# ELECTROCHEMICAL SYNTHESIS OF COPPER(II)-CIPROFLOXACIN/DECANOIC ACID AND COPPER(II)-DICLOFENAC/DECANOIC ACID COMPLEXES AND INHIBITORY EFFECT ON BREAST CANCER CELL LINES

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**by**

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## LIST OF SYMBOLS

| | |
|---|---|
| $\delta$ | Chemical shift |
| $^{o}$C | Degree Celsius |
| µL | Microliter |
| µM | Micrometre |
| × | Multiply |
| µg/ml | Microgram per millilitre |
| % | Percentage |
| ± | Plus–minus |

## LIST OF ABBREVIATIONS

| | |
|---|---|
| 3D7 | Plasmodium falciparum |
| A20 | Murine lymphoma |
| A 549 | Human lung tumour cell lines |
| A-431 | Human skin epidermoid carcinoma |
| A-431 | Human skin epidermoid carcinoma |
| ATR-FTIR | Fourier transform infrared spectroscopy – attenuated total reflectance |
| Aq | Aqueous |
| ATCC | American Type Culture Collection |
| Au | Gold |
| B16-F10 | Murine melanoma |
| BINAP | Organophosphorus Compound |
| BOP | Benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate |
| C | Carbon |
| Cu | Copper |
| CCRF-CEM | Human acute lymphoblastic leukemia cells |
| $CH_2Cl_2$ | Dichloromethane |
| CI | Chlorine |
| $CI^-$ | Chloride |
| Cm | Centimeter |
| $cm^{-1}$ | Per centimeter |
| $^{13}C$ NMR | Carbon-13 nuclear magnetic resonance |
| CP | Ciprofloxacin |
| CP/DA | Ciprofloxacin/Decanoic Acid |
| CT | Calf Thymus |
| $Cu^+$ | Copper(I) ion |
| $Cu^{2+}$ | Copper(II) ion |

| | |
|---|---|
| CuNPs | Copper nanoparticles |
| $CuSO_4$ | Copper sulfate |
| Cu(II)-CP/DA | Copper(II)-Ciprofloxacin / Decanoic Acid |
| Cu(II)-DF/DA | Copper(II)-Diclofenac / Decanoic Acid |
| DA | Decanoic Acid |
| DC | Direct Current |
| DCM | Dichloromethane |
| DF | Diclofenac |
| DF/DA | Diclofenac / Decanoic Acid |
| DHA | Docosahexaenoic |
| D-MEM/F-12 | Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 |
| DMSO | Dimethyl sulfoxide |
| DNA | Deoxyribonucleic acid |
| Dppz | Dipyrido[3,2-a:2′,3′-c]-phenazine |
| EDX | Energy Dispersive X-ray |
| Eq | Equation |
| ER | Estrogen receptor |
| ESDT | Ethylsarcosinedithiocarbamate |
| eV | Electron volt |
| F | Fluorine |
| FA | Fatty acid |
| FBS | Fetal Bovine Serum |
| FESEM | Field emission scanning electron microscopy |
| Fig. | Figure |
| g | Gram |
| h | Hours |
| HaCaT | Human epidermal keratinocytes |
| HDL | High-density lipoprotein |
| HeLa | Human cervical carcinoma cells |
| HepG2 | Human liver cancer cell line |

| | |
|---|---|
| HIV | Human immunodeficiency virus |
| $^{1}H$ NMR | Proton nuclear magnetic resonance |
| $H_2O$ | Water |
| $H_2SO_4$ | Sulfuric acid |
| HT-29 | Colon adenocarcinoma cells |
| HTC-116 | Human colorectal carcinoma |
| $IC_{50}$ | Half maximal inhibitory concentration |
| IL-6 | Interleukin 6 |
| K562 | Human myeloid leukaemia |
| Kg | Kilogram |
| $KNO_3$ | Potassium nitrate |
| kV | Kilovolt |
| $LD_{50}$ | Median lethal dose |
| LDL | Low-density lipoprotein |
| LNCaP | Lymph Node Carcinoma of the prostate |
| M | Meter |
| M | Molar |
| MCF 7 | Human breast cancer cell line |
| MCF 10A | Non-tumorigenic epithelial cell line |
| MDA-MB-231 | Epithelial of human breast cancer cell line |
| MDR | Multidrug resistance |
| MeOH | Methanol |
| MHz | Megahertz |
| Mg | Milligram |
| Mg $kg^{-1}$ | Milligram per kilogram |
| $MgSO_4$ | Magnesium sulphate |
| mL | Mililiter |
| Mm | Millimeter |
| Mol $L^{-1}$ | Mole per litre |
| MTT | 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium Bromide |

| | |
|---|---|
| N | Nitrogen |
| NaOH | Sodium hydroxide |
| NF-κB | Nuclear factor kappa light chain enhancer of activated B cells |
| $NH_3$ | Ammonia |
| $NH_2CH_2CH_2NH_2$ | Ethylenediamine |
| Nm | Nanometer |
| NMR | Nuclear magnetic resonance |
| $NO_3$ | Nitrate |
| NPs | Nanoparticles |
| NRF2 | Nuclear factor erythroid 2-related factor 2 |
| NSAID | Non-steroidal anti-inflammatory drug |
| O | Oxygen |
| oc | Octahedral |
| OD | Optical Density |
| o-3FAs | Omega-3 fatty acids |
| PC-3 | Human prostate cancer cell line |
| pH | Potential hydrogen |
| phen | Phenanthroline |
| RNA | Ribonucleic acid |
| ROS | Reactive oxygen species |
| RPM | Revolutions Per Minute |
| Ru | Ruthenium |
| Ru(II) | Ruthenium(II) |
| Ru(III) | Ruthenium(III) |
| Sp | Square planar |
| SGC-7901 | Human gastric cancer cell line |
| SW480 | Human primary colon cancer |
| SW620 | Human metastatic colon cancer |
| TB | Tuberculosis |
| tbp | Trigonal bipyramidal |
| TEM | Transmission electron microscopy |

| | |
|---|---|
| tptz | 2,4,6-tris(2-pyridyl)-1,3,5-triazine |
| tpy | 2,2′:6′,2″-terpyridine |
| TSCs | Thiosemicarbazones |
| UV-Vis | Ultra Violet-Visible |
| V | Applied Voltage |
| VLFAs | Long-chain fatty acids |
| v/v | Volume/volume |
| $\nu_{as}$ | Asymmetrical |
| $\nu_{s}$ | Symmetrical |
| XRD | X-Ray diffraction |
| Zn | Zinc |
| Zn(II) | Zinc(II) |

## LIST OF APPENDICES

**SINTESIS ELEKTROKIMIA KOMPLEKS KUPRUM(II)-CIPROFLOXACIN/ASID DEKANOIK DAN KUPRUM(II)-DIKLOFENAK/ASID DEKANOIK DAN KESAN RENCATAN TERHADAP TITISAN SEL KANSER PAYUDARA**

