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HARRACHE Rabiaa

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Synthesis, characterisation, antioxidant, anti-inflammatory and antibacterial effects of bis- ketone 1,3-Bis (2 acetylphenoxy)-2- propanol: *in silico*, *in vitro* and *in vivo* studies

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THESE

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Par

HARRACHE Rabiaa

THÈME

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Soutenue le 13/12/2025

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Dedicated

To those who sacrificed much of their time

for my happiness and my success

My dearest parents who encouraged me throughout

my studies

To my brothers: **Nassim**, **Abdelmalek**

To my sisters: **Assia**, **Imene**, **Ryma**

To the memory of my grandfather,

To all **my family**

To all **my colleagues** and **my friends**, to whom I wish all the

happiness

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LIST OF CONTENTS

Materials and methods

Results and discussion

List of figures

List of tables

List of abbreviations

ADMET: Absorption, Distribution, Metabolism, and Excretion.

BHT: Butylated Hydroxy Toluen.

Bis-AcPh: 1,3-Bis(2-acetylphenoxy)-2-propanol .

COX-2: cyclooxygenase-2.

^{13}C NMR: Carbon-13 Nuclear Magnetic Resonance.

DMSO-d6: Deuterated dimethyl sulfoxide.

DFT: Density functional theory.

DEET: N,N-diethyl-mtoluamide.

DPPH: 2, 2-diphenyl-picrylhydrazyl

EMSA: Electrophoretic Mobility Shift Assay.

FMOs: frontier molecular orbitals.

FRAP: Ferric Reducing Antioxidant Power.

FTIR : Fourier Transforme Infrared Sprctroscopy.

HOMO: Highest Occupied Molecular.

LPS: Lipopolysaccharide.

LUMO: Lowest Unoccupied Molecular Orbitals.

MDS: Molecular Dynamics Simulation.

MMPBSA: Molecular Mechanics Poisson Boltzmann Surface Area.

NMR: Nuclear Magnetic Resonance.

SAR: structure-activity relationship.

SD: Standard deviation.

SEM: standard error of the mean.

SN2: Substitution Nucleophilic Bimolecular.

^{1}HNMR: Proton Nuclear Magnetic Resonance.

UV-Vis : Ultraviolet-Visible Spectroscopy.

VitC: Vitamin C (Ascorbic acid)

General introduction

The invention in the research of developing various pharmacological and biological activities in substances that enhance the patient's quality of life and provide minimal-reported side effects in patients represents the main concern for synthetic organic chemists [1]. One of such compounds are acetophenones and their derivatives that are useful compound in chemistry, development of drug, medicine, etc. Their diverse functional groups allow them to display specific biological activities like anticancer, antitubercular, antimicrobial, antifungal, antibacterial, anti-inflammatory, antiviral, and anti-hyperglycemic. These characteristics make them useful in various studies as well as in therapeutic applications [2]. Acetophenones derivatives are attractive model molecules when used as foreign substrates for biotransformation, since a readily detectable enantiomer can be formed, and easily determined. These substances have been successfully applied in the building blocks for pharmacological asymmetric synthesis [3]. 2-Hydroxyacetophenone are thought to be crucial intermediates in the synthesis of numerous biological active compounds, especially in the formation of benzopyran compounds for example the compounds of flavonoids, coumarins derivatives or chromones, these being privileged structures for searching new drug [4].

Ether moieties find application in various pharmaceutical, and biological fields [5].This group of compounds is characterized by an oxygen atom united to two alkyl or aryl group. Their structures depends on the groups associated with other oxygen [6]. For synthesis chemist such C-O bond formation, which brings two organic segments, allows for one of the most powerful technologies with various applications in chemical science such as covalent linkages, solid support and essential motifs in biologically active compounds. It is also important in the manufacturing of intermediates and products relying on Williamson ether synthesis [7].

Williamson ether synthesis has been widely used in pharmaceutical research, such as the synthesis of anti-influenza drugs, because it involves bimolecular nucleophilic substitution on saturated carbons [8]. It include the coupling between alkoxides and alkyl halides through a SN2 reaction under basic conditions. Developed by the chemist Alexander Williamson over 160 years ago [9]. It is considered as one of the reactions classes most often used in synthetic organic chemistry and is still the best versatile method for the synthesis of

unsymmetrical and symmetrical ethers [10].

Alcohol-functional dialdehydes have been previously synthesized via the Williamson ether synthesis, they are used as predecessor for the synthesis of macrocyclic ligand ported a pendent arm, because they may frequently be induced to undergo Schiff base cyclization reactions when they react with diamines. These pendent arms macrocycles ligand have attracted considerable attention in bioinorganic chemistry as models for biomolecules, and in model chemical science such as in magnetic resonance imaging [11].

In the present study, Williamson ether reaction of 2'hydroxyacetophenone with 1,3-diChloropropanol in 1:2 mole ratio was used to generate the required bis-ketone 1,3-Bis(2-acetylphenoxy)-2-propanol (denoted as **Bis-AcPh**) containing two acetophenoxy groups and ported hydroxyl pendant arm.

In the first part of this thesis, we have focused on the theoretical research about molecules similar to our prepared compound. This theoretical part contain also two part; the first part is an overview of some biological activities of natural and synthesized derived 2'-hydroxyacetophenones molecules. Whereas the second part is also an overview of pharmacological activities of some synthesized agents bearing a phenoxy group, as our prepared compound bearing an alcohol function, and contain a ketones and a phenoxy groups.

In the second part of this thesis, we have developed firstly the various instruments and protocols used for the synthesis, the characterization, and the biological evaluations of the prepared bis- ketone, then we have detailed all the results and the discussions obtained. The structure of the synthesized bis-ketone was determined by FTIR, UV-Vis, and NMR spectroscopic methods, and their biological properties were evaluated by antioxidant, anti-inflammatory, cytotoxicity, and antibacterial activities, In Addition, the compound was subjected to computational studies to confirm the experimental results. The biological activities were undertaken on the following objectives:

- The antioxidant effect of the synthesized bis-ketone was assessed by performing DPPH radical scavenging and reducing power method.
- The in anti-inflammatory effect was estimated using the in vitro anti-inflammatory activity through egg albumin denaturation test, while the in vivo anti-inflammatory effect was investigated through xylene and croton oil-induced ear oedema in mice model.
- The cytotoxicity was tested using hemolytic test against red blood cells.

- The bacteriological activities were tested against two Gram-positive strains: *Staphylococcus aureus*, *Bacillus cereus*, and against two Gram-negative strains: *Escherichia coli,* and *Salmonella Enteritidis.*

The computational studies were evaluated throw:

- The chemical reactivity prediction using Density Functional Theory (DFT) study.
- Docking prediction to evaluate the binding interactions between the synthesized compound Bis-AcPh and the cyclooxygenase-2 (COX-2) enzyme.
- ADMET study to evaluate the pharmacokinetic and toxicity profiles of the synthesized compound Bis-AcPh in comparison with established anti-inflammatory agents.
- Molecular Dynamics Simulation to further evaluate the stability and the dynamic behavior of the protein–ligand complexes obtained from molecular docking.
- MMPBSA calculation to quantitatively evaluate the binding affinity of the synthesized compound Bis-AcPh with COX-2

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A review study over some biological activities of hydroxyacetophenone derivatives

I.1. Introduction

Acetophenones and their derivatives play a crucial part in various fields like chemistry, medicine, and drug synthesis. Due to their diverse functionality, these compounds display a range of biological activities including antimicrobial, antibacterial, antifungal, anticancer, antitubercular, anti-inflammatory, antiviral, antihyperglycemic properties [1]. Additionally, they have also been discovred to possess analgesic, antiaggregatory, antioxidant, antimicrobial, and cytotoxic properties [2].

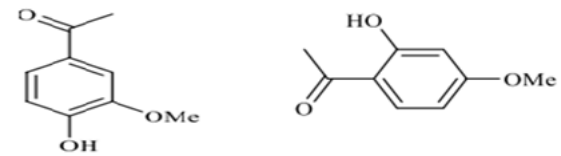
As derivatives, Hydroxyacetophenones are known for their antioxidant, antifungal, and hypolipidemic properties. They also serve as a synthetic building block for ephedrine, which has both direct and indirect effects on heart and blood vessel tone [3]. 2-Hydroxyacetophenones play a key role as intermediates in the creation of various biologically active compounds. They are particularly important in the synthesis of benzopyrans, which include flavonoids, chromones, and coumarin derivatives structures that are highly valued in drug discovery [4].

I.2. Natural-derived hydroxyacetophenones

These days, pharmaceutical companies are leaning more towards discovering drugs from natural sources rather than relying on synthetic options. In fact, about 75% of the drugs that received approval between 1981 and 2014 were derived from natural origins or semi-synthetic versions, and they are used to treat various diseases, such as cancer [5]. Acetophenones are a fascinating group of phenolic compounds that many plants from different families produce. One of the reasons for this production is to help repel insects [6]. The remarkable ability of plants rich in acetophenone to ward off pests and insects has opened up exciting possibilities for utilising acetophenone derivatives as natural pesticides. With crops facing devastating losses from pest infestations and diseases, and growing public concern about the use of chemical pesticides, acetophenone is emerging as a promising eco-friendly alternative to synthetic options [7].

Plant-based acetophenones are cricial building blocks in the production of various type of drugs. For instance, they are essential in the synthesis of many pharmaceutical compounds. Acetophenone derivatives including apocynin and paeonol (**Figure 1**) exhibit anti-

inflammatory properties without any adverse effects, making them ideal candidates for drugs synthesizing [8].



Apocynin Paeonol

Figure 1. Apocynin and Paeonol structures.

