Synthesis and biological characterization of Diclofenac based S-Stabilized Nanocomposites

Tuseef Rizwan[1], Freeha Hafeez[1*], Muhammad Suleman[1], Maqsood Ahmad[2], Muhammad Asad Ali[2], Muhammad Mohsin[3], Muhammad Mateen Iqbal[1], Muhammad Awais Aslam[3], Komal Sana[1]
[1]Department of Chemistry, Riphah International University Faisalabad
[2]Department of Physics, Riphah International University Faisalabad
[3]Department of Chemistry, University of Education, Lahore, Faisalabad campus

Abstract: A class of chemical compounds known as Diclofenac has the general formula: $C_{14}H_{11}Cl_2NO_2$. Nanoparticles based diclofenac are special chemical compounds synthesized from the reaction of nanoparticles with diclofenac derivatives. The most common method for creating nanoparticles based diclofenac derivatives involves converting of diclofenac to Hydrazide and oxadiazole thiol and finally reacting with Zn and Mg nanoparticles in various chemical solvents like butanol as ethanol. Diclofenac are used for a variety of things, including medications, the production of polymers and adhesives, industries, and many other things. Acid-catalyzed cyclization of N-(2, 6-dichlorophenyl)-α-(methylsulfinyl) acetanilide or α-chloro-N-(2, 6-dichlorophenyl)-α-(methylthio) acetanilide resulted in desulfurization and hydrolysis of the resulting 1-(2, 6-dichlorophenyl)-3-(methylthio) oxindole, which was used to make diclofenac, a powerful anti-inflammatory drug. Diclofenac has long been used to treat antibacterial infections.. For a long time, bacterial infections have been treated using medications based on nanoparticles based diclofenac derivatives. The product was cleaned by recrystallization with ethanol after being separated by filtering. The products **119-120** obtained 70-80% yield. Antibacterial infections have traditionally been treated with diclofenac. For a long time, bacterial infections have been treated using medications based on nanocomposite based diclofenac

INTRODUCTION

Nanotechnology has been instrumental in advancing potent antimicrobial solutions. Numerous metal and metal oxide nanoparticle varieties have shown encouraging antibacterial qualities. Among the alumina, cerium oxide, zinc oxide, and copper oxide nanoparticles have surfaced as viable choices for biological applications (Islam et al., 2022). These nanoparticles are utilized in various ways, serving as carriers for drug delivery, components in skin disease ointments, food additives, as well as preventative measures in for tissue injuries. Nanoparticles of ZnO, in particular, have been thoroughly investigatesd and demonstrated to have significant inhibitory effects on the growth of several pathogenic bacteria. Intriguingly, formulations combining these nanoparticles of metal and oxides, such as unstructured ZnO, with other similar particles or antibiotic medications with biodegradable substances have been stated. These innovative combinations are aimed at enhancing both material and biological applications. Researchers have reported that the blend of ZnO and/or silver with TiO_2 nanoparticles and chitosan, used as covering and adorning materials respectively, exhibits an enhanced antimicrobial effect. Specifically, the combination of ZnO nanoparticles has demonstrated heightened synergistic bioactivity when paired with β-lactam antibiotics (Seil & Webster, 2012).

Diclofenac sodium, a potent NSAID, falls within the phenylacetic acid derivative class, featuring a group of phenyl acetic with secondary amino group, a ring of phenyl housing ortho and chloro groups. This category of medications is widely employed to address inflammation, pain and fever. Two-(2,6-dichloro anilino) phenylacetic acid is its chemical designation, which is where the term "diclofenac" comes from. Through extensive research among 200 analogs, diclofenac sodium structure was finalized by Rudolf Pfister and Alferd Sallmann, which entered the market in 1973 under the trade name VoltarenVR. From the last 35 years, diclofenac has been authorized in more than 115 countries and ranks 30th among the top more than 200 drugs. It is utilized in treating various conditions such as gout attacks, polymyositis, rheumatoid arthritis, and arthritis, as well as severe migraines (Islam et al., 2022). Additionally, it plays a crucial role in alleviating pain following surgery triggered by inflammatory response and certain little soreness in the muscles conditions. Despite its proven effectiveness, diclofenac sodium does have Limitations and unfavorable consequences, notably hepatotoxicity, ulceration, and gastrointestinal discomfort. Other prominent part of the phenylacetic acid that ads Lumiracoxib and Aceclofenac, a famous drug that is similar to diclofenac sodium (Sayen, Carlier, Tarpin, & Guillon, 2013), (Figure 1).