## ABSTRAK

Dalam kajian ini, kuprum digunakan sebagai logam, dan ligan ialah ciprofloxacin/asid dekanoik dan diklofenak/asid dekanoik. Kompleks kuprum(II)-ciprofloxacin/asid dekanoik dan kompleks kuprum(II)-diklofenak/asid dekanoik disintesis dengan menggunakan teknik elektrokimia, di mana elektrolisis dijalankan dengan kerajang kumprum sebagai anod dan rod grafit sebagai katod dalam larutan elektrolit 0.01 M kalium nitrat. Kompleks kuprum(II)-ciprofloxacin/asid dekanoik dan kompleks kuprum(II)-diklofenak/asid dekanoik yang disintesis telah dicirikan menggunakan spektroskopi inframerah pemindahan fourier, resonans magnetik nuklear proton dan karbon-13, spektroskopi UV-Vis, Pembelauan X-Ray dan X-ray Penyebaran Tenaga. Keputusan pencirian membuktikan bahawa kompleks kuprum(II)-ciprofloxacin/asid dekanoik dan kompleks kuprum(II)-diklofenak/asid dekanoik telah berjaya disintesis menggunakan teknik elektrokimia (elektrolisis). Untuk mengkaji kesan keadaan elektrolisis terpada saiz zarah kuprum, morfologi kompleks kuprum(II)-ciprofloxacin/asid dekanoik dan kompleks kuprum(II)-diklofenak/asid dekanoik dengan nilai voltan terpakai yang berbeza (1 V, 5 V dan 10 V), kepekatan elektrolit sokongan (0.01 M, 0.1 M, 0.5 M) dan pH awal (3, 4.5, 7, 9 dan 11) telah disiasat dengan menggunakan mikroskop elektron penghantaran dan mikroskop elektron pengimbasan pelepasan medan. Kompleks kuprum(II)-

ciprofloxacin/asid dekanoik (ditandakan sebagai CuCP1) mempunyai saiz 2.31 nm ± 12.49 nm dan kompleks kuprum(II)-diklofenak/asid dekanoik (ditandakan sebagai CuDF1) 1.77 nm ± 4.77 nm ialah bersaiz kecil telah dibentuk dengan menggunakan voltan terpakai 1 V , 0.01 M kalium nitrat sebagai elektrolit penyokong dan larutan awal pH 4.50 semasa proses elektrosintesis. Kemudiannya, penyiasatan kesitotoksikan dilakukan pada sel normal epitelium payudara manusia (MCF 10A) dan kanser payudara manusia (MCF 7) menggunakan zarah nano yang berbeza iaitu CuCP1 (2.31 nm ± 12.49 nm), CuCP2 (65.21 nm ± 179.31 nm), CuCP3 (836.15 nm ± 1479.44 nm), CuDF1 (1.77 nm ± 4.77 nm), CuDF2 (93.03 nm ± 273.73 nm), dan CuDF3 (26.34 nm ± 249.21 nm). Daripada keputusan yang diperolehi, saiz terkecil CuCP1 (2.31 nm ± 12.49 nm) dan CuDF1 (1.77 nm ± 4.77 nm) menunjukkan kesan perencatan yang rendah pada sel MCF 10A iaitu hampir 90% daripada sel yang masih hidup diperhatikan, manakala ia memberikan kesan perencatan yang tinggi terhadap sel MCF 7 iaitu CuCP1 (31% dan 13%) dan CuDF1 (7% dan 18%) selepas 72 jam rawatan dengan 25 µM dan 100 µM kompleks kuprum(II)-ciprofloxacin/asid dekanoik dan kompleks kuprum(II)-diklofenak/asid dekanoik, masing-masing. Kesimpulannya, dengan membandingkan kesan perencatan kedua-dua sel semasa rawatan dengan CuCP1 dan CuDF1, CuDF1 adalah lebih berkesan untuk membunuh sel kanser berbanding kompleks CuCP1 kerana saiz CuDF1 (1.77 nm ± 4.77 nm) lebih kecil daripada CuCP1 (2.31 nm ± 12.49) kompleks. Oleh itu, kuprum(II) nanozarah yang bersaiz sesuai adalah boleh digunakan untuk meningkatkan kesan perencatan terhadap sel kanser (MCF 7).

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## ABSTRACT

In the present study, copper is used as the metal, and the ligands are ciprofloxacin/decanoic acid and diclofenac/decanoic acid**.** The copper(II)-ciprofloxacin/decanoic acid and copper(II)-diclofenac/decanoic acid complexes are synthesized using an electrochemical technique, where electrolysis is conducted with a copper foil as the anode and a graphite rod as the cathode in a 0.01 M potassium nitrate electrolyte solution. The synthesized copper(II)-ciprofloxacin/decanoic acid and copper(II)-diclofenac/decanoic acid complexes was characterized using attenuated total reflectance-fourier transform infrared spectroscopy, proton and carbon-13 nuclear magnetic resonance, UV-Vis spectroscopy, X-Ray Diffraction and Energy Dispersive X-ray analysis. The characterization results proved that copper(II)-ciprofloxacin/decanoic acid and copper(II)-diclofenac/decanoic acid complexes has been successfully synthesized using electrochemical technique (electrolysis). To study the effect of electrolysis conditions on the copper particle size in copper(II)-ciprofloxacin/decanoic acid and copper(II)-diclofenac/decanoic acid complexes morphologies, different values of applied voltages (1 V, 5 V and 10 V), supporting electrolyte concentrations which is potassium nitrate (0.01 M, 0.1 M, 0.5 M) and initial pH (3, 4.5, 7, 9 and 11) were investigated using transmission electron microscopy and field emission scanning electron microscopy. A small-sized copper(II)-

ciprofloxacin/decanoic acid (denoted as CuCP1) 2.31 nm ± 12.49 nm and copper(II)-diclofenac/decanoic acid (denoted as CuDF1) 1.77 nm ± 4.77 nm was formed by using 1 V applied voltage, 0.01 M potassium nitrate as supporting electrolyte and initial solution pH of 4.50 during the electrosynthesis process. The cytotoxicity investigation was then performed on human breast epithelial normal (MCF 10A) and human breast cancer (MCF 7) cells using NPs with different particles which are CuCP1 (2.31 nm ± 12.49 nm), CuCP2 (65.21 nm ± 179.31 nm), CuCP3 (836.15 nm ± 1479.44 nm), CuDF1 (1.77 nm ± 4.77 nm), CuDF2 (93.03 nm ± 273.73 nm), and CuDF3 (26.34 nm ± 249.21 nm). From the results obtained, the smallest size of CuCP1 (2.31 nm ± 12.49 nm) and CuDF1 (1.77 nm ± 4.77 nm) shows low inhibitory effect on MCF 10A cells which is almost 90% of viable cells were observed while it gives high inhibitory effect on MCF 7 cells for CuCP1 (31% and 13%) and CuDF1 (7% and 18%) after 72 hr treatment with 25 μM and 100 μM of copper(II)-ciprofloxacin/decanoic acid and copper(II)-diclofenac/decanoic acid complexes, respectively. In conclusion, by comparing to the inhibition effect of both cells upon treatment with CuCP1 and CuDF1, the CuDF1 is more effective to kill cancer cell compared to CuCP1 complex due to the size of CuDF1 (1.77 nm ± 4.77 nm) is smaller than CuCP1 (2.31 nm ± 12.49) complex. Therefore, appropriately sized copper(II) nanoparticles could be utilised to enhance the inhibitory effects of cancer cells (MCF 7).