Chen et al. investigated the chemical constituents of *Acronychia oligophlebia* plant, and identified seven novel acetophenone-derived compounds. These products known as acrolione were all identified as the cause behind the antioxidant activity of their host plants because they were confirmed to have the correlated effects, through the DPPH radical-scavenging ability and the FRAP processes, and some of them showed anti-inflammatory effect against the LPS-stimulated RAW 264.7 cells [9].

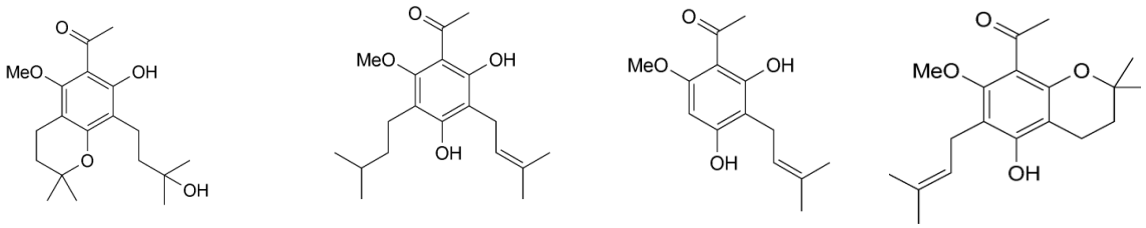


Figure 2. Novel Acronychia oligophlebia acetophenone derivatives.

Zhang et al. carried out the extraction of two novel acetophenones derivatives (1): 2,4-dihydroxy-6-methoxyl-methylene-3-methylacetophenone, and (2): 2-dihydroxy-6-methoxyl-methylene-3-methyl-acetophenone-4-O-β-Dglucopyranoside from the roots of Euphorbia ebracteolata Hayata, and clarified their chemical structure. Both of the compounds were discovered to be selectively cytotoxic to some human tumor cell lines, such as Hela-60, A-549, and MCF7. The activity of the tested compounds on A-549 and MCF-7 was comparatively higher with higher activity measured with compound (2) against cells and the reverse is true with compound (1) and Hela-60 cells. The cytotoxicity bioassays demonstrated that both compound 1 and 2 exhibit greater biological selective activity against

partial human tumour cell lines, they expect to administer a future generation of drugs to treat tumour cells [10].

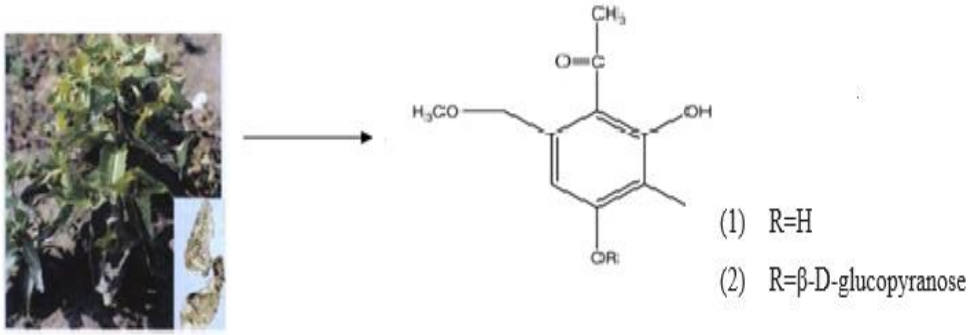


Figure 3. Acetophenone derivatives from the roots of *Euphorbia*.

Kim et al [11] explored the acaricidal effects of acetophenone derivatives as potential natural acaricides specifically targeting house dust and stored food mites. The active part extracted from root of *C. paniculatum*, and prepared derivatives of acetophenone containing a hydroxyl and methoxy groups were compared with N,N-diethyl-m-toluamide (DEET) and widely known compound benzyl benzoate. The findings support that the variation on acaricidal activity was based on the type and location of hydroxyl and methoxy on the structure of acetophenone. The most active compounds were that with a one methoxy group (2′-, 3′-, and 4′-methoxyacetone).

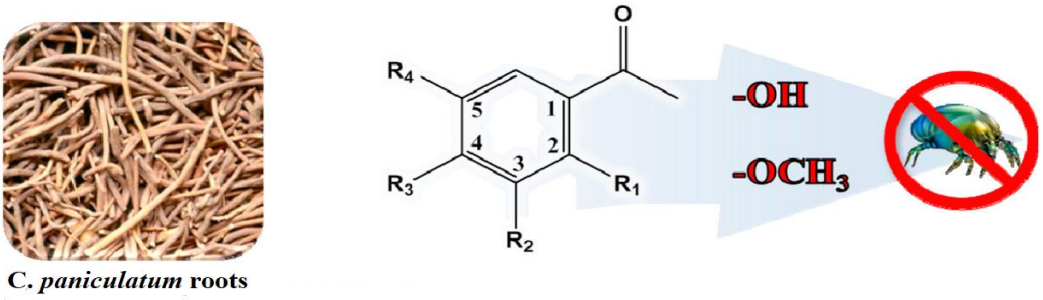


Figure 4. Active compound extracted from *C. paniculatum roots*.

I.3. Biological activities of synthesized hydroxyacetophenone derivatives

I.3.1. Antioxidant activity

The quantum mechanical computation (QM) of hydroxyacetophenone derivatives studied by Bentes et al [12] suggested that the existing of carbonyl group in phenolic derivatives could help stabilize the radical produced during oxidation, allowing for an extension of conjugation

through resonance effects. Additionally, Rezket al [13] have also pointed out the possible antioxidant activity of 2, 6-dihydroxyacetophenone, which might be due to its ability to stabilize the radicals formed during hydrogen abstraction.

Emami et al [14] synthesized acetophenone benzoylhydrazones that have a phenolic group and reported their antioxidant properties with exploring their SAR (**Figure 5**).The data gathered from the compound (5g): 2,4-dihydroxyacetophenone-derived benzoylhydrazone, along with its corresponding parent acetophenone compound, showed that adding a hydrazone group significantly boosts both antioxidant strength and metal-binding capabilities of the acetophenone structure. The structure-activity relationship (SAR) study also indicated that the position of hydroxy and methoxy groups on acetophenone benzoylhydrazone framework may determine the antioxidant activity of these prototype compounds.

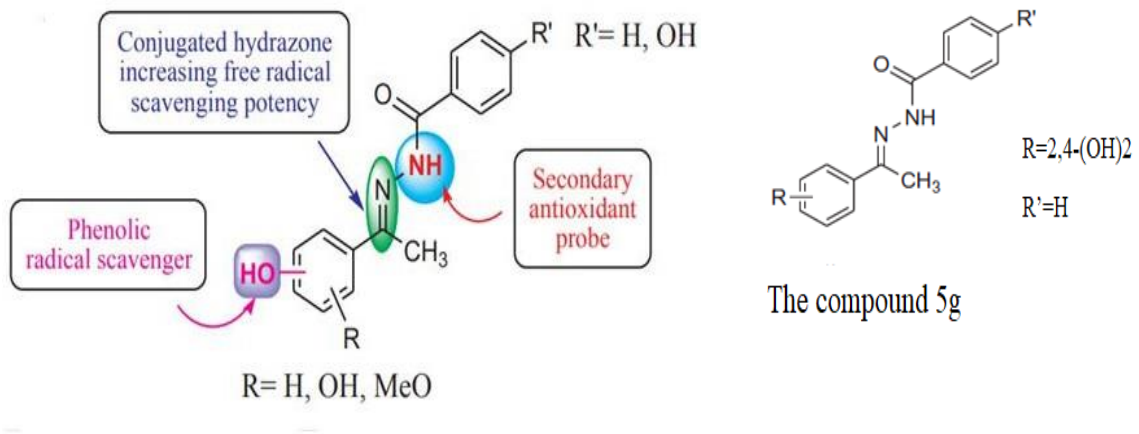


Figure 5. Designing acetophenone benzoylhydrazones as antioxidants agents.

Chalcones are interesting compounds which are obtained by the reaction of substituted aromatic compounds with simple or different substituted acetophenones, all in the presence of an alkali [15]. They are a well-known intermediates serve for creating a range of heterocyclic compound derivatives. Essentially, chalcones are aromatic compounds where the two aromatic rings are linked through a three-carbon α, β-unsaturated carbonyl unit [16].

Chalcones have diverse biological functions such as anti-bacterial, anti- malarial, anti-fungal, anti- microbial etc. On screening these chalcone moieties to determine antioxidant activity, they exhibited excellent antioxidant activity. [17].The antioxidant activity of chalcones and its derrivatives is attributed to the presence of the reactive keto and vinylenic group [16].

Shaifali and al [18] developed a new series of chalcones by using 2-hydroxyacetophenone and substituted aldehyde and tested their in- vitro anti-oxidant activity using 2, 2-diphenyl-1- picrylhydrazyl method (DPPH). Among the various derivatives of chalcones, those substituted with 4- nitrobenzaldehyde, 2-methoxy, 4-methoxy, 2, 4-dimethoxy and 2, 4-dichloro, demonstrated notable antioxidant activity. with respect to antioxidant properties, the most efficient was the 2, 4- dimethoxybenzaldehyde derivative, while the others, including 2, 4-dichloro , 2-methoxy, 4-methoxy, and 4-nitro substituted chalcones, exhibited a moderate level of antioxidant activity.