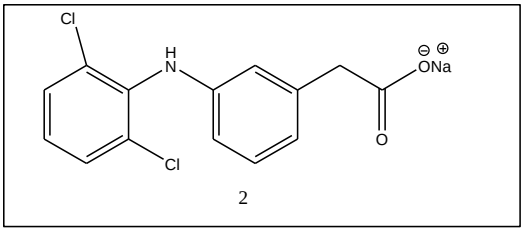


Figure 1: *Structure of Diclofenac Sodium*

Considering these aspects and as a component of our continuous investigation into producing physiologically active nanoparticles, we used a successful procedure to synthesis diclofenac based on nanocomposites.
We then assessed the target molecules' pharmacological properties using thrombolysis and hemolysis.

MATERIALS ANDMETHOD

General Information

Only solvents and reagents of the highest caliber were employed in this synthesis sequence. The melting point (MP) of the target derivatives was determined using a Gallenkamp melting point apparatus (Snizhko et al., 2022). Bruker spectrometers were used to record NMR (Nuclear Magnetic Resonance) spectra at 400 MHz for 1H-NMR and 100 MHz for 13C-NMR (Kumar et al., 2021).
Parts per million, or ppm, were used to quantify chemical changes, whereas hertz (Hz) was used to assess coupling constant J values (Metz, 2016). FT-IR (Fourier transform-infra red) spectra were acquired using a Bruker Fourier Transform IR spectrometer (Pascale et al., 2022). To track
the development of the reaction, silica gel plates were subjected to TLC (Thin Layer Chromatography). However, the UV (ultra violet) lamp caused spots to appear (Booyens & Thantsha, 2014)

