), *Klebsiella pneumonia* (MTCC 109) and *Candida albicans* (MTCC 3017), that were procured from the Institute of Microbial Technology and Gene bank (IMTECH), Chandigarh, India. *Staphylococcus aureus* and *Cryptococcus neoformans* were obtained from the University Department of Pharmaceutical Sciences, Utkal University. Ampicillin and fluconazole were used as reference antibiotics.

The antimicrobial diffusion test was performed using a cell suspension of approximately 1.5×10^6 CFU mL^{-1} employing a McFarland turbidity standard No. 0.5. The antimicrobial activities of the novel 4-HC analogues (**3a–3b**) and the reported complexes (**4a–4g**) were determined by the agar well diffusion method using sterile molten nutrient agar (antibacterial activity) preparations of the compounds and Sabouraud dextrose agar (antifungal activity) preparations of compound **4d** and their respective complexes.[9]

Minimum inhibitory concentration (MIC)

A 1 mg mL^{-1} stock solution of each of the synthesized compounds and reference antibiotic was prepared using DMF. Further, five different concentrations (500–31.25 μg mL^{-1}) were prepared by the serial dilution method. The different concentrations for the respective compounds were loaded into the wells and incubated at 37 °C for 18–24 h. After incubation, the MIC was determined.[9]

Acute toxicity study

The experiment was carried under the guideline of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and approved by the Institutional Animal Ethical Committee (IAEC), School of Pharmaceutical Sciences. Siksha 'O' Anusandhan University, Bhubaneswar, Odisha, India, with registration number 1171/C/08/CPCSEA and Ref. No. 60/SPS/IAEC/SOAU. The said synthesized compounds were subjected to an acute oral toxicity study to establish their safety dose. Healthy young female Wister rats that were 8–12 weeks of age were selected. OECD guideline No. 420 (2000) for the acute oral toxicity fixed dose procedure of the test compounds (**3a–3b** and **4a–4h**) was followed. To conduct the sighting study, a single animal was provided with suspensions of the synthesized compounds having a specific dose, viz. 5 mg, 50 mg, 300 mg and 2000 mg/kg body weight, with the aid of an intubation canula. A period of 24 h was maintained between each dosing. In the main study, another 4 animals were administered 2000 mg/kg. The animals were observed for a period of 14 days. However, the acute toxic symptoms and the behavioural changes produced by the test compounds were observed continuously at an interval of 4 h up to 24 h.

Statistical analysis

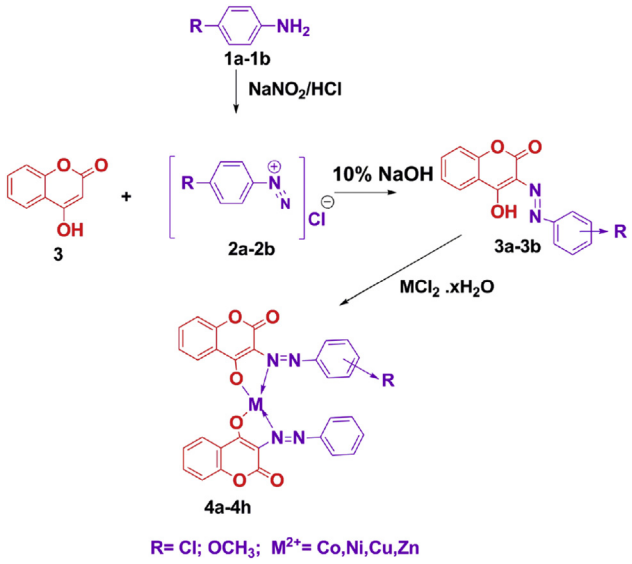
The observed data on the zone of inhibitions were subjected to a one way analysis of variance. The mean zone of inhibition for each compound on each strain was compared with the reference antibiotic through a Dunnett Post Hoc test (https://www.statstodo.com/SSizAOV_Pgm.php). The test of significance was performed at the 5% level of type one error. The research hypothesis was 'the zone of inhibition for the test compound was higher than the reference antibiotics' against the hypothesis of no difference (null hypotheses), which states that there is no significant difference between the zone of inhibition of the test compound and the reference antibiotics.

Sample size determination

A minimum sample size of five was calculated taking the probability of type 1 error (d) = 0.05, Power (1-β) = 0.8, Number of groups = 13 within group SD = 2. However, a sample size of six has been taken in the study for each compound against each strain.

Results

The metal complexes of 3-aryl-azo-4-hydroxy coumarin analogues were synthesized by refluxing different hydro alcoholic solutions of metal chlorides with 3-(4-chloro phenyl/4-methoxy phenyl)-azo-4-hydroxy coumarin analogues (Scheme 1) and finally re-crystallized from diethyl ether. The structures of the prepared compounds are confirmed by different instrumental methods of analysis. The physical characteristics of the synthesized compounds are reported in Table 1. The FT/IR spectra of synthesized ligands **3a** and **3b** showed strong vibration bands at 3479–3445 cm^{-1}, 1619–1603 cm^{-1}, 1726–1745 cm^{-1}, 1298–1246 cm^{-1} and 1529–1505 cm^{-1} due to presence of functional groups such as OH str., C=C str., C=O str., C–O str. and N=N str., respectively. The strong vibration bands of compound **3a** at 1726, 1619 and 1298 cm^{-1} may be due to presence of a lactone carbonyl of C=O str., C=C str. and =C–O str., respectively, and are illustrated in Figure 1. The frequencies of all the complexes (**4a–4h**) assigned at 1629–1613 cm^{-1}, 1729–1721 cm^{-1}, 1296–1248 cm^{-1} and 1529–1524 cm^{-1} correspond to C=C str., C=O str., C–O str. and N=N str., respectively. The IR