# CHAPTER 1

# INTRODUCTION

## 1.1 Copper Complexes

Copper (Cu) is the 29$^{th}$ chemical element in the Periodic Table and belongs to Group 11 metals in the first row, with the electronic configuration $3d^{10}4s^{1}$. Hence, the Cu(I) ion has a full-filled of $3d^{10}$ shell, whereas the Cu(II) ion, lost two electrons and it called as a partially filled d-block $3d^{9}$ shells configuration, behaving like a common transition metal [1]. Cu was initially utilised by humans roughly 10,000 years ago. Since Cu was mostly mined on the island of Cyprus during the Roman Empire, Cyprium, or "metal of Cyprus," eventually became known as Cuprum, from which the chemical symbol Cu was derived [1]. Cu is a trace metal that is found in all living things and is important for growth, development, and redox chemistry [2]. Numerous types of proteins and enzymes, such as cytochrome oxidase, ascorbate oxidase, and tyrosinase requires it to function. It is also indispensable for deoxyribonucleic acid (DNA) synthesis and respiration. Moreover, the primary processes of Cu containing biological substances involve oxidation-reduction reactions, in which free radicals are produced when Cu contains biological particles are directly interact with oxygen particles [3,4].

The redox chemistry in a catalytic cofactor of iron absorption and mitochondrial respiration, Cu plays a crucial part in cell physiology [5]. Alterations in Cu levels are linked to disorders like cancer gastrointestinal ulcers, diabetes, epilepsy, rheumatoid arthritis similar to Menkes syndrome and Wilson disease, which are inherited [3]. Previous studies of Menkes and Wilson diseases have uncovered novel information about Cu homeostasis, specifically about the molecular mechanisms underlying the

intracellular trafficking and distribution of Cu [3]. Cu is toxic due to its capability which can generate the directly cleave deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). There are many different classifications of human malignancies, such as prostate, colon, breast, lung, and brain tumors, had been linked to increased Cu levels [6]. Also, it seems that a certain amount of Cu in the area is needed for angiogenesis to happen [7].

Cu being an essential element, is likely to have a lower toxicity level compared to non-essential metals including platinum. A few Cu complex types have been investigated as potential anticancer treatments in past few years [1]. Cu complexes have gained attention because they behave differently from cisplatin which covalently binds to DNA, even though the chemical foundation of their mechanism of action is still dimly understood. Consequently, Cu complexes could theoretically offer a wider range of antitumor behaviour [1]. All higher plants and animals require trace amounts of Cu, for proper growth and development. It is mostly present as a cofactor in several enzymes and bloodstream [8]. However, Cu is toxic and can even be lethal if large doses of Cu and it cause harm to living things [9].

In additions, bivalent ion in a Cu formation is extremely toxic to lower organisms. For instance, microorganisms or bacteria that cause deterioration perish of copper vessel in water and Cu compounds inhibit the formation of algae. Like how it can become harmful for cells in people when quantities are high. Since Cu has a higher affinity for binding DNA than any other divalent cation, it encourages DNA oxidation. Therefore, proteins, polynucleotides, DNA, and biomembranes all can have their conformational structures changed by the binding to Cu ions at particular locations [10]. This binding are influenced by the geometry of the generated adduct, the charge electron affinity, and the size of the Cu. Therefore, previous studies mentioned

that copper ions in the form of $Cu^{+}$ and $Cu^{2+}$ can alter the redox potential and make it easier for electron transfer processes to take place in blue Cu proteins. Through transition metal ions, which are capable of performing a valence change in redox processes, it can achieve the selectivity in a higher degree of molecular recognition [11].

Therefore, Cu based complexes have been studied that endogenous metals are less harmful. The type of metal ion that bound to the donor atoms and ligands are heavily influences the characteristics of Cu coordinated compounds, either in organometallic compounds, traditional inorganic coordination complexes, or bioinorganic model compounds [12,13]. Furthermore, the metal of stabilized in oxidation states are Cu(I), Cu(II), and Cu(III) but only few instances of Cu(III) compounds have been discovered [13]. Besides, Cu(I) complexes are typically colourless solids due to the closed shell of electronic configuration ($d^{10}$), and it preferentially favour soft donor atoms (aromatic amines) with ligands. The characteristic electronic configuration ($d^{9}$) of Cu(II) derivatives shows *d-d* transitions, results in intensely coloured species. The chelation, geometry, donor set, and substituent steric effect all have a significant role in the redox capacity of the physiologically available of Cu(I)/Cu(II) pair, which changes mostly based on the interactions of ligands [14]. Additionally, any such electron transfer invariably results in significant changes to the relevant oxidized/reduced complexes' stereochemistry. The Cu(I)/Cu(II) in physiological media is incredibly complicated, as shown by this property as well as ability to release the groups of coordinations continuously, for instance, from octahedral Cu(II) to tetrahedral Cu(I) species.

Additionally, denticity is a factor in the classification of ligands. The term "denticity" of the ligand refers to how many atoms link the ligand to the central metal.

It comes in the following varieties which are unidentate ligands, bidentate ligands, polydentate ligands and multidentate ligand. Unidentate ligands are defined as having only one coordination site through one donating site to metal. For instance, water ($H_2O$), chloride ($Cl^-$), and ammonia ($NH_3$) [15]. While bidentate ligands are those that have two coordination sites that connect to the metal through two distinct coordinating sites to produce two coordinate bonds. For instance, Bipyridyl, (R)-BINAP, (S)-BINAP, (R), and $NH_2CH_2CH_2NH_2$ (en). Besides, polydentate ligands are those that have more than two coordinating sites that can form bonds of coordination with the metal. Tridentate, tetradentate, pentadentate, and hexadentate ligands all fall under this category [16].

There are few types of coordination complex. The first type of complex is a mononuclear complex, which defined as one in which the coordination sphere contains only one central metal cation, and it commonly a transition metal. The complex is polynuclear due to the presence of numerous central metal cations in the coordination sphere, the cations may be from the same transition metal or from different metals [17]. Moreover, some coordination sphere, which includes the main metal ion and the ligands as a single unit, is known as a neutral complex because it has no net charge. This might be because of neutral ligands themselves participating or a charge balance brought on by an equal amount of positive and negative charges in the complex [18].

### 1.2 Metal-based Anticancer Agent

Gold(III)-dithiocarbamato complexes have previously get increased interest as efficiency antitumor agents due to potent tumour cell growth–inhibiting impacts of complex, which are typically accomplished by the mechanism of different cisplatin [19]. A goal of research is to lower the adverse effects in specific nephrotoxicity which

is platinum-based drugs that brought on by clinically and combining with anticancer characteristics of the gold(III) metal centre with the efficiency chemoprotective function of coordinated dithiocarbamates [19]. Dithiocarbamates are bidentate chelating ligands, and the resultant complexes are expected to be relatively stable due to the chelate effect. In this setting, $Au^{III}Br_2(ESDT)]$, ESDT = ethylsarcosinedithiocarbamate demonstrated to exert exceptional and promising anticancer activity *in vitro*, as well as to beat existing obstacles inherent resistance to cisplatin that was demonstrated by some types of tumours [19]. As a continuation of earlier work, the results of extensive *in vivo* trials in rodents, including tests for toxicity, nephrotoxicity, anticancer activity against three transplantable mouse tumour models, and histological analysis. Surprisingly, the gold(III) complex having a stronger antitumor efficacy compared to cisplatin towards all of the mouse tumour models studied, resulting in up to 80% tumour growth suppression. Additionally, it exhibits reduced nephrotoxicity and lower the acute toxicity levels (lethal dose, $LD_{50}$ = 30 mg $kg^{-1}$). Overall, these results validate the gold(III)-dithiocarbamato derivative as clinical trials [19].