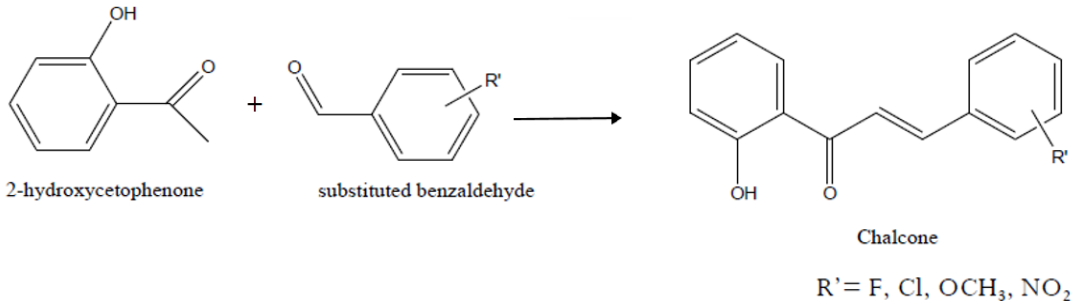


Figure 6. Reaction scheme for synthesizing chalcone by Shaifali et al.

I.3.2. Antifungal activity

Some 2'hydroxyacetophenone derivatives have been demonstrated antifungal activity, which make them antifungal agent capable of preventing plants diseases caused by fungal pathogens. An example of antifungal compounds is 2-hydroxy-4,6-dimethoxyacetophenone xanthoxylin (1), a component of the medicinal plant *Melicope borbonica (Meliaceae*), and 2,4-dihydroxy-5-methylacetophenone (2), a component of the higher fungus *Polyporus picipes* (*Polyporaceae*), respectively [19].

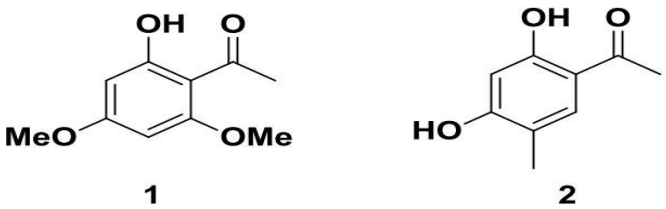


Figure 7. Antifungal agents derived from 2'hydroxyacetophenone.

Shi W et al [19] found that in a synthetic series of new acetophenone analogs, the alkyl ketone derivatives showed a wide range of antifungal activities against various plant pathogens. They discovered that as the chain length of the linear alkyl ketones increased, their antifungal effectiveness also improved. Interestingly, branched alkyl ketones demonstrated even

stronger activity compared to their linear counterparts. Notably, compound 2 g (**Figure 8**), which features an isobutyryl group, exhibited impressive in vitro antifungal activity, making it an attractive candidate for the development of new fungicides. The compound **2** exhibited *in vitro* significant growth inhibitory activity, and SAR studies also indicated the importance of 2,4-dihydroxy groups for antifungal activity.

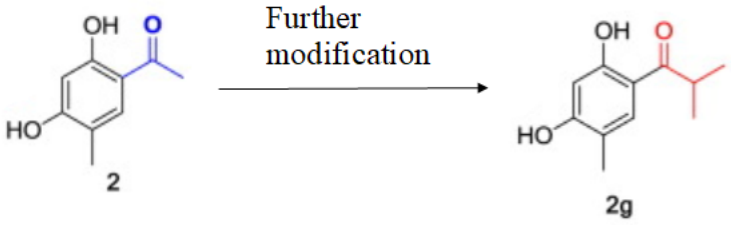


Figure 8. Derived alkyl ketone as antifungal agent.

1.3.3. Anticancer activity

Li Wang et al. [20] explored how 2′-hydroxy-5′-methoxyacetophenone affects aldose reductase, collagenase enzymes, and α-amylase. Their molecular docking studies showed that this compound has a strong affinity for these target enzymes. They also compared the biochemical activity of 2′-hydroxy-5′-methoxyacetophenone against these enzymes through additional modeling calculations.it is also worth noting that 2′-hydroxy-5′-methoxyacetophenone demonstrated impressive antioxidant properties and showed promise in combating ovarian cancer. It exhibited significant cytotoxic effects toward several ovarian cancer cell lines, including PA-1, SK-OV3 and Caov-3. The research suggests that the compound's ability to fight ovarian cancer may be linked to its antioxidant capabilities.

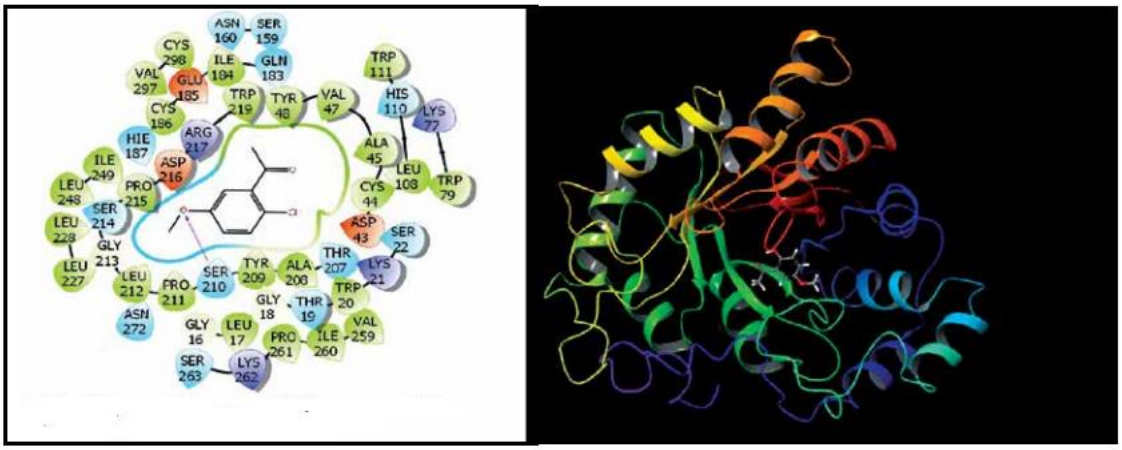


Figure 9. The interactions of methoxyacetophenone with aldose reductase.

I.3.4. Anti-inflammatory activity

Zang et al. [21] analysed the anti-inflammatory activity of 2'-Hydroxy-5-Methoxyacetophenone (2H5M) toward lipopolysaccharides (LPS)-stimulated BV-2 cells and RAW 264.7 cells.

It was found that 2'-Hydroxy-5′- Methoxyacetophenone has the ability to lower nitric oxide (NO) levels by reducing the expression of inducible nitric oxide synthase (iNOS). It also has a negative effect on (COX-2), TNF-α (Tumor Necrosis Factor-alpha), and the formation of reactive oxygen species (ROS). Additionally, when it comes to signaling pathways, 2H5M inhibits the phosphorylation of p65, IκB (Inhibitor of κB), and ERK (Extracellular signal-regulated kinase). Moreover, EMSA (Electrophoretic Mobility Shift Assay) results demonstrated that 2H5M directly interferes with NF-κB (Nuclear Factor-κB) DNA binding activity, and molecular modeling studies suggest that 2H5M can interact with NF-κB. These findings indicate that 2H5M might be potentiallya good anti-inflammatory agent [21].

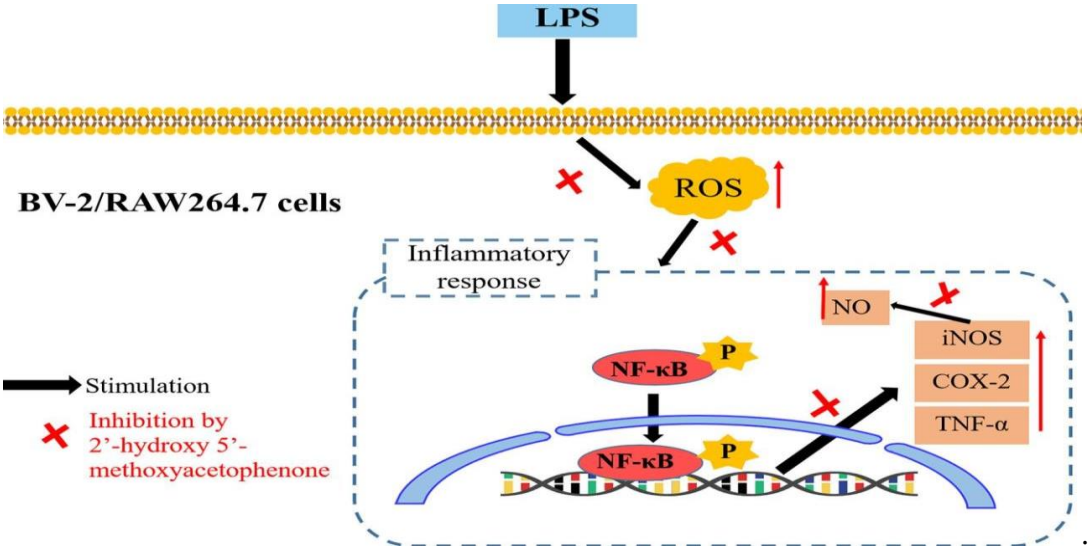


Figure 10. Graphical abstract of the process of anti-inflammatory effect of 2'-Hydroxy- 5′-Methoxyacetophenone.