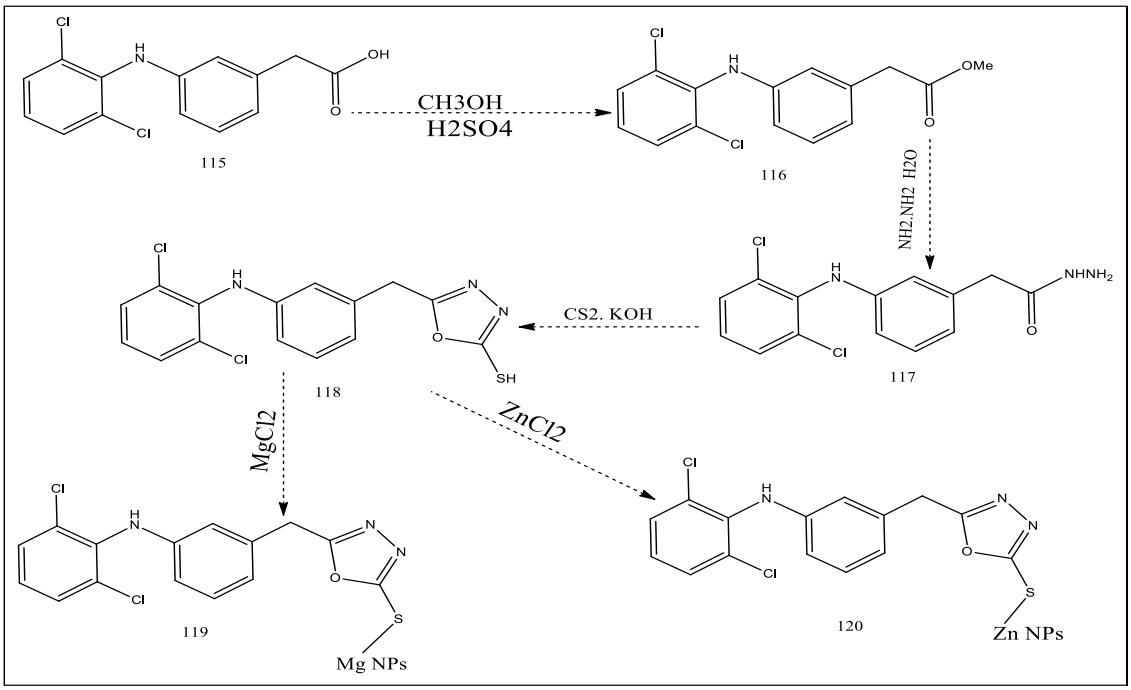


Figure 2: Synthesis of diclofenac based Nanocomposites

Synthesis of diclofenac methyl Ester

Diclofenac acid was dissolved in methanol while stirring, and sulfuric acid was added drop by drop with constant stirring. After that, the mixture was cooked under reflux for a while. After the reaction was finished (as verified by TLC analysis), the mixture was allowed to cool to ambient temperature before being poured over crushed ice to form a crystalline solid (Shah et al., 2018).

Synthesis of diclofenac based Hydrazide

The diclofenac ester solution was refluxed with hydrazine hydrate for a suitable duration. Subsequently, the reaction mixture was cooled, and the resulting solid precipitate was washed with water, dried, and subjected to recrystallization from ethanol (Judge et al., 2013).

Synthesis of diclofenac Oxadiazole Thiol

When the hydrazide was refluxed with CS_2 in absolute ethanol and KOH was added drop wise at room temperature, and the reaction was allowed to reflux for 24 hours. Upon completion of the reaction (confirmed by TLC analysis), product was obtained. This salt was then acidified with concentrated HCl under stirring conditions to yield a solid product (Belkadi & Othman, 2006).

Synthesis of Zn metal Complex of Oxadiazole thiol

Zn metal complex was created using a standard process. The oxadiazole analogue solution MeOH was combined with a solution of the Zinc chloride in an ethanol water mixture. After one hour of reflux with continuous stirring, the resultant liquid was filtered out and twice recrystallized with cold ethanol to afford pure crystals (Reddy, Harinath, Suneetha, Seshaiah, & Reddy, 2011).

Synthesis of Mg metal Complex of Oxadiazole thiol

The complex was synthesized by mixing 0.001 mol of ligand and 0.001 mol of salt $MgCl_2.H_2O$ in 5 milliliters of ethanol, then refluxing the mixture for three hours while TLC watched the process. After that, unreacted salts or ligand were removed from the precipitate by filtering it and washing it with ethanol or aqueous ethanol. The precipitated complexes were then dried (Merdas, 2022).

Synthesis of Nanocomposites

A metal salt; a dopant; and, in some cases, an oxidant were added to aqueous solutions (typically10 mL) with oxadiazole concentration of 0.1M.Ammonium peroxy disulfate or benzoyl peroxide was used as the oxidant, with a concentration of 0.02 M. The metal salts, AgNO3 and HAuCl4, were added at a concentration of up to 0.1M. The dopant used was either HCl (for the Au composites) or HNO3(for the Ag composites). The dopant concentration was 0.3 M in all cases. In experiments where an oxidant was not employed, aniline and HAuCl4 were added to final concentrations of 0.1and 0.02M, respectively. The suspensions were centrifuged at 5000 rpm for10 min and filtered, and then the powders were washed with water, methanol, and ether to remove unreacted precursors, oligomers, and other byproducts.

