Ariane Schiavenin

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Dissertação Apresentada à Universidade de Caxias do Sul, para obtenção do Título de Mestre em Ciências da Saúde.

Caxias do Sul

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Ariane Schiavenin

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Dedicatória

Dedico esta conquista à minha família:

Aos meus amados pais, Olir e Vera, que desde muito cedo, ensinaram o valor do estudo e do conhecimento. Meus pais me deram asas, permitiram que eu sonhasse e fizeram tudo o que estivesse no alcance para ajudar na concretização destes sonhos.

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Sumário

Esta dissertação de Mestrado Acadêmico Stricto Sensu é apresentada no formato exigido pelo Programa de Pós-Graduação em Ciências da Saúde da Universidade de Caxias do Sul. A mesma é constituída da secção de “Introdução com referências bibliográficas”, a inclusão do artigo original submetido/publicado em periódico Qualis A na classificação da Coordenação de Aperfeiçoamento de Pessoal em Nível Superior (CAPES), e as “Considerações Finais e Perspectivas”.

1. INTRODUÇÃO

O desenvolvimento de novos medicamentos desde a pesquisa básica até a formulação final é uma missão desafiadora, haja visto o longo tempo, devido a todas as fases necessárias, bem como o valor a ser investido. Pesquisas estimam que o valor investido para a obtenção de um novo medicamento ultrapasse 2 bilhões de dólares (1). Além disso, há uma baixa taxa de sucesso, sendo que a grande maioria dos medicamentos avaliados em ensaios clínicos não chega ao mercado devido à falta de eficácia ou a presença de efeitos colaterais inaceitáveis (2,3).

Desta forma, a maioria das moléculas candidatas a fármacos são descartadas durante as fases de testes I e II da pesquisa clínica, em razão de sua toxicidade. Esse é considerado o principal fator que contribui para o alto custo no processo de desenvolvimento de medicamentos. Estatísticas mostram que apenas 0,1% das moléculas sintetizadas ou obtidas de fontes naturais possuem potencial para se tornar um fármaco comercial. As perdas ao longo do processo de desenvolvimento de um novo fármaco são elevadas, apenas 15% dos medicamentos que são testados nas fases clínicas chegam ao mercado (2,4). Assim, entre as estratégias recentemente utilizadas para diminuir os riscos atrelados ao desenvolvimento de fármacos, está o bioisosterismo, que o processo de modificar quimicamente compostos já conhecidos para uma mesma aplicação farmacológica (5,6).

Entre as estratégias para obtenção de bioisósteros, está a síntese de complexos metálicos. Para isso, algumas características das moléculas a serem complexadas são importantes, como por exemplo, serem bases de Lewis (caracterizada pela presença de funções: amina, carboxilato, álcool, sulfeto, entre outras), as quais podem ser exploradas como ligantes em reações de complexação com cátions metálicos (ácidos de Lewis), os quais possuem orbitais *d* vazios que podem acomodar os pares de elétrons doados pelos ligantes. O número máximo de ligações coordenadas que um átomo metálico comportará vai depender do número de orbitais vazios de energia mínima adequada para a ligação ocorrer (7). Estes compostos podem ser descritos como um sistema coordenado com um átomo metálico e um ligante, que pode ser de natureza orgânica ou inorgânica (8,9). Em

resumo, os complexos ou compostos de coordenação são definidos como resultado de um mecanismo doador-aceptor ou de uma reação ácido-base de Lewis entre dois ou mais compostos químicos diferentes (10).

A manutenção das funções biológicas está diretamente relacionada a presença de metais, os quais atuam como cofatores enzimáticos, em sítios ativos de enzimas e nas metaloproteínas. Entre os metais mais encontrados estão o ferro, mangnésio, cobre, molibdênio, zinco, cobalto e níquel (11). Entre os complexos de coordenação com função biológica destacada, pode-se citar os complexos biológicos derivados do grupo porfirina, entre os quais citamos a clorofila *a*, o grupamento heme e a vitamina B12, ou cianocobalamina. As clorofilas apresentam um centro reativo denominado magnésio II-porfirina, além de serem os pigmentos fotossintetizantes dos seres autotróficos, são responsáveis pela produção de todo oxigênio atmosférico (12). Complexos de ferro II-porfirina são formados pelas metaloproteínas carreadoras de hemoglobina e mioglobina. Estes complexos são responsáveis pelo transporte de oxigênio às células e pela remoção de gás carbônico produzido pela respiração celular (13). Por fim, a enzima cianocobalamina, ou vitamina B12 é composta por um centro ativo composto por uma estrutura complexada de cobalto III-porfirina. A cianocobalamina é sintetizada pelos microrganismos e sua presença nos tecidos humanos ocorre através da cadeia alimentar. A cianocobalamina é uma enzima essencial para a eritropoiese (14).

Entre os metais de transição, o zinco ocorre naturalmente na forma oxidada Zn^{+2} na forma de sais. O zinco é componente estrutural e/ou funcional de várias metaloenzimas e metaloproteínas, participando de muitas reações do metabolismo celular, incluindo processos fisiológicos, tais como função imune, defesa antioxidante, crescimento e desenvolvimento. Desta forma, este metal é predominante tanto para as funções metabólicas nos sistemas biológicos como para aplicações como reagente em química. O íon zinco age como um ácido de Lewis forte, pois é capaz de aceitar um par de elétrons, fazendo com que seja um íon

estável, ligando-se preferencialmente com bases de Lewis fortes, como óxido, sulfeto e bases nitrogenadas (15,16).

Os fármacos anti-inflamatórios não-esteroides ou AINEs (NSAIDs do inglês) compreendem uma família de moléculas inibidoras das enzimas ciclooxigenases (COX) mediadoras de processos inflamatórios (17). Esta classe pode ainda ser dividida com base na sua seletividade para as isoformas (COX-1 e COX-2) em AINEs não seletivos e AINEs seletivos com inibição preferencial da COX-2 (18). O mecanismo geral de ação desta classe de fármacos baseia-se na inibição competitiva das COX e bloqueiam a conversão do ácido araquidônico em mediadores inflamatórios, como as prostaglandinas. Esta classe de fármacos representa a maior porção do mercado farmacêutico, sendo os medicamentos sintéticos mais consumidos da história (19,20). Os AINEs mais comumente utilizados são paracetamol, aspirina, diclofenaco, ibuprofeno e naproxeno (21). No entanto, a toxicidade gastrointestinal associada ao amplo uso de AINEs provou ser uma das principais desvantagens durante a terapia de longo prazo (21,22). As complicações gastrointestinais são mediadas principalmente pela inibição da (COX-1) e consequente supressão da produção de prostaglandinas. São atribuídos ao uso prolongado de AINEs, condições como úlcera péptica, perfuração e sangramento gastrointestinal (18).

O ibuprofeno ou ácido (R,S)-2-(4'-isobutilfenil)propanoico, é um dos analgésicos-antipiréticos-AINEs mais utilizados em todo o mundo (23). Este é um dos fármacos mais seguros utilizados no momento para tratamento da dor, inflamação e febre. O extensivo uso clínico deste fármaco o tornaram uma das histórias de sucesso da indústria farmacêutica (24). Enquanto isso, o 2-(2-((2,6-diclotofenil)amino)fenil)acetato ou diclofenaco é um fármaco AINE da família dos acetatos com larga aplicação por via oral, nas formas farmacêuticas sólida ou líquida, geralmente sob a forma de sal de Na^+, K^+, dietilamina e epolamina além de compor preparações injetáveis via intramuscular sendo um dos mais potentes AINEs (25). Ibuprofeno e diclofenaco são AINEs derivados dos ácidos acético e propiônico respectivamente, com a função carboxilato como grupo farmacofórico em comum. Estes fármacos apresentam propriedades químicas para serem

utilizados como compostos de coordenação com íons metálicos por ligação covalente coordenada.

Para avaliar a viabilidade de uma molécula alguns ensaios são necessários, uma vez que, surpreendentemente, mais da metade dos AINEs introduzidos na clínica desde a década de 1970 foram retirados do mercado devido, principalmente, a toxicidade apresentada (24). Assim, estimar a citotoxicidade é primordial no desenvolvimento de um fármaco e está compreendida ainda na fase I da pesquisa clínica (26). O ensaio de citotoxicidade com culturas de células de mamíferos é uma metodologia padronizada para o estudo da citotoxicidade de xenobióticos. A determinação da citotoxicidade pode ser através de avaliação qualitativa ou quantitativa (27). Além disso, a interação entre a farmacocinética, toxicidade e potência é crucial para a avaliação da efetividade da droga. A evolução das abordagens computacionais, para aperfeiçoar as propriedades farmacocinéticas e de predição de toxicidade permite a predição do perfil da molécula de forma eficaz e rápida para os candidatos a medicamentos (28,29).

Em síntese, há inúmeras razões para o uso de bioisosterismo no desenvolvimento de novos medicamentos, incluindo a necessidade de melhorar a atividade farmacológica, obter seletividade por determinado receptor ou subtipo de isoforma enzimática – com redução simultânea de certos efeitos adversos - ou mesmo otimizar a farmacocinética (30). Considerando o bioisosterismo como uma excelente estratégia de modificação molecular, é evidente sua importância na construção de compostos congêneres projetados como candidatos a novos medicamentos.

Considerando a importância da obtenção de compostos de coordenação derivados de medicamentos, entende-se que a busca por complexos organometálicos viáveis, ou seja, os quais apresentem propriedades farmacológicas aceitáveis como maior potência e seletividade, e menor toxicidade é de extrema relevância científica. Desta forma, o presente trabalho teve como foco a obtenção de quatro compostos de coordenação derivados de ibuprofeno e diclofenaco ligados ao íon zinco, sua caracterização química, avaliação da

citotoxicidade, avaliação da capacidade de quebra de DNA plasmidial e testes *in silico* visando o desenvolvimento de novos protótipos de drogas, possivelmente a serem utilizados como anti-inflamatórios.

A discussão e resultados deste trabalho serão apresentados na forma de artigo científico a ser submetido à revista "J*ournal of Inorganic Biochemistry*" - Fator de impacto 3.4.

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3. ARTIGO

Journal of Inorganic Biochemistry

ZINC ORGANOCOMPLEXES CONTAINING NON-STEROID ANTI-INFLAMMATORIES AND PLANE AROMATIC DIIMINES: NEW POTENTIAL DRUGS

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Abstract

Strategies have been developing to obtain new medicines. Bioisosterism represents an approach used by the medicinal chemist for the rational modification of lead compounds into safer and more clinically effective agents. Evidently, the synthesis of organocomplexes has been an important strategy in the planning of new drugs with examples of great improvements in therapeutic efficacy. Thus, this work aimed at the synthesis of new zinc complexes with nonsteroidal anti-inflammatory drugs (NSAIDs), as well as the chemical characterization and the previous toxicity by cytotoxicity, and evaluating the ability of these compounds to interact with DNA. As a result, four new zinc II ternary complexes containing the NSAIDs diclofenac (Diclof) and ibuprofen (Ibup) and zinc neutral linker were obtained by the two-step solvent metalligand complexation method (1. Zn-Ibup-Bipy, 2. Zn-Ibup-Phen, 3. Zn-Diclof-Bipy, 4. Zn-Diclof-Phen). Molecular structures were determined by NMR, FTIR and HR-MS which demonstrated that complexes are binuclear systems of general formula [Zn($RCOO^{-}$)$_2$N-binder]. *In silico* studies were performed to toxicity and physicochemical properties and biological target prediction. Cytotoxic was determined by MTT assay. Results indicated a relative absence of toxicity to these organometallic zinc derivatives. It is also observed that compound Zn-Diclof-Bipy and Zn-Diclof-Phen showed high potential to be submitted to studies of evaluation of biological activity, without potential theoretical toxic effects. Plasmidi DNA breakdown capacities were evaluated by producing single and double breaks (DNA FII and FIII) from plasmid incubation with complex solutions in the concentration range 0 to 400 $\mu mol \cdot L^{-1}$ in experiments with the presence and absence of light. Both experiments did not show significant differences ($P \leq 0.05$) in induced DNA cleavage activity between the maximum study concentrations (400 $\mu mol \cdot L^{-1}$) and the negative controls for both complexes. The types of complex 1 and 2 interactions with the secondary DNA structure were determined by titrating a CT-DNA solution with complex solutions and monitored by circular dichroism spectrometry. Complex 1 was not cytotoxic at the concentrations tested against the Vero cell line. The results showed that both complexes interact with the grooves of the secondary structure of CT-DNA by electrostatic attraction, but without evidence of alteration in the primary structure. Thus, four new compounds were synthesized, characterized and had their previous toxicities determined. These compounds are promising new drugs, with the next step being evaluations of their activity.

Key words: Zinc complexes; Bioisosterism; NSAIDs; Anti-inflammatory activity.

1. Introduction

The process of creating a new drug is complex, long and highly expensive. A study estimated that the cost of bringing a new drug to the market was over U$ 2 billion dollars (1). Several factors affect the cost and the failure of drug development, but basically the reasons are: lack of efficacy, adverse effects, presence of toxicity and market reasons(2–4).

Strategies have been developing to obtain new medicines. Biosterism represents one approach used by the medicinal chemist for the rational modification of lead compounds into safer and more clinically effective agents. The success of this strategy in developing new substances which are therapeutically attractive has observed a significant growth in distinct therapeutic classes, being amply used by the pharmaceutical industry to discover new analogs of therapeutic innovations commercially attractive (me-too), and also as a tool useful in the molecular modification(5,6).

In the historical context, cisplatin was the first molecule purely inorganic to be used as cancer treatment. Today cisplatin is the first-line medication for the treatment of ovarian and esophageal cancer. Its mechanism of action occurs by intercalating the alpha helix of the DNA by complexation, usually to the guanine bases, inducing double chain breaks and, consequently, apoptosis of the cell. After the advent of cisplatin in 1978, many kinds of research were developed to obtain new complexes analogous to cisplatin for the treatment of other types of cancer (7,8).

Diclofenac and Ibuprofen are widely used NSAIDs (a non-steroidal anti-inflammatory drug), therapeutically used in inflammatory and painful conditions of rheumatic and non-rheumatic origin (3,9). The success of NSAIDs in treating various inflammatory conditions such as rheumatoid arthritis and osteoarthritis is due to inhibition of the enzyme prostaglandin H synthase (PGHS) or cyclooxygenase (COX) as a highly suitable target in anti-inflammatory therapies (10–12). However, the gastrointestinal (GI) toxicities associated with widespread NSAID use proved to be a major concern during long term therapy(11).

Thus, for increasing the efficiency of NSAIDs, several strategies have been developed, such as the synthesis of metal complexes, once some studies have been suggested that the anti-inflammatory activity of NSAIDs is enhanced by coordination with metals. The synthesis of metal complexes is a strategy that can increase potency and bioavailability and at the same time, decreasing the toxicity. Synthesis and study of metal complexes with anti-inflammatory drugs as ligands is a research area of considerable interest as an approach to new development (13–16).

Diclofenac and Ibuprofen which has carboxylic acid as the main functional group, have a chemical affinity for metal ions, such as zinc II and copper II, to form coordination complexes with varied biological activity (15). The biological role of zinc is well-established since zinc is a biometal with relatively high abundance in the human body. Zinc II is required by several proteins and enzymes. Zinc homeostasis is highly regulated in all cells and organisms to optimize availability (14,17,18).

There are two commonly nitrogen binders used for synthesis and study of metal complexes. Bipyridine (Bipy) and Phenanthroline (Phen). Phen is considered a ligand with the following characteristics: a rigid, flat, hydrophobic and electron-poor heteroaromatic system, whose nitrogen atoms are placed to act cooperatively in the cation binding. These structural features determine its coordination ability toward metal ions. Bipy has been extremely used as binders for different applications, since it is slightly polar and insoluble in water and, due to the non-binding electron pairs of nitrogen atoms in heteroaromatic rings (19,20).

In this context, this work aims to synthesize and characterize four ternary complexes of Diclofenac and Ibuprofen with Zn and Phen and Bipy as a nitrogen ligand, which can be explored as anti-inflammatory. For this purpose, the compounds were characterized by spectroscopic properties 1H and 13C NMR, FTIR, HRMS. *In silico* studies were conducted to predicted toxicity, physicochemical properties, and biological target prediction. Biological activity was evaluated by MTT assay. The ability of these compounds to interact with DNA was also evaluated.

2. Material and Methods

The chemicals 1,10-phenanthroline, 2,2'-bipyridine, anhydrous zinc chloride, zinc nitrate heptahydrate, potassium diclofenac and (*R*,*S*)-ibuprofen acid form were obtained from Sigma-Aldrich. Absolute ethanol and *N*,*N'*-dimethylformamide were purchased from Vetec Chemicals.

2.1 Physical measurements

^{1}H and ^{13}C for NMR analysis was obtained for by Fourier 300 spectrometer (Bruker) (300.18 MHz from ^{1}H and 75.49 MHz from ^{13}C with 5 mm probe). Infrared analysis was obtained by Perkin Elmer Spectrum 400 FTIR spectrometer from KBr pellet method. HRMS analysis were performed on Bruker MicroTOF-QII with electrospray source by positive ions mode.