Next is Ruthenium (Ru) complexes as anticancer agents. According to previous study, preparations of Ru complexes were made to improve the activity of cisplatin, especially on resistant tumours, or to minimise host toxicity at therapeutic levels. Since a long time ago, several scientific groups have been working actively in the field of inorganic antitumor medications [20]. They have created a variety of Ru(II) and Ru(III) complexes, which have been demonstrated to have strong antitumor and, more importantly, antimetastatic capabilities against animal models. In the field of medicinal chemistry, Ru complexes are receiving a lot of attention as anticancer medicines with targeted antimetastatic characteristics and low systemic toxicity from

previous investigation. Moreover, Ru compounds are able to pass through the tumour cells and bind to DNA successfully [20].

According to earlier research, Zinc (Zn) is the next most common essential element in the human body and most significant microelements required for human physiology. [21]. It is found in thousands of enzymes and proteins because of its chemical properties, particularly its capacity to support many coordination geometries, accelerate ligand exchange, and exhibit little redox activity. In a manner comparable to that of other trace elements, an imbalance in its homeostasis can precipitate the development of a variety of disorders, and in some instances may even be linked to the progression of cancer [21]. Moreover, Zn have positive treatment and preventative effects on infectious diseases as well as to its physiological function. Zn(II) complexes have frequent adverse effects than other metal-based medications according to previous study [21]. Previous study reported that, ligand is based on nature because DNA intercalation characteristics and inherent toxicity, which can increase the metal effect [21]. Thus, planar aromatic quinoline, 2,2′-bipyridine, and 1,10-phenanthroline ligands have frequently been the ligands for medicinal chemists [21].

### 1.3 Problem statement

In recent years, heightened attention has been directed toward the exploration and advancement of anticancer drugs. This surge in interest is driven by the identification of novel synthetic compounds that show promise in various *in vitro* and preclinical studies. Despite these advancements, some drug candidates, upon reaching clinical trials, exhibit substantial toxicity on normal cell lines while displaying lower toxicity on cancer cell lines.

Previous research indicates that the conjugation of anticancer ligands which means the conjugates are intended to deliver an extremely toxic carrier directly to the target cancer cells. They consist of a tumor-homing carrier that connected by a linker to a cytotoxic substance, and this may result in diminished effectiveness. Consequently, the current study aims to modify anticancer drugs ciprofloxacin and diclofenac with decanoic acid, anticipating an enhancement in cytotoxicity activity against cancer cells and reduced toxicity to normal cells. The choice of Cu as a metal center stems from its reported lower toxicity for normal cells compared to cancer cells.

Building on prior investigations, the therapeutic efficacy of metal-based anticancer agents is influenced by metal in the complex that bound to both bioactive ligand and the metal center itself. The selection of donor atoms in bioactive ligands particularly impacts the chemical and biological characteristics of the synthesized complex. Hence, a strategy to augment the cytotoxicity activity of organic anticancer drugs involves the chemical modification of existing anticancer drugs with fatty acids.

The size of metal particles in the compound presents a significant challenge in determining the cytotoxic effects of metal-based drugs for cancer treatment. Nanoparticles (NPs) play a crucial role in facilitating direct drug delivery to the active site due to their superior ability to permeate cell membranes compared to larger particles, resulting in increased bioavailability and therapeutic effectiveness. However, the inability to precisely control the size of synthesized NPs poses a major hurdle, particularly in conventional chemical synthesis methods, which also contribute to environmental challenges through chemical waste.

To address these issues, an electrochemical technique is proposed for NP synthesis, allowing for precise control over particle size by manipulating electrolysis

parameters such as applied voltage, concentration of supporting electrolyte, and pH solution. This approach minimizes chemical consumption, as electrons serve as the primary reagent for redox reactions, presenting a more sustainable alternative to conventional chemical synthesis methods.

## 1.4 Objectives

The objectives of this study are as follows:

1. To characterize the synthesized Cu(II)-CP/DA and Cu(II)-DF/DA by using electrochemical method.
2. To examine the effect of applied voltage, supporting electrolyte concentration ($KNO_3$) and solution pH on Cu particle size and surface morphology in the synthesis of Cu(II)-CP/DA and Cu(II)-DF/DA complexes.
3. To investigate the inhibition effect of electrochemically synthesized Cu(II)-CP/DA and Cu(II)-DF/DA with different particles size on MCF 7 and MCF 10A cells.

## 1.5 Scope of study

In this study, Cu is employed as the metal, and the ligands are CP/DA and DF/DA. The Cu(II)-CP/DA and Cu(II)-DF/DA complexes are synthesized using an electrochemical technique, where electrolysis is conducted with a Cu foil as the anode and a graphite rod as the cathode in a 0.01 M potassium nitrate ($KNO_3$) electrolyte solution. Various techniques, including Attenuated Total Reflectance (ATR-FTIR), Nuclear Magnetic Resonance (NMR), Ultraviolet-Visible (UV-Vis) and X-ray Diffraction (XRD) were employed for characterization of synthesized complexes. The ATR-FTIR helps in identifying the specific functional groups present in the CP, DF, DA, CP/DA, DF/DA, Cu(II)-CP/DA and Cu(II)-DF/DA. By analyzing the vibrational

frequencies of the bonds, it were determine the chemical groups that involved in the interaction between the ligand (CP/DA and DF/DA) and complex (Cu(II)-CP/DA and Cu(II)-DF/DA). In this study, NMR was studied to identify the specific proton and carbon environments and the molecular structures in CP, DF, DA, CP/DA and DF/DA compounds. Then, UV-Vis spectroscopy was used to observe electronic transitions within the Cu(II) ion, particularly d-d transitions. These transitions can provide insights into the coordination environment of the Cu(II) ion and the ligands (CP/DA and (DF/DA). The XRD analysis was investigated the crystalline structure materials, including CP, DF, DA and their complexes with Cu(II) which are Cu(II)-CP/DA and Cu(II)-DF/DA. The XRD analysis was confirmed the formation of Cu(II)-CP/DA and Cu(II)-DF/DA complexes by comparing the diffraction patterns of the complexes with those of the individual components (CP, DF, DA, and Cu(II) complexes). The appearance of new diffraction peaks or changes in peak positions or intensities can indicate the formation of a new crystalline complex. Next, FESEM analysis was used to study the surface morphology of Cu(II)-CP/DA and Cu(II)-DF/DA) complexes, particularly under different electrolysis conditions. FESEM analysis were provides high-resolution images of the surface of the Cu(II)-CP/DA and Cu(II)-DF/DA complexes, revealing detailed information about their morphology. It can observe the size, shape, and distribution of particles or aggregates on the surface. This is important in this research because the surface characteristics can significantly influence the material's properties, such as reactivity, stability, and interaction with biological systems. While FESEM analysis primarily focuses on morphology, it was coupled with EDX to provide elemental analysis of the surface. This allows to map the distribution of elements on the surface of the Cu(II) complexes. The TEM analysis was used to identify the size and internal structure of nanoparticles in Cu(II)-CP/DA and

