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Synthesis of New Dicoumarol Based Zinc Compounds and their *Invitro* Antimicrobial Studies

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Abstract. The dicoumarol derivatives were reacted with Zn (II) salt yielding the complexes (**1-10**) where metal centre was seen to be coordinated with dicoumarols through hydroxyl and carbonyl sites of attachments. All the synthesized compounds were studied spectroscopically using ^{1}H, $^{13}C\{^{1}H\}$-NMR, infrared spectroscopic method, and analytically using $ES^{(+,-)}$-MS, elemental analyses and conductance studies. The combined NMR and mass spectral data suggested the attachment of two ligands to the zinc (II) centre. Hydroxyl site is deprotonated and take part in charge neutralization of metal center. The synthesized zinc based dicoumarol compounds were screened for antimicrobial activities against Gram negative bacteria *Escherichia coli, Salmonella typhus*, *Agrobacterium tumefaciens*, *Erwinia carotovora, Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, Gram positive bacteria *Staphylococcus aureus, Bacillus subtilis*, *Bacillus atrophaeus* and fungal Strain *Candida albicans*. All the compounds shown exceptional antimicrobial and antifungal activities.
Key words: Dicoumarols, zinc compounds, spectral analysis, antimicrobial, antifungal activities

Resumen: Derivados del dicoumarol se hicieron reaccionar con sales de Zn (II) para formar los complejos (1-10), en donde el centro metálico se encuentra coordinado con la moléculas de dicumarol a través de los grupos hidroxilos y carbonilos. Todos los compuestos sintetizados fueron estudiados espectroscópicamente, utilizando: RMN de ^{1}H, $^{13}C\{^{1}H\}$, espectroscopia de infrarrojo; y analíticamente, utilizando: espectrometría de masas con ionización por electroespray $ES(^{+,-})$-MS, análisis elemental y conductancia. Los datos de RMN y de espectrometría de masas sugieren que existen dos ligandos unidos al centro de Zn (II). Los grupos hidroxilo se encuentran desprotonados y contribuyen a la neutralización de la carga del metal central. Los compuestos de dicoumarol base Zinc, sintetizados en este trabajo, fueron evaluados en su actividad antimicrobiana con bacterias Gram Negativas: *Escherichia coli, Salmonella typhus, Agrobacterium tumefaciens, Erwinia carotovora, Pseudomonas aeruginosa, Klebsiella pneumoniae*; bacterias Gram positivas: *Staphylococcus aureus, Bacillus subtilis, Bacillus atrophaeus* y la cepa fungica de *Candida albicans*. Todos los compuestos mostraron actividades excepcionales, tanto antimicrobianas como anti fúngicas.
Palabras clave: Dicoumarol; compuestos de zinc; análisis espectroscópico; actividad antimicrobiana; actividad antifúngica

Introduction

Zinc is one of the essential metal ion found in all forms of life and is involved in many regulatory activities inside body which includes growth and development of the body, normal brain functioning and gene expressions. An average adult human being has around 3 g of zinc in the body. It also play vital role in preventing many diseases like pneumonia, malaria, common cold and cancers etc [1,2].

Zinc if combined with coumarin derivatives may function as good inhibitor of many enzymes, cure diseases by inhibiting the activities of many pathogenic microbes and recently tested for the anticancer activities. Through coordination of dicoumarols with zinc (II) ion a library of compounds can be synthesized with new type of zinc binding groups (ZBG's). Though the ZBG of hydroxamic acid and other heterocyclic ZBG's were also synthesized but the present library of ZBG's provide insight into the natural product based zinc (II) binders [2-5].

Coumarins can act as effective drugs in treating many diseases like cancer, neuronal diseases, AIDS, etc because they are regarded as good chelator for the iron in haem or inside proteins[6]. For example epigallocatechin-3-gallate (EGCG) is an excellent remedy for neuronal diseases. Structurally it is comprised of coumarin unit that neutralizes iron (III) safeguarding the cell from the oxidative stress of iron (III) [7]. Since the discovery of metal ion role inside body the metal based chemistry is becoming attractive field for the scientific community. Metal ions like zinc, copper, iron, and cobalt are crucial for the proper functioning of cells inside the living matrix, and any disruption can lead to serious neuropsychiatric diseases such as Alzheimer's, Menke's, Wilson's and Parkinson's diseases, Friedreich's ataxia and Hallervorden–Spatz syndrome [8].