Scheme 1: Synthesis of coumarin based transitional metal complexes.

spectral bands of all metal complexes appearing at 451–441 and 538–531 cm^{-1} are assigned to (M–N) and (M–O), respectively. The ^1H NMR spectra of the ligands showed a broad singlet at δ 16.81–16.86 ppm towards the presence of the enolic OH group of 4-hydroxy coumarin. The ^1H NMR spectrum of compound **3b** showed a sharp singlet at δ 3.83 ppm corresponding to the proton of $-OCH_3$ (Figure 2). All of the complexes showed signals at a range of (6.90–7.26)–(7.23–7.37) ppm corresponding to {m, 8H, $(C_6H_4)_2$}.

The magnetic susceptibility of the Co(II) complex is 5.09 BM, which is nearer to the reported value for octahedral symmetry.[21] The Ni(II) complex showed a magnetic moment of 2.94 BM, whereas the Cu(II) complex showed a magnetic moment of 1.98 BM, suggesting an octahedral geometry. Compounds **3a–3b** act as bidentate ligands by coordinating to the transitional metal ions through their azo and enolic hydroxyl moiety. Based upon the study of magnetic susceptibility values of the complexes, the probable structure is given in Scheme 1.

The predicted molecular weights of the synthesized compounds were confirmed by LC–MS and the results are summarized in Table 1. Compound **3a** possesses a molecular ion peak at 301.2 *m/z* that strongly reveals the predicted molecular formula $C_{15}H_9ClN_2O_3$ that is reported in Figure 4.

The UV spectroscopic analyses of the synthesized ligands and complexes revealed that complexes **4a** and **4e** demonstrated a good bathochromic shift at 463 and 450 nm, whereas ligands **3a** and **3b** possessed a λ_{max} at 409 and 413 nm, respectively. The solvent effect of the ligands and their respective complexes are spectrally presented in Figure 3.

The X-ray diffraction (XRD) technique provides the most definitive structural information. The study of the XRD pattern of the synthesized complexes was performed using a Cu Kα X-ray source and a step of 0.02 (2θ) and run at 2θ = 2–80° using a Shimadzu XRD 7000 instrument at a scanning speed of 2.000 (deg/min). Using Origin data analysis software, the structures of the obtained complexes were interpreted. The pattern and the number of reflections reported in Figure 5 clearly declared the structural difference of compounds **4e** and **4f**.

The results of the antimicrobial activity of the newly synthesized 3-aryl-azo-4-hydroxy coumarin analogues and their complexes compared with reference antibiotics (RA) ampicillin and fluconazole (as antibacterial and antifungal standard drugs, respectively) expressed in mean ± SD are reported in Table 2. The reported results revealed that compounds **4a** and **4e** showed significant antimicrobial activity in comparison to standard drugs ($p < 0.05$) against *E. coli, K. pneumonia, S. aureus, C. albicans* and *C. neoformans*. All compounds except for **4d** and **4h** showed significant antibacterial activity against *S. aureus*. However, complex **4a** showed highest mean zone of inhibition (mm) against *K. pneumonia* and *C. neoformans*, 19 ± 1.1 and 27.5 ± 1.64, respectively. The largest mean zones of inhibition exhibited by complex **4e** against *S. aureus* and *C. neoformans* was 23 ± 2.28 and 28.17 ± 1.72, respectively. The anti-biogram pattern of compound **3a**, **3b** and their complexes against different fungal strains are illustrated in Figure 6. The graphical representation of complexes **4a** and **4e** is illustrated in Figure 7.

The inhibitory property of the compounds was determined in terms of MIC (μg mL^{-1}). The Co^{++} complexes of both the ligands showed the antimicrobial activity against all the selected strains at MIC level 31.25 μg mL^{-1}. All compounds except for **4d** and **4h** showed a zone of inhibition at the MIC level 31.25 μg mL^{-1} against *C. albicans* (Table 3).

No mortality was found for all of the test compounds per the results of the toxicity study. The synthesized compounds were safe up to 2000 mg/kg body weight. No significant change in the body weight of the animals was observed. No toxic symptoms and gross behavioural changes were observed in the animals.