Cu(II)-DF/DA complexes, especially when synthesized under different electrolysis conditions. By comparing TEM images of Cu(II) complexes formed under different electrolysis conditions, it can assess how these parameters influence particle size. For example, different conditions might lead to the formation of smaller or larger nanoparticles, which can impact the overall effectiveness of the complexes in their intended applications. Next, the MTT assay was employed to evaluate the inhibitory effects of Cu(II)-CP/DA and Cu(II)-DF/DA complexes, with varying Cu nanoparticle sizes (denoted as CuCP1, CuCP2, CuCP3, CuDF1, CuDF2, and CuDF3), on the human breast cancer cell line (MCF 7) and the non-tumorigenic epithelial cell line (MCF 10A). This assay measures cellular metabolic activity as an indicator of cell health, proliferation, and viability. The size of the nanoparticles plays a critical role in their inhibitory effects on MCF 7 and MCF 10A cells. Smaller nanoparticles tend to exhibit higher cellular uptake, faster drug release, and greater inhibitory effects, making them potentially more effective in targeting cancer cells like MCF 7. In contrast, larger nanoparticles may offer more controlled drug release, reduced uptake by non-cancerous cells, and potentially less aggregation, which could enhance the selectivity and safety profile of the treatment. Therefore, optimizing the balance between effective cancer cell inhibition and minimal impact on healthy cells can be achieved by manipulating the particle size.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Fatty acid (FA)

Fatty acid (FA) is a carboxylic acid that is a structural component of fats, oils, and all other types of lipids, which are fat-soluble compounds that can be found in plant and animal tissues. Fatty acids can be classified into short, medium, and long-chain. The short-chain fatty acids are those with aliphatic tails of five or fewer carbon atoms, medium-chain fatty acids, which contain six to twelve carbon atoms, and long-chain fatty acids, which contain fourteen to twenty carbon atoms. The very long-chain fatty acids (VLFAs), which include 22 carbon atoms or more, are prevalent as brain lipid's components [22]. Most fatty acids that can be found in nature, that have a chain structure.

Previous research has demonstrated that FA have antifungal, antimicrobial, and anticancer properties [23]. By conjugating existing cancer treatments with a targeting moiety, it is possible to make chemotherapy more efficacious to cancer cell and less toxicity to normal cells. Numerous studies highlight the beneficial effects of saturated and unsaturated fatty acids utilized to change various heterocyclic compounds on the compound's selectivity for aim the tumour cells and cytotoxic efficiency, while also demonstrating a reduced cytotoxic effect against normal cell lines [24, 25].

Moreover, fatty acids also increase the efficiency of anticancer medications, which has been shown in several *in vivo* and *in vitro* research [26]. For this study, figure 2.1 shows decanoic acid (DA) that will be used as a FA component for chemical modification with an anticancer drugs which are CP and DF. A considerable amount

of literature has been published on the *in vitro* cytotoxicity studies of decanoic acid (DA). Human colorectal carcinoma (HTC-116), human skin epidermoid carcinoma (A-431) and human mammary gland adenocarcinoma (MDA-MB-231) are all inhibited from proliferating by DA, according to study of Narayanan et al. [27] DA was utilised as a ligand in Cu(II) complexes in earlier research, which led to modest cytotoxicity effects on the A549 cells and human cervical carcinoma cells (HeLa). Through chemical modification in anticancer drugs with DA, it is expected to enhance the cytotoxicity activity of Cu(II)-CP/DA and Cu(II)-DF/DA complexes compared to Cu(II)-DA from previous studies [27].

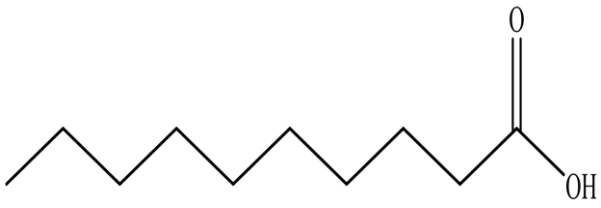


Figure 2.1 Chemical structure of decanoic acid

From previous investigation, several human cancer cell lines including, those from colon, prostate, pancreatic and breast cancer, showed reduced proliferation and increased apoptosis after being treated with omega-3 fatty acids (o-3FAs). Previous research has shown that o-3FAs acts synergistically with chemotherapeutic drugs and it also has the potential to be utilised to increase the radiosensitivity of tumours [28]. In addition, the presence of o-3FAs in biological membranes changes the profile of lipid mediators produced during inflammatory reactions. Furthermore, 3FAs inhibit the transcription of nuclear factor kappa B (NF-$_k$B) dependent genes by acting as ligands of nuclear peroxisome proliferator-activated receptors.

The ability of 3-FA and its derivatives, such as conjugated eicosapentaenoic acid, can serve as significant nutritional adjuvant treatments in the treatment of a variety of human cancer disorders, as well as the influence of 3-FA on cancer prevention [28]. The C-2′ location of the second-generation taxoids that could defeat multidrug resistance (MDR) brought on by overexpressed ATP-binding cassette (ABC) transporters was connected to polyunsaturated fatty acids such docosahexaenoic acid (DHA) and linoleic acid [29]. In previous vivo tests showed the novel that conjugates to have potent action against drug-resistant colon cancer and drug-sensitive ovarian cancer xenografts in mice. It was discovered that two of the novel conjugates, DHA–SB-T-1214 and DHA–SB-T-1213, were able to achieve the whole regression of drug-resistant and drug-sensitive tumours, respectively, in the animal models with a significantly reduced amount of systemic toxicity [29].

## 2.2 Ciprofloxacin (CP)

Ciprofloxacin (CP), (Figure 2.2) [30] is an antibiotic that belongs to the class of drugs known as 1-cyclopropyl-6-fluoro-1-4-dihydro4-oxo-7-(1-piperazinyl)-3-quinoline-carboxylic acids, which having position C-6 of fluorine atom and position C-7 of piperazine or methyl piperazine [30]. Quinolones or fluoroquinolones are broad category of synthetic antimicrobial substances which are strongly treat the many types of infectious disorders, especially in bacterial infections for example bone, skin, soft tissue and respiratory system [31]. According to previous studies, these antibiotics have an extensive range of potential against Gram-positive and Gram-negative bacteria because of fluorine atom at position C-6 and the piperazine or methyl piperazine at position C-7. Thus, it was frequently utilized to treat both human and veterinary disorders [32]. Moreover, the DNA gyrase of bacterial topoisomerase I or maybe the topoisomerase IV in Gram-positive species are blocked by antibacterial agents

(quinolone), which prevent the tertiary bacterial of DNA (super coiling). Perhaps the interaction of quinolones with DNA-gyrase causes this effect, and the resulting complex quickly turns bactericidal [33].