Carbonic anhydrase (CAs) is one of the important enzymes involved in the conversion of carbon dioxide to carbonate and proton, a very simple but essential step to overcome the carbon dioxide concentration inside cells. This process is slow without the presence of a suitable metallic catalyst [9]. CAs evolved independently at least five times, with five genetically distinct enzyme families known to date: the α-, β-, γ-, δ-, and ζ-CAs [9-15]. All of them are metalloenzymes with their own distinctions. The α-, β-, and δ- CAs use Zn(II) ions at the active

site, the γ-CAs are probably Fe(II) enzymes (but they are also active when bounded to Zn(II) or Co(II) ions), whereas the ζ-class uses Cd(II) or Zn(II) to perform the physiologic reaction catalysis [13-16].

Metallo thioneins, the ZIP and ZnT families of proteins distribute the zinc ions for specific activities. Therefore zinc is very essential metal ion either as mobile or chelatible and much attention was paid by the scientists all round the world. The role of zinc can be seen in many zinc enriched tissues of hippo campus, pancreas, and most important prostate [17]. Intra cellular zinc trafficking therefore is attracting much attention, hence our work aimed to synthesize the coordination complex of biologically important coumarins and use them for the in vitro antimicrobial studies [18]. The active dicomarols reported earlier were used for the synthesis of zinc based derivatives [19].

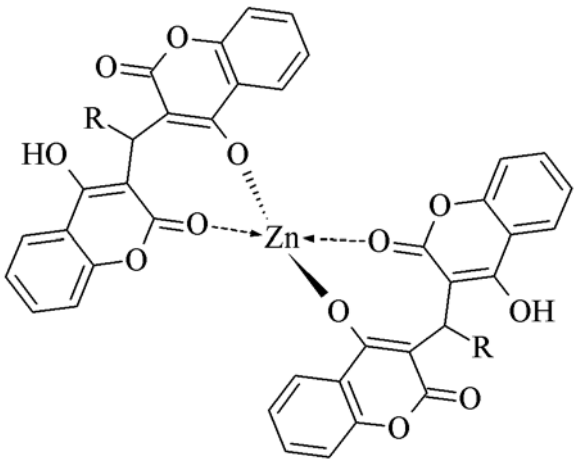
2. Results and discussion

2.1 Spectral analyses

Dicoumarols derived from the condensation of different aldehydes with 4-hydroxycoumarin were reacted with $[Zn\{N(SiMe_3)_2\}_2]$ [21] to get the tetrahedral complexes in 1:2 molar metal to ligand ratio. These complexes were characterized unambiguously using different spectroscopic and analytical techniques. All the ligands were found to be anionic in nature and coordinating to the zinc center through lactons and hydroxyl site of attachments [19].

The high resolution ES^+-MS analyses revealed the specific molecular ion peaks with reasonable abundance, exceptions were seen for the **4** and **10** metal complexes. The reason may be due to the unstable nature of these two complexes in solution formation. The compositions of all the zinc based metal complexes (**1-10**) of dicoumarols were also confirmed by elemental analyses.

Further structural studies was done using techniques like ^1H and $^{13}C\{^1H\}$ NMR along with infrared spectral studies.