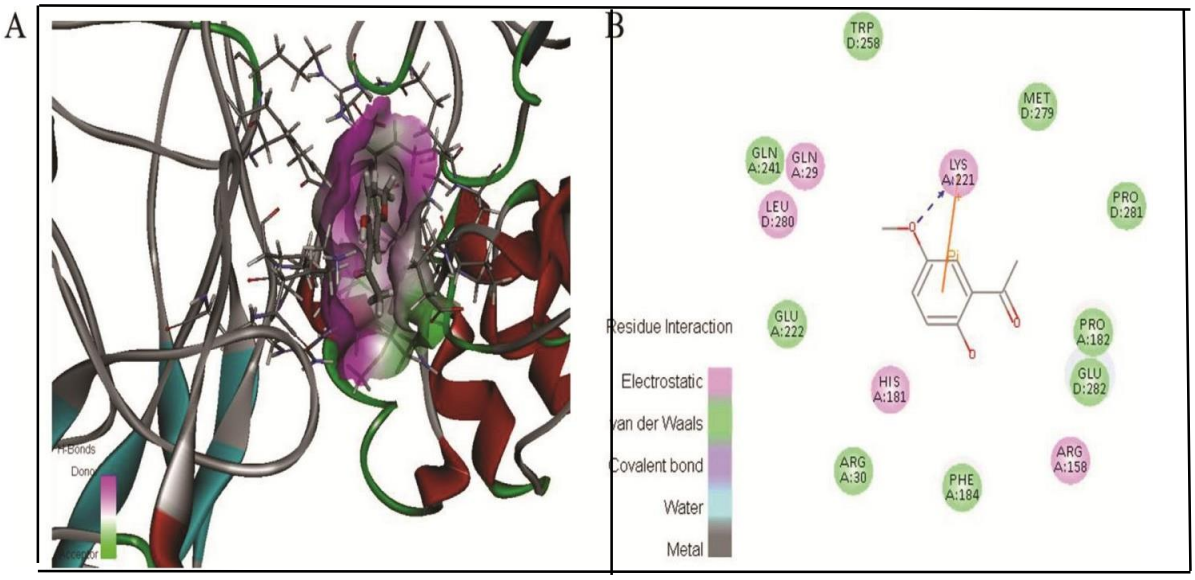


Figure 11. (A): the binding pose of 2H5M with NF-κB. (B): The binding residues that resulted between 2H5M and NF-κB; the blue arrow represent the hydrogen bonding occurred to LYS 221.

I.3.5. Antimicrobial activity

Hydroxyacetophenones have the ability to show a superb activity on bacterial and fungal pathogen. To take an example, 2′-hydroxy-3′,4′,6′-trimethoxyacetophenone is one of the antibacterial agents that have a strong efficacy on *Pseudomonas aeruginosa* and *Staphylococcus aureus* [22].

Disli et al [23] prepared a series of new hybrid compounds, in which 2-hydroxyacetophenone and N-substituted thiadiazoles interacted by coupling thiotetrazole with methylene spacers of varying lengths. The title compounds were subjected to antimicrobial activity towards clinical isolates of Gram-positive and Gram negative bacterial and fungal strains. All the compounds were found to possess a wide range of antimicrobial assay and exhibited inhibition against the entire bacteria and fungi assays, and the compound **4a** and **5d** were the most active antibacterial derivatives.

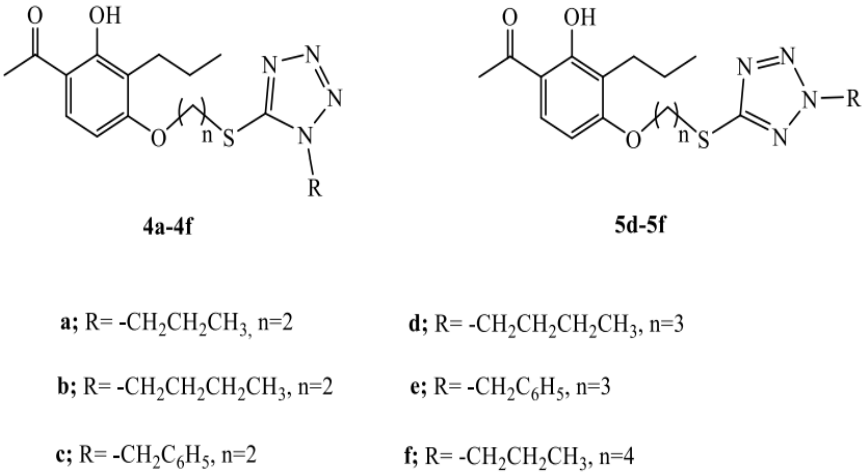


Figure 12. The hydroxyacetophenones derivatives coupled to N-alkylated thiotetrazoles via methylene spacers.

I.4. Biological activity of certain Schiff base complex Derived of 2'-hydroxyacetophenone

Metals and their complexes have a fascinating array of roles in biological systems. These metals and inorganic compounds boast a diverse set of properties, making them valuable in numerous biological and medical applications. A number of metal complexes are clinically used to treat a range of diseases (with the most successful example being cisplatin) and even more are being tested in either clinical or preclinical trials. Metal complexes are well-recognized for their crucial role in drug efficacy and are integral to how these drugs work. The ligands that could be used in potential metal-based drugs can vary widely, ranging from synthetic organic compounds to natural products [24]. Additionally, hydroxy acetophenone is a ligand known for its impressive ability to form various complexes with both transition and non-transition metal ions [25].

The antimicrobial research on Co^{2+} and Cu^{2+} ion complexes formed with a tetradentate Schiff base ligand, created from 2- hydroxyacetophenone and *o*-phenylenediamine and, shows that each of the synthesized complexes exhibit stronger activity against the strain *Bacillus cereus*, *Staphylococcus aureus*, and *Escherichia coli* compared to the free ligand. Notably, the Co^{2+} complexes outperform the Cu^{2+} complexes in terms of effectiveness [26].

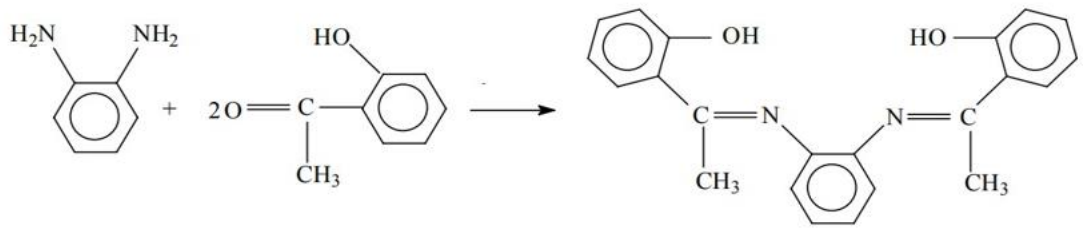


Figure 13. Synthesized Schiff base from hydroxyacetophenone and o-phenylenediamine .

A Schiff base ligand (L) and its metal complexes; Cu(II), Mn(II), Ni(II), Co(II), Fe(III), Cd(II) and Mg(II), Zn(II), have been formed through the reaction of diethylenetriamine and 2-hydroxyacetophenone; the obtained compounds were subjected to antibacterial and antifungal screening. Most them showed moderate to good antimicrobial effects against four gram (-) bacteria; *Acinetobacter baumannii , Escherichia coli*, *Salmonella typhimurium*, and *Citrobacter freundii* along with two gram (+) bacteria; *Enterococcus faecalis* and *Listeria monocytogenes*. They were also found as effective against various pathogenic fungal species, such as *Candida albicans*, *Aspergillus niger*, *Aspergillus flavus* and *Fusarium oxysporum* [27].

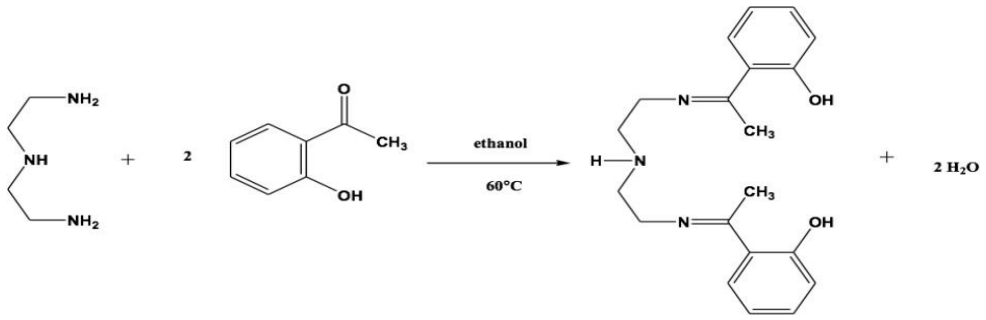


Figure 14. Synthesis of Schiff base ligand (L).

Another research based on the antioxidant activity of a range of synthesized Schiff base derived from tryptamine and various compounds like 5-methoxy-2- hydroxyacetophenone, 5-methyl-2-hydroxyacetophenone, and 2-hydroxyacetophenone, along with their copper(II) and nickel(II) complexes, showed that these ligands are actually more effective as antioxidants compared to their metal complexes, and this effectiveness increases with concentration. They primarily function as hydrogen atom transferring antioxidants during oxidative processes [28].

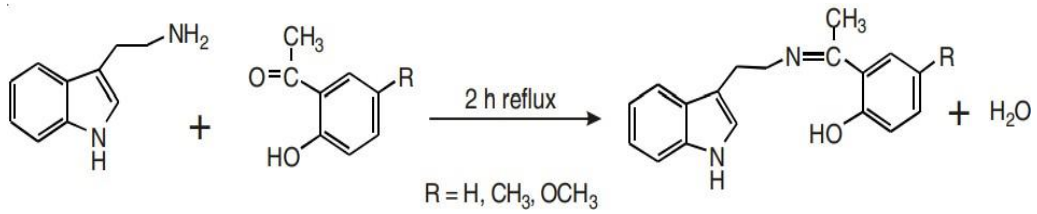


Figure 15. Proposed structures of the Schiff base derived from 5-methoxy-2-hydoxyacetophenone and tryptamine.