Discussion

The FT/IR spectra of synthesized ligands **3a** and **3b** showed vibrations at 1619–1603 cm^{-1}, 1726–1745 cm^{-1}, 1298–1246 cm^{-1} and 1529–1505 cm^{-1} corresponding to C=C str., C=O str., C–O str. and N=N str., respectively, which were also observed in the synthesized complexes (**4a–4h**). The only exception is the bands due to OH str. at 3479–3445 cm^{-1} exhibited in the ligands that are not observable in the complexes. In metal Cu(II), Ni(II) Co(II) and Zn(II) complexes of **3a** and **3b**, the FT/IR absorption band due to the hydroxyl group at 4-HC has been diminished and indicates the metal co-ordination of the OH group via deprotonation due to liberation of hydrochloric acid. In addition to these FT/IR spectral data, stretching of the M–N and M–O bonds of the complexes, which appear in the higher frequency wavenumber region in the range 451–441 and 538–531 cm^{-1}, also indicates that complexation occurred through nitrogen and oxygen atoms from the azo-enolic ligands.[22,23] The ^1H NMR spectra of ligands **3a** and **3b** showed the enolic OH peak at 16.81 and 16.86 ppm. The disappearance of the enolic OH group in all the complexes (**4a–4h**) may be due to the interaction of metal chlorides

Table 1: Physical characteristic data of the synthesized 4-hydroxy coumarin analogues and their complexes.

Comps.	Substitution	M. formula	*m/z* (Calc)	*m/z* (Found)	Rf	M. P. (°C)	Colour	Yield (%)	UV λ_{max} DMSO	Elemental Analysis Cal. (Found)		
										C	H	N
3a	4-Chlorophenyl HL_1	$C_{15}H_9ClN_2O_3$	300.03	301.2	0.7	235–40	Off yellow	91	409	59.91 (59.93)	3.02 (3.04)	9.32 (9.36)
3b	4-Methoxyphenyl Hl_2	$C_{16}H_{12}N_2O_4$	296.08	297.0	0.8	205–10	Brick red	93	413	64.86 (64.91)	4.08 (4.11)	9.46 (9.43)

Comps.	Synthetic components	M. formula	*m/z* (Calc)	*m/z* (Found)	Rf	M. P. (°C)	Colour	Yield (%)	UV λ_{max}	C	H	N	Co	Ni	Cu	Zn
4a	Cobalt complex of HL_1	$C_{32}H_{24}ClCoN_4O_6$	654.07	655.0	0.8	245–50	Greenish yellow	39	463	58.68 (58.64)	3.69 (3.71)	8.55 (8.52)	9.00 (9.03)	–	–	–
4b	Nickel complex of HL_1	$C_{32}H_{24}ClN_4NiO_6$	653.07	654.01	0.8	245–50	Light green	37	440	58.70 (58.68)	3.69 (3.72)	8.56 (8.58)	–	8.56 (8.54)	9.63	–
4c	Copper complex of HL_1	$C_{32}H_{24}ClCuN_4O_6$	658.07	657.03	0.6	240–45	Light green	46	432	58.27 (58.31)	3.67 (3.64)	8.49 (8.51)	–	–	9.63 (9.65)	–
4d	Zinc complex of HL_1	$C_{32}H_{24}ClN_4O_6Zn$	659.07	661.2	0.9	250–55	Greenish yellow	33	421	58.11 (58.14)	3.66 (3.64)	8.47 (8.50)	–	–	–	9.89 (9.87)
4e	Cobalt complex of HL_2	$C_{33}H_{27}CoN_4O_7$	650.12	651.0	0.7	245–50	Brown	43	450	60.93 (60.94)	4.18 (4.15)	8.61 (8.65)	9.06 (9.09)	–	–	–
4f	Nickel complex of HL_2	$C_{33}H_{27}N_4NiO_7$	650.28	651.01	0.5	235–40	Brick red	39	439	60.95 (60.97)	4.19 (4.17)	8.62 (8.61)	–	9.03 (9.11)	–	–
4g	Copper complex of HL_2	$C_{33}H_{27}CuN_4O_7$	654.12	655.03	0.8	245–50	Brown	47	430	60.50 (60.54)	4.15 (4.14)	8.55 (8.59)	–	–	9.70 (9.71)	–
4h	Zinc complex of HL_2	$C_{33}H_{27}N_4O_7Zn$	655.12	655.07	0.9	235–40	Brown	41	420	60.33 (60.37)	4.14 (4.16)	8.53 (8.51)	–	–	–	9.96 (9.92)