2.2 *Synthesis of zinc (II) complexes*

Figure 1 presents a summary of the synthesis of the compounds.

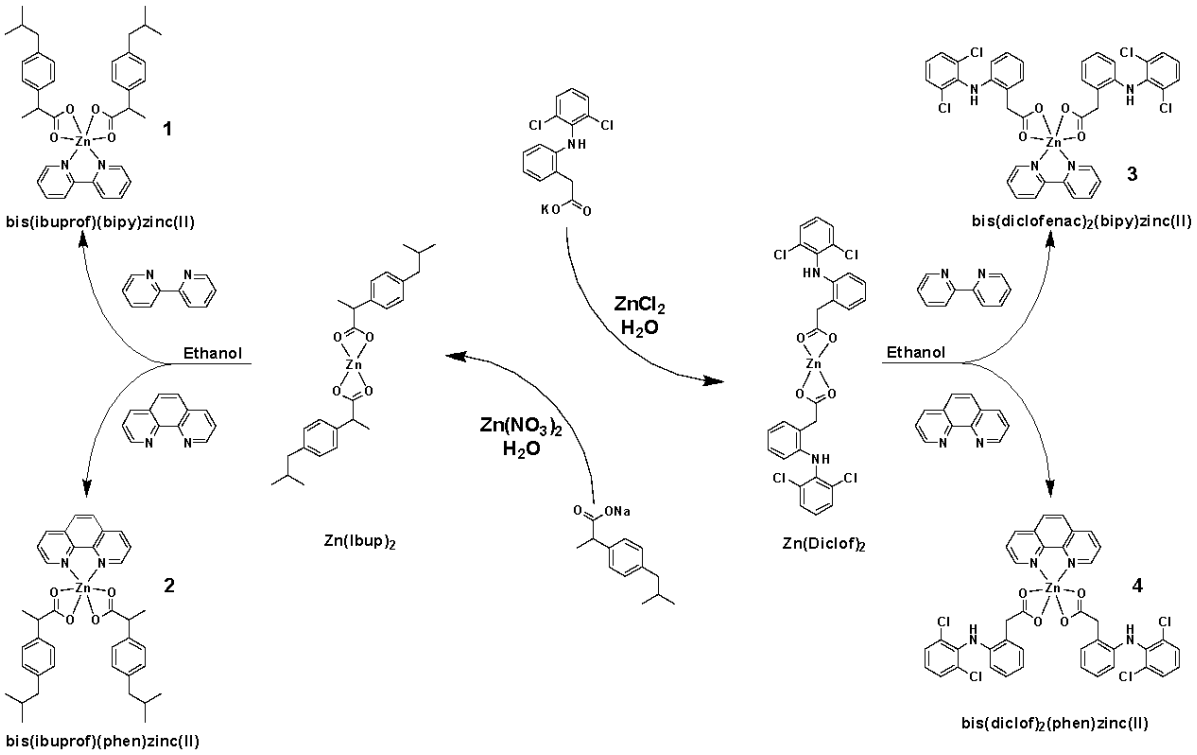


Figure 1. Scheme of synthetic routes to obtain four ternary complexes contain NSAIDs drugs, zinc and planar aromatic diimines.

2.2.1 Synthesis of bis[(R,S)-2-{4-(methypropyl)phenyl}propanoate] zinc(II) – Zn(ibup)$_2$

The binary salt was prepared according Abu Ali, *et al*, (2016) method with a few modifications, (21) briefly: the (*R,S*)-2-{4-(methypropyl)phenyl}propanoic acid (0.825 g, 4.0 mmol) was added over water (50 mL) and neutralized with Na_2CO_3 (water solution 0.5 mol.L^{-1}) to pH 8.9 over stir at room temperature. $Zn(NO_3)_2.6H_2O$ water solution (0.594 g, 2 mmol, 20 mL) was added dropwise to the first solution by vigorous stir. An amorphous white solid was immediately formed, recovered by filtration after 24 h and dried over freeze draying for 24 h. Yield: 0.630 g, (66 %); MP: 78° C; FTIR (cm^{-1}, KBr pellet): 3215.5 (*H2O*), 2954.1 (*Assym CH3*), 2930.9 (*Assym CH2*), 2868.2 (*Sym CH3*), 1684.6, 1545.2 (*Assym COO*), 1511.5, 1458.5, 1411.7 (*Sym COO*), 1365.9, 1292.1, 1067.9, 85.6, 785.4, 757.4, 717.4, 598.8, $\Delta_{(Assym-Sym\ COO^-)}$ 128; ^{1}HNMR (δ-ppm, DMSO-d_6): 0.83 (d-6H, $2CH_3$, J_{H-H} = 6.6Hz), 1.28 (d-3H, CH_3, J_{H-H} = 6.9 Hz), 1.78 (sep-1H, CH, J_{H-H} = 6.6Hz), 2.38 (d-2H, CH_2, J_{H-H} = 7.9Hz), 3.49 (q-1H, CH, J_{H-H} = 7.2Hz), 7.01 (d-2H, 2CH, J_{H-H} = 8.1Hz), 7.17 (d-2H, 2CH, J_{H-H} = 8.1Hz); ^{13}CNMR (δ-ppm, DMSO-d_6): 20.1 (CH_3), 22.3 (CH_3), 29.79 (CH), 44.39 (CH), 45.93 (CH_2),

127.32 (CH), 128.62 (CH), 138.69 (C), 140.87 (C), 179.61 (COO^-). HRMS (positive ions): *m/z* 1155.4811 ($C_{65}H_{87}O_{10}Zn_2$) $[2M+Ibup+2H]^+$, 949.3534 ($C_{52}H_{69}O_8Zn_2$) $[2M+H]^+$, 761.2359 ($C_{39}H_{53}O_7Zn_2$) $[2M-Ibup+H_2O]^+$, 743.2323 ($C_{78}H_{102}O_{12}Zn_4$) $[3M+Zn]^{2+}$, 506.1439 ($C_{52}H_{68}O_8Zn_3$) $[2M+Zn]^{2+}$, 745.1825 ($C_{26}H_{35}O_4Zn$) $[M+H]^+$, 287.0620 ($C_{13}H_{19}O_3Zn$) $[M-Ibup+H_2O]^+$, 229.1201 ($C_{13}H_{18}NaO_2$) $[Ibup+Na+H]^+$.

2.2.2 Synthesis of bis[2-{2-[(2,6-dichlorophenyl)amino]phenyl}acetate]zinc(II) – Zn(diclof)$_2$

Binary salt [$Zn(diclof)_2$] was prepared adapting Abu Ali & Jabali (2016) (16) and Dos Santos, et al, (2020) (15) methods. Diclofenac potassium (0.668 g, 2.0 mmol) in 30 ml of water was added dropwise to $ZnCl_2$ solution (2.87 g, 1.0 mmol, 10 mL of water) at room temperature. A white colloidal suspension was formed immediately, and was kept stirring for 12 h. The solid was then filtered, washed with ultrapure water, dried over freeze drying for 12 h and stored in dark flask. Yield: 0.655 g (87%); MP: 244° C; IR (cm^{-1}, KBr pellet): 3270.8 *(N-H)*, 2969.0 *(Assym CH2)*,1576.1 *(Assym COO)*, 1558.1, 1501.4, 1472.4, 1452.2, 1399.6 *(Sym COO)*,1303.2,1201.0, 1153.8, 869.3, 838.9, 769.0, 747.8, 716.0, 673.1, 612.3, $\Delta_{(Assym-Sym\ COO)}$ 177. ^{1}HNMR (δ-ppm, DMSO d_6): 3.59 (s-4H, $2CH_2$), 6.29 (d-2H, 2CH, J_{H-H} = 7.5Hz), 6.82 (dt-2H, 2CH, $J1_{H-H}$ = 7.5Hz, $J2_{H-H}$ = 0.9Hz), 7.01 (dt-2H, 2CH, $J1_{H-H}$ = 7.5Hz, $J2_{H-H}$= 1.5Hz), 7.08 (t-2H, 2CH, J_{H-H} = 7.8Hz), 7.15 (dd-2H, 2CH, $J1_{H-H}$ = 7.5Hz, $J2_{H-H}$ = 1.2Hz), 7.43 (d-4H, 4CH, J_{H-H} = 7.8Hz), 8.31 (s-2H, 2N-H); ^{13}CNMR (δ-ppm, DMSO d_6): 40.8 (CH_2), 116.4 (CH), 120.8 (CH), 124.6 (C), 126.6 (CH), 126.9 (C), 128.9 (CH), 129.1 (CH), 130.6 (CH), 137.6 (C), 142.9 (CCl), 177.4 (COO); HRMS (positive ions): m/z 1326.8945 ($C_{56}H_{40}Cl_8N_4NaO_8Zn_2$) $[2M+Na]^+$, 1304.8890 ($C_{56}H_{41}Cl_8N_4O_8Zn_2$) $[2M+H]^+$, 1009.8808 ($C_{42}H_{30}Cl_6N_3O_6Zn_2$) $[2M-diclof]^+$, 683.9134 ($C_{56}H_{40}Cl_8N_4O_8Zn_3$) $[2M+Zn]^+$, 652.9533 ($C_{28}H_{21}Cl_4N_2O_4Zn$) $[M+H]^+$, 505.4474 ($C_{42}H_{31}Cl_6N_3O_6Zn_2$) $[2M-diclof+H]^{2+}$, 357.9375 ($C_{14}H_{10}Cl_2NO_2Zn$) $[M-diclof]^+$, 318.0055 ($C_{14}H_{11}Cl_2NNaO_2$) $[diclof+Na+H]^+$, 296.0233 ($C_{14}H_{12}Cl_2NO_2$) $[diclof+2H]^+$.

2.2.3 Synthesis of bis[2-(4-(methylpropyl)phenyl)propanoate](bipy)zinc(II) (1)

Complex 1 was prepared according Abu Ali, *et al*., (2016) (21). The precursor salt $Zn(Ibup)_2$ (0.4761g, 1.0 mmol) was dissolved in absolute ethanol (10 mL), filtered and add dropwise over a stirred solution of 2,2'-bipy (0.1561g, 1.0 mmol). A clear solution was than stirred at room temperature for 2 hours. The white crystals was than obtained after 24 hours, filtered and dried over freeze dryer for 12 hours. Yield: 79%. (FTIR, cm^{-1}) 2957.9 (*Assym CH3*), 2934.3 (*Assym CH2*), 2863.9 (*Sym CH3*), 1612.3 and 1586.7 (*Assym COO*) 1566.0, 1509.6, 1443.5, 1382.8(*Sym COO*), 1355.8, 1314.8, 1156.6, 1062.1, 1025.5, 891.0, 850.5, 770.9, 733.8, 712.6, 653.3, 635.0, 603.6, $\Delta_{(Assim\text{-}Sym\ COO)}$ 229 cm^{-1}; (δ-ppm, DMSO-d_6): 0.824 (d-12H, $4CH_3$, $J_{H\text{-}H}$ = 6.6Hz), 1.216 (d-6H, $2CH_3$, $J_{H\text{-}H}$ = 7.2 Hz), 1.768 (sep-2H, 2CH, $J_{H\text{-}H}$ = 6.6Hz), 2.351 (d-4H, $2CH_2$, $J_{H\text{-}H}$ = 7.2Hz), 3.405 (q-2H, 2CH, $J_{H\text{-}H}$ = 6.9Hz + 2.5 H_2O (signal overlap)), 6.949 (d-4H, 2CH, $J_{H\text{-}H}$ = 8.1Hz), 7.105 (d-4H, 4CH, $J_{H\text{-}H}$ = 8.1Hz), 7.567 (t-2H, 2CH, $J_{H\text{-}H}$ = 6.3Hz), 8.099 (t-2H, 2CH, $J_{H\text{-}H}$ = 7.2Hz), 8.488 (d-2H, 2CH, $J_{H\text{-}H}$ = 7.8Hz), 8.654 (dd-2H, 2CH, $J1_{H\text{-}H}$ = 4.8Hz, $J2_{H\text{-}H}$ = 0.9Hz); ^{13}CNMR (δ-ppm, DMSO-d_6): 20.1 (CH_3), 22.3 (CH_3), 29.7 (CH), 44.4 (CH), 46.3 (CH_2), 127.2 (CH), 128.5 (CH), 138.4 (C), 141.2 (C), 149.1 ($CH_{(Bipy)}$)179.3 (COO^-); HRMS (positive ions): *m/z* 1529.5330 ($C_{85}H_{101}N_4O_{10}Zn_3$) $[2M+Ibup+Zn]^+$, 1055.3626 ($C_{59}H_{67}N_4O_6Zn_2$) $[2M-Ibup]^+$, 899.2914 ($C_{49}H_{59}N_2O_6Zn_2$) $[2M-Ibup-Bipy]^+$, 653.2317 ($C_{36}H_{42}N_2NaO_4Zn$) $[M+Na]^+$, 581.1885 ($C_{33}H_{33}N_4O_2Zn$) $[M-Ibup+Bipy]^+$, 425.1199 ($C_{23}H_{25}N_2O_2Zn$) $[M-Ibup]^+$.

2.2.4 Synthesis of bis[2-(4-(methylpropyl)phenyl)propanoate](phen)zinc(II) (2)

The precursor salt $Zn(Ibup)_2$ (0.4761g, 1.0 mmol) was dissolved in absolute ethanol (10 mL), filtered and add dropwise over a stirred solution of 1,10-phen.H_2O (0.1979g, 1.0 mmol). A clear solution was than stirred at room temperature for 2 h. The white crystals were carried-out after 24 h, filtered and dried over freeze dryer for 12 h. Yield: 72%. (FTIR, cm^{-1}): 2961.8 (*Assym CH3*), 2925.1 (*Assym CH2*), 2867.8 (*Sym CH3*), 1622.9 and 1581.4 (*Assym COO*) 1560.2, 1515.8, 1463.8, 1425.2(*Sym COO*),1404.9, 1338.9, 1141.7, 1102.6, 1063.1, 893.4, 849.0, 725.1, 643.2, $\Delta_{(Assim\text{-}Sym\ COO)}$ 198; ^{1}HNMR (δ-ppm, DMSO d_6): 0.79 (d-12H, $4CH_3$, $J_{H\text{-}H}$ = 6.6Hz), 1.17 (d-6H, $2CH_3$, $J_{H\text{-}H}$ = 7.2 Hz), 1.73 (sep-2H, 2CH, $J_{H\text{-}H}$ = 6.6Hz), 2.32 (d-4H, $2CH_2$, $J_{H\text{-}H}$ = 7.2Hz), 3.37 (q-2H, 2CH, $J_{H\text{-}H}$ = 6.9Hz), 3.49 (s-6H, 3.0 H_2O), 6.88 (d-

4H, 4CH, J_{H-H} = 8.1Hz), 7.02 (d-4H, 4CH, J_{H-H} = 8.1Hz), 7.88 (dd-2H, 2CH, J_{H-H} = 8.1Hz), 8.10 (s-2H, 2CH), 8.70 (dd-2H, 2CH, $J1_{H-H}$ = 8.1Hz, $J2_{H-H}$ = 1.5Hz), 8.89 (dd-2H, 2CH, $J1_{H-H}$ = 4.8Hz, $J2_{H-H}$ = 1.5Hz); ^{13}CNMR (δ-ppm, DMSO d_6): 19.3 (CH_3), 22.3 (CH_3), 29.7 (CH), 44.3 (CH), 46.4 (CH_2), 125.1 (CH), 126.8 (CH),127.1 (CH), 128.3 (CH), 138.3 (C), 139.0 (CH), 140.6 (C), 141.3 (C), 149.4 (CH), 179.3 (COO^-); HRMS (positive ions): *m/z* 1103.3636 ($C_{63}H_{67}N_4O_6Zn_2$) $[2M\text{-Ibup}]^+$, 923.2954 ($C_{51}H_{59}N_2O_6Zn_2$) $[M+Ibup+Zn]^+$, 677.2315 ($C_{38}H_{42}N_2NaO_4Zn$) $[M+Na]^+$, 629.1890 ($C_{37}H_{33}N_4O_2Zn$) $[M\text{-Ibup+Phen}]^+$, 449.1213 ($C_{25}H_{25}N_2O_2Zn$) $[M\text{-Ibup}]^+$.