Phenoxy moiety as a crucial structural motif in Bioactive compounds

II.1. Introduction

Phenoxy group is an important part of the pharmacophore of a number of current medications, including viral medicine [29], and prostate relaxants [30]. Moreover, molecules carrying this group showed anti-inflammatory activity [31]. An increasing number of studies showing how the phenoxy moiety is relevant to the biological activity of the compound. The phenoxy moiety in the vast majority of cases provided the opportunity to fit the target, and the selectivity, the π–π interaction or increased the formation of hydrogen bonds due to the oxygen ether atom's. A new generation of drugs with a phenoxy group might be the future of pharmacotherapy [32].

II.2. New agent bearing phenoxy group

II.2.1. Neurological Disorders

More and more, neurologic disorders are being seen as one of the main caused of death and disability around the word. In 2016, the second most prominent cause of death was neurological disorders. There are depressive conditions, neuroses, phobias, on the other hand, neurodegenerative diseases [32]. **Figure 16** shows the new possible agents of the neurological disorders with a phenoxy group.

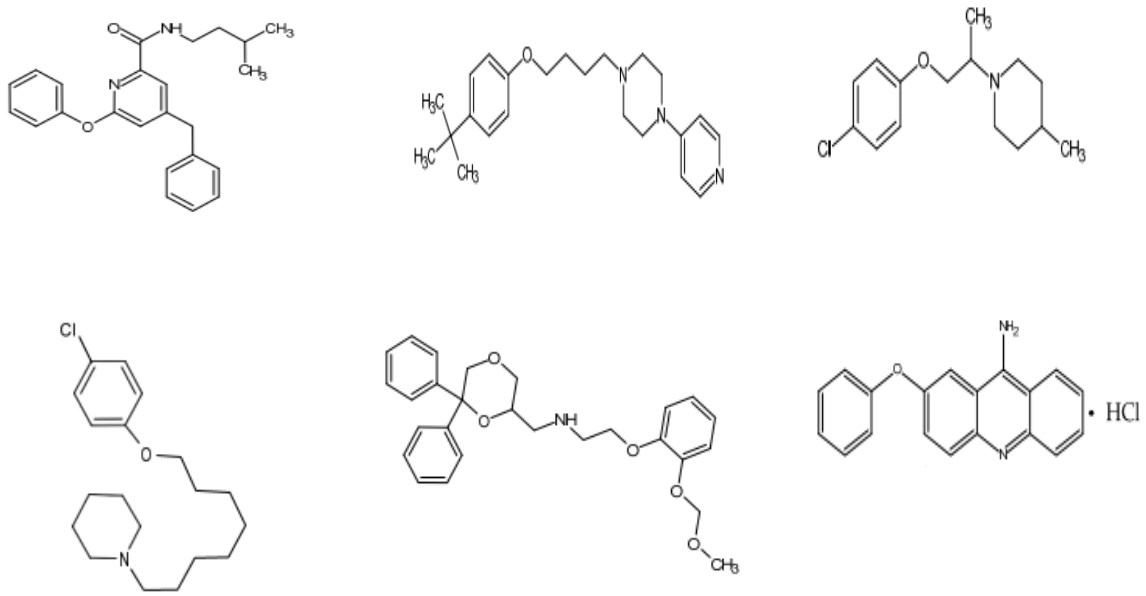


Figure 16. Some recent potential agents for a neorological desorder bearing a phenoxy group.

Szczepan SK et al. designed a family of phenoxyalkylamine analogs and evaluated their activity in silico, in vitro and in vivo against the Human H3 receptor (H3R) [33]; H3R contributes to the regulation of the level of the histamine and other neurotransmitters, therefore it is a potential target for treating neurological and psychiatric disorders [34].

The results showed that the compound contained 4-tert-butylphenoxy group was the most active. The researchers discovered that the oxygen atom in the phenoxy group forms hydrogen bonds with a particular amino acid in the receptor [33].

II.2.2. Anticancer activity

Cancer is a disease that occur when abnormal cells divide uncontrollably destroying body tissues. The currently available cancer drug do not work as effectively. High-quality chemotherapeutic drugs is needed, and this has been the principal challenge of researchers over the previous years [35]. The novel potential anticancer compounds that have a phenoxy group are illustrated in the figure below.

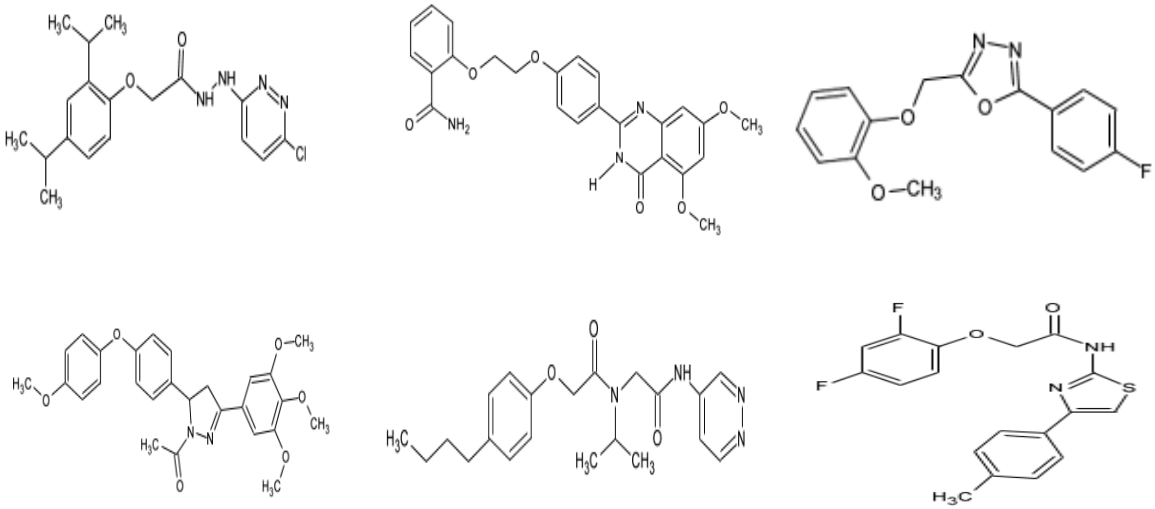


Figure 17. New possible anticancer agent family bearing a phenoxy group.

The study of et al [36]. focused on designing new pyrazoline compounds that incorporated the phenoxy moieties, which is present in several FDA-approved VEGFR inhibitors (drugs officially approved by the U.S Food and Drug Administation (FDA) to treat specific cancers by inhibiting Vascular Endothelial Growth Factor Reseptors (VEGFRs) like axintinib and tivozanib). Among the prepared molecules, the compound 1-(5-(4-(4-

methoxyphenoxy)phenyl)-3-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone exhibited potent anticancer activity against ovarian and breast tumorcidal cell, outperforming the standard drug the staurosporin. Substitution of the phenoxy group with morpholino group led to a decrease in the activity of the compound prepared and this showed that the phenoxy moiety plays a significant role in enhancing the anticancer effects.

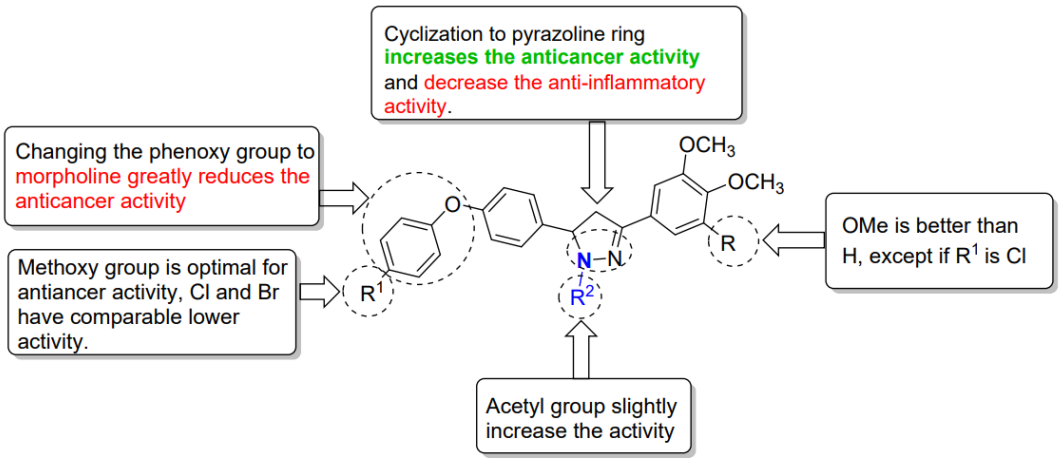


Figure 18. SAR of the prepared pyrazoline derivatives [36].

II.2.3. Antimicrobial activity

There is some even refer to an antibiotic therapy crisis do to only a few number of antibiotics being effective againt certain pathogens infections. The most famous ones include methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus*, drug-resistant *Streptococcus pneumoniae*, drug-resistant *Mycobacterium tuberculosis*, carbapenem-resistant *Enterobacteriaceae* (CRE), and multi-drug-resistant (MDR spectrum) *Pseudomonas Acuginobeta* [37]. There is a similar issue with antifungal drugs. A similar issue with anti-fungal medication. The figure depicts the new potential antimicrobial drugs that bear phenoxy group.