Biological Evaluation

Hemolysis

Red blood cells (RCBs) were separated by centrifuging a 50 mL blood sample. After being purified and treated with a DMSO sample solution, the resultant supernatant was rinsed with PBS. After 60 minutes of incubation at 37°C, the tubes were centrifuged once more. Once the sample was diluted with phosphate buffer saline, the absorbance at 517 nm was determined. Three runs of the tests were conducted, and a formula was used to determine the percentage of RCB lysis (Antonio et al., 2015; Cao et al., 2021).

Thrombolysis

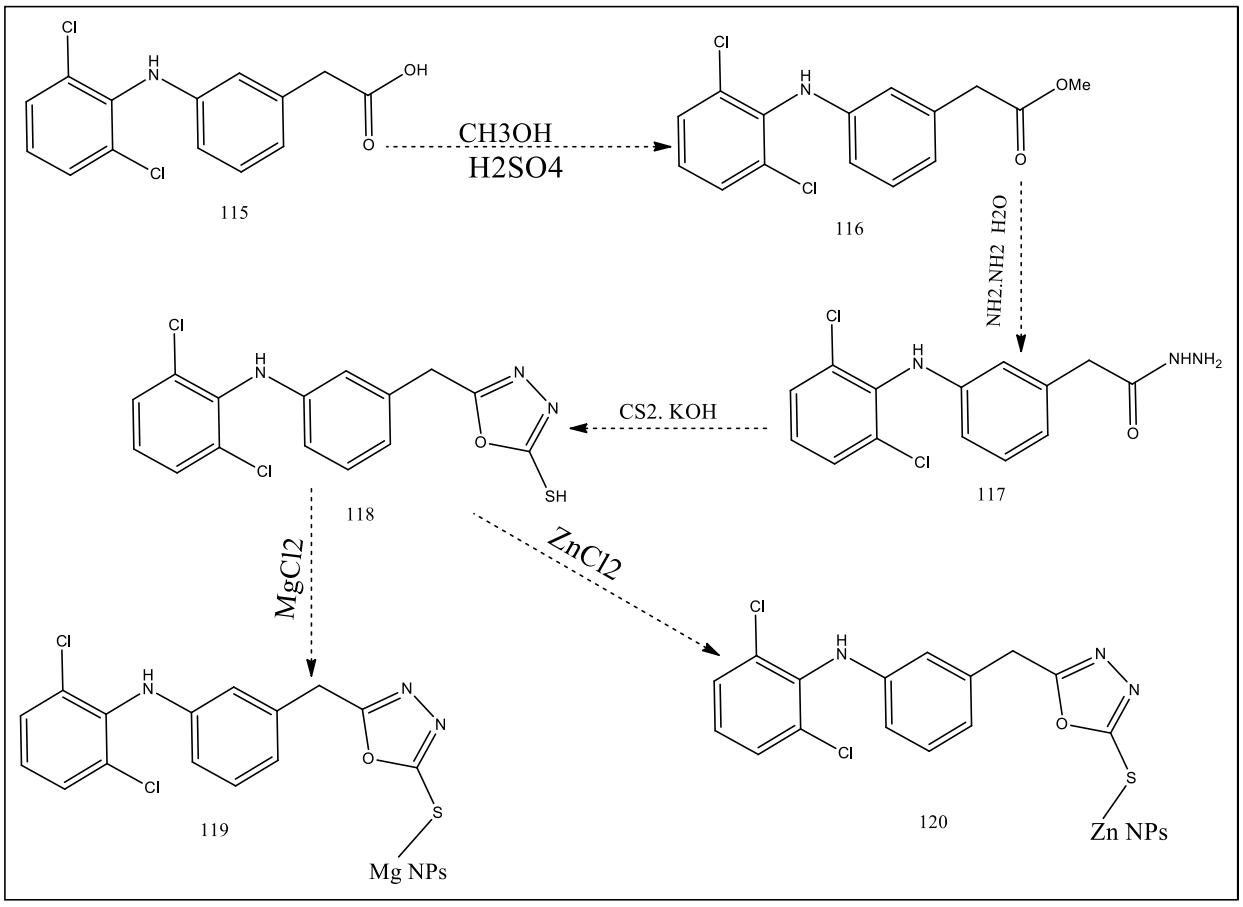
Experimented on thrombolysis using accepted techniques. A blood sample was given, and blood was placed into 500 μL Eppendorf tubes. For one hour, the tubes were incubated at 37ºC to see the development of clots. After removing the serum, the tube holding the clot was weighed. To determine the proportion of cell lysis, a sample solution in DMSO was added to the clot in triplicate trials (Absar et al., 2013; Migliavacca et al., 2024).