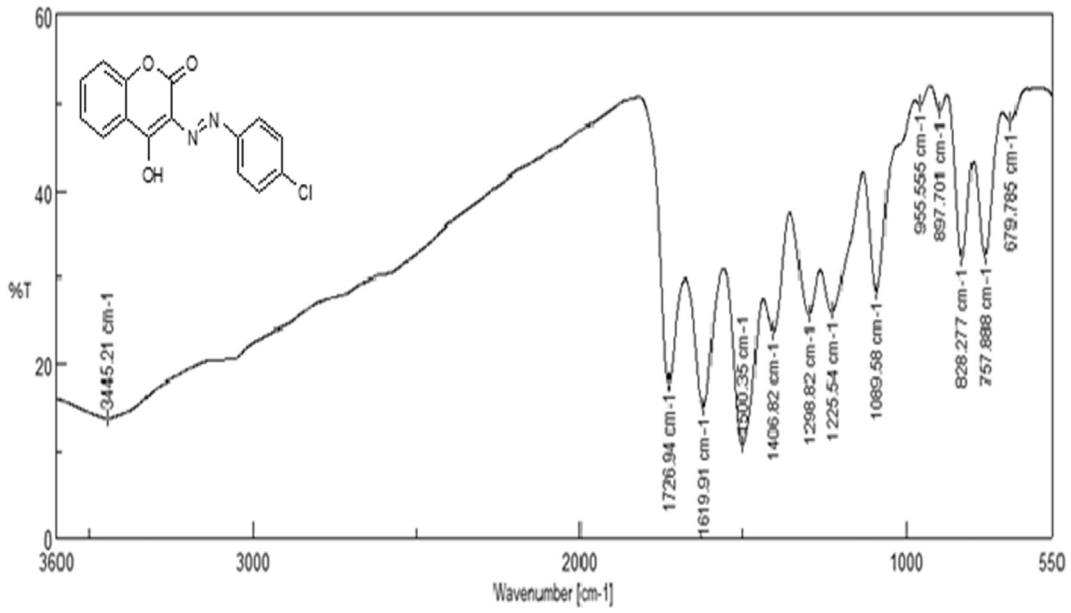


Figure 1: FT/IR spectra of 3-((4-chlorophenyl) diazenyl)-4-hydroxy-2H-chromen-2-one (**3a**).

with the hydroxyl group of the ligands resulting in deprotonation and complexation of the ligands to the metal ions. Ligand **3b** shows three identical, similar-environment protons at 3.83 ppm, which indicates the presence of an $-OCH_3$ functional group. The good bathochromic shift at 463 and 450 nm exhibited by complexes **4a** and **4e** may be due to the attachment of transition metal ion Co^{++} to the respective ligands **3a** and **3b**. Most of the compounds showed good antimicrobial activity in comparison to ampicillin. The enhanced antimicrobial activity exhibited by complexes **4a, 4b, 4e** and **4f** relative to their ligands against *E. coli* may be due to coordination of Co^{++} and Ni^{++} ions to the respective ligands **3a** and **3b**. However, complexes **4a** and **4e** showed considerably enhanced antimicrobial activity against most of the pathogenic strains relative to their ligands. Complexation reduces the polarity of the metal ion by coordinating with ligands and increases the lipophilicity of the metals.[24] Thus, it facilitates the penetration of the novel synthesized complexes into the lipoid cell membrane of microorganisms and inhibits their growth.

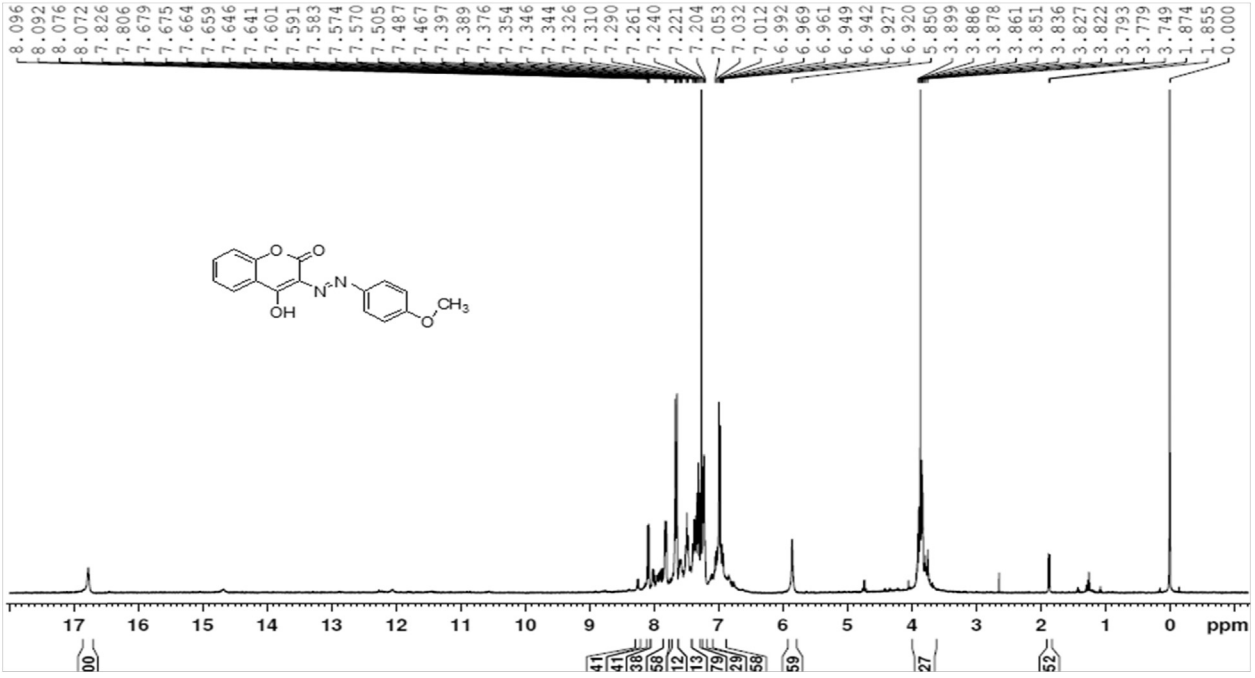


Figure 2: ^{1}H NMR spectra of 4-hydroxy-3-((4-methoxyphenyl)diazenyl)-2H-chromen-2-one (**3b**).