2.2.5 Synthesis of bis[2-[2-[(2,6-dichlorophenyl)amino]phenyl]acetate](bipy)zinc(II) (3)

The precursor salt $Zn(Diclof)_2$ (0.6556g, 1.0 mmol) was dissolved in absolute ethanol (20 mL), filtered and add dropwise over a stirred solution of 2,2'-bipy (0.1561g, 1.0 mmol, absolute ethanol 10 mL). White solid was immediately formed with the mixture keeped stirring for 1 h. The white powdered product was than obtained after 96 h, filtered and dried over freeze dryer for 12 h. Yield: 86%. (FTIR, cm^{-1}): 3275.2 $_{(Assym\ NH)}$, 3074.1, 3030.2, 2984.9 $_{(Assym\ CH2)}$, 1612.7, 1602.1, 1577.6, 1562.1$_{(Assym\ COO)}$, 1501.9, 1472.0, 1451.2$_{(Sym\ COO)}$, 1406.8, 1363.5, 1302.3, 1283.9, 1150.4, 1087.7, 866.9, 836.5, 759.4, 744.4, 730.9, 713.6, 653.8, 610.9, $\Delta_{(Assym\text{-}Sym\ COO)}$161; ^{1}HNMR (δ-ppm, DMSO d_6): 3.28 (s-2.5H, $1H_2O$), 3.55 (s-4H, $2CH_2$), 6.24 (d-2H, 2CH, J_{H-H} = 8.1Hz), 6.78 (t-2H, 2CH, J_{H-H} = 7.5Hz), 6.98 (t-2H, 2CH, J_{H-H} = 7.5Hz), 7.06 (t-2H, 2CH, J_{H-H} = 8.1Hz), 7.10 (d-2H, 2CH, J_{H-H} = 7.2Hz), 7.38 (d-4H, 4CH, J_{H-H} = 7.8Hz), 7.57 (t-2H, 2CH, J_{H-H} = 5.4Hz), 8.13 (t-2H, 2CH, J_{H-H} = 7.2Hz), 8.32 (s-2H, 2NH), 8.52 (d-2H, 2CH, J_{H-H} = 8.1 Hz), 8.68 (d-2H, 2CH, J_{H-H} = 4.8 Hz); ^{13}CNMR (δ-ppm, DMSO d_6): 126.5 (CH), 128.8 (CH), 137.5 (C), 142.7 (CCl), 148.7 (C), 155.5 (C), 177.1 (COO); HRMS (positive ions): *m/z* 1165.9522 ($C_{52}H_{38}Cl_6N_5O_6Zn_2$) $[M+Zn+ibup]^+$, 831.0024 ($C_{38}H_{28}Cl_4N_4NaO_4Zn$) $[M+Na]^+$, 670.0757 ($C_{34}H_{26}Cl_2N_5O_2Zn$) $[M\text{-diclof+Bipy}]^+$, 514.0067 ($C_{24}H_{18}Cl_2N_3O_2Zn$) $[M\text{-diclof}]^+$, 357.9375 ($C_{14}H_{10}Cl_2NO_2Zn$) $[Diclof+Zn]^+$.

2.2.6 Synthesis of bis[2-[2-[(2,6-dichlorophenyl)amino]phenyl]acetate](phen)zinc(II) (4)

The precursor salt $Zn(Diclof)_2$ (0.6557g, 1.0 mmol) was dissolved in absolute ethanol (20 mL), filtered and add dropwise over a stirred solution of 1,10-phen.H_2O (0.1984g, 1.0 mmol, ethanol 20 mL). Pale yellow solid was immediately formed with the mixture kept stirring for 1 hour. The powdered product was than obtained after 96 h, filtered and dried over freeze dryer for 12 h. Yield: 85%. (FTIR, cm^{-1}): 3235.6$_{(Assym\ NH)}$, 3060.1, 3013.8, 2985.9$_{(Assym\ CH2)}$, 1584.8$_{(Assym\ COO)}$, 1571.8, 1558.7, 1514.9, 1449.8, 1425.2 $_{(Sym\ COO)}$, 1305.1, 1286.3, 1167.3, 1104.6, 847.1, 762.3, 751.2, 724.7, 715.5, 664.4, $\Delta_{(Assym\text{-}Sym\ COO)}$ 159; ^{1}HNMR (δ-ppm, DMSO d_6): 3.265 (s-8H, $4H_2O$), 3.509 (s-4H, $2CH_2$), 6.192 (d-2H, 2CH, $J_{H\text{-}H}$ = 7.8Hz), 6.762 (t-2H, 2CH, $J_{H\text{-}H}$ = 7.2Hz), 6.955 (dt-2H, 2CH, $J1_{H\text{-}H}$ = 7.5Hz, $J2_{H\text{-}H}$ = 1.5Hz), 7.034 (t-2H, 2CH, $J_{H\text{-}H}$ = 8.1Hz), 7.067 (d-2H, 2CH, $J_{H\text{-}H}$ = 6.9Hz), 7.347 (d-4H, 4CH, $J_{H\text{-}H}$ = 8.1Hz), 7.887 (dd-2H, 2CH, $J_{H\text{-}H}$ = 8.1Hz), 8.184 (s-2H, 2CH), 8.350 (s-2H, 2NH), 8.760 (d-2H, 2CH, $J_{H\text{-}H}$ = 8.1 Hz), 8.958 (d-2H, 2CH, $J_{H\text{-}H}$ = 4.2 Hz); ^{13}CNMR (δ-ppm, DMSO d_6): 128.73 (C), 137.51 (C), 142.76 (C); HRMS (positive ions): *m/z* 1009.8869 ($C_{42}H_{30}Cl_6N_3O_6Zn_2$) $[Zn_2(Diclof)_3]^+$, 855.0022 ($C_{40}H_{28}Cl_4N_4NaO_4Zn$) $[M+Na]^+$, 652.9551 ($C_{28}H_{21}Cl_4N_2O_4Zn$) $[M\text{-}Phen+H]^+$, 538.0061 ($C_{26}H_{18}Cl_2N_3O_2Zn$) $[M\text{-}diclof]^+$.

2.3 Cell Culture

VERO (African green monkey kidney) cell line was purchased from American Type Culture Collection (ATCC), Manassas, VA, USA. Cells were cultured in DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% heat-inactivated FBS. Cells were maintained in a humidified atmosphere at 37 °C, in 5 % CO_2, and 95 % air. VERO cells were grown in DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% fetal bovine serum (Gibco BRL; Life Technologies) and 1% of penicillin-streptomycin in a humidified atmosphere at 37 °C with 5% CO_2.

2.4 MTT Assays

Cytotoxicity was assessed by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) according Denizot & Lang method (1986) (22). The cells were incubated in 96-

well microplates in concentrations of 8x104 mL^{-1}. After 24 h incubation, the cells were treated with increasing concentrations of pyrazole compounds. Aliquots of complexes were solubilized in 90/10% saline solution/DMSO and filtered through a sterile 20 µm membrane. Complex solutions were diluted in culture medium to obtain concentrations of 0.0, 5.0, 10.0, 15.0 and 25.0 µmol L^{-1}, followed by application on cell cultures. Subsequently, the MTT solution was removed and the product was dissolved in DMSO. The absorbance was measured at 570 nm. The control represented 100% viability. The percentage growth inhibition was calculated using the equation (absorbance of experimental wells/absorbance of control wells) × 100. Each experiment was performed in triplicate. The data were expressed as means of at least three independent experiments.

2.5 Spectrophotometric UV-Vis DNA Interaction Assay

Absorption titration measurements were done by varying the concentration of CT DNA but keeping the metal complexes in 10% ACN and 10 mmol L-1 HEPES buffer pH 7.5 concentration as constant (50 µmol L^{-1}) and using the concentrations of 0.00, 4.98, 9.90, 14.78, 19.61, 24.39, 29.13, 33.82, 38.46, 43.06, 47.62, 52.13, 56.60 and 61.03 µmol L-1 of CT-DNA. The base line was performed with the mixed solvent and parallel measurements with the solvent and CT-DNA to eliminate the absorbance of DNA itself. The solutions were allowed to incubate for 60 minutes before the absorption spectra were recorded. The experiments were repeated three times and the results obtained were plotted in tables and shown on graphics.

2.6 DNA Interaction Activity: Plasmid Cleavage Activity Assay

Plasmid DNA pBSK II (Stratagene) was obtained and purified according to standard techniques (23). The DNA cleavage ability of the ternary complexes 1, 2, 3 and 4 diluted in water:acetonitrile 20%, were examined in order to establish the influence of compound concentration on the conversion of pBSK II supercoiled DNA (F I) to the open circular (F II) and linear DNA (F III) using agarose gel electrophoresis to separate the cleavage

products [23]. Exploring experiments were designed in accordance to the proceedings calculated using the OriginPro® 2016 (b9.3.226, evaluation version). In general, 300 ng of pBSK II DNA (30.0 μM bp) in 10.0 mM of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer pH 7.0 was treated with Zn(II) complexes at concentrations of 125 and 250 μM in a final concentration of 20% acetonitrile at 37 ºC in the absence of light (AL) for 12 h and UVB light (UV) for 60 s using a BIORAD® transilluminator UV 302 T26M apparatus with a UVB peak ranging from ≈ 300 to 312 nm at 100% light power. All the assays were conducted using freshly prepared solutions and included one negative control reaction with Milli-Q® water:acetonitrile 80:20 (reference of spontaneous plasmid DNA fragmentation). Thereafter, each reaction was quenched by adding 4 μL of a loading buffer solution (50.0 mM of tris(hydroxymethyl)aminomethane-HCl pH 7.5, 0.01% bromophenol blue, 50% glycerol, and 250.0 mM EDTA) and then subjected to electrophoresis on a 1.0% agarose gel containing 0.3 μg mL-1 of ethidium bromide in 0.5 × tris-borate-EDTA (TBE) buffer (44.5 mM Tris pH 8.0, 44.5 mM boric acid, and 1.0 mM EDTA) at 90 volts (V) for 1.5 h. The resulting gels were visualized and digitized using a DigiDoc-It gel documentation system (UVP) (KODAK). The proportion of plasmid DNA in each band was quantified using GelAnalyzer version 2010a software (freeware). The quantification of supercoiled DNA (F I) was corrected by a factor of 1.47, since the ability of ethidium bromide to intercalate into this DNA topoisomeric form is decreased relative to open circular and linear DNA (21). The results are expressed as graphic representations of the best correlation of the concentration in order to maximize F III (linear) plasmid DNA.

2. 7 *In silico* Studies

2.7.1 Toxicity and *Pharmacokinetics studies*

Compounds were submitted to evaluation of theoretical risk to cause toxic effects using two *in silico* toxicity programs, Osiris Property Explorer and pKCSM. Osiris Property Explorer web server was used to determine the potential mutagenic, tumorigenic, irritant,

and toxicant reproductive system effects. pKCSM was used to investigate the theoretical hepatotoxicity and Genotoxicity (Ames Assay) effects (24).

SwissADME and pKCSM were used to study the potential biological properties related to biodistribution and CYP interaction in human organism (if substrate or inhibitor)(24,25). SwissADME provide data about CNS penetration, human intestinal absorption probability.

2. 7. 2 Biological Target Prediction

Compounds 1-4 were designed in a computational chemistry program (ChemDraw Ultra 8, Perkin Elmer Informatics) and had their description in smiles language converted prior to perform the prediction studies of potential biological targets. The analysis of similarity and suggestion of biological targets was carried out on a web server Go SEA (Similarity Ensemble Approach) available in web server http://sea.bkslab.org/ (26). All results are showed as name of target, the P value (related to probability) and MaxTC (similarity criterion).

3. Results and Discussion

The search for bioisosteres of compounds with consolidated pharmaceutical activity can shorten the time in the search for more selective and effective compounds, as well as with fewer side effects. Thus, the formation of organocomplexes has been a strategy highlighted in the planning of new drugs with examples of significant improvements in therapeutic efficacy.

Following this strategy, in this study due to the bioisosterism technique it was possible to obtain potentially molecules that further could be use as anti-inflammatory drugs.

3.1 Zinc complexes

Synthesis and study of metal complexes with anti-inflammatory drugs as ligands is a research area of considerable interest (12).

The precursors $Zn(Ibup)_2$ and $Zn(Diclof)_2$ were recovered with 66% and 87% yields respectively, according Abu Ali, *et al.*, (2016) (21) and Abu Ali & Jabali (2016) (16). The ^{1}HRMN analyzes for both structures show the hydrogens corresponding to the respective starting materials ibuprofen and diclofenac. HRMS spectra indicate that both structures are binary salts of type $M[R\text{-}COO]_2$ structure showing proton adducts $[M + H]^+$ at m/z 475.1875 and m/z 652.9533. Stable oligomers detected as 2M and 3M denotes possible molecular chain conformation in both cases.

Complex 1 was obtained according Hijazi Abu Ali, et al, (2016) (21) but absolute ethanol was used as a solvent instead acetone. The changed method promotes 79% yield, compared to the 33% yield reported by the author, characterizing a significant improvement in methodology. The data obtained by FTIR, ^{1}HRMN and ^{13}CRMN corroborate with reference and show that complex 1 has a molecular formula of type $M[R\text{-}COO]_2L$. The HRMS spectrum shows molecular ion as sodium adduct $[M + Na]^+$ in m/z 653.2317 and molecular clusters with the formula $[2M+ibup+Zn]^+$ in m/z 1529.5330, $[2M\text{-}ibup]^+$ in m/z 1055.3626 and $[2M\text{-}ibup\text{-}Bipy]^+$ in m/z 899.2914, showing the tendency of Zn (II) complexes to form molecular chains in solid phase and in aqueous solution.

Complex 2 was obtained according Omar (2017) (27) when absolute ethanol was used as solvent instead acetone. The yield obtained of 72% is lower than the value of 90% reported by the reference, but using a more environmentally friendly solvent. The structural characterization by FTIR, ^{1}HRMN and ^{13}CRMN corroborates the reference description, with formula $M[R\text{-}COO]_2L$ just like the complex 1. The HRMS analysis presents the molecular ion as a sodium adduct $[M+Na]^+$ in m/z 677.2315, molecular rearrangement $[M\text{-}ibup+Phen]^+$ in m/z 629.1890 and biuclear cluster $[2M\text{-}Ibup]^+$ in m/z 1103.3636.

Complexes 3 and 4 were obtained according Abu Ali and Jabali, (2016) (16) methodology using absolute ethanol as a solvent instead acetone. Complex 3 presents

86% yield and the spectral data of FTIR, ^{1}HRMN and ^{13}CRMN corroborates with reference data, showing molecular formula type M[R-COO]$_2$L. The HRMS spectrum shows the molecular ion as sodium adduct $[M+Na]^+$ in m/z 831.0024, molecular rearrangement $[M\text{-diclof+Bipy}]^+$ in m/z 670.0757 in addition to fragments with a mass greater than the mass of the molecular ion also detected in the precursor Zn(diclof)$_2$, denoting the preferential loss of the Phen ligand. Complex 4 was obtained according Abu Ali and Jabali, (2016) (16) using absolute ethanol as a solvent instead acetone. The 85% yield obtained in absolute ethanol is superior to the reference method, which recovered 75% in acetone. The spectroscopic data of FTIR, ^{1}HRMN and ^{13}CRMN corroborates with the reference data. The HRMS spectrum presents the molecular ion as a sodium adduct $[M+Na]^+$ in m/z 855.0022 and molecular rearrangements with $[M\text{-diclof+phen}]^+$ in m/z 718.1742 as main ions. Therefore, the molecular analyzes of the complexes show that the structures correspond to the literature data.

3.2 *Cytotoxicity by MTT*

Cytotoxicity by MTT assay was evaluated only for complex 1, since the other complexes were not soluble under the conditions tested. The percentage inhibition of cell viability was greater at 15 µM and 25 µM, representing 20.2 and 19.4%, respectively. The 5 µM concentration inhibited cell viability by 13.8% and the 10 µM concentration by 15.4%. Only the highest concentrations showed a statistical difference in the percentage of cell viability inhibition compared to the control ($p \leq 0,05$) (Figure 2).

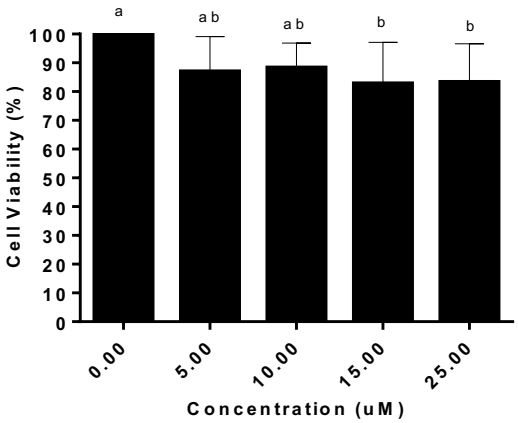


Figure 2. Cytotoxicity of Complex 1 against the VERO cell line, after 24h of exposure compared to vehicle control (0.00). Different letters correspond to the statistical difference using ANOVA-Tukey ($p \leq 0.05$).