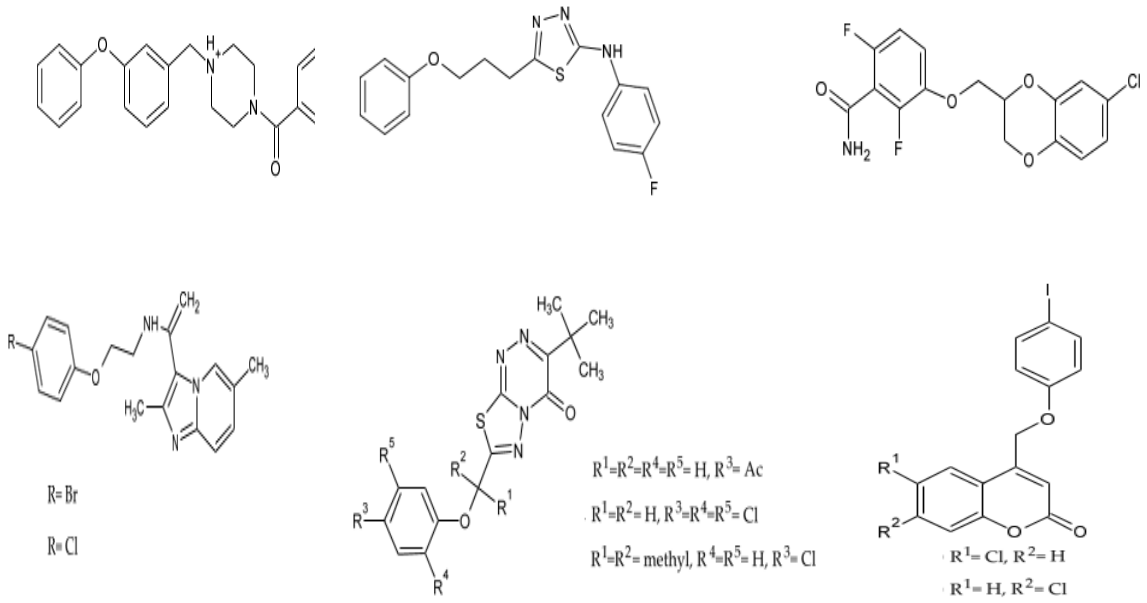


Figure 19. Several new potential antimicrobial agents containing a phenoxy group.

Chiodini et al [38] constructed a new derivative of three substituted 2,6-difluorobenzabides as potent inhibitors of bacterial cell division. The S- enantiomer of one of these derivatives displayed strong antibacterial activity towards *S. aureus,* and showed better results than the reference compound,2,6-diluoro-3-nonyloxybenzamide. The greatest activity was attributed to the presence of 3-amido2,4-difluorophenoxy moiety.

Nehra et al [39] have reported the synthesis of new bioactive 1,2,3, triazole hybrids. The most active derivative exhibited considerable antifungal activity against *C. tropicalis* and *A. terreus,* and it performed better than the reference drug fluconazole. According to molecular docking studies, the phenoxy part is very important in hydrophobic interaction. The most active was that bearing 4chlorophenoxy substituent.

Wu et al. [40] prepared a set of new N(2-phenoxyethyl)imidazo[1, 2-a]pyridine-3-carboxamides.One derivative demonstrated superior activity against *Mycobacterium tuberculosis* H37RV compared to the standards drugs rifampicin and isoniazid. Another compound revealed notable activity against both multidrug-resistant *M. tuberculosis strains.* Interesting to note that the two strains are resistant to the isoniazid and rifampicin. According to the authors, the oxyethyl linker of the phenoxy moiety was more active than the aminoethyl moiety. The most active derivatives were those incorporated either a 4-bromophenoxy or 4-chlorophenoxy substituent.

II.2.4. Antiparasitic activity

Parasitic diseases cause a major public health problem and the increasing drug resistance has limited the effectiveness of existing treatments. The diseases are located primarily in the lower-income countries [41]. Figure 20 shows the novel antiparasitic agents with a phenoxy group.

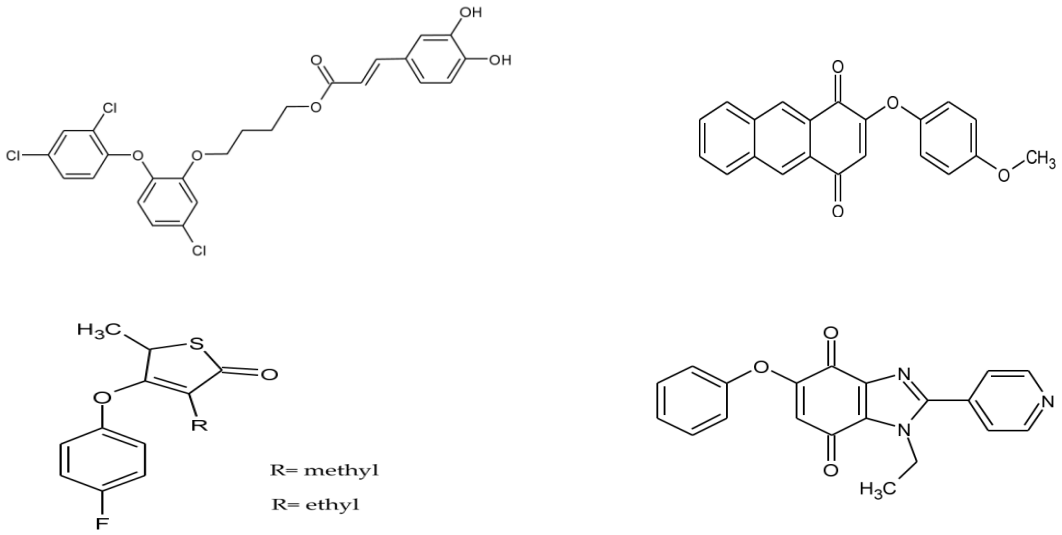


Figure 20. Novel antiparasitic agents with a phenoxy group.

Otero et al [42] prepared novel triclosancafeic acid analogs.The proposed drug had an anti-leishmanial effect against *L. (V.) panamensis* and *T. cruzi* when compared to the standard medications Triclosan and Benznidazole. The compound that included the 2-(2,4-dichlorophenoxy)-5-chlorophenoxy moiety was the most active.

II.2.5. Analgesic effect

Pain is among the most universal indicators, which take patients seeking clinical care. Despite the revolutionary finding of opioids, salicylic acid, etc., several decades ago, the first line drugs lack long term effects. As an example, the long term use of opioids causes hyperalgesia. The medical chemist would have a great difficulty with the complexity of pain pathomecanism, hence it is necessary to keep on searching for potential drug agents that will provide a response to this problem [43] The novel potential analgesic agents bearing a phenoxy group are described in the **Figure 21**.

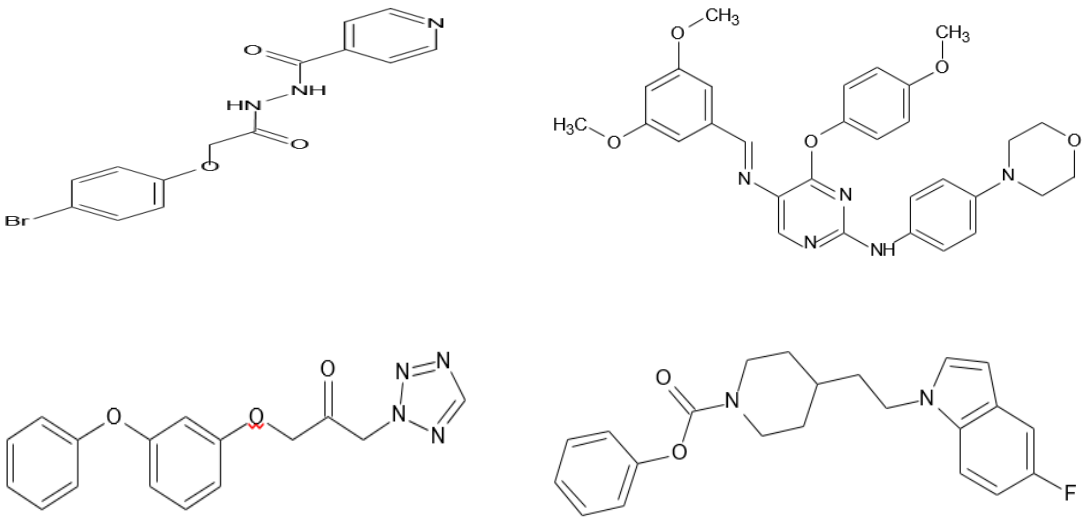


Figure 21. The novel potential analgesic agents contain a phenoxy group.

Cyclooxygenases 1 and 2 (COX-1 and COX-2) are important enzymes in health maintenance and injury. COX is found in biosynthesis of prostaglandins (PG) and thromboxane (TX) that are responsive to pain and inflammation. Inhibition of COX therefore lead to an analgesic and anti-inflammatory properties [44].

Pallavi et al. developed the new derivatives of N-(2-phenoxyacetyl) isonicotinohydrazide as analgesic and anti-inflammatory substitutes. The most promising activity against the COX-1 and COX-2 was that of the compound w. According to authors, the introduction of the bromo substituents in the para position of the phenoxy group led to the better selectivity index and it was found in the most active compounds. A molecular docking study of the binding w with COX-2 indicated that there is a π- π interaction between TRY385 and TRY387 and the phenoxy group [45].

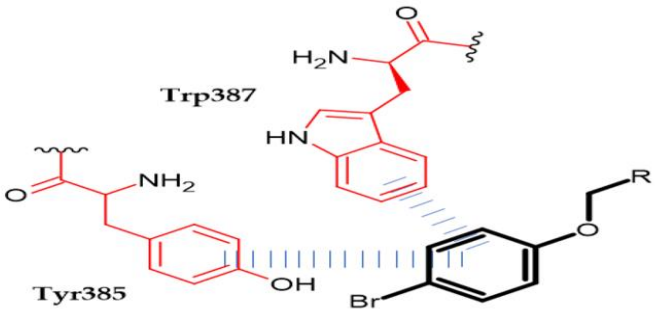


Figure 22. The representation of the phenoxy group that creates the π–π interaction interaction (blue line), R representing the remainder of the molecule

II.2.6. Anti-Diabetic activity

Diabetes can be described as a disorder that affect the release of insulin leading to hyperglycemia. Uncontrolled diabetes may result in coma or even death from ketoacidosis [46].Figure shows some potential anti-diabetic agents that bear a phenoxy group.