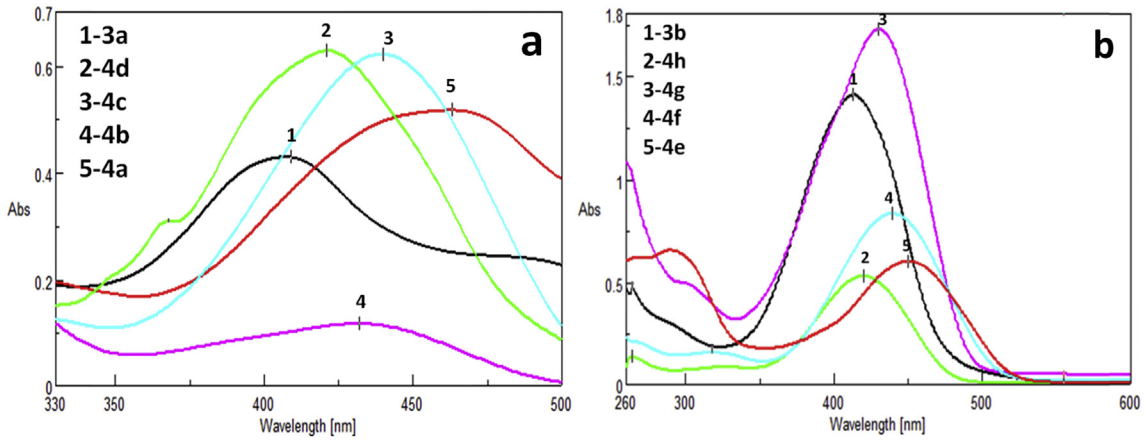


Figure 3: Solvent effect of the complexes of **3a** its metal complexes (plate **a**) and **3b** its metal complexes (plate **b**) using DMSO.

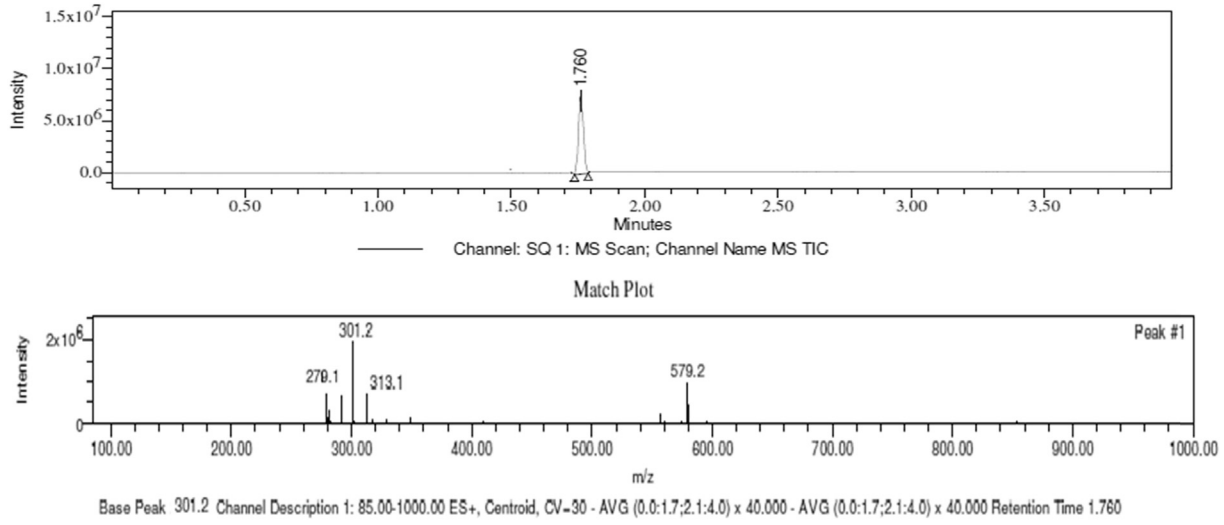


Figure 4: LCMS of (*E*)-3-((4-chlorophenyl)diazenyl)-4-hydroxy-2H-chromen-2-one (HL_1): (**3a**).