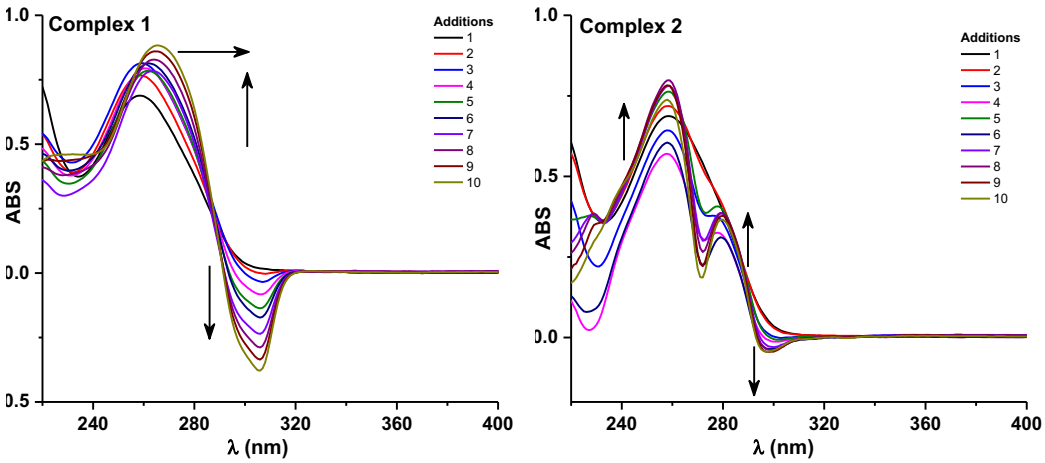
These results indicate that the complex 1 presented low cytotoxicity against the non-tumoral cell line of monkey kidney at concentrations 15 and 25 μM compared to the vehicle control. In the study by Matos et al. (2019) (28), zinc complexes showed cytotoxic effect against the non-tumor line V79 (fibroblasts) in concentrations ranging from 6.78 to 33.6 μM. However, the complexes showed selectivity for the A2780 ovarian tumor cell line. Non-Steroidal Anti-Inflammatory Drugs re a group of drugs that work to prevent the development of cancer (Banti & Hadjikakou, 2016) (29).

Thus, studies that evaluate the cytotoxicity of these compounds aim to evaluate the antitumor activity, making it difficult to compare this study with others. The determination of cytotoxicity in vitro is one of the most important biological indicators to assess the toxicity of a drug. Assays using cell cultures are widely used and can predict toxicity in vivo, avoiding the use of animal tests. The low in vitro cytotoxicity combined with the absence of toxicity (on reproductive system and hepatotoxicity), irritability, mutagenicity and non-tumorigenic in computational models is a strong indicator of of the possible reduction of toxicity in vivo. These results suggest that the compound Zn-Ibup-Phen is a promising candidate for further tests for the development of a new drug with anti-inflammatory activity and reduced toxicity and side effects.

3. 3 DNA Interactions

3.3.1 UV – Vis Spectrophotometric Assay

Results obtained in UV – Vis Spectrophotometric Assays are shown on Figure 3. Both 1 and 2 presented the characteristic bands of absorption of Phen and Bipy, respectively at δ 310 and 280 nm. Complex 1 presented no significant decrease of absorbance at 310 nm (hypochromism on δ 310 nm), means structural changes on complex structure with consequent hyperchromism and red shift on DNA main band (258 nm). Complex 2 presents hyperchromism at δ 258 and 280 nm with week red shift on DNA main band (258 nm) denoting less DNA interaction comparing to complex 1. Complexes 3 and 4 present hyperchromism in both cases without spectral shifts, denoting no visible interactions between CT-DNA and diclofenac complexes (2 and 3). The hyperchromism can be resulted of concomitant DNA-diclofenac absorption once these two molecules have the same UV peak.



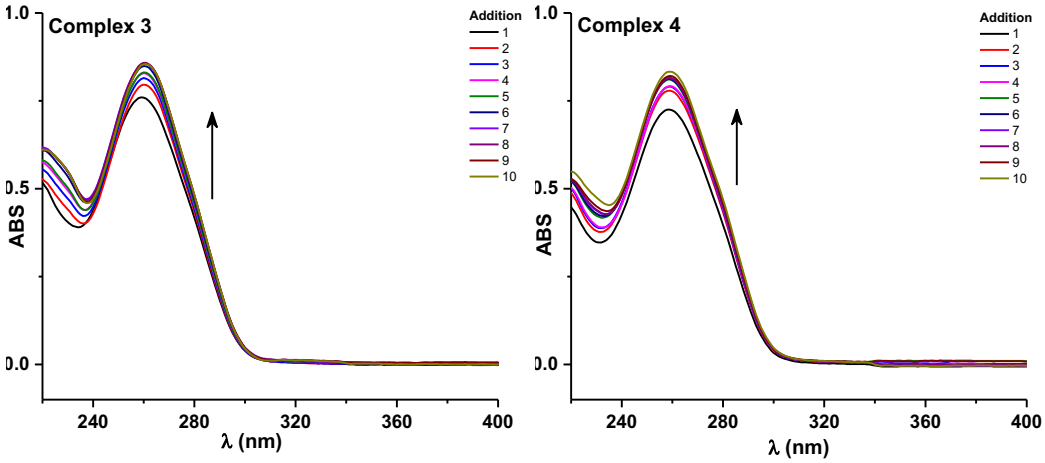


Figure 3. Stack spectra by complex-DNA interactions measured in acetonitrile.

Interactions of DNA with the metal complexes of the NSAIDs such as ibuprofen, mefenamic acid, lornoxicam, isoxicam, meloxicam, naproxen and diclofenac have been studied for several researchers (17,30–32).

The results expressed in the present study are in agreement with the literature data. In a similar study, zinc (II) complexes mefenamic acid also suggests binding to CT DNA probably by intercalation (31).

3.3 .2 DNA Interaction Activity: Plasmid Cleavage Activity Assays

As a first step the complexes 1, 2, 3 and 4 were tested in respect to their ability to cleave supercoiled plasmid DNA (F I), forming circular open (F II), linear (F III) or even breaking the DNA almost completely. Two concentrations of each complexes (125 and 250 μM) was tested only by classical methods in the absence of light (Dark test) and UVB light (photoactivation test). In the same experiment the solvent (NC) used in the reactions was tested against water (nc) to certify that it doesn't modify the proportion of the DNA forms and no significative result was observed (Figure 4). In dark conditions, around 10% of double breaks (DNA FIII) was observed in every cases. Single breaks was observed in every case with amounts of 50% of total start DNA, but there are no difference with the negative control (NC). For UV conditions, we can observe that every samples present activation ability under UV radiation. Around 50% of single breaks was detected in all samples with around 10% of double breaks.

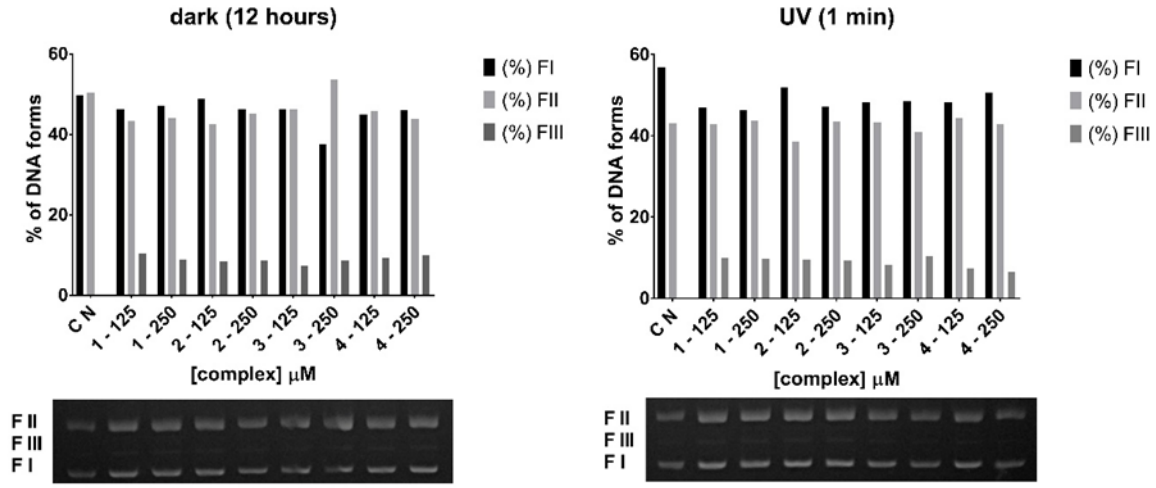


Figure 4. Induced break of plasmidial DNA by temperature and UV radiation

3. 4 In silico Studies

The organometallic zinc derivatives (1-4) were submitted to the evaluation of potential toxicological and also the probable biological target aiming to suggest their theoretical safety and pharmacological applications. This evaluation is carried out through the application of *in silico* screening, which consists in the use of high-performance computing to analyze large databases of chemical compounds to identify possible drug candidates (33).

In the first step, the physicochemical and pharmacokinetic properties such as human intestinal absorption, CNS penetrant possibility, and also the cytochrome P isoforms (CYP) interaction were studied. All results are showed in table 1.

Table 1. Pharmacokinetics properties and *in vitro* toxicity of molecules calculated using pkCSM* and SwissADME**.

Entry	CNS*	Human Intestinal Absorption*	CYP Interaction and Substrate Probability** (substrate/Inhibitor)
1	No	Low absorbed	CYP2D6 (inhibitor)

			CYP1A2 (No interaction)
			CYP3A4 (substrate/inhibitor)
2	No	Highly absorbed	CYP 2D6 (inhibitor)
			CYP1A2 (No interaction)
			CYP3A4 (substrate/inhibitor)
3	No	Low absorbed	CYP 2D6 (No interaction)
			CYP1A2 (Inhibitor)
			CYP3A4 (substrate)
4	No	Low absorbed	CYP 2D6 (inhibitor)
			CYP1A2 (inhibitor)
			CYP3A4 (substrate)

Table 2. ADMET properties and prediction toxicity of molecules calculated using Osiris Property Explorer* and pKCSM**

Entry	Mutagenic*	Tumorigenic*	Effect on reproductive system*	Irritant*	Hepatotoxicity**	Genotoxicity (Ames Assay)**
1	No	No	No	No	No	Yes
2	No	No	No	No	No	No
3	No	No	No	No	No	No
4	No	No	No	No	No	No

All compounds studied were submitted to *in silico* toxicity and biological properties prediction aiming to generate the information about their potential toxic effects and biodistribution. These data will be used to indicate which compounds should demonstrate the low toxic effect and good properties, and therefore may be indicated to be used in future studies. Only the compound 1 showed theoretical genotoxicity, and no mutagenic, tumorigenic, irritant, or cause effect on the reproductive system, hepatotoxicity was predicted to all compounds studied (Table 1). These results indicated

a relative absence of toxicity to these organometallic zinc derivatives. Compared with others, these results corroborate to a previous work (34) , were authors designed and synthesized new molecules. *In silico* toxicity risk assessment and drug likeness predictions were also conducted by Osiris Property Explorer. The study revealed that only one out of 9 complexes showed high risk of mutagenic and medium risk of tumorgenic effects, the other eight compounds are supposed to be non-mutagenic, non-tumorgenic, non-irritant with no reproductive effects.

All compounds were evaluated by SwissADME web server and they showed no trespassing the central nervous system penetration (CNS). Compound 2 showed high absorption considering the human Intestinal Absorption. Compounds 3 and 4, however, showed a prediction of low absorption in human intestine (Table 1). Considering the pKCSM software (if the substrate and inhibition) the compounds 1 and 2 present prediction of CYP2D6 inhibition and act as substrate and inhibitor of CYP3A4. The molecules 3 and 4 demonstrate to be the only substrate of CYP3A4, and derivative 3 showed also theoretical inhibition of CYP1A2 inhibition. The inhibition of isoform 1A2, 2D6, and 3A4, can result in the drug-drug interactions and in the case of CYP3A4 an accumulation of parent drug concentrations turns this at increased risk for side effects and possible toxicity.

The prediction of potential biological targets of compounds 1 – 4 showed the potential results only to 3 and 4, which are complexed with diclofenac. The main target suggested was CXCL8 structure which is receptor to interleukin-8 (IL-8) involved in inflammatory events (35). IL-8 plays a role as an important pro-inflammatory mediator. IL-8 is responsible for initiating and increasing the inflammatory response in the presence of specific pathogens, causing activation and migration of neutrophils from peripheral blood to tissues. Thus, both inhibition of action as well as regulation of IL-8 production would be an interesting pharmacological action (36). The anti-inflammatory properties of metal complexes have been studying for several authors. In a review about NSAIDs in metal complexes, researchers conclude that the majority of the complexes of Mn II, Fe II, Co II, Ni II, and Zn II showed anti-inflammatory properties superior to those of the parent drugs, indicating that these properties are enhanced upon

coordination to the metal ions (29). Another study prepared complexes of Mn(II), Fe(III), Fe(II), Co(II), Ni(II), and Pd(II) with diclofenac in order to investigate their chemical behavior and anti-inflammatory activity (12). The anti-inflammatory activity of these complexes, their inhibitory effects on rat or mouse paw edema induced by carrageenan, con-A, nystatin, and baker's yeast were assessment and compared with those of diclofenac. The results suggested that all complexes except the complex of Fe(III) exhibited a strong inhibitory effect on carrageenin-induced edema suggesting that they interfere with the release of histamine and serotonin and/or prostaglandin syntheses.

The values of P-Max and MaxTC were 8.176e-38, and 0.41 to complex 3 and 6.429e-43 and 0.43 to complex 4, which indicated the number 4 as the better agent to Interact with the target predicted. Since the results generated it is observed that complexes 3 and 4 showed high potential to be submitted to studies of evaluation of biological activity, without potential theoretical toxic effects.

4. Conclusion

In summary, in this work were synthesized and characterized by FTIR, ^{1}H NMR, ^{13}C NMR spectroscopy and HRMS analysis of four ternary complexes of Zn-Diclof and Zn-Ibup with Zn as metal, Phen and Bipy as a nitrogen ligand. The evaluation trough *in silico* programs of potential toxicological and probable biological targets suggests theoretical safety. The additional studies of circular dichroism and Plasmidial DNA interactions assays as well the prediction of cytotoxicity by MTT Assays indicate the potential to be applied as an anti-inflammatory agents with improved characteristics compared to parent drugs. In conclusion, trough the results generated it is observed that these complexes are promising candidates with the pharmacological potential to be applied as an anti-inflammatory agents. As perspectives, further assays should be explored in vitro or in vivo to identify activity, bioavailability, and toxicity of this drug prototype.

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4. CONSIDERAÇÕES FINAIS E PERSPECTIVAS

Os compostos de coordenação obtidos e quimicamente caracterizados neste trabalho se apresentam como moléculas promissoras ao desenvolvimento de novos medicamentos.

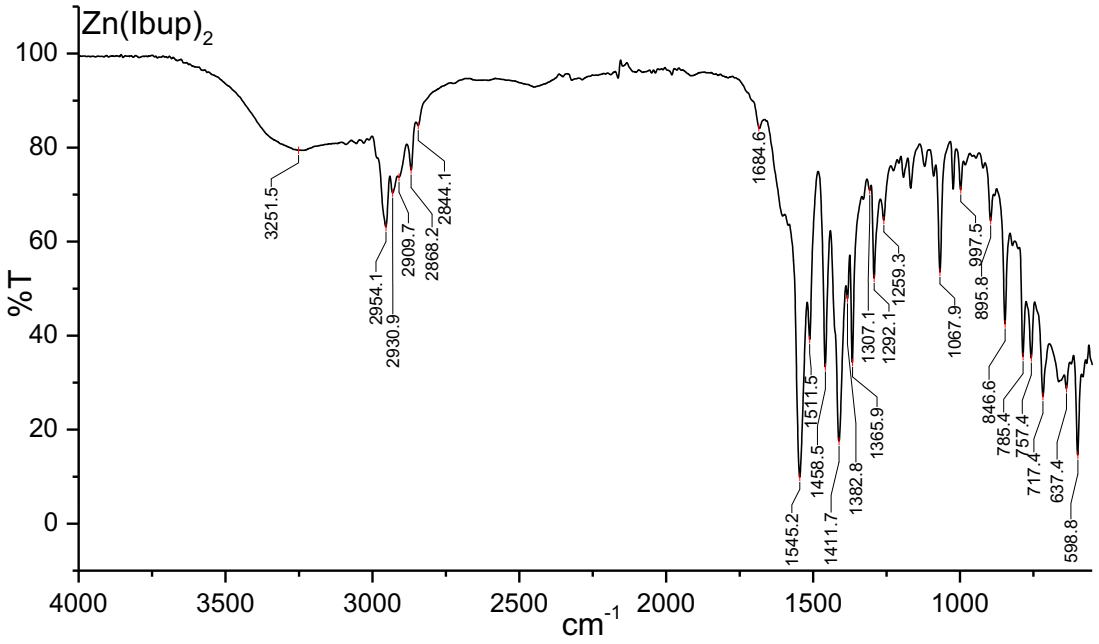
A partir dos ensaios conduzidos evidenciamos que os quatro organocomplexos destacam-se por apresentar ausência teórica de toxicidade. Os ensaios computacionais *in silico* sugerem o mecanismo de ação sobre a estrutura CXCL-8, que é receptora da inlerleucina-8 (IL-8) envolvida em eventos inflamatórios. É bem descrito na literatura o papel da IL-8 no processo inflamatório, desta forma, uma ação sobre a inibição desta proteína seria uma alternativa muito interessante a ser explorada pela indústria farmacêutica. As propriedades físicio-químicas iniciais nos sugerem a veiculação desta molécula em formas farmacêuticas semi-sólidas como geis, pomadas e cremes.