Li et al [47] prepared new catechol derivatives as a specific inhibitors of PTP1B (protein tyrosine phosphatase 1B) the key negative regulator in both insulin and leptin signaling pathways, and thus participate in the glucose and lipid methabolisme modulation. Hence, it present an attractive molecular target in diabetes type 2 and obesity treatments. The authors state that the hydrophobic arm of the phenoxy group was highly selective and offered a new generation of non-insulin-dependent drugs in the treatment of type 2 diabetes having a high level of membrane permeability. The 4-tert-butylphenoxy moiety was found to be the most active compound.

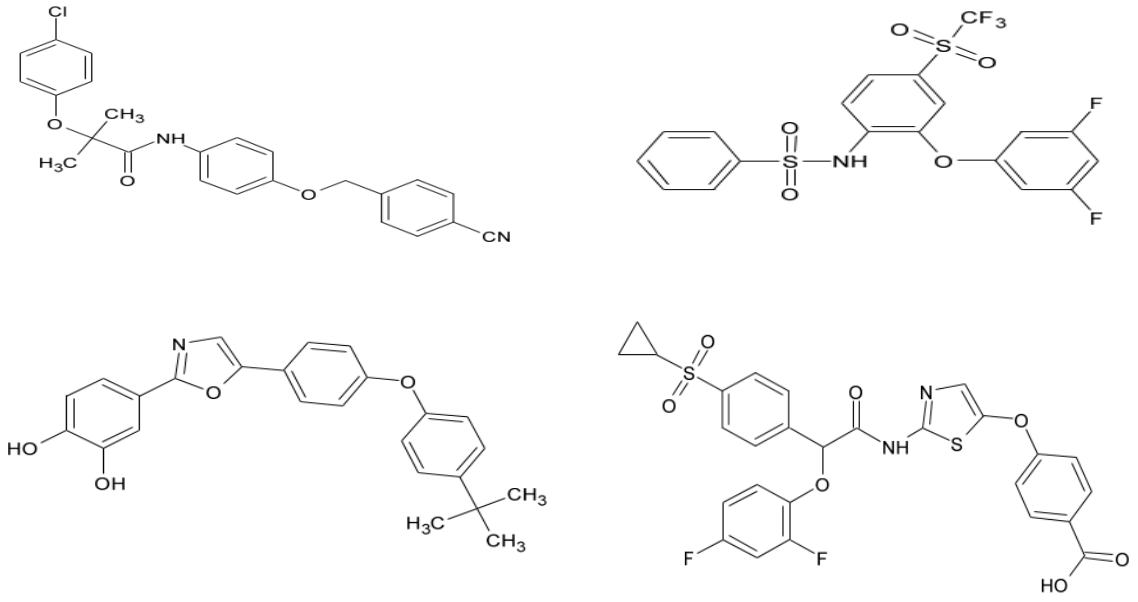


Figure 23. Some compounds bearing a phenoxy moiety and have the potential to be novel anti-diabetic agents.

II.3. Drugs and Auxiliary substances bearing a functional phenoxy group

II.3.1. ***Drugs***

The figure bellow lists some currently drugs used in treatment, categorized by biological activity.

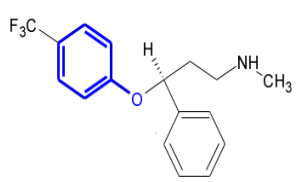
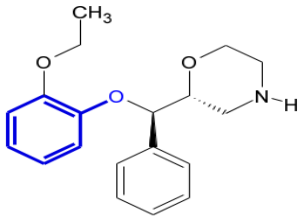
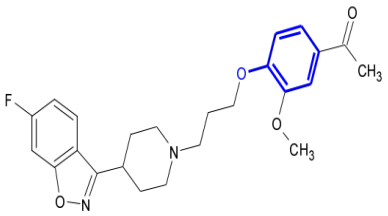
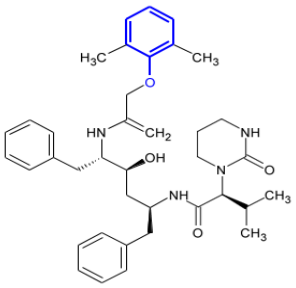
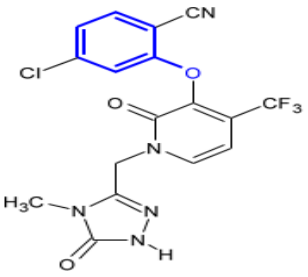
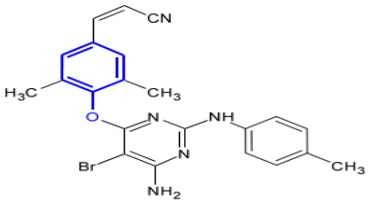
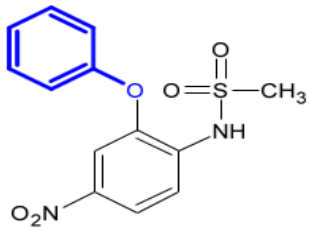
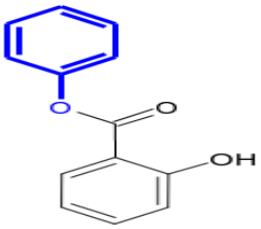
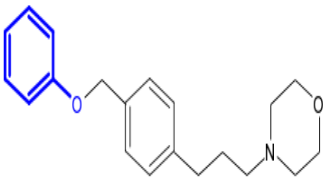
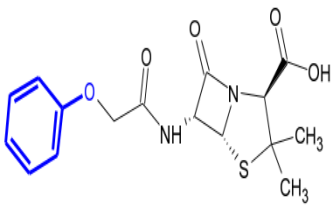
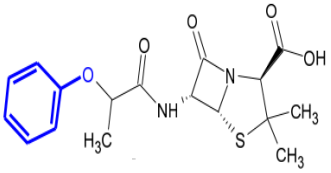
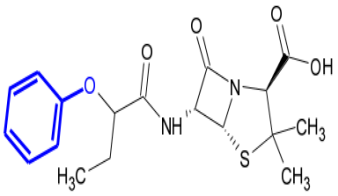
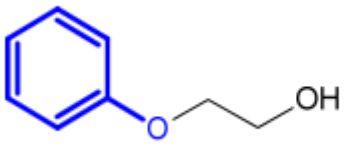
Neurological disorder drugs		
 Fluoxetine	 Reboxetine	 Iloperidone
Antiviral drugs		
 Lopinavire	 Doravirine	 Rilpivirine
Analgesic drugs		
 Nimesulid	 Phenylsalicylate	 Fomocaine
Antimicrobial drugs		
 Pencillin V	 Feneticillin	 Propicillin

Figure 24. Currently drugs used in treatment of some disease.

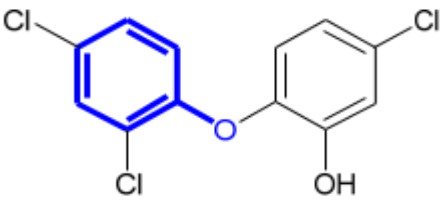
II.3.2. Preservatives

Preservatives are present in the synthetic food that we consume, cosmetics, vaccinations, and medicines. This maintains their qualities and effectiveness for a longer duration. As example, 2-phenoxyethanol and Triclosan both are commonly used as antimicrobial agents [48,49]

Due to its antimicrobials activity, phenoxyethanol has been applied as a preservative since many decade in several products including vaccines, hands disinfecting biocidal up to 5% concentration [48].



2-phenoxyethanol



Triclosan

Figure 25. Preservatives bearing a phenoxy group.

II.4. References

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Materials and methods

III.1. Introduction

We present in this section the various instruments and experimental protocols used for the synthesis and the characterization of the bis ketone 1,3-Bis (2 acetylphenoxy)-2-propanol (Bis- AcPh), to insure accurate identification of the synthesized molecule. The biological potential of the compound was assessed through in vivo and in vitro assays designed to evaluate their antioxidant, anti-inflammatory and cytotoxicity properties. in addition to the experimental investigation, computational approaches using DFT, molecular docking and MDS simulations have been integrated to provide a deeper understanding of the molecular behavior and to complement the experimental findings.

III.2. Materials

III.2.1. Chemicals and reagents

All chemicals and reagents used in this work were procured from Sigma Aldrich; The reagents 1,3 dichloropropan2-ol (98%), 2'-hydroxyacetophenone (98%), NaOH (99%), were used for the synthesis of the bis-ketone. Whereas, Indomethacin, Butylated Hydroxy Toluen (BHT), DPPH, ascorbic acid, xylene, croton oil and other reagent were used for biological tests.

III.2.2. Animals

In this experience, the animal handling procedures included in this experience followed the principles of the Animal Welfare Act, and were done as stated the guide for the care and the use of laboratory animals of the " Institut Pasteur d'Algérie."

Before carrying out the tests, healthy whites mice (female mice, 25-30 g weight) were kept in clean cages for fifteen days with acclimatization and under controlled conditions of 12h light: 12h dark cycle and 24 ± one °C with free access to standard rodent diet and clean water before doing the tests. The experimental assays were authorized by the Committee of the 'Association Algérienne des Sciences en Expérimentation Animale' (http://aasea.asso.dz/articles) in compliance with law No. 88- 08/1988, which pertains to veterinary medical practices and the protection of animal health (N° JORA: 004/1988).