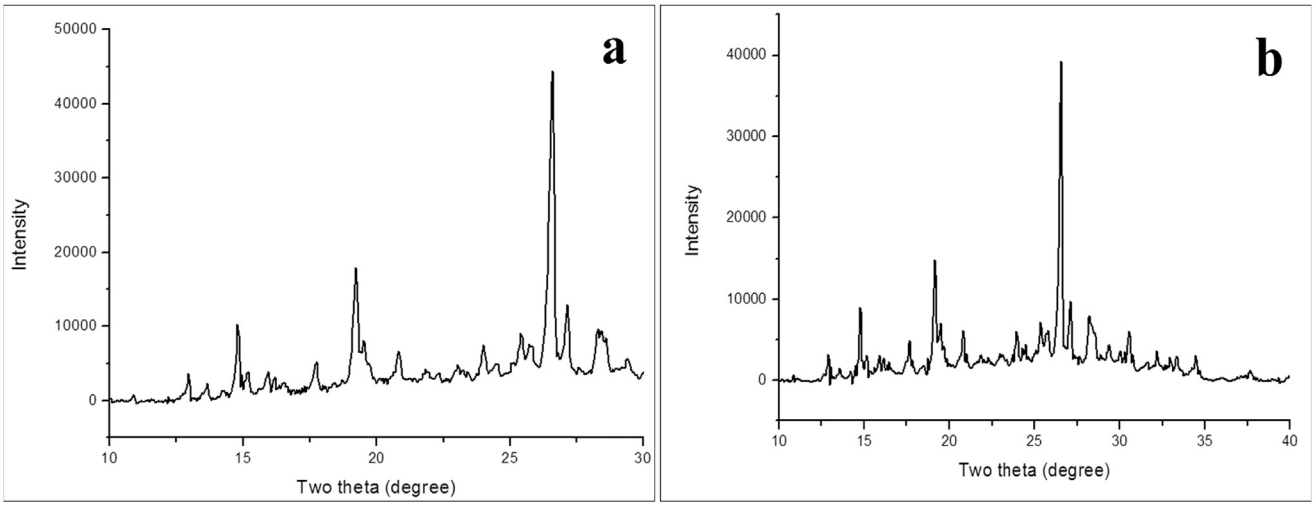


Figure 5: XRD of **4e** and **4f** respectively in plate **a** & **b**.

Table 2: Antimicrobial activity (Zone of inhibition in mm) of the newly synthesized 4-hydroxy coumarin analogues and their complexes against different microbial strains (Mean ± S.D.) at a concentration of 1 μg μL^{-1}.

Comps.	*E. coli*[a]	*K. pneumonia*[b]	*S. aureus*[c]	*C. albicans*[d]	*C. neoformans*[e]
3a	17.33 ± 1.63*	16.67 ± 2.42	15.33 ± 2.34	20.67 ± 1.86	25.17 ± 1.84
3b	19.17 ± 2.56*	18.5 ± 3.27	18.83 ± 1.47*	21.83 ± 1.84	26.83 ± 2.64*
4a	19.17 ± 2.4*	19 ± 1.1*	18.33 ± 2.34*	23.67 ± 2.16*	27.5 ± 1.64*
4b	19.17 ± 1.72*	18 ± 1.1	18.83 ± 3.25*	20.83 ± 2.56	14 ± 0.89
4c	–	14.17 ± 2.71	17.5 ± 1.64*	18.33 ± 1.63	26.67 ± 1.03*
4d	–	11.5 ± 0.84	12.67 ± 1.51	12.5 ± 2.35	11.5 ± 1.64
4e	21.17 ± 2.71*	21.17 ± 4.45*	23 ± 2.28*	25.5 ± 2.07*	28.17 ± 1.72*
4f	23.17 ± 2.23*	19.83 ± 4.07*	21.5 ± 1.76*	22.33 ± 3.27	15.83 ± 1.84
4g	–	14.83 ± 2.14	16.83 ± 2.71*	25.17 ± 1.6*	27 ± 2.1*
4h	–	12.33 ± 1.51	12.67 ± 2.25	13 ± 3.1	10.83 ± 1.33
RA	12.67 ± 1.51	15.33 ± 1.97	13 ± 1.67	19.33 ± 4.68	24.17 ± 1.94

Results are expressed as the Mean ± S.D. (n = 6). The data were analysed by one-way ANOVA followed by Dunnett's Post Hoc test. (Statistical significance at *$p < 0.05$ in comparison to RA (Reference Antibiotic): ampicillin (antibacterial); fluconazole (antifungal)); –, No zone of inhibition; [a], *Escherichia coli*; [b], *Klebsiella pneumonia*; [c], *Staphylococcus aureus*; [d], *Candida albicans*; [e], *Cryptococcus neoformans*.

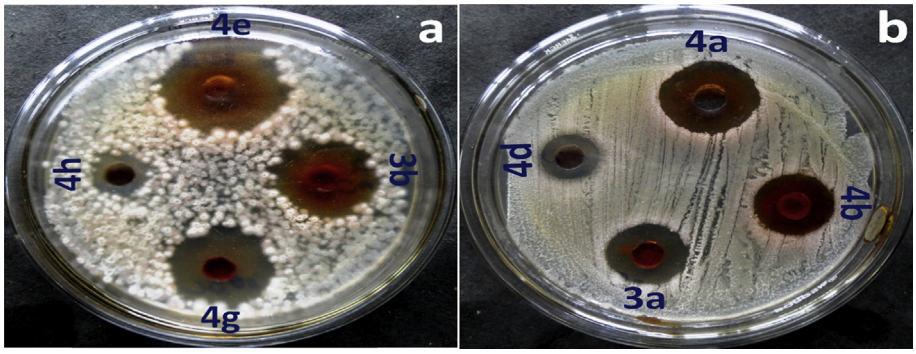


Figure 6: Antifungal activity of **3a** its metal complexes (plate **a**) and **3b** its metal complexes (plate **b**) against *C. neoformans* and *C. albicans* respectively.

The limitations of our study include only the investigation of antimicrobial activity of the novel synthesized complexes against a few bacterial and fungal strains. Furthermore, it is necessary to investigate the antimicrobial activities of the novel complexes against multidrug-resistant microorganisms. A literature survey revealed that the metal complexes serve as vehicles for activation of the ligands as principal cytotoxic species. Therefore, these complexes may be implemented for the investigation of cytotoxic activity against different cancer cell lines.