Perspectivas futuras:

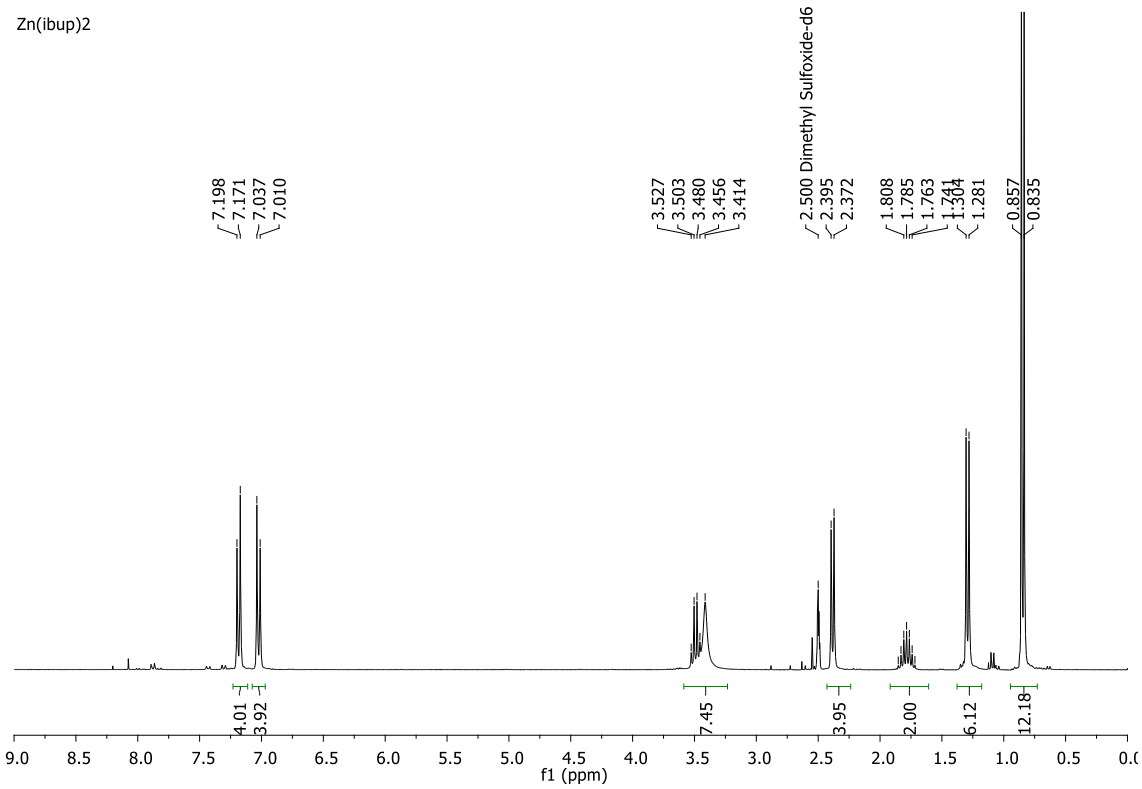
- Estudos adicionais *in vitro* ou *in vivo* para fins de avaliação e elucidação de propriedades farmacocinéticas adicionais como: absorção, metabolização, distribuição, biodisponibilidade e toxicidade desse protótipo de medicamento.
- Teste de inibição Cox1 e Cox 2.
- Testes para elucidação e confirmação do mecanismo de ação destes complexos.
- Estudos de ligação às proteínas plasmáticas.
- Veiculação dos complexos obtidos em formas farmacêuticas a fim de obter um protótipo de medicamento.

5. ANEXOS

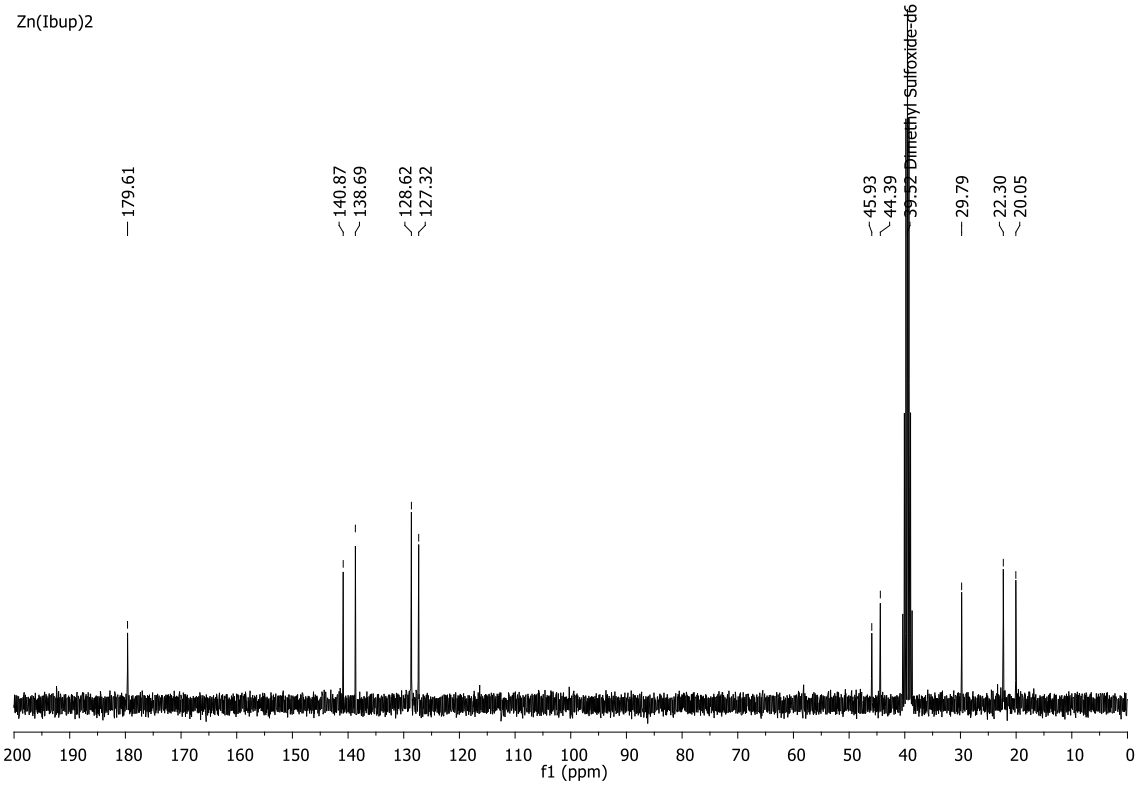
5.1 Experimental data of precursor 1 $Zn(ibup)_2$



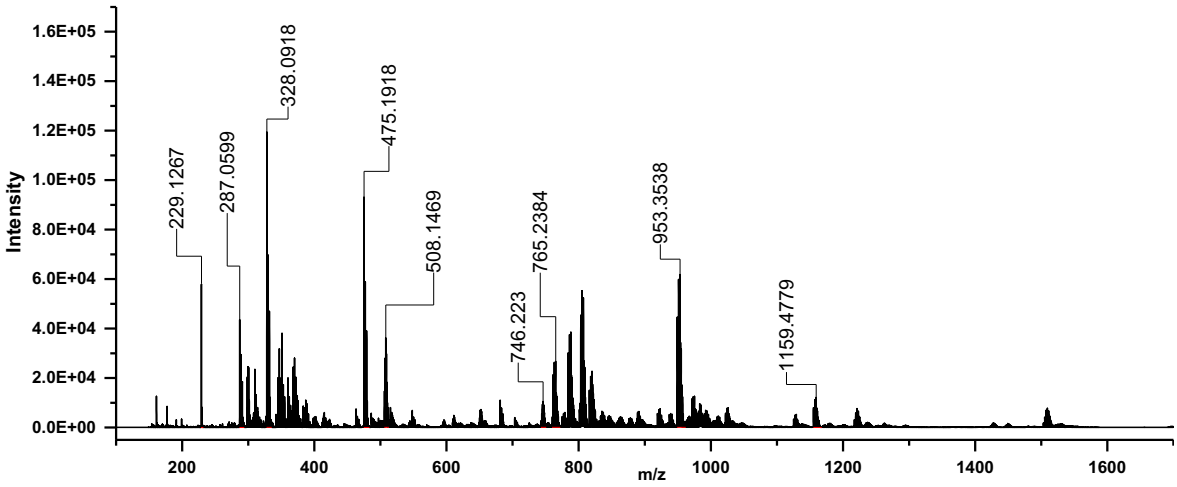
SM Fig. 1. Infrared spectrum for $Zn(Ibup)_2$.



SM Fig. 2. ^{1}HNMR spectrum for Zn(Ibup)$_2$



SM Fig. 3. ^{13}CNMR spectrum for Zn(Ibup)$_2$

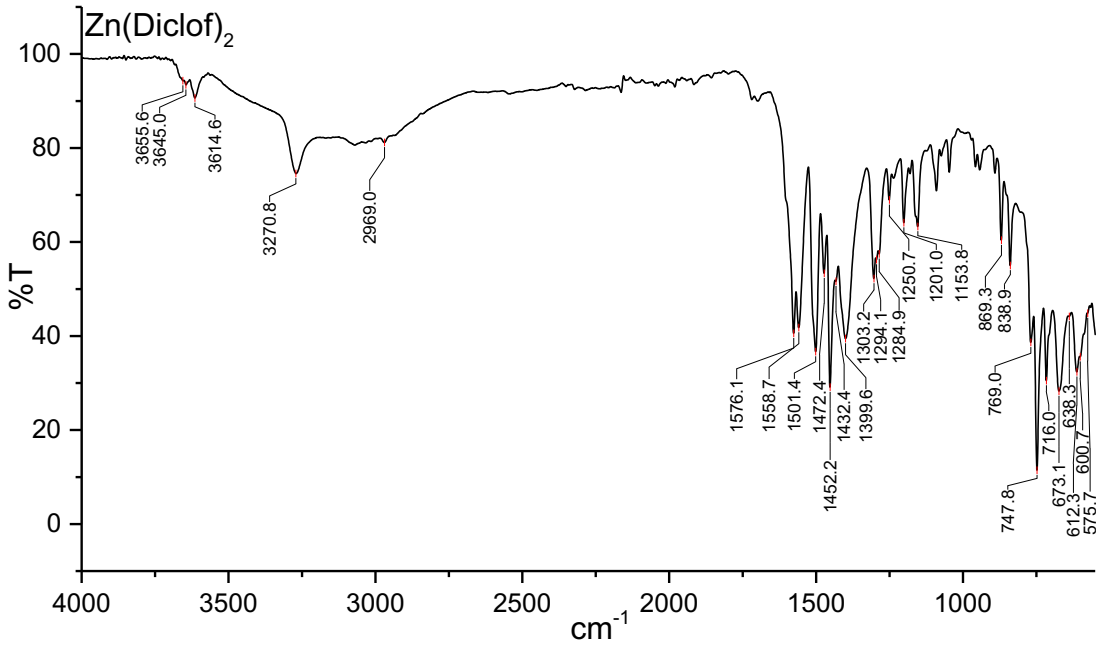


SM Fig. 4. HRMS spectrum of Zn(Ibup)2 (positive ions)

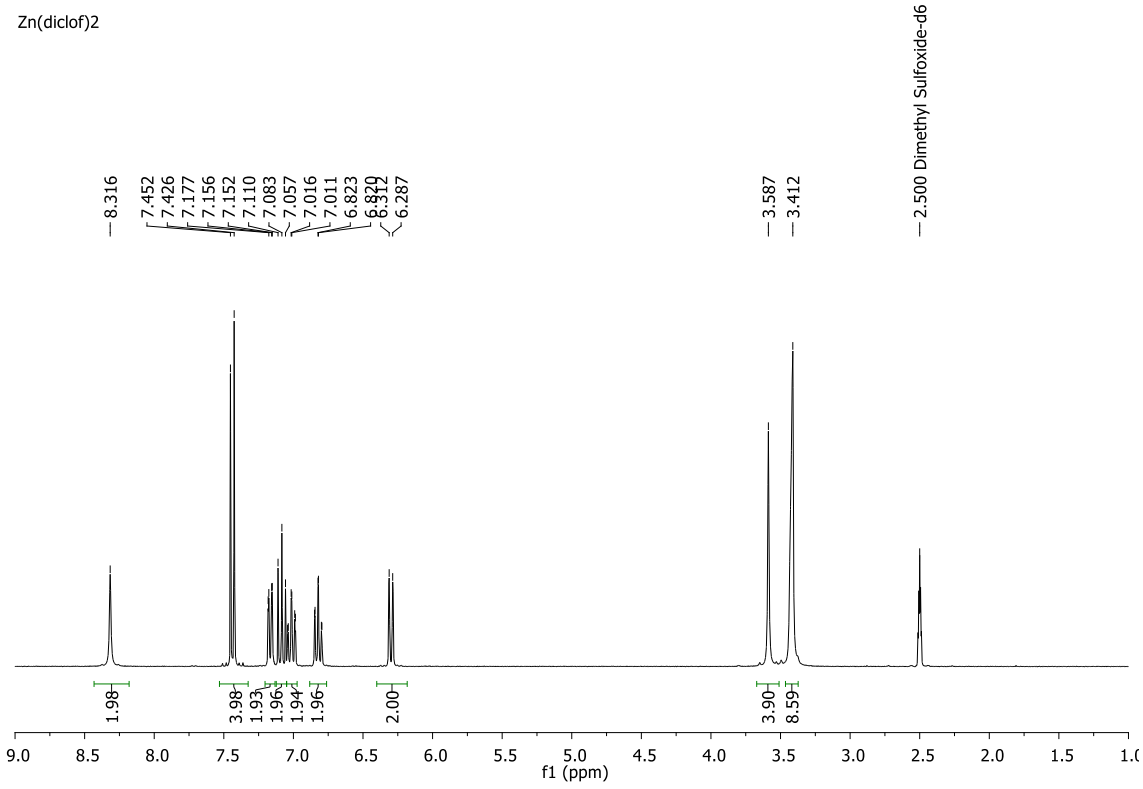
SM Table 1. HRMS data of $Zn(ibup)_2$

m/z	Calculated	Error (ppm)	Formula	Íon
1155,4811	1155,4877	5,7	$C_{65}H_{87}O_{10}Zn_2$	$[2M+ibup+2H]^+$
949,3534	949,3570	3,8	$C_{52}H_{69}O_8Zn_2$	$[2M+H]^+$
761,2359	761,2369	0,9	$C_{39}H_{53}O_7Zn_2$	$[2M-ibup+H_2O]^+$
743,2323	743,2268	1,7	$C_{78}H_{102}O_{12}Zn_4$	$[3M+Zn]^{2+}$
506,1439	506,1394	5,8	$C_{52}H_{68}O_8Zn_3$	$[2M+Zn]^{2+}$
475,1825	475,1821	0,8	$C_{26}H_{35}O_4Zn$	$[M+H]^+$
328,0891	328,0856	9,1	$C_{13}H_{23}NNaO_3Zn$	$[M-ibup+NaH+NH_4OH]^+$
287,0620	287,0620	0,2	$C_{13}H_{19}O_3Zn$	$[M-ibup+H_2O]^+$
229,1201	229,1199	0,9	$C_{13}H_{18}NaO_2$	$[ibup+Na+H]^+$

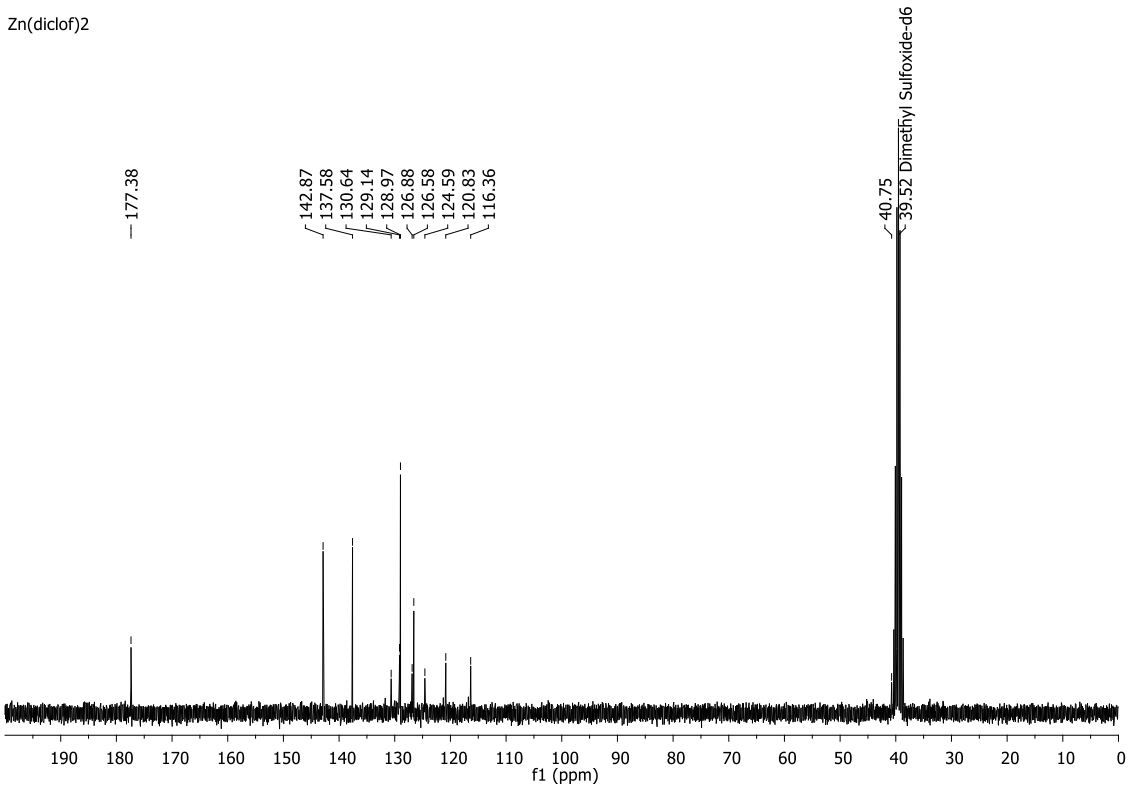
5.2 Experimental data of precursor 2 $Zn(diclof)_2$