where R = aldehydes with different substitutions

Fig. 1. Proposed structure of Zinc (II) complexes of dicoumarols

Multinuclear NMR and infrared studies confirm the formulation revealed from elemental analyses and MS spectral studies. The resonances caused by the $N(SiMe_3)_2$ were replaced by dicoumarols. The deprotonation of strong hydrogen bonded phenols is therefore very easily attained. In previous studies such deprotonation was obtained with sodium metals or sodium methoxide which is also confirmed by us in our sodium work in the field of the same ligands [20]. The unambiguous and successful deprotonation along with successful approach to break the strong intramolecular hydrogen bonds is therefore one of beneficial aspects of the $[Zn\{N(SiMe_3)_2\}_2]$. The CH proton found at the linking site of the two coumarin groups was found to be resonating at 5.3-5.9 ppm depending upon the variation in the aromatic aldehydes [19]. All the complexes show multiplates in the region of 6-9 ppm, assigned to the resonances caused by aromatic protons. The hydroxyl resonance, observed at 11-17 ppm in the free ligands, was found completely absent in the ^1H-NMR spectra of all the zinc complexes. The $^{13}C\{^1H\}$-NMR also support the ^1H-NMR spectral analyses, the hydroxyl based carbon and lactone carbonyl resonances were found low field compared to the neat ligand ($\Delta\delta = \sim 30$ ppm). Therefore all the observations were found completely in line with the complexation behavior for the dicoumarol ligands coordinating through these two sites of attachments. In an impure NMR spectra of the samples show a singlet at 0 ppm which was assigned to the $HN(SiMe_3)_2$. On purification such resonances were completely removed. The infrared spectra also established the attachment of dicoumarol ligands through lactone and phenolic sites. The vibration caused by hydroxyl group was completely diminished by the complexation to zinc centre whereas in other cases it is broadened to a very large extent compared to the neat ligand. The lactonic stretch was found misplaced by $\Delta\upsilon = 40\text{-}70\ cm^{-1}$, suggesting its participation in coordination. The general structure of the produced complexes is shown in Fig. 1.

2.2 Antimicrobial activities

All the synthesized zinc complexes were subjected to their antimicrobial activities against selected pathogenic Gram positive bacteria, Gram negative bacteria and a fungal strain. The activities of all the synthesized compounds were compared with a standard drug already used for stopping the pathogenic activities of tested microorganisms. As may be depicted from table 1, all the compounds were found much more active against the *Bacillus atrophaeus* except **6**. By comparing these results with the bare dicoumarol ligands (as given in table 2) [19] it can be concluded that metal complexation make the ligands much more active. The reason for the enhanced activities may be due to the increase in hydrophobicity in complexes. The results for the antimicrobial activities of these compounds against *Bacillus subtilis* and *Bacillus atrophaeus* are very close. Therefore we can conclude that all the zinc based metal complexes of dicoumarols are showing potential antimicrobial activities against *Bacillus* species of Gram positive bacteria. On the other hand all the zinc based metal complexes of dicoumarol were found moderately active against Gram negative

bacteria viz *Klebsiella pneumoniae*, *Salmonella typhus*, *Pseudomonas aeruginosa*, and *Escherichia coli* and Gram positive bacteria like *Staphylococcus aureus*. Strong antimicrobial activities were observed against *Agrobacterium tumefaciens*, *Erwinia carotovora,* and *Candida albican*. All the compounds were found much more active than the bare ligands. These results show that zinc which is also essential metal for many body functions can also be used as potential antimicrobial therapeutic agent for decreasing or completely vanishing the functions of the microbes in the form of metal complex of dicoumarol ligand.

Compound **1** was found more active than the parent dicoumarol ligand against all the tested microbes except *Candida albican* and *Erwinia carotovora*, compound **2** was more active than the dicoumarol ligand except the *Pseudomonas aeruginosa*, similar activities were seen for all the compounds from **3**-**10**. Compounds like **3**, **5**, **7**, **8**, & **9** were found much active against *Bacillus atrophaeus* than the standard drug erythromycin. Compounds **2** & **10** showed similar activities against *Bacillus atrophaeus* which are aslo comparable to the drug. Compound **2** was found much active than the standard drug in inhibiting the activities of *Bacillus subtilis*. None of the metal based compound was found active against the *Klebsiella pneumoniae*, *Salmonella typhus*, *Pseudomonas aeruginosa*, *Escherichia coli, Erwinia carotovora,* and *Staphylococcus aureus*. Except compound **1**, **4** and **6** all the tested compounds showed greater activities against the *Candida albican* as compared to the standard drug. Similarly all the tested compounds reveal greater activities against *Agrobacterium tumefaciens* except the compound **6**.