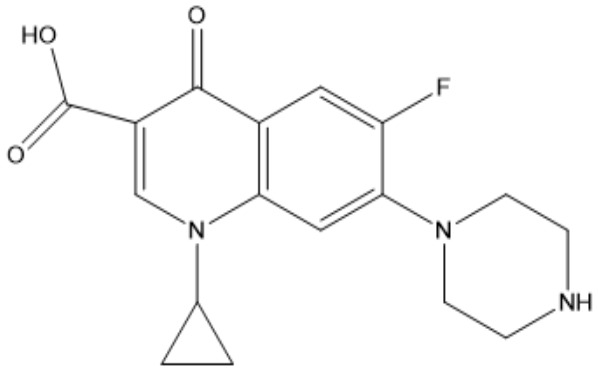


Figure 2.2 Chemical structure of ciprofloxacin or quinolone derivative

Many derivatives of ciprofloxacin are demonstrated different characteristics of biological, such as such as anti-fungal, anti-bacterial, antitumor, anti-malarial, anti-ischemic and anti-TB activities including ureases inhibitory have been discovered in the last 30 years, and the anti-bacterial properties always a major research field of ciprofloxacin derivatives [34]. It has been proven that the 3-oxo-4-carboxylic acid core is the active DNA-gyrase binding site, and that replacing the carboxylic acid group leads to an inhibition in the anti-bacterial action [35]. Furthermore, the ciprofloxacin hybrids with 4-thiazolidinones replacing the carboxylic acid of ciprofloxacin demonstrated limited antibacterial activity, confirming that modification of the carboxylic acid at the C-3 position led to a reduction of efficacy [36]. It was interestingly discovered that, the ciprofloxacin of carboxylic acid was substituted with an aromatic amide group, considerable increases the drug's efficacy against organisms were obtained [37].

Based on the previous observation that quinolone antibiotic drugs block DNA gyrase and topoisomerase IV [38], several types of derivatives of fluoroquinolones

have been investigated as anticancer agents [39-42]. Enzymes known as type II topoisomerases are essential for preserving and regulating the conformations needed for transcription and DNA replication [43]. In fact, the enzyme DNA topoisomerase II catalyses the breakage of DNA's double strands, allowing strands to flow through and regulating DNA shape [44]. Targets of chemotherapeutic intervention in anticancer and antibacterial therapy, DNA topoisomerases are present in both prokaryotic and eukaryotic cells [45]. Furthermore, it was demonstrated that they could also block the polymerization of tubulin and mammalian topoisomerase, therefore performing as strong anti-cancer drugs [46].

Tests have been conducted on CP derivatives against tumour cell lines and HIV. Based on previous study, a series of 1-[(sub)]-6-fluoro3-[(sub)]-1,3,4-oxadiazol-2-yl-7-piperazino-1,4-dihydro 4-quinolinones and synthesised compounds were tested for their antiproliferative properties against human lung tumour cell lines (A549) [47]. A combination of fluoroquinolone and oxadiazole, 1-cyclopropyl-6-fluoro-3-[5(4-nitrophenyl)-1,3,4-oxadiazol-2-yl] -7-piperazino-1,4-dihydro-4-quinolinone were discovered to be the most active compound *in vitro* with an $IC_{50}$ of 9.0 µg/ml [48]. Previous study of Sriram et al., (2007) reported that, created a number of novel tetracycline derivatives by reacting the proper tetracyclines with formaldehyde and the piperazino (secondary amino) function of fluoroquinolones. These compounds were then assessed for their anti-HIV and antimycobacterial integrase enzyme of inhibition properties [49]. Based on previous study, Azéma et al., (2009) reported that several CP derivatives with different lipophilicities and also examined the activity of five human cancer cell lines [50]. They did this because they were aware of the antiproliferative and apoptotic activities in several cancer cell lines of CP and the significance of increasing the lipophilicity to improve anticancer efficacy. Among the

derivatives that were prepared in previous study, the two compounds (X & Y, Figure 2.3) exhibited as significant *in vitro* anticancer activities [48].

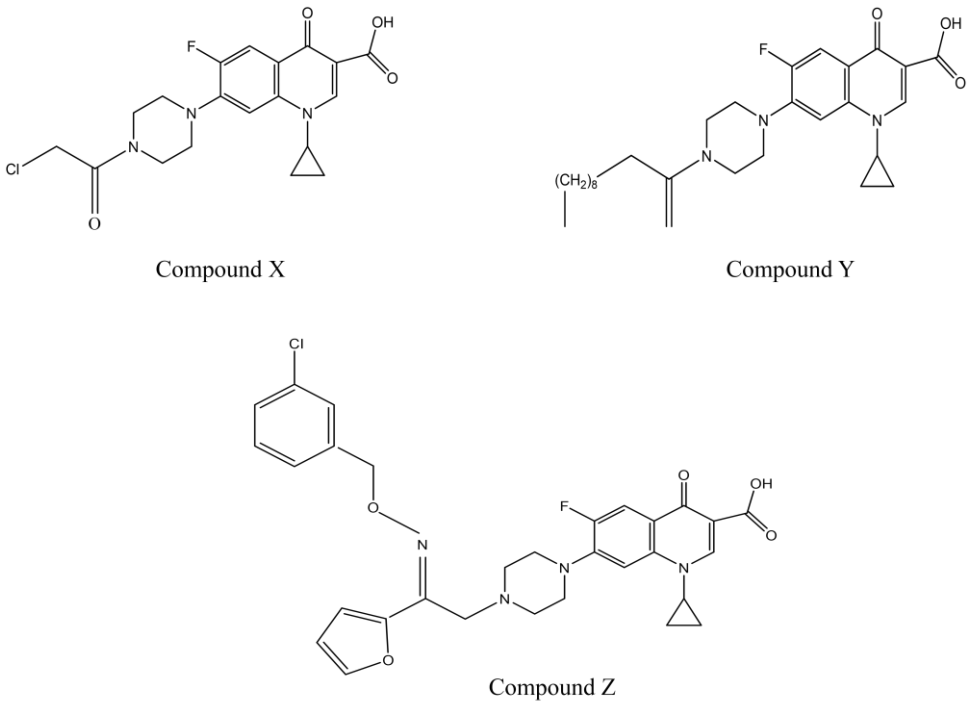


Figure 2.3 Anticancer compounds with the proposed structure of ciprofloxacin derivatives

Nevertheless, the lipophilic properties of the substituent did not exert any influence on this activity. With these findings, the previous authors described how adding straightforward substituents to the piperazinyl group can positively influence the cytotoxicity of CP. Previous investigations of Foroumadi et al., (2011) carried on with their derivative synthesis work, but researchers examined the compounds' cytotoxic potential against human breast tumour cell lines [51]. The study demonstrated the synthesis and assessment in a range of *N*-pipearzinyl quinolones with an *N*-2-(furyl-2yl)-2-(chlorobenzyloxyimino) ethyl moiety's cytotoxic activity. Compound (Z; Figure 2.3) from this series exhibited notable cytotoxic effect against human breast tumour cell lines ($IC_{50}$ = 5, as opposed to $IC_{50}$ = 12 of tamoxifen) [48].

According to previous findings, the cytotoxic activity and alterations at positions 2, 3, or 4 of the phenyl rings are associated. The energy and range of cytotoxic activity are significantly influenced by substitution at the 3-chlorophenyl site [48].

According to Akhtar et al., (2020) research, they made a new *N*-acylated ciprofloxacin scaffolds by reacting different carboxylic acids and sulfonyl halides with ciprofloxacin methyl ester. This was done to learn more about how strong anticancer fluoroquinolones [52]. It was found that several of the synthetic derivatives had a significant anticancer effect when tested against the MCF 7 tumour cell line for their cytotoxic effects. Moreover, the 7- [4-(2-bromoacetyl) piperazin-1-yl] -1-cyclopropyl-6-fluoro-4-oxo-1, 4-dihydroquinoline-3-carboxylate compound shows more potent of anticancer effects with $IC_{50}$ 2.0 μg/mL [52]. This drug inhibits topoisomerase II with a higher affinity and appears to be a promising lead molecule for the development of strong anticancer medicines in the future. The inhibition of topoisomerase II was examined via induced fit docking [52].