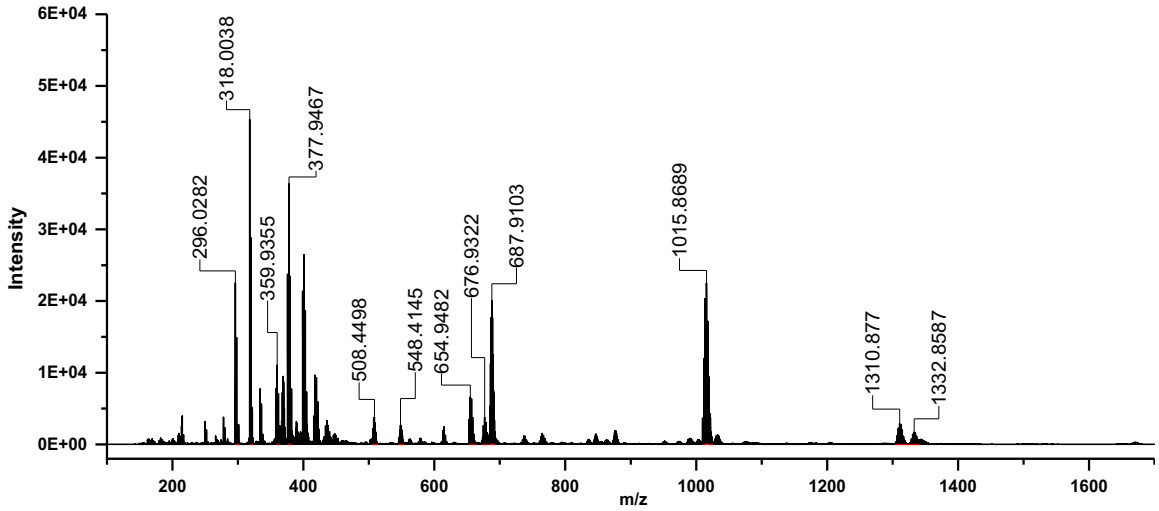
SM Fig. 5. Infrared spectrum for $Zn(Diclof)_2$



SM Fig. 6. ^{1}HNMR spectrum for $Zn(Diclof)_2$



SM Fig. 7. ^{13}CNMR spectrum for Zn(Diclof)$_2$



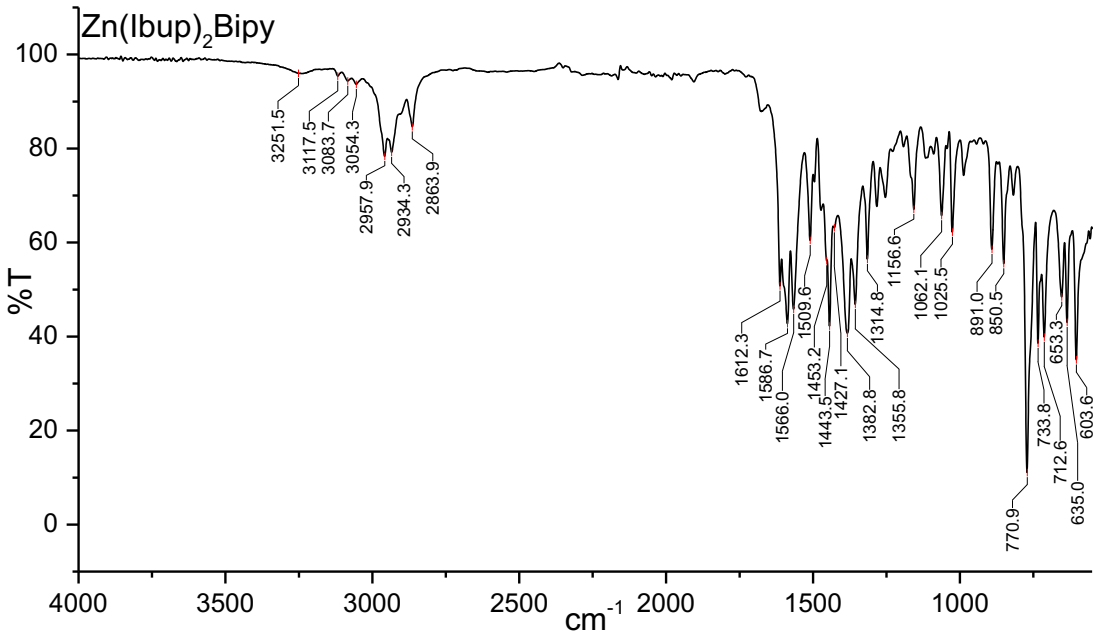
SM Fig. 8. HRMS spectrum of Zn(diclof)$_2$ (positive ions).

SM Table 2. HRMS data of Zn(diclof)$_2$

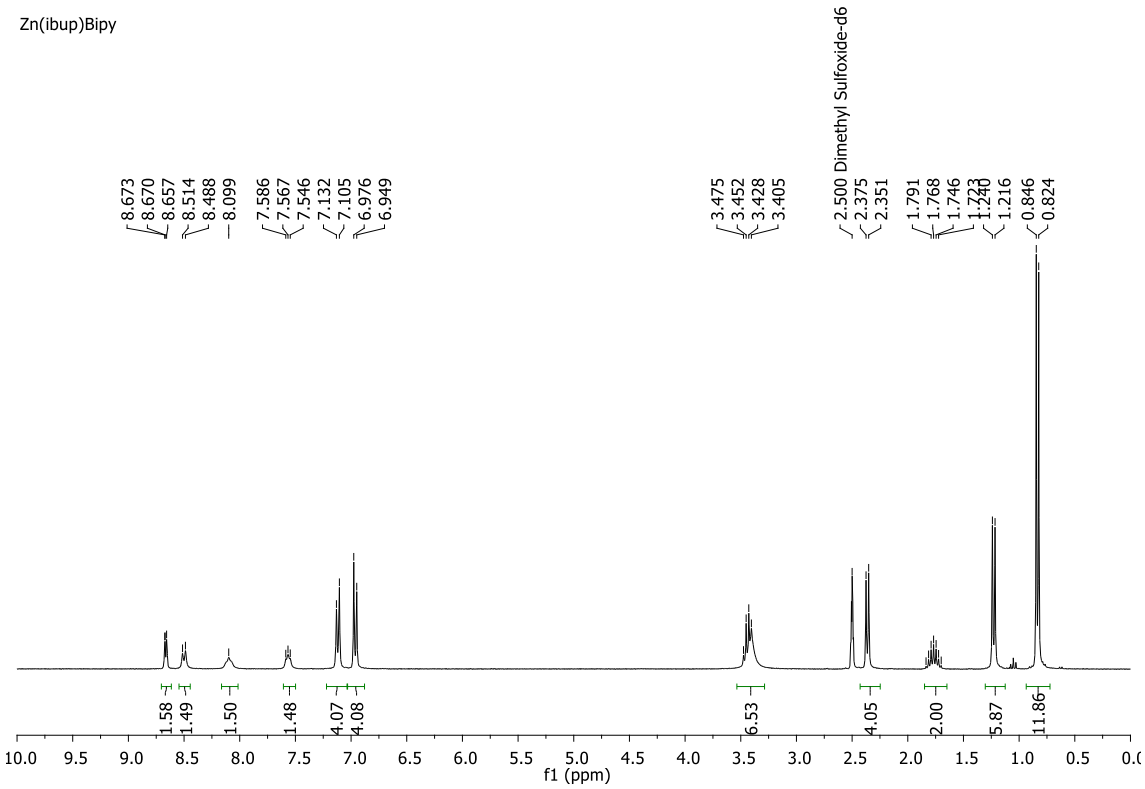
m/z*	Calculated	Error (ppm)	Formula	Ion
1326,8945	1326,8830	8,7	$C_{56}H_{40}Cl_8N_4NaO_8Zn_2$	$[2M+Na]^+$
1304,8890	1304,9010	7,4	$C_{56}H_{41}Cl_8N_4O_8Zn_2$	$[2M+H]^+$
1009,8808	1009,8843	3,5	$C_{42}H_{30}Cl_6N_3O_6Zn_2$	$[2M\text{-diclof}]^+$
683,9134	683,9114	3,7	$C_{56}H_{40}Cl_8N_4O_8Zn_3$	$[2M+Zn]^{2+}$
671,9417	671,9428	1,2	$C_{56}H_{41}Cl_8N_4NaO_9Zn_2$	$[2M+OH+Na]^{2+}$
652,9533	652,9541	1,3	$C_{28}H_{21}Cl_4N_2O_4Zn$	$[M+H]^+$
545,4074	545,4081	0,8	$C_{42}H_{31}Cl_6N_3O_7Zn_3$	$[2M\text{-diclof}+Zn+OH]^{2+}$
505,4474	505,4458	2,8	$C_{42}H_{31}Cl_6N_3O_6Zn_2$	$[2M\text{-diclof}+H]^{2+}$
375,9477	375,9480	0,8	$C_{14}H_{12}Cl_2NO_3Zn$	$[M\text{-diclof}+H_2O]^+$
357,9375	357,9375	0,0	$C_{14}H_{10}Cl_2NO_2Zn$	$[M\text{-diclof}]^+$
318,0055	318,0059	1,4	$C_{14}H_{11}Cl_2NNaO_2$	$[diclof+Na+H]^+$
296,0233	296,0240	2,3	$C_{14}H_{12}Cl_2NO_2$	$[diclof+2H]^+$

*Monoisotopic mass of ^{12}C, ^{1}H, ^{14}N, ^{16}O, ^{35}Cl, ^{64}Zn, ^{23}Na

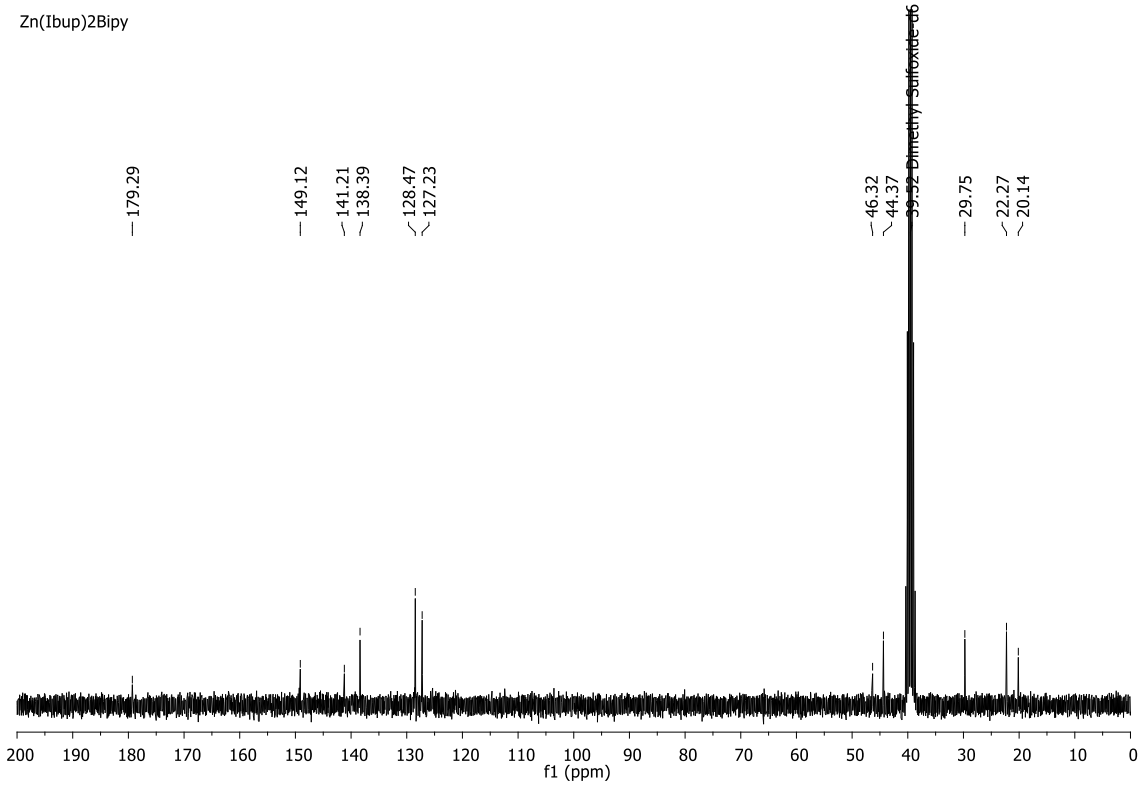
5.3 Experimental data of complex 1



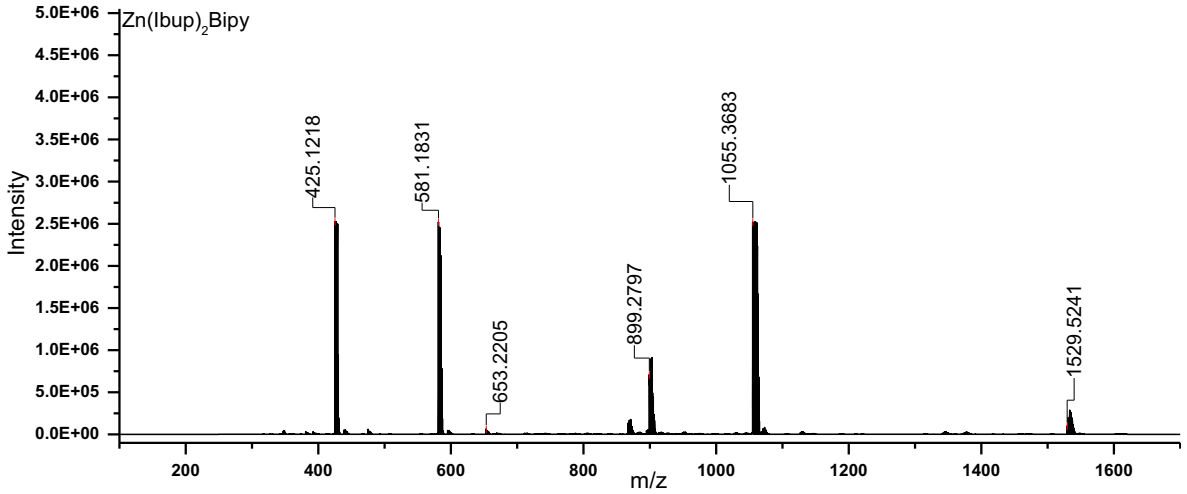
SM Fig. 9. Infrared spectrum for complex 1.



SM Fig. 10. ^{1}HNMR spectrum for complex 1.



SM Fig. 11. ^{13}CNMR spectrum for complex 1.



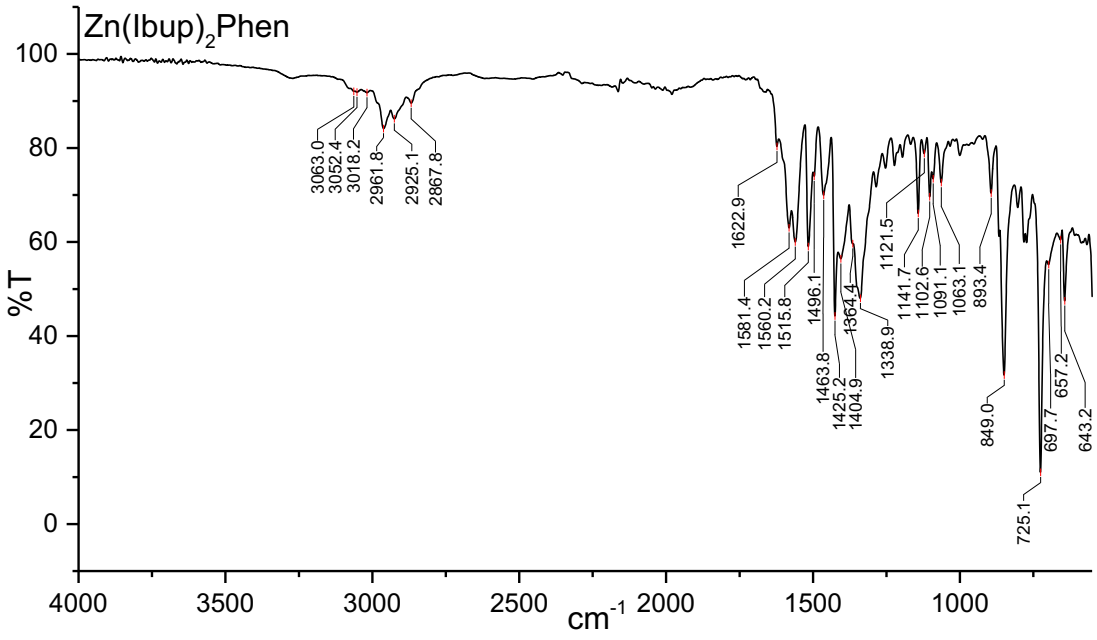
SM Fig. 12. HRMS spectrum for complex 1.