Table 1. In Vitro antimicrobial activities of zinc complexes dicoumarols against different animal and plant pathogens

Compounds	*Bacillus atrophaeus (mm)*	*Bacillus subtilis (mm)*	*Klebsiella pneumoniae (mm)*	*Salmonella typhus (mm)*	*Pseudomonas aeruginosa (mm)*	*Escherichia coli (mm)*	*Staphylococcus aureus (mm)*	*Candida albican (mm)*	*Agrobacterium tumefaciens (mm)*	*Erwinia carotovora (mm)*
1	24	24	16	16	---	15	16	---	22	---
2	25	28	19	12	---	24	15	22	26	15
3	27	22	12	13	21	14	22	23	24	16
4	22	22	16	---	16	16	17	---	22	15
5	26	22	09	13	22	25	19	24	22	14
6	19	15	11	---	20	24	21	19	13	15
7	29	20	---	12	20	22	19	21	26	15
8	29	22	---	---	21	25	21	25	24	18
9	27	25	14	20	20	19	21	23	27	22
10	25	20	20	10	20	20	21	24	26	16
Standard	25	26	29	42	36	38	34	16	15	26

Gram positive bacteria: *Bacillus atrophaeus*, *Bacillus subtilis*, *Staphylococcus aureus*, standard used was erythromycin in 6 µM
Gram negative bacteria: *Klebsiella pneumoniae*, *Salmonella typhus*, *Pseudomonas aeruginosa*, *Escherichia coli, Agrobacterium tumefaciens*, *Erwinia carotovora,* standard used was ciprofloxacin in 6 µM
Fungal Strain: *Candida albican,* standard used was clothrimazol in 6 µM

Table 2. In Vitro antimicrobial activities of dicoumarols against different animal and plant pathogens*

Compounds	*Bacillus atrophaeus (mm)*	*Bacillus subtilis (mm)*	*Klebsiella pneumoniae (mm)*	*Salmonella typhus (mm)*	*Pseudomonas aeruginosa (mm)*	*Escherichia coli (mm)*	*Staphylococcus aureus (mm)*	*Candida albican (mm)*	*Agrobacterium tumefaciens (mm)*	*Erwinia carotovora (mm)*
L_1	25	22	---	---	20	14	16	22	22	15
L_2	12	09	09	06	09	---	16	12	10	10
L_3	26	22	---	---	16	---	09	15	20	---
L_4	17	17	---	---	18	12	__	12	16	11
L_5	15	12	---	11	15	10	13	30	20	---
L_6	13	15	---	---	18	---	13	19	27	13
L_7	21	22	---	12	18	12	11	20	24	17
L_8	16	20	11	---	20	09	15	20	20	---
L_9	21	21	12	10	19	19	09	21	20	15
L_{10}	22	25	---	---	15	12	12	---	28	11
Standard	25	26	29	42	36	38	34	16	15	26

[19] Rehman, S.; Ikram, M.; Baker, R. J.; Zubair, M.; Azad, E.; Min, S.; Riaz, K.; Mok, K. H.; Rehman, S.-U-.; *Chem. Cent. J.,* **2013**, *7*, 68.

By comparison of the activities of dicoumarols and their zinc based metal complexes it becomes clear that the zinc complexation make the ligands much active against the pathogenic microbes.

3. Conclusion

Zinc metal complexes of the dicoumarols were synthesized *in-situ* by reacting $[Zn\{N(SiMe_3)_2\}_2]$ with the ligands in THF as a medium. All the metal complexes (**1-10**) were assigned geometries using various spectro-analytical techniques. *In vitro* antimicrobial activities revealed that all the metal complexes (**1-10**) except **6** are more active against Gram positive bacteria and Candida albican, whereas moderate activities were observed against Gram negative bacteria. The most important aspect of these metal complexes is the presence of nontoxic and bioregulatory zinc ion, which can make them very useful as effective pharmaceutical drug.

4. Experimental

4.1 Materials and methods

All chemicals, buffers and solvents used were of analytical grade. Benzaldehyde, 4-nitrobenzaldehyde, 4-chlorobenzaldehyde, and N,N-dimethyl-4-benzaldehyde were obtained from Fluka whereas 3-pyridinecarboxaldehyde, 3-indolecarboxaldehyde, 4-methoxybenzaldehyde, vaniline, 2-hydroxynaphthaldehyde, salicyldehyde, dimethylsilazane and butyllithium were obtained from Sigma Aldrich and were used as such without further purification. Zinc chloride was obtained from fluka, and solvents were obtained from local suppliers of Sigma Aldrich, Merck or Fluka and were distilled at least twice before use. Unless otherwise stated, all reactions were carried out under a dinitrogen atmosphere.