III.2.3. Microorganisms strains

The reference microorganisms utilized in the current study are members of pathogenic species and were acquired at American Type Culture Collection (ATCC). The bacteria were

consisted of two Gram-positive strains: *Staphylococcus aureus* (ATCC25923), *Bacillus cereus* (ATCC1086),and two Gram-negative strains: *Escherichia coli* (ATCC25922), and *Salmonella Enteritidis* (ATCC13076). The strains employed are the ones recommended by the quality control and compliance laboratory Biotechnology Research Center (CRBT Constantine Algeria. All the bacterial strains were subcultured from the original culture, stored at −20 ° C, maintained on Muller-Hinton (MH) agar plates at 4°C, and grown at 37°C when required.

III.2.4. Thin layer chromatography

The possibility to break down a mixture into its separate chemical components is critical in a large number of experiments. This aids in the isolation of a given compound or determining the purity of the mixture. Thin layer chromatography (TLC) is one of the most universal, easy ways to accomplish this. It is economic, simple to use, fast at development, sensitive and Attainable with good reproducibility [1]. To keep track of the reactions, we used TLC. By placing TLC sheets under UV light, we could monitor the progress of the reactions and assess the purity of the compounds throughout the study.

III.2.5. Fourier-transform infrared (FTIR) spectroscopy

FTIR absorption spectroscopy is a powerful technique, which analyzes the response of various vibration frequencies of hetero-atomic bonds toward infrared radiation. Usually, FTIR spectra are given as a graph of absorbance intensity verses the wave number that is measured in cm^{-1}. It allows as proposing the structure of ours compounds. Each bound exhibits characteristic vibration of a well-defined frequency. [2]. Fourier transform infrared spectra of the synthesized compound was recorded with a Shimadzu FTIR-8400S spectrophotometer in the wave number range 4000-400cm^{-1}.

III.2.6. Electronic adsorption spectroscopy

UV/Vis spectroscopy forms one of the more common analytical and characterization tools of science. It has a simple linear dependency on the absorbance and concentration of the absorber; that is why it has been especially attractive in quantitative measurements. This method is crucial for exploring the electronic properties of various materials and their precursors, in both fundamental research and in the development of practical application [3].

Electronic spectroscopy studies in this work was performed on a SHIMADZU UV –650 (JASCO visible spectrophotometer) in the range 200 –900 nm.

III.2.7. Nuclear Magnetic Resonance (NMR)

In order to obtain ^{1}HNMR and ^{13}C NMR spectra, a Bruker DPX-500 MHz spectrometer (Bruker Topspin, Berlin, Germany) was used. The solvent was DMSO-d6 (Deuterated dimethyl sulfoxide) and the internal standard TMS (Tetramethylsilane). Chemical shifts are expressed in δ units, while coupling constants (J values) are reported in hertz.

III.2.8. Melting point

A Stuart scientific melting point apparatus was employed for measuring the melting point of the compound Bis-AcPh. HCl

III.3. Experimental methods

III.3.1. Synthesis of 1,3-Bis(2-acetylphenoxy)-2-propanol (Bis-AcPh)

The compound 1,3-bis(2-acetylphenoxy)-2-propanol (**Bis-AcPh**) was prepared using Williamson ether synthesis method, as described by Lindoy and Armstrong [4].

100 ml of aqueous solution of sodium hydroxide (2.0 g,0.05mol) was stirred and then added to an ethanolic solution (6.75 g, 0.05 mol) of 2-hydroxyacetophenone. After warming, an ethanolic solution of 1, 3-dichloro-2-propanol (3.16 g, 0.025 mol) was then added. Sufficient ethanol (50 ml) was subsequently added to this mixture to form a homogenous solution. The solution was refluxed for 72h, and then cooled at 0°C. The solid formed was recrystallized using ethanol-water mixture and the resulting brown crystals were filtered and then dried in a vacuum, (yield 44%, melting point: 103°C).

III.3.2. Biological activities of the synthesized compound Bis-AcPh

III.3.2.1. Evaluation of the antioxidant activity

III.3.2.1.1. DPPH radical-scavenging assay

This assay was performed to identify the hydrogen donating and/or electron transfer potential of the **Bis-AcPh** using a stable free radical, DPPH [5]. In this experiment, 250 µL of methanolic solution of the tested compound **Bis-AcPh** was added to an equal volume of a methanolic solution of a freshly prepared DPPH (0.004% w/v). As a comparative standard, the commercially available antioxidant butylated hydroxytoluene (BHT) that exhibits 100% activity was employed, and the resulting composition was incubated in darkness for a half-

hour at room temperature. This test was carried out in triplicate and the results were recorded spectrophotometrically at 517 nm against a blank using spectrophotometer. The decrease in absorbance indicates an increase in radical scavenging activity [6].The outcomes of Bis-AcPh as well as the standard BHT preparation were given as an IC50 values; which signify the concentration that inhibits 50% of the free radical in the mixture. The percentage of DPPH scavenging was computed using the following equation:

DPPH scavenging activity (%) = [Abs $_{control}$ − Abs $_{test\ sample}$ /Abs $_{control}$] X 100…. (1)

III.3.2.1.1. Reducing power assay

The reducing power test is depended on the reduction of Fe (III) to Fe (II), which is considered as a significant indicator of antioxidant capacity of the studied sample. According to the following protocol [7]. An aliquot volume of 400 µL of the compound Bis-AcPh with different concentrations was reacted with the same amount of both potassium ferricyanide (1%) and phosphate buffer (0.2 M, pH = 6.6). The resulting mixture was then incubated in a water bath at 50°C for 20 min. After this period, the reaction was halted by adding a volume of 400 µL of the reagent trichloroacetic acid (10%), and then followed by centrifuging this mixture at 3000 rpm for 10 min. At last, 400 µL of distilled water and 80 µL of ferric chloride solution (0.1%), where added to 400 µL the upper layer of this solution after 10 min of incubation. The absorption of this mixture was observed at 700 nm, and ascorbic acid (VitC) was regarded as the reference drug. The increased reaction mixture absorbance is an indication of stronger reducing power.

III.3.2.2. Evaluation of the anti-inflammatory activity

III.3.2.2.1. *In vitro* anti-inflammatory assay by egg albumin denaturation

According to the following protocol [8] The albumin of fresh eggs of hens was diluted to 1% with Tris-HCl (20 mM, pH 6.87) buffer solution (v/v), stirred for 10 min and then filtered through a strip of agarose. A volume of 500 µL of this prepared albumin solution, was added to a volume of 250 µL of both Bis-AcPh and Aspirin. Aspirin was the reference drug while the buffer TrisHCl served as the negative control. The absorbance was read at 650 nm and the rate of prevention of protein denaturation is based on the following formula:

$$\% \text{ inhibition of protein denaturation} = (A_C - A_S) \times 100 / A_C \quad \text{.....(2).}$$

Were A_C = absorbance of the control sample, and A_S = absorbance of the test sample.

III.3.2.2.2. *In vivo* topical anti-inflammatory assay

c) Xylene induced ear edema test

In the xylene-induced ear edema test, animals were randomized into three groups of six animals each group comprised six animals as follows: Group 1 (the positive control) was topically treated by the standard drug indomethacin at a dose of 2 mg/ear. Group 2 (the negative control) was treated topically by xylene (30 µL/ear), and also group 3 received the same dose (2 mg/ear) of Bis-AcPh that was applied at the time immediately following xylene application. All the treatments were applied to both the inner and the outer surface of the right earlobe; the left ear served as a control. The thickness of the ears was assessed both before and two hours after the final dose of treatment using a digital calliper that was placed in the vicinity of the tip of the ear slightly proximal to the cartilaginous ridges. The formula bellow is employed to calculate the mean oedema inhibition rate (%).

$$\text{Inhibition rate (\%)} = 100\times (Dn-D) / Dn \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

Where D is the thick variation of ears edema in the treated group; whereas, Dn is the thick variation of ear edemas in the negative group [9].

d) Croton oil induced ear edema test

In accordance with similar protocol of topical inflammation caused by xylene; in croton oil-induced ear edema assay, mice randomly assigned into three groups of six mice each. Croton oil solution with about 80 µg dissolved 15 µL of a mixture of acetone/water (1:1, v/v), was at the initial stage topically applied on both the inner and the outer part of each mouse's right ear. At the same time point, 15 L of the ethanol-water mixture with 2 mg of Bis-AcPh or 2 mg of the indomethacin was also topically tested in the same area of the edema of the ear in each of the mice, the negative control also have been stimulated topically using croton oil. Ear thickness, measured in micrometer (µm), was determined using a digital caliper placed close to the ear's tip just distal from the cartilaginous ridges. The variation in thickness of mice ear edema before application of the treatment and after 6 hours of inflammation induced by croton oil treatment was calculated according to the equation (3) [10].

Figure 26. Topical anti-inflammatory activity

III.3.2.3. In vitro cytotoxicity assay

The technique employed in the toxicity assessment of the Bis-AcPh was on the capacity of RBCs to liberate hemoglobin. Human Erythrocytes are obtained by centrifuging peripheral blood of a healthy donor based on the standard operating procedures of the International Federation of Blood Donor Organizations (IFBDO). Blood received in a heparinized tube was centrifuged at 3000 rpm/10 min. The erythrocyte suspension was obtained by washing the cells with 0.9% of sterile saline solution. After each wash, the pellets that contained the