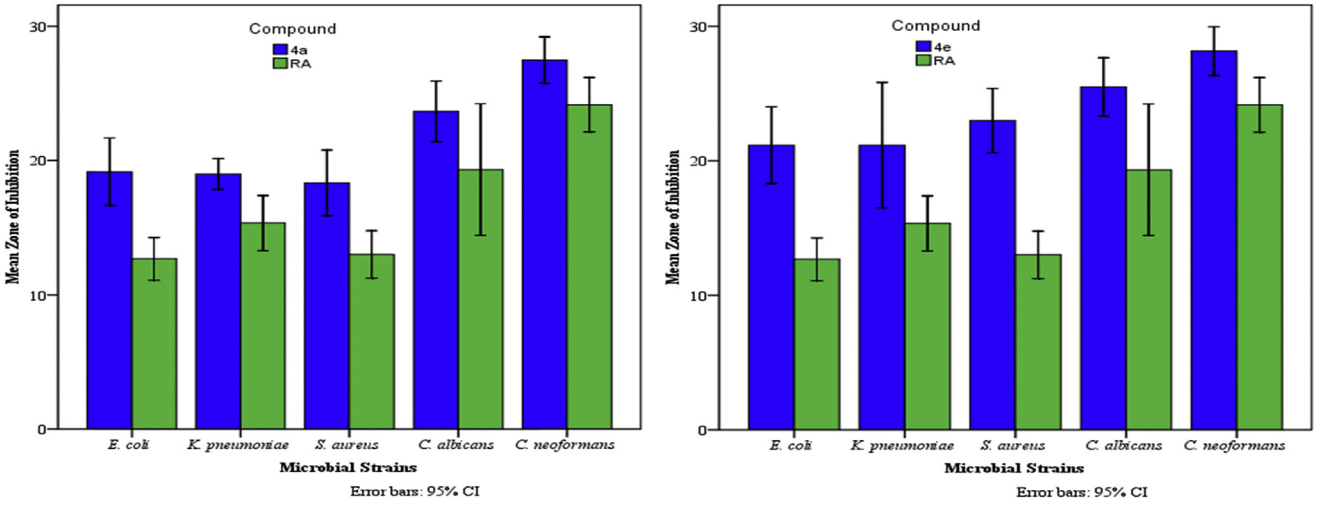


Figure 7: Graphical presentation of antimicrobial activity of **4d** and **4e**.

Table 3: Minimum inhibitory concentration MIC (μg mL^{-1}) of the newly synthesized 4-hydroxy coumarin analogues and their complexes against different microbial strains against different microbial strains.

Comps.	*E. coli*[a]	*K. pneumonia*[b]	*S. aureus*[c]	*C. albicans*[d]	*C. neoformans*[e]
3a	31.25	31.25	250	31.25	31.25
3b	31.25	31.25	31.25	31.25	31.25
4a	31.25	31.25	31.25	31.25	31.25
4b	31.25	31.25	31.25	31.25	250
4c	>500	250	31.25	31.25	31.25
4d	>500	500	500	500	500
4e	31.25	31.25	31.25	31.25	31.25
4f	31.25	31.25	31.25	31.25	250
4g	>500	500	250	31.25	31.25
4h	>500	>500	>500	500	>500

[a] *Escherichia coli.*
[b] *Klebsiella pneumonia.*
[c] *Staphylococcus aureus.*
[d] *Candida albicans.*
[e] *Cryptococcus neoformans.*

Conclusions

In the present work, metal complexes from 3-aryl-azo-4-hydroxy coumarin analogues were synthesized. The spectral characterization of the complexes confirmed their structural environment. It is suggested that the pronounced antimicrobial activity executed by the complexes (**4a** and **4e**) relative to their ligands permits their recommendation as a new chemical class of antimicrobial agents.

Conflict of interest

The authors have no conflict of interest to declare.

Authors' contribution

The conceptualization of this research work was designed by SKP. The experimental work, interpretation of the data and drafting of the manuscript were performed by JS. Both authors substantially contributed to fulfilling the requirements.

Acknowledgements

The authors are thankful to the Dean, School of Pharmaceutical Sciences, Siksha 'O' Anusandhan University, Director NISER, Director IMMT, Bhubaneswar, India, Director Piramal Healthcare, Ahmadabad, India. We also express our heartiest gratitude to Dr. Bijaya Bhusan Nanda, Dy. Director, Regional Institute for Planning, Applied Economics and Statistics, Bhubaneswar, India for his sincere guidance during the course of statistical interpretation.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jtumed.2016.10.004.