4.2 Instrumentation

Elemental analyses were carried out on Varian Elementar II. Melting points were recorded on a Gallenkamp apparatus. IR spectra were recorded using Shimadzu FTIR Spectrophotometer Prestige-21. ^{1}H-NMR were measured with Bruker DPX 400MHz (400.23 MHz) whereas, $^{13}C\{^1H\}$NMR were recorded on Bruker AV 400MHz (150.9 MHz) spectrometers in CD_3OD at room temperature. Chemical shifts are reported in ppm and standardized by observing signals for residual protons. Molar conductance of the solutions of the metal complexes was determined with a conductivity meter type HI-8333. All measurements were carried out at room temperature with freshly prepared solutions. Mass spectra were recorded on a LCT Orthogonal Acceleration TOF Electrospray mass spectrometer.

4.3 Antimicrobial activity

About 2.8 g/L nutrient agar and nutrient broth were prepared in deionized water and kept in autoclave set at 1.5 Pounds pressure for about 15 min. The nutrient agar media were poured aseptically into sterilized petri dishes in laminar flow under inert atmosphere. The petri dishes were kept in inverted position for about 24 hrs at 37 ºC. Bacterial cultures were adjusted to 0.5 McFarland turbidity standards and *Candida albican* was adjusted to 108 cfu/ml. Sterile filter paper of diameter 6mm was used for bacterial strains whereas its thickness ranged upto 13 mm for fungal strains. These filter papers were in the form of discs and were seeded with 0.5 McFarland and 106 cfu/ml cultures of bacteria and fungi respectively. Solutions (0.5 mM) of the synthesized compounds were applied to the prepared discs and incubated for 18 hr at 37 ºC. Subsequent measurements of the zone of activity were carried out [21].

4.4 Synthesis of dicoumarols

Synthesis of dicoumarols has been described by Sadia et al. 2013 [19]. The sequence of codes $\mathbf{L_1}$-$\mathbf{L_{10}}$ used in this manuscript is following the sequence as described earlier by us [19].

4.5 Synthesis of zinc compounds

Zinc derivatives of the coumarin ligands [19] were prepared by following the same procedure. $[Zn\{N(SiMe_3)_2\}_2]$ was synthesized according to the literature procedure [22]. 10 mmol of butyllithium in n-hexane was added to the 50 mmol dimethyl silazane dissolved in degassed dry diethyl ether and the reaction mixture stirred for one hour at 0 ºC in completely inert atmosphere. 20 mmol of synthesized yellow liquid $Li\{N(SiMe_3)_2\}_2$ was added to the 10mmol ethereal solution of zinc chloride and the mixture stirred under argon for one hour. White floating powder of $[Zn\{N(SiMe_3)_2\}_2]$ was obtained after purifying the sample by washing and recrystallizing from diethyl ether.

10 mmol of $[Zn\{N(SiMe_3)_2\}_2]$ was added to 5 cm^3 THF and stirred at 0 ºC, 5 mmol of dicoumarol ligand dissolved in minimum amount of THF was added to this solution and the mixture stirred for 4-5 hour at room temperature. After the formation of powder, the mixture was filtered and washed many times with diethyl ether and THF and dried in vacou.