SM Table 3. HRMS data of complex 1.

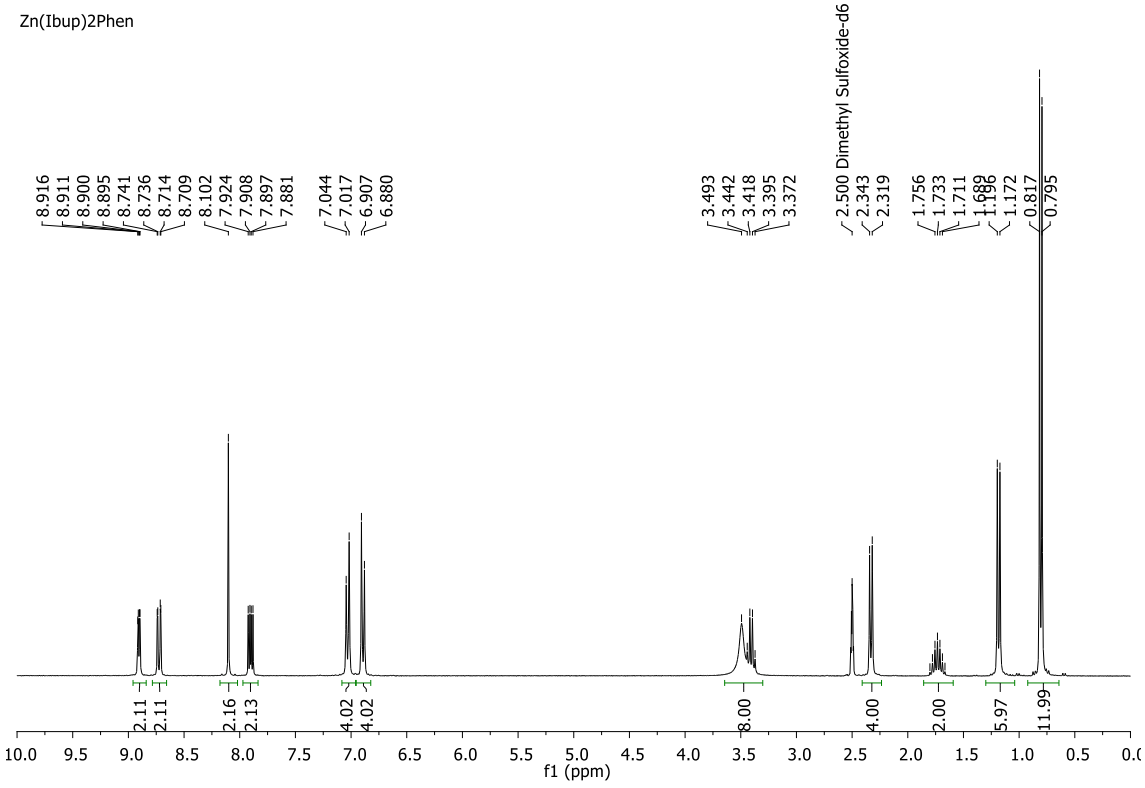
m/z*	Calculated	Error	Chemical formula	Ion
1529.5330	1529.5392	3.7	$C_{85}H_{101}N_4O_{10}Zn_3$	$[2M+ibup+Zn]^+$
1055.3626	1055.3644	1.2	$C_{59}H_{67}N_4O_6Zn_2$	$[2M-ibup]^+$
899.2914	899.2956	4.1	$C_{49}H_{59}N_2O_6Zn_2$	$[2M-ibup-Bipy]^+$
653.2317	653.2334	1.6	$C_{36}H_{42}N_2NaO_4Zn$	$[M+Na]^+$
581.1885	581.1895	0.7	$C_{33}H_{33}N_4O_2Zn$	$[M-ibup+Bipy]^+$
425.1199	425.1207	0.7	$C_{23}H_{25}N_2O_2Zn$	$[M-ibup]^+$

*Monoisotopic mass of ^{12}C, ^{1}H, ^{14}N, ^{16}O, ^{64}Zn, ^{23}Na

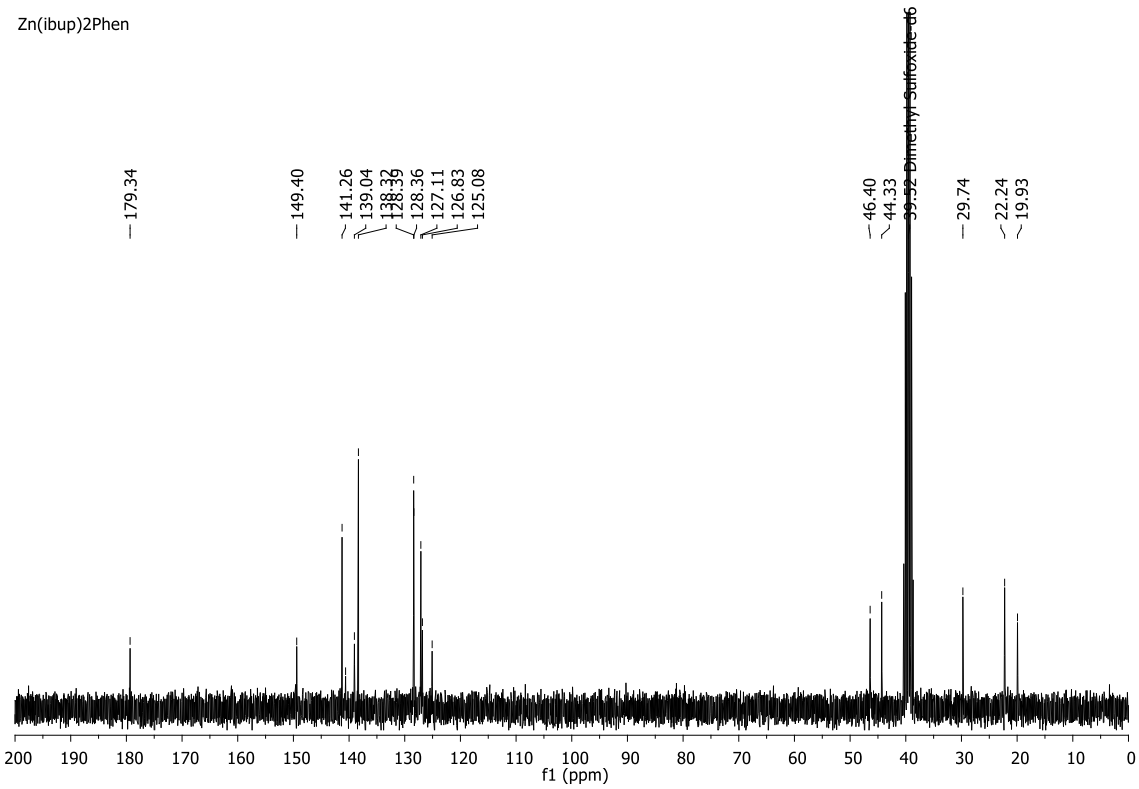
5.4 Experimental data of complex 2



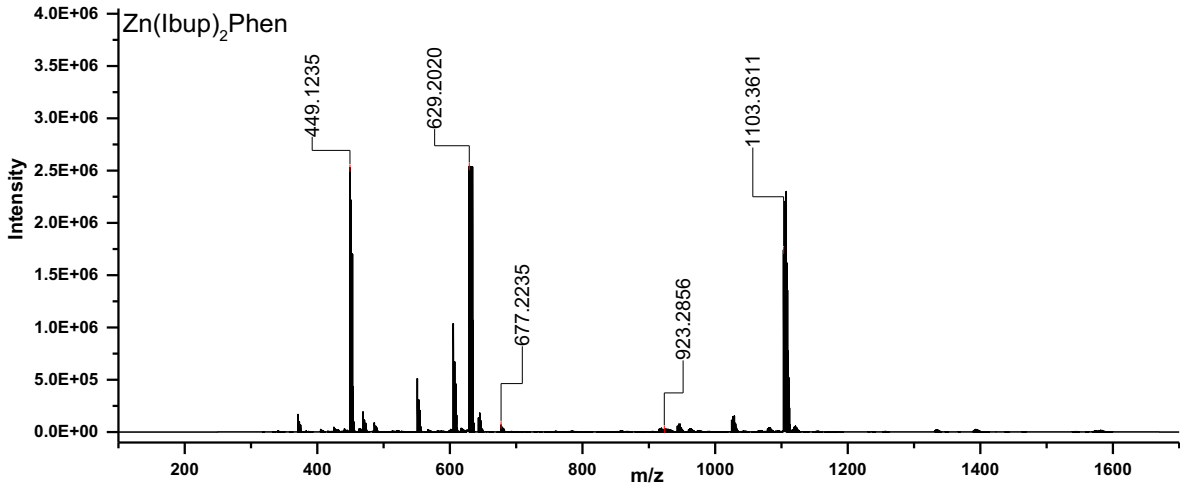
SM Fig. 13. Infrared spectrum for complex 2.



SM Fig. 14. 1HNMR spectrum for complex 2.



SM Fig. 15. ^{13}CNMR spectrum for complex 2.



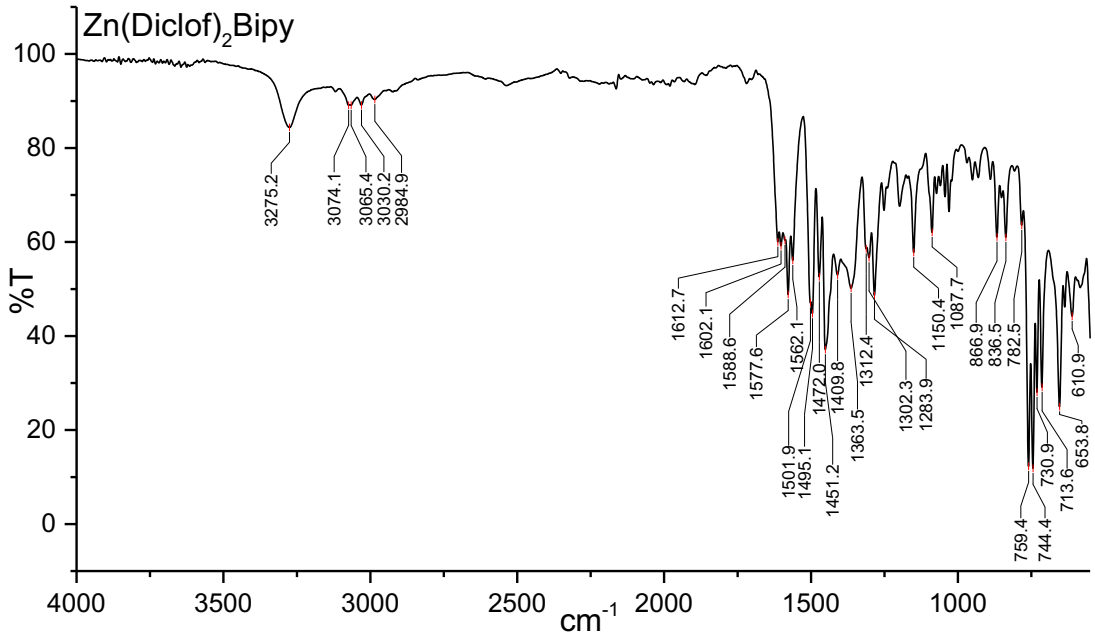
SM Fig. 16. HRMS spectrum for complex 2.

SM Table 4. HRMS data of complex 2.

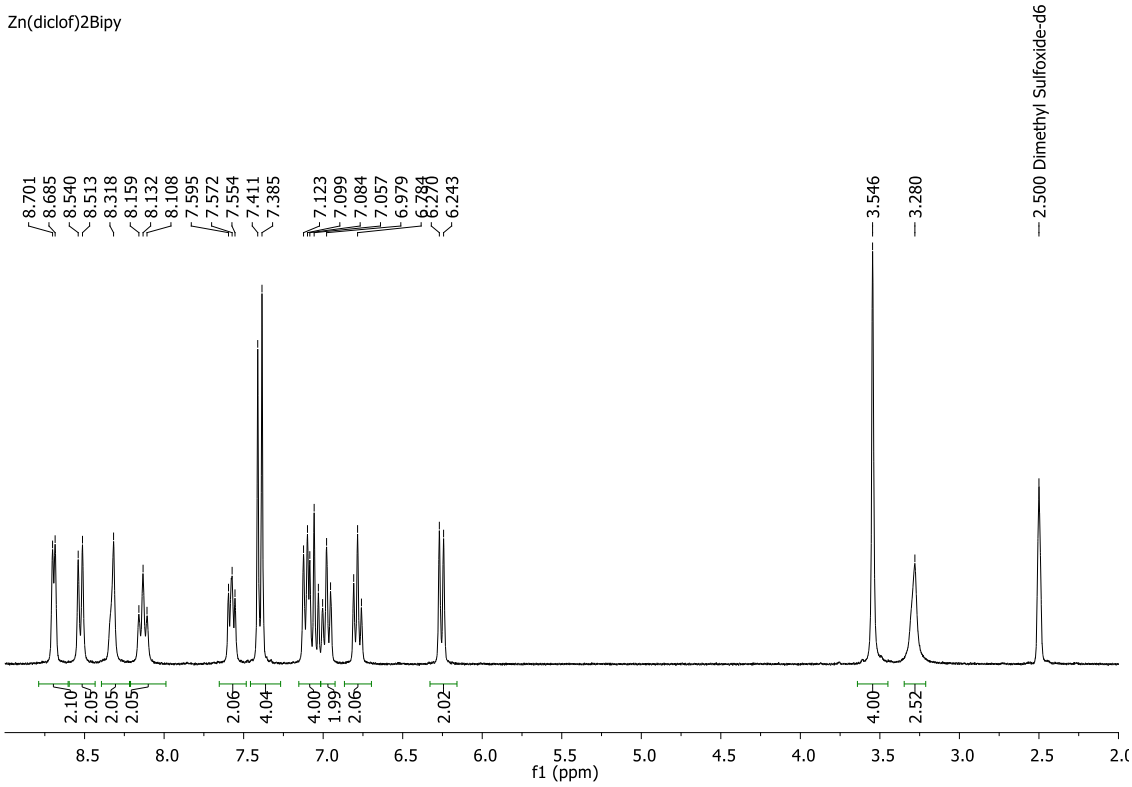
m/z*	Calculated	Error (ppm)	Chemical formula	Ion
1103.3636	1103.3644	0.2	$C_{63}H_{67}N_4O_6Zn_2$	[2M-ibup]+
923.2954	923.2956	0.4	$C_{51}H_{59}N_2O_6Zn_2$	$[M+ibup+Zn]^+$
677.2315	677.2334	1.9	$C_{38}H_{42}N_2NaO_4Zn$	$[M+Na]^+$
629.1890	628.1895	0.1	$C_{37}H_{33}N_4O_2Zn$	$[M-ibup+Phen]^+$
449.1213	449.1207	2.5	$C_{25}H_{25}N_2O_2Zn$	$[M-ibup]^+$