References

1. Desai NC, Bhatt N, Hardik S, Trivedi A. Synthesis, antimicrobial and cytotoxic activities of some novel thiazole clubbed 1,3,4-oxadiazoles. **Eur J Med Chem 2013**; 67: 54–59.
2. Browing CH, Cohen JB, Ellingworth S, Gulbransen R. The antiseptic properties of the aminoderivatives of styryl and anil quinoline. **J Stor 1926**; 100: 293–325.
3. Sahoo J, Paidesetty SK. Biological evaluation and spectral characterization of 4-hydroxy coumarin analogues. **J Taibah Univ Med Sci 2015**; 10: 306–319.
4. Garg HG, Praksh C. Potential anti-diabetics, 11 preparation of 4-arylazo-3,5-disubstituted-(2*H*)-1,2,6-thiadiazine 1,1-dioxides. **J Med Chem 1971**; 14(2): 175–176.
5. Child RG, Wilkinson RG, Tomcu-Fucik A. Effect of substrate orientation of the adhesion of polymer joints. **Chem Abstr 1977**; 87: 6031.
6. Rudyk H, Knaggs MH, Vasiljevic S, Hope J, Birkett C, Gilbert IH. Synthesis and evaluation of analogues of Congo red as potential compounds against transmissible spongiform encephalopathies. **Eur J Med Chem 2003**; 38(6): 567–579.
7. Carceller E, Salas J, Merlos M, Giiral M, Ferrando R, Escamilla I, Ramis J, Garciia Rafanell J, Forn J. Novel azo derivatives as prodrugs of 5-aminosalicylic acid and amino derivatives with potent platelet activating factor antagonist activity. **J Med Chem 2001**; 44(18): 3001–3013.
8. Chinnagiri TKK, Keshavayya J, Rajesh NT, Peethambar KS, Shoukat Ali AR. Synthesis, characterization, and biological activity of 5-Phenyl-1, 3, 4-thiadiazole-2-amine incorporated azo dye derivatives. **Org Chem Int 2013**: 1–7.
9. Sahoo J, Paidesetty SK. Antimicrobial, analgesic, antioxidant and *in silico* study of synthesized salicylic acid congeners and their structural interpretation. **Egypt J Basic App Sci 2015**; 2: 268–280.
10. Rohini RM, Kalpana D, Simi D. Synthesis of novel azo chalcone derivatives for antitubericular, anti inflammatory and antioxidant activity. **Der Pharm Chem 2015**; 7(1): 77–83.
11. Tonelli M, Vazzana I, Tasso B, Boido V, Sparatore F, Fermeglia M, Paneni MS, Posocco P, Pricl S, La Colla P, Ibba C, Secci B, Collu G, Loddo R. Antiviral and cytotoxic activities of aminoarylazo compounds and aryltriazene derivatives. **Bioorg Med Chem 2009**; 17: 4425–4440.
12. Thoraya AF, Abdallah AZ. Synthesis, azo-hydrazone tautomer ism and antitumor screening of N-(3-ethoxycarbonyl-4,5,6,7-tetrahydro-benzo [b]thien-2-yl)-2-arylhydrazono-3-oxobutana mide derivatives. **ARKIVOC 2008**; xvii: 295–305.

13. Abou-DobaraI MI, El-Sonbati AZ, Diab MA, El-Bindary AA, Morgan SM. Thermal properties, antimicrobial activity of azo complexes and ultra structure study of some affected bacteria. **J Microb Biochem Tech 2014**; S3. 006, http://dx.doi.org/10.4172/1948-5948.S3-006.
14. Esin. The synthesis, characterization, electrochemical character, catalytic and antimicrobial activity of novel, azo-containing Schiff bases and their metal complexes. **Dyes Pigment 2009**; 82: 13–19.
15. Refat SM, Mohamed YES, Abdel Majid AA. Cu (ii), Co (ii) and Ni (II) complexes of new Schiff base ligand: synthesis, thermal and a spectroscopic characterizations. **J Mol Str 2013**; 1038: 62–72.
16. Petering DH. *Metal ions in biological systems*. New York: Marcel Dekker; 1973.
17. Hankare PP, Patil RK, Chavan SS, Jagtap AH, Batase PS. Synthesis, characterization and biological studies of some bivalent metal complexes of Schiff bases derived from aminothiazole and substituted azosalicylaldehyde. **Ind J Chem 2001**; 40A: 1326–1329.
18. Siddiqi ZA, Khalid M, Kumar S, Shahid M, Noor S. Antimicrobial and SOD activities of novel transitional metal complexes of pyridine- 2,6 dicarboxylic acid containing 4-picoline as auxiliary ligand. **Euro J Med Chem 2010**; 45(1): 264–269.
19. Sahoo J, Mekap SK, Paidesetty SK. Synthesis, spectral characterization of some new 3- Heteroaryl azo 4-Hydroxy coumarin derivatives and their antimicrobial evaluation. **J Taibah Univ Sci 2015**; 9: 187–195.
20. Abdallah SM. Metal complexes of azo compounds derived from 4-acetamidophenol and substituted aniline. **Arab J Chem 2012**; 5: 251–256.
21. Sivasankaran MN, Arish D, Johnson J. Synthesis, characterization and biological studies on some metal complexes with Schiff base ligand containing pyrazolone moiety. **J Saudi Chem Soc 2016**; 20: S591–S598.
22. Nakamoto K. *Infrared of inorganic and coordination compounds*. 6th ed. New York: John Wiley; 1997.
23. Yousifa EA, Majeeda AS, Salihb NA. Synthesis, characterization and preliminary *in vitro* antibacterial screening activity of metal complex derivatives of 2-[(5-styryl-[1,3,4]thiadiazol-2-ylimino)-methyl]-phenol. **J Taibah Univ Sci 2014**; 8: 26–30.
24. Sheikh J, Juneja H, Ingle V, Ali P, Hadda TB. Synthesis and *in vitro* biology of Co(II), Ni(II), Cu(II) and Zinc(II) complexes of functionalized beta-diketone bearing energy buried potential antibacterial and antiviral O, O pharmacophore sites. **J Saudi Chem Soc 2013**; 17: 269–276.

How to cite this article: Sahoo J, Paidesetty SK. Antimicrobial activity of novel synthesized coumarin based transitional metal complexes. J Taibah Univ Med Sc 2017;12(2):115–124.