In addition, Chrzanowska et al., (2020) studies reported that, CP has been demonstrated to have a cytotoxic effect on certain malignant cells, but only in extremely high doses [53]. Adding natural fatty acids to a medication could boost its cytotoxicity because of the characteristics of natural fatty acids, which include greater cellular absorption by cancer cells, biocompatibility, and biodegradability. Thus, researcher was examined the cytotoxicity effects, apoptosis-inducing effects, and IL-6 release inhibition of CP with saturated and unsaturated fatty acids in human primary colon cancer (SW480) and human metastatic colon cancer (SW620), human metastatic prostate cancer (PC3), and normal (HaCaT) cell lines [53]. The PC3 cell line responded most strongly conjugates that were observed. The $IC_{50}$ value for oleic acid conjugate **(d)** was 7.7 μM, which is 12 times less than the value for pure CP (101.40 μM) [53].

All of the investigated derivatives caused late apoptosis in cancer cell lines but not in normal cells. The conjugate **(d)** was the most effective inducer of apoptosis, as evidenced by the largest proportion of PC3 cells in late apoptosis of 81.5% ± 3.9, followed by elaidic acid amide **(e)** with 75% ± 4.8. Moreover, the compounds **(b)** and **(e)** seemed to be the most beneficial in SW620 cell lines, while conjugates of Docosahexaenoic (DHA) **(h)** and sorbic **(b)** acids showed the most pro-apoptic effects on SW480 cells [53]. To determine the cytotoxic action mechanism of derivatives **(b)**, **(d)**, and **(e)** the interleukin-6 (IL-6) level was determined. In particular, IL-6 secretion from cancer cells was dramatically suppressed by the compounds having the highest lethal potential. Besides, the antibacterial efficacy of each conjugation was also tested in vitro. The most effective short chain amides against *Staphyloccoci* were crotonic **(a)** and sorbic **(b)**. The second-mentioned amide has demonstrated potent *antistaphylococcal* and antitumor effect [53].

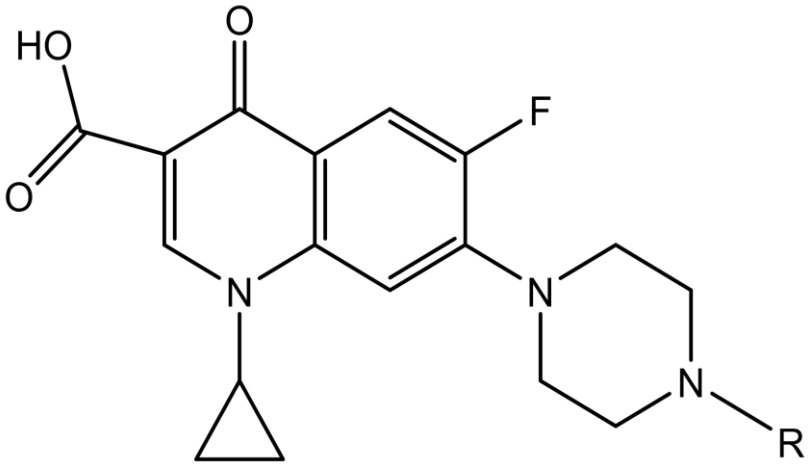


Figure 2.4 CP conjugates with different compound from **a**-**i**.

Table 2.1 studies of cytotoxicity effects ($IC_{50}$, μM) on different compound **a-i** [20]

| Compound | R | SW480 ($IC_{50}$) | SW620 ($IC_{50}$) | PC3 ($IC_{50}$) |
|---|---|---|---|---|
| a | $C_3H_5C(O)$- | 51.9 ± 4.7 | 54.5 ± 5.2 | 93.9 ± 3.4 |
| b | $C_5H_7C(O)$- | 20.1 ± 2.1 | 21.5 ± 3.1 | 11.7 ± 1.8 |
| c | $C_9H_{15}C(O)$- | 32.8 ± 2.4 | 38.2 ± 3.1 | 73.4 ± 2.7 |
| d | $C_{17}H_{33}C(O)$- | 79.5 ± 5.4 | 37.9 ± 4.7 | 7.7 ± 2.1 |
| e | $C_{17}H_{33}C(O)$- | 35.7 ± 2.6 | 33.9 ± 1.8 | 15.3 ± 5.3 |
| f | $C_{17}H_{29}C(O)$- | 64.6 ± 3.1 | 45.8 ± 5.6 | 34.4 ± 2.4 |
| g | $C_{21}H_{41}C(O)$- | 118.6 ± 5.6 | 65.8 ± 4.3 | 76.4 ± 3.3 |
| h | $C_{21}H_{31}C(O)$- | 26.2 ± 4.3 | 40.1 ± 3.7 | 27.8 ± 1.9 |
| i | $C_{15}H_{31}C(O)$- | 82.4 ± 6.3 | 93.3 ± 6.9 | 51.1 ± 4.5 |

*Human primary colon cancer (SW480)

*Human metastatic colon cancer (SW620)

*Human metastatic prostate cancer (PC3)

Additionally, previous research of Cozzarelli NR et al., (1980) demonstrated the potency of zinc complexes of the second-generation quinolone lomefloxacin against the breast cancer cell line (MCF7) [54]. Furthermore, several cancer cell lines, including A20 (murine lymphoma), B16-F10 (murine melanoma), and K562 (human myeloid leukaemia), were evaluated with the gold complexes of levofloxacin and sparfloxacin, the fourth-generation fluoroquinolones. According to the findings of Abd El-Halim HF et al., (2011), metal complexes have a greater degree of action against tumour cells than free ligand drug of molecules [54]. Recent research has also shown that the anticancer effect of polyoxometalates with quinolone ligands on their surface was greater than that of the free drug molecules [54]. Moreover, a study using MCF 7 cell lines shown that the antitumor activity of pipemedic acid (quinolone) Zn(II)

complexes introduced onto SiW12 polyoxoanion was significantly higher than that of the parent compound without metal complex. Similar to Patitungkho et al., (2011) study, the Cu(II) compounds of enrofloxacin having polyoxoanions in combination exhibit greater anticancer effects against SGC7901 lines [55].

### 2.3 Diclofenac (DF)

Figure 2.5 shows diclofenac (DF) with the IUPAC name of 2-[2-(2,6-dichloroanilino)phenyl] acetic acid, is a usually used as a non-steroidal anti-inflammatory drug (NSAID) for the treatment of reducing inflammatory and pain sensation, includes joints and bone problems such as rheumatoid arthritis and osteoarthritis. DF has regularly ranked top in the NSAIDs market for more than 10 years as a new type of powerful non-steroidal anti-inflammatory drug. In 1998, DF had a market share of 27.4 %, which increased to 29.7 % in 2001 and is over 30 % in 2018 [56]. Oral dosing of NSAIDs, including DF, has been reported in causing a gastrointestinal disturbance, together with significant gastrointestinal, cardiovascular, and renal side effects, as well as other deleterious effects, including high blood pressure, depending on the dosage taken [57]. As to enhance the safety profile of pharmaceuticals including to reduce the adverse effects on human health, synthetic methods based on chemical alteration have been employed.