4.5.1 Bis[3,3'-(1H-indole-3-ylmethanediyl-4-hydroxy-2H-chromen-2-one)]zinc (II) (1)

IR: 3500(bd), 2980(w), 2612(w), 1689(s), 1636(w), 1584(w), 1518(w), 1483(s), 1417(s), 1260(s), 1241(w), 1222(w), 1107(w), 1073(s), 1025(w), 900(w), 848(s), 791(w), 749(w), 695(w), 660(w) cm^{-1}, ^{1}H-NMR (400.23 MHz, CD_3OD, 303k): δ = 6.93 (s, 1H, methyl), 7.21 (m, 1H), 7.22 (m, 1H), 7.23 (dd, J_{HH} = 8.77 Hz, 1H), 7.25 (m, 1H), 7.48 (dd, J_{HH} = 8.71 Hz, 1H), 7.78 (s, 1H), 7.80 (s, 1H) ppm, $^{13}C\{^1H\}$-NMR (150.9 MHz, CD_3OD, 303k): δ = 67.03 (CH, pyrrole), 105 (C, pyrrole), 112-138 (CH, aromatic), 155 (C, phenolic), 164 and 165 (C=O, lactone) ppm, Elemental Analysis ($C_{54}H_{36}N_2O_{12}Zn$), Calc. C: 66.84%, H: 3.74%, N: 2.89%, Zn: 6.74%, Exp. C: 66.44%, H: 3.69%, N: 2.90%, Zn: 6.34%, EI-MS: *m/z* (%) 968.1554 (100%) [$C_{54}H_{36}N_2O_{12}Zn^+$].

4.5.2 Bis[(3,3'-(4-chlorophenyl)methanediyl-4-hydroxy-2H-chromen-2-one)]zinc(II) (2)

IR: 3420(bd), 2924(w), 2890(w), 1703(w), 1649(s), 1605(s), 1524(s), 1411(s), 1347(s), 1277(w), 1182(s), 1107(s), 1045(s), 921(s), 894(s), 831(s), 797(s), 758(s), 742(s), 712(s), 699(s), 669(s) cm^{-1}, ^{1}H-NMR (400.23 MHz, CD_3OD, 303k): δ = 5.9 (s, 1H, methyl), 7.18-8.0 (aromatic protons) ppm, $^{13}C\{^1H\}$-NMR (150.9 MHz, CD_3OD, 303k): δ = 103 (CH, methyl), 114.8-132 (Aromatic carbons), 152 (C, Chloro), 154 (C, Phenolic), 162 & 164 (C, lactone) ppm. Elemental Analysis ($C_{50}H_{28}Cl_2O_{12}Zn$), Calc. C: 62.75%, H: 2.95%, Zn: 6.83%, Exp. C: 62.14%, H: 3.09%, Zn: 6.91%, EI-MS: not observed.

4.5.3 Bis[(3,3'-(4-hydroxyphenyl)methanediyl-4-hydroxy-2H-chromen-2-one)]zinc(II) (3)

IR: 3700(bd), 2980(bd), 2800(bd), 1704(s), 1649(s), 1605(s), 1526(s), 1469(s), 1414(s), 1245(s), 1182(s), 1107(s), 1042(s), 921(s), 892(s), 831(w), 799(w), 758(s), 743(s), 712(s), 669(s) cm^{-1}, ^{1}H-NMR (400.23 MHz, CD_3OD, 303k): δ = 5.34 (s, 1H, methyl), 6.63-7.81 (m, aromatic protons) ppm, $^{13}C\{^1H\}$-NMR (150.9 MHz, CD_3OD, 303k): δ = 104.6 (CH, methyl), 109-142 (aromatic carbons), 164 (C, lactone) ppm, Elemental Analysis ($C_{50}H_{30}O_{14}Zn$), Calc. C: 65.26%, H: 3.29%, Zn: 7.11%, Exp. C: 65.80%, H: 5.10%, Zn: 8.89%. EI-MS: *m/z* (%) 918.0921 (40%) [$C_{50}H_{30}O_{14}Zn^+$].

4.5.4 Bis[(3,3'-(4-nitrophenyl)methanediyl-4-hydroxy-2H-chromen-2-one)]zinc(II) (4)

IR: 3420(bd), 2924(w), 2890(w), 1703(w), 1649(s), 1605(s), 1524(s), 1411(s), 1347(s), 1277(w), 1182(s), 1107(s), 1045(s), 921(s), 894(s), 831(s), 797(s), 758(s), 742(s), 712(s), 699(s), 669(s) cm^{-1}, ^{1}H-NMR (400.23 MHz, CD_3OD, 303k): δ = 5.9 (s, 1H, methyl), 7.18-8.0 (aromatic protons) ppm, $^{13}C\{^1H\}$-NMR (150.9 MHz, CD_3OD, 303k): δ = 103 (CH, methyl), 114.8-132 (Aromatic carbons), 152 (C, nitro), 154 (C, Phenolic), 162 & 164 (C, lactone) ppm. Elemental Analysis ($C_{50}H_{28}N_2O_{16}Zn$), Calc. C: 61.39%, H: 2.89%, N: 2.86 %, Zn: 6.69%, Exp. C: 63.01%, H: 2.11%, N: 2.89 %, Zn: 5.93%, EI-MS: *m/z* (%) 976.0724 (5%) [$C_{50}H_{28}N_2O_{16}Zn^+$].