*Monoisotopic mass of ^{12}C, ^{1}H, ^{14}N, ^{16}O, ^{64}Zn, ^{23}Na

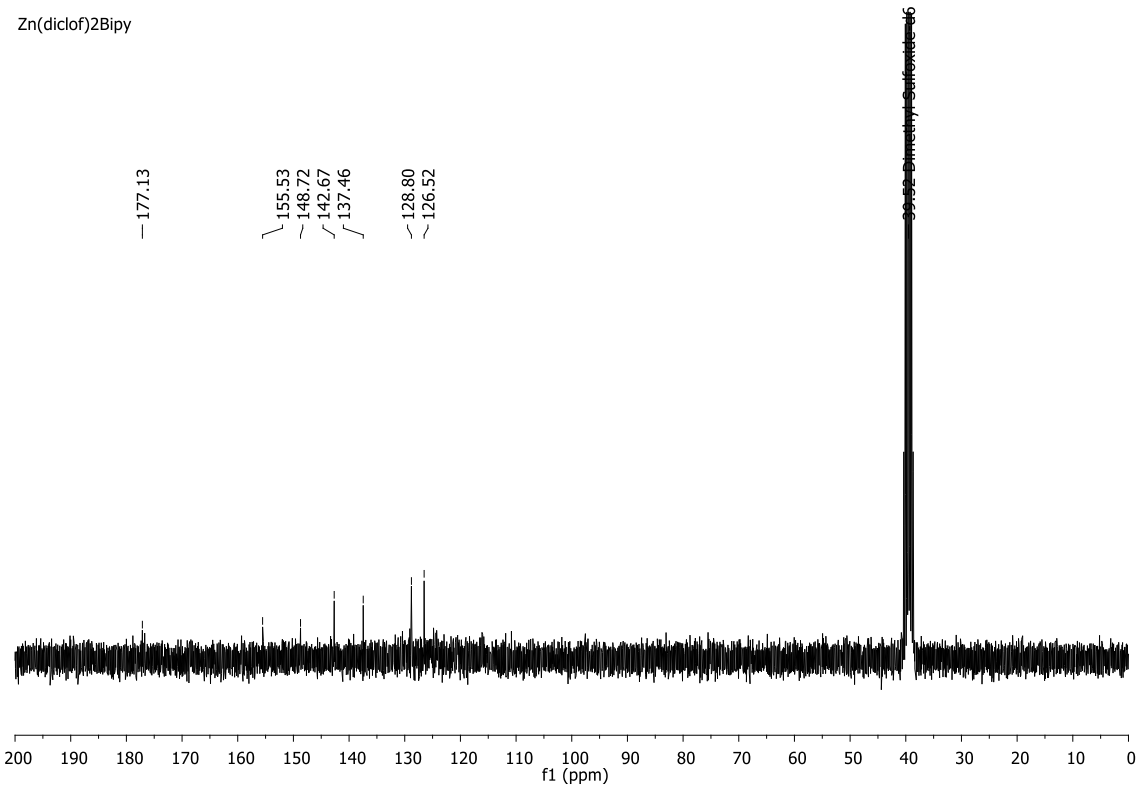
5.5 Experimental data of complex 3



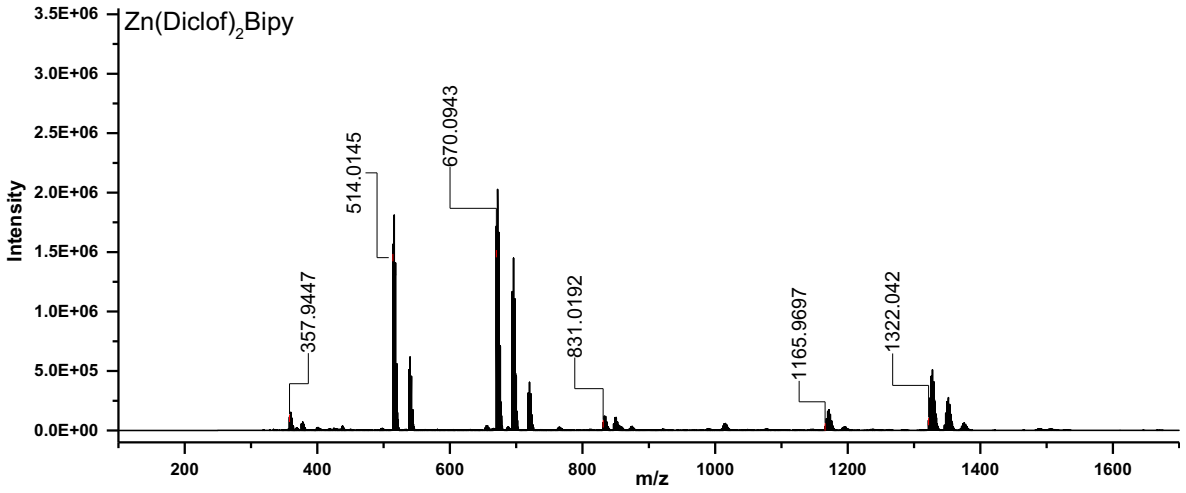
SM Fig. 17. Infrared spectrum for complex 3.



SM Fig. 18. ^{1}HNMR spectrum for complex 3.



SM Fig. 19. ^{13}CNMR spectrum for complex 3.



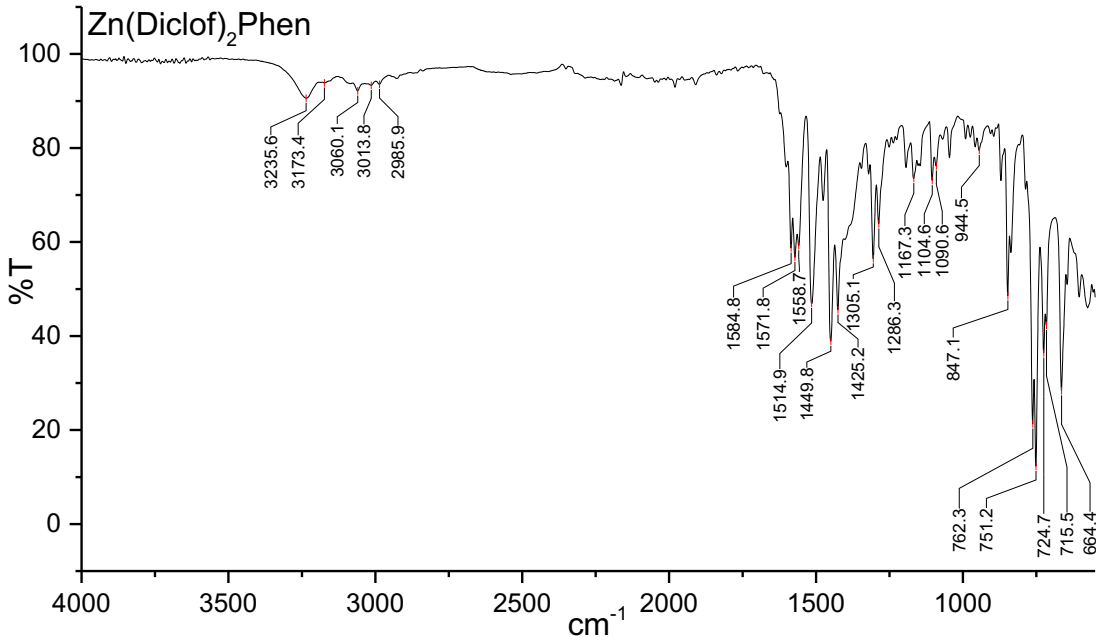
SM Fig. 20. HRMS spectrum for complex 3 (positive ions).

SM Table 5. HRMS data of complex 3.

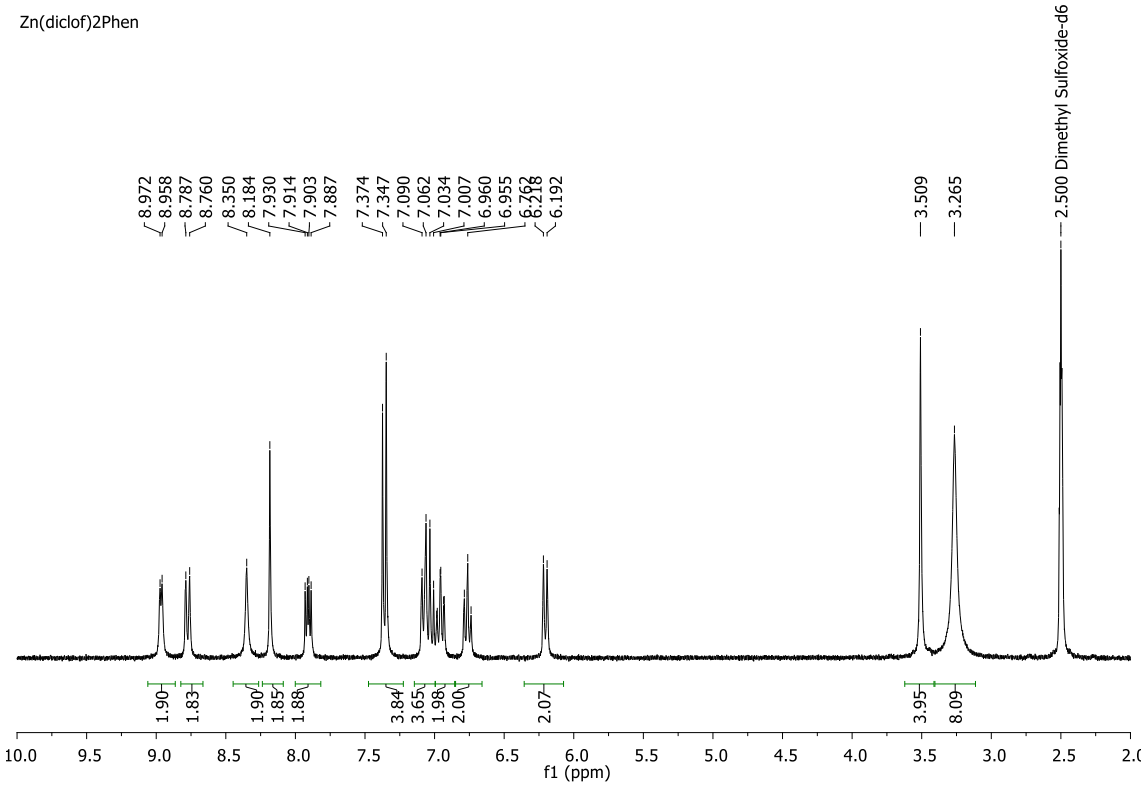
m/z*	Calculated	Error	Chemical formula	Ion
1346.0153	1346.0200	3.1	$C_{62}H_{47}Cl_6N_7NaO_6Zn_2$	$[2M\text{-diclof} + NaH]^+$
1165.9522	1165.9536	0.7	$C_{52}H_{38}Cl_6N_5O_6Zn_2$	$[M+Zn+\text{diclof}]^+$
831.0024	831.0054	3.0	$C_{38}H_{28}Cl_4N_4NaO_4Zn$	$[M+Na]^+$
670.0757	670.0755	1.0	$C_{34}H_{26}Cl_2N_5O_2Zn$	$[M\text{-diclof+Bipy}]^+$
514.0067	514.0068	1.0	$C_{24}H_{18}Cl_2N_3O_2Zn$	$[M\text{-diclof}]^+$
357.9380	357.9380	0.0	$C_{14}H_{10}Cl_2NO_2Zn$	$[M\text{-diclof-Bipy}]^+$

*Monoisotopic mass of ^{12}C, ^{1}H, ^{35}Cl, ^{14}N, ^{16}O, ^{64}Zn, ^{23}Na

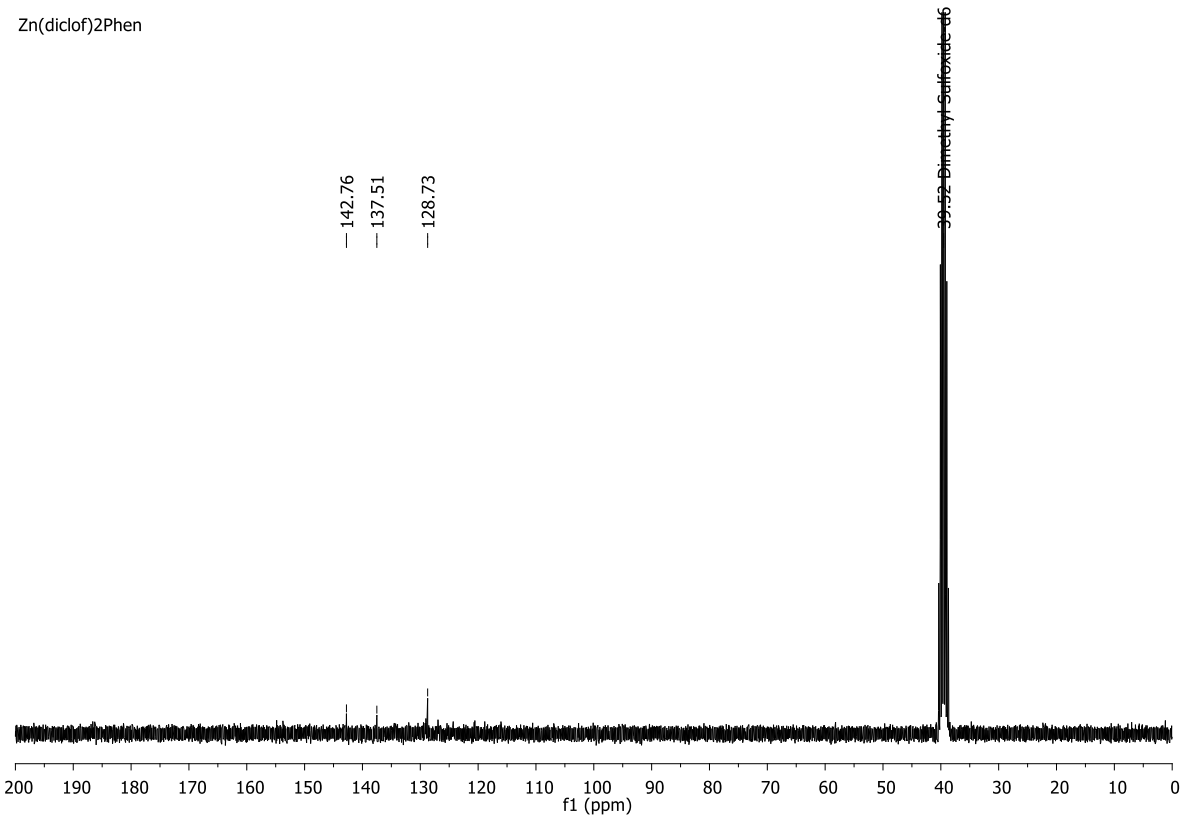
5.6 Experimental data of complex 4



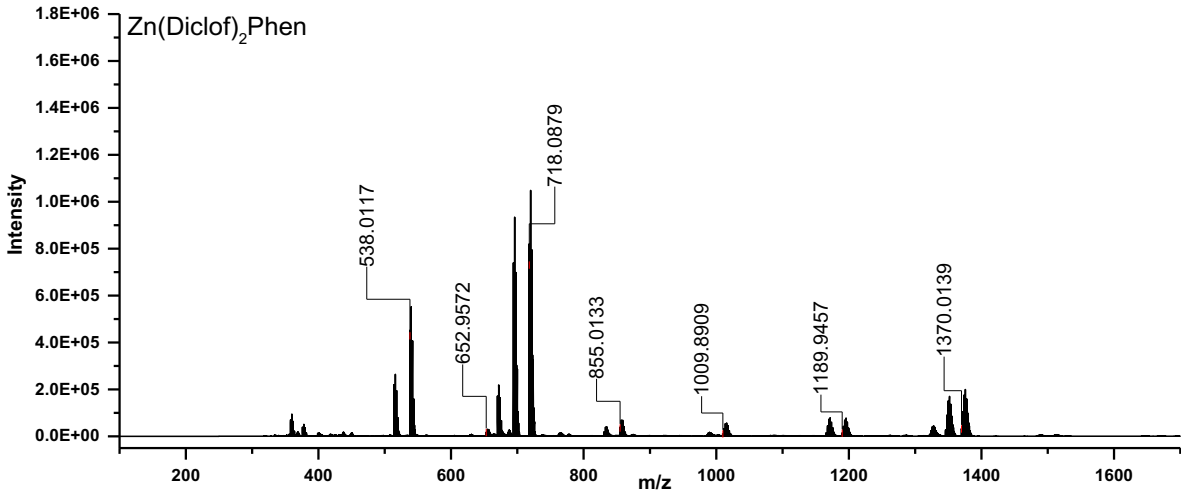
SM Fig. 21. Infrared spectrum for complex 4.



SM Fig. 22. ^{1}HNMR spectrum for complex 4.



SM Fig. 23. ^{13}CNMR spectrum of complex 4.



SM Fig. 24. HRMS spectrum for complex 4 (positive ions).

SM Table 6. HRMS data of complex 4.

m/z*	Calculated	Error (ppm)	Chemical formula	Ion
1189.9494	1189.9536	3.1	$C_{54}H_{38}Cl_6N_5O_6Zn_2$	$[M+diclof+Zn]^+$
1009.8869	1009.8849	2.6	$C_{42}H_{30}Cl_6N_3O_6Zn_2$	$[Zn_2(diclof)_3]^+$
855.0022	855.0054	3.1	$C_{40}H_{28}Cl_4N_4NaO_4Zn$	$[M+Na]^+$
718.0742	718.0755	1.0	$C_{38}H_{26}Cl_2N_5O_2Zn$	$[M-diclof+Phen]^+$
652.9551	652.9547	1.4	$C_{28}H_{21}Cl_4N_2O_4Zn$	$[M-Phen+H]^+$
538.0061	538.0068	0.3	$C_{26}H_{18}Cl_2N_3O_2Zn$	$[M-diclof]^+$

*Monoisotopic mass of ^{12}C, ^{1}H, ^{35}Cl, ^{14}N, ^{16}O, ^{64}Zn, ^{23}Na