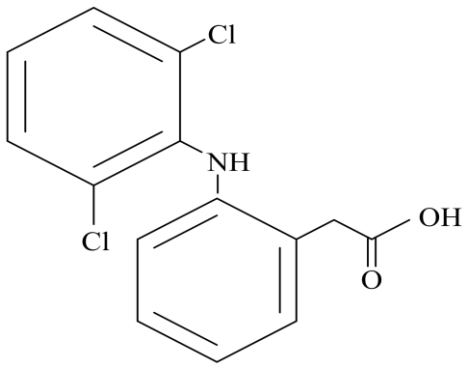


Figure 2.5 Chemical structure of diclofenac

In poor, medium, and high-income nations, DF is the most often used NSAID, and it can be accessed without a prescription in most countries [58]. According to a cohort study carried out by Shin et al., (2016) [59], the risk of cardiovascular diseases from taking DF medication is compared with other NSAIDs medication were studied. NSAID usage was formerly thought to be risk-free when it is used for short periods and at low doses. However, this study had shown that even at low dosages of DF, the danger of cardiovascular problems is prevalent compared with the same dosages of other NSAIDs. In addition, they also discovered that DF initiators had a risk of upper gastrointestinal haemorrhage that was double that of ibuprofen initiators. Hence, it is necessary to acknowledge the possible health risks of pure DF and as well as strengthen the effort on modifying the DF structure using the chemical synthetic technique to diminish the side effects on human health.

The compound of carboxylic acid with NSAIDs has been derivatized, resulting in an increase in anti-inflammatory activity and a decrease in ulcerogenic activity as compared to the parents' NSAIDs [60]. Additionally, according to Sriram et al., 2006 [61], modification of DF with acid hydrazones and amides possesses better anti-mycobacterial activities toward *Mycobacterium tuberculosis* than pure NSAIDs like DF and CP by lowering the bacterial content in the lung and spleen tissues. Derivatives of NSAIDs with N-acyl hydrazone also proved to exhibit equal anti-inflammatory and analgesic properties as their respective parent NSAIDs but with lower gastrotoxicity [62].

According to the study conducted by Poku et al., (2020), [63], the complex of DF with docosahexaenoic acid (DHA) which is a type of polyunsaturated fatty acid performs greater anticancer activity than their parent compound alone, which the complex can be further explored for the complementary treatment of lung cancer. The

complex of DF conjugating with oleanolic acid oximes has better anti-cancer activity [64]. The DF complex decreases the activation of oleanolic acid derivative conjugates of Nrf2 and NF-κB, the signalling pathways inducing inflammation, which leads to the inauguration of apoptosis and a decrease in cell proliferation. In situ complex of DF with bismuth synthesized using capillary electrophoresis exhibits a higher disintegrated rate than the same amount of DF alone, the administration of the bismuth DF complex is also associated with lesser gastrointestinal ulceration than the sodium DF administration, which demonstrates the quality antiulcer action of the complex [65].

The complexes of 1,10-phenanthroline (phen)-DF with different alkali metals such as lithium and potassium were successfully synthesized by [66]. These complexes were characterized using NMR, UV-Vis spectrometry and mass spectrometry. When compared to normal mouse fibroblasts, all the phen-DF alkali metal complexes demonstrated notable cytotoxic action against the oral and lung cancer cell lines. In addition, against Gram-negative bacteria such as *A. baumannii* and *E. coli* the complexes showed considerable antibacterial and antibiofilm action. Not only with metal, but DF also can be combined with vitamins to expand its application in medical fields.

For instance, the complex of DF with vitamin B showed significant properties of pain relief, anti-inflammatory and synergic neuro-regenerative action, thus it is recommended to be used in acute lower back pain treatment, where its effectiveness had been proved in various clinical studies using different vitamin B such as B1, B6 and B12 [67]. Moreover, the DF-vitamin B complex is proven to reduce the cardiotoxic consequences of the medication of sodium DF by modulating both the level of low-density lipoprotein (LDL) and high-density lipoprotein (HDL) hence

serving as a preventative agent against DF toxicity-induced heart tissue damage, revealed by [68].

Calf Thymus DNA (CT DNA) is a kind of natural DNA that has been broadly employed in the research of DNA-binding agents and anticancer drugs that regulate the DNA structures and functions. There are a total of five Cu-DF complexes synthesized by Kumar et al., (2018) [69] are proved to attached more tightly than a free sodium DF as revealed by the UV-Visible spectroscopy, viscosity measurement as well as cyclic voltammetry, thus exhibiting better anticancer properties than the pure NSAIDs. Another research carried out by Dimiza et al., (2011) [70] has shown that the Cu(II)-DF complex with pyridine as the nitrogen donor heterocyclic ligand, can bond to the CT DNA by the binding mode of intercalative, by measuring the viscosity of the DNA solution. The complex also has a high binding constant value and affinity toward the bovine serum albumin protein.

In addition, the Cu(II)-DF-piperaquine complex demonstrates substantial suppression on the bacterial strains, particularly *Staphylococcus aureus*, at 1 % weight by weight with inhibiting a zone of 100 mm, which is several folds more than the effect of pure DF and ciprofloxacin with an inhibition zone of 25 mm and 92 m respectively [71]. Moreover, the complex also displays superior antioxidant properties with $IC_{50}$ of 382.7 µg/mL, in comparison with ascorbic acid, a representative antioxidant with 7526 µg/mL. Anticancer medications are known to target DNA as one of the most prevalent biological targets. Theodorou et al., (1999) [72] have found that the Cu(II) complexes with DF successfully causes the disintegration of the plasmid DNA which could be used in the treatment of cancer and tumour, while the parent DF showed no effect while treating with plasmid DNA.

A Cu(II)-DF complex synthesized in a complete water medium without any coorganic ligand showed biological effects on two healthy and two tumoral human cell lines respectively, and in the case of tumoral cell lines, the complex has a stronger activity than the parent molecule alone [73]. Apart from exhibiting anti-inflammatory activities, the copper-DF complexes also showed other pharmacological properties such as antiarthritic, anticancer, antidiabetic, as well as antiulcer [74]. However, research carried out by Rehman et al., (2010) [75] found that the copper-DF complex possesses no anti-malarial properties against Plasmodium falciparum 3D7 and low antibacterial activity against *S. typhi* and *B. subtilis*. Aside from contributing to the medicinal treatment, the copper-DF complexes combined with pyridine act as a catalyst by speeding up the reaction rate of the oxidation of 3,5-di-tert-butylcatechol to 3,5-di-tertbutylquinone, at a significant concentration of substrate [76].

## 2.4 Nanoparticles

Nanoparticles (NPs) can be found in the natural world, and they can also be manufactured by humans through various processes. They have unique material characteristics because it manufactured different type of submicroscopic size in nanoparticles [77]. NPs are typically defined as solid, colloidal particles with sizes between from 10 nm to 1000 nm [78]. The range of nanoparticles is beyond our ability to see with the human eye. They can have a variety of significant physical and chemical features also. They are all connected to their more substantial material counterparts [79]. In the last few decades, nanoparticles have gotten a lot of attention in many areas, such as biomedicine, sensors, energy storage, and catalysis because they have unique physical and chemical properties [80].