4.5.5 Bis[3,3'-[(3-methoxy-4-hydroxyphenyl)methanediyl-4-hydroxy-2H-chromen-2-one)]zinc(II) (5)

IR: 3300(bd), 2980(bd), 1704(s), 1649(s), 1605(s), 1526(s), 1469(s), 1414(s), 1245(s), 1182(s), 1107(s), 1042(s), 921(s), 892(s), 831(w), 799(w), 758(s), 743(s), 712(s), 669(s) cm^{-1}, ^{1}H-NMR (400.23 MHz, CD_3OD, 303k): δ = 2.51 (br, s, 2H, methyl), 6.55-6.64 (m, 1H), 7.19 – 7.31 (m, 2H), 7.50 (t, $^3J_{HH}$ = 7.78 Hz, 1H), 7.82 (d, $^3J_{HH}$ = 8.03Hz, 1H) ppm, = Elemental Analysis ($C_{52}H_{34}O_{16}Zn$), Calc. C: 63.72%, H: 3.50%, Zn: 6.67%, Exp. C: 62.23%, H: 4.10%, Zn: 6.89%. EI-MS: *m/z* (%) 978.1132 (23 %) [$C_{52}H_{34}O_{16}Zn^+$].

4.5.6 Bis[3,3'-(pyridin-3-ylmethanediyl-4-hydroxy-2H-chromen-2-one)]zinc(II) (6)

IR: 3700(bd), 2980(bd), 2800(bd), 1704(s), 1649(s), 1605(s), 1526(s), 1469(s), 1414(s), 1245(s), 1182(s), 1107(s), 1042(s), 921(s), 892(s), 831(w), 799(w), 758(s), 743(s), 712(s), 669(s) cm^{-1}, ^{1}H-NMR (400.23 MHz, CD_3OD, 303k): δ = 5.34 (s, 1H, methyl), 6.63-7.81 (m, aromatic protons) ppm, $^{13}C\{^1H\}$-NMR (150.9 MHz, CD_3OD, 303k): δ = 104.6 (CH, methyl), 109-142 (aromatic carbons), 164 (C, lactone) ppm, Elemental Analysis ($C_{48}H_{28}N_2O_{12}Zn$), Calc. C: 62.40%, H: 3.17%, N: 3.15%, Zn: 7.35%, Exp. C: 62.43%, H: 3.79%, N: 3.98%, Zn: 7.06%, EI-MS: *m/z* (%) 888.0928 (100%) [$C_{48}H_{28}N_2O_{12}Zn^+$].

4.5.7 Bis[3,3'-[(4-methoxyphenyl)methanediyl-4-hydroxy-2H-chromen-2-one)]zinc(II) (7)

IR: 2980(w), 1622(s), 1597(s), 1577(s), 1556(s), 1521(s), 1481(s), 1452(s), 1402(s), 1340(s), 1271(s), 1242(s), 1209(s), 1153(w), 1105(s), 1072(s), 1024(s), 979(s), 947(s), 881(s), 815(s), 765(s), 750(s), 717(s), 688(s), 677(s), 610(w), 526(s) cm^{-1}, $^{13}C\{^1H\}$-NMR (150.9 MHz, CD_3OD, 303k): δ = 55.57 (CH, N-CH_3), 103.73 (CH, methyl), 114.85-146.99 (aromatic region), 152.34 (C, phenolic), 164.82 & 167.82 (C, lactone) ppm, Elemental Analysis ($C_{52}H_{34}O_{14}Zn$), Calc. C: 65.87%, H: 3.61%, Zn: 6.90%, Exp. C: 66.32%, H: 3.83%, Zn: 7.03%, EI-MS: *m/z* (%)946.1234 (7%) [$C_{52}H_{34}O_{14}Zn^+$].