Statistical Analysis

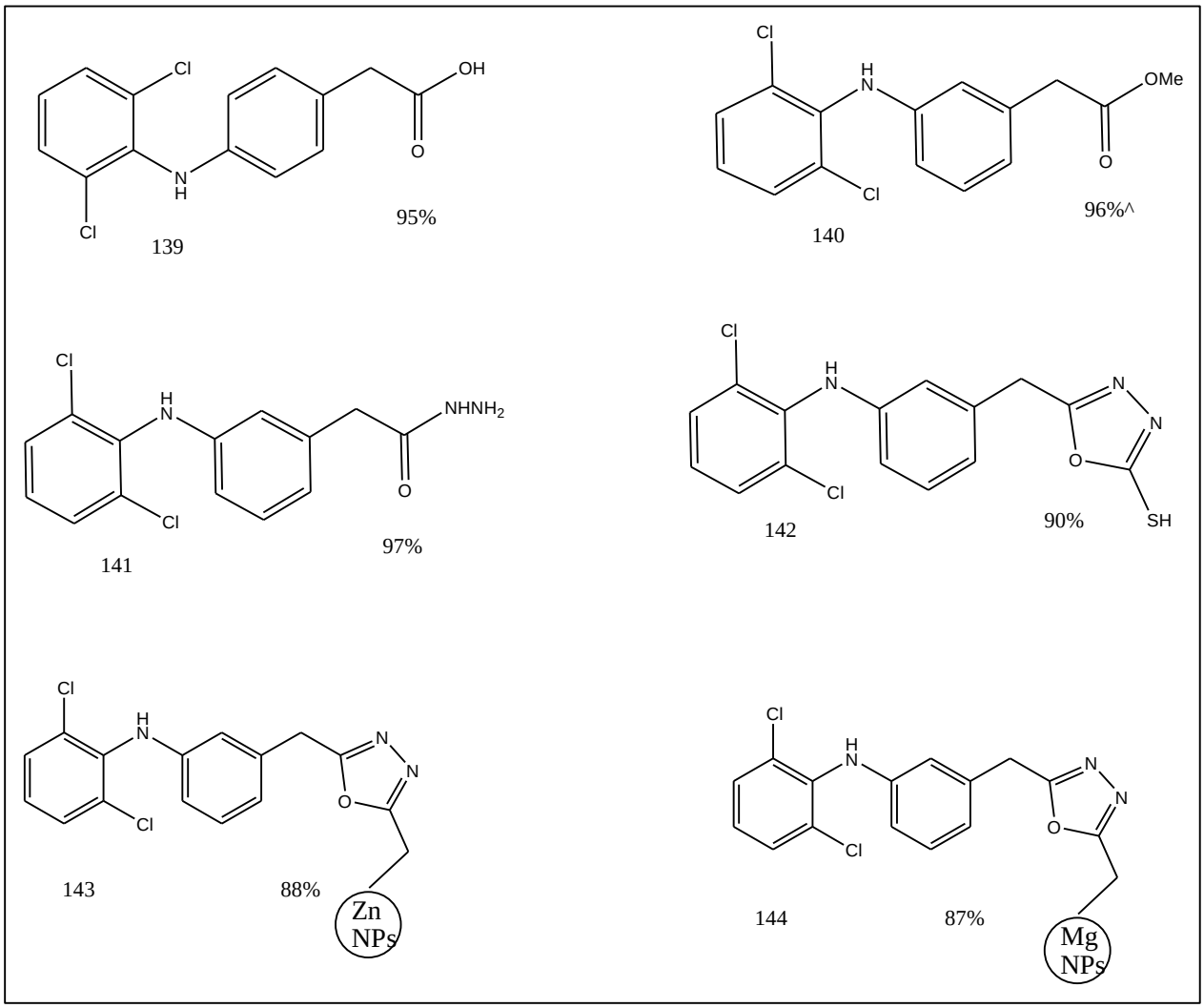
The statistical analysis was performed using Microsoft Excel 2010 and each experiment was conducted three times. The data for cell viability are shown as the mean± standard deviation (Sengupta et al., 2014).