4.5.8 Bis[3,3'-(phenylmethanediyl-4-hydroxy-2H-chromen-2-one)]zinc(II) (8)

IR, : 3400(bd), 3026(w), 2980(w), 1598(s), 1516(s), 1446(s), 1405(s), 1366(s), 1356(w), 1277(w), 1250(w), 1211(w), 1107(s), 1084(s), 1058(w), 937(w), 837(s), 757(s), 727(s), 693(s) cm^{-1}, ^{1}H-NMR (400.23 MHz, CD_3OD, 303k): δ = 6.98-8.11 (aromatic protons) ppm, $^{13}C\{^1H\}$-NMR (150.9 MHz, CD_3OD, 303k): δ = 103.9 (CH, methyl), 111.9-141.89 (aromatic), 166.7 & 168 (C=O, lactone) ppm, Elemental Analysis ($C_{50}H_{30}O_{12}Zn$), Calc. C: 67.61%, H: 3.40 %, Zn: 7.36%, Exp. C: 67.21%, H: 3.76 %, Zn: 7.99 %, EI-MS: *m/z* (%) 886.1023 (60%) [$C_{50}H_{30}O_{12}Zn^+$].

4.5.9 Bis[(3,3'- (4-N,N-dimethylaminophenyl)methanediyl-4-hydroxy-2H-chromen-2-one)]zinc(II) (9)

IR: 3414(bd), 3242(w), 2980(w), 1622(s), 1597(s), 1577(s), 1556(s), 1521(s), 1481(s), 1452(s), 1402(s), 1340(s), 1271(s), 1242(s), 1209(s), 1153(w), 1105(s), 1072(s), 1024(s), 979(s), 947(s), 881(s), 815(s), 765 (s), 750 (s), 717 (s), 688 (s), 677 (s), 610 (w), 526 (s) cm^{-1}, $^{13}C\{^1H\}$-NMR (150.9 MHz, CD_3OD, 303k): δ = 55.57 (CH, N-CH_3), 103.73 (CH, methyl), 114.85-146.99 (aromatic region), 152.34 (C, C-N-$(CH_3)_2$), 164.82 & 167.82 (C, lactone) ppm, Elemental Analysis ($C_{54}H_{40}N_2O_{12}Zn$), Calc. C: 66.57%, H: 4.14%, N: 2.88%, Zn: 6.71, Exp. C: 66.57%, H: 4.14%, N: 2.88%, Zn: 6.71, EI-MS: *m/z* (%)972.1867 (100%) [$C_{54}H_{40}N_2O_{12}Zn^+$].

4.5.10 Bis[(3,3'- (2-hydroxy-1,2-dihydronaphthalen-1-yl-4-hydroxy-2H-chromen-2-one)]zinc(II) (10)

IR: 2980(w), 1622(s), 1597(s), 1577(s), 1556(s), 1521(s), 1481(s), 1452(s), 1402(s), 1340(s), 1271(s), 1242(s), 1209(s), 1153(w), 1105(s), 1072(s), 1024(s), 979(s), 947(s), 881(s), 815(s), 765(s), 750(s), 717(s), 688(s), 677(s), 610(w), 526(s)cm^{-1}, $^{13}C\{^1H\}$-NMR (150.9 MHz, CD_3OD, 303k): 103.73 (CH, methyl), 114.85-146.99 (aromatic region), 152.34 (CH, phenolic), 164.82 & 167.82 (C, lactone) ppm, Elemental Analysis ($C_{58}H_{34}O_{14}Zn$),

Calc. C: 68.28%, H: 3.36%, Zn: 6.41%, Exp. C: 68.31%, H: 3.87%, Zn: 6.12%. EI-MS: *m/z* (%)1018.1234 (10%) [$C_{58}H_{34}O_{14}Zn^{+}$].

Conflict of interest

The author declares no conflict of interest.

Author's contribution

Both the authors M. Ikram and S. Rehman equally contributed to the content of this manuscript and equal first authors.

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