RESULTS

Zn and Mg nanoparticles based diclofenac derivatives will be synthesized by passing through a series of reactions. The starting material diclofenac acid 115 will be prepared by diclofenac sodium. After that it will be converted into diclofenac ester 116 which will be reacted with hydrazine monohydrate and diclofenac hydrazide 117 will be formed. Diclofenac hydrazide is reacted with CS_2 and KOH and diclofenac oxadiazole thiol 118 will be formed. In the final step diclofenac oxadiazole thiole will be reacted with $ZnCl_2$ and $MgCl_2$ to form final product . The structure was recognized through the application of various spectroscopic methods.



Scheme4.1*: General outline for* 119-120



Biological Evaluation

Hemolysis Analysis

Out of all the compounds tested, compound 118 caused the less lysis of red blood cells, 3.1%. Moreover, it was the less cytotoxic. With rates of 1.45% and 2.80%, respectively, compounds 119 and 120 also significantly induced hemolysis.
The kind and position of the compounds' functional groups had a substantial impact on their hemolytic activity, indicating possible anticancer uses (Antonio et al., 2015; Cao et al., 2021).

Thrombolysis Analysis

We evaluated the potential for thrombolysis of each produced molecule. These compounds had relatively little thrombolytic action overall. Compounds 119 and 120 had more thrombolytic capability in comparison to the standard control, ABTS, whereas other derivatives shown intermediate capacity. Comparing Compound 119 to ABTS, Compound 119 had the highest lysis rate at 65.1%. The lowest lysis rate, however, was recorded by Compound 118, at 40.2%. In this the impact of functional groups that positively induce thrombolysis is examined.
(Absar et al., 2013; Migliavacca et al., 2024).

Table 1: Target molecule hemolysis and thrombolysis potential mean ± standard deviation (3a-f)

Compounds	Hemolysis (% ± SD)	Thrombolysis (% ± SD)
118	3.1 ± 0.121	40.2 ± 0.081
119	1.45 ± 0.004	65.1 ± 0.031
120	2.80 ± 0.005	53.2 ± 0.011
ABTS(Positive control)	95.9	86

Correlation is established between the ability of compounds to cleave blood clot and destroy red cell membrane for hemolysis, formation of yellow rings at end point with substitution on thiophene ring that influence both inhibitory actions. Compounds 118 and 120 showed moderate hemolytic (HL) activity but appreciated thrombolytic activities in comparison to reference drug, making them promising leads.

CONCLUSION

This endeavor may be a little effort to provide information on potential medication candidates and to modify existing structures for additional, more promising functions. Researchers are becoming more interested in diclofenac derivatives since it has been shown to have health-promoting properties. Due to their anti-cancer, anti-viral, analgesic and anti-inflammatory properties, they have been recognized as a rich source of biologically active compounds that can support human health and prevent the growth of many illnesses. A future interest is to work on finding the other biological activities of diclofenac. Researching diclofenac other biological functions is something that interests me in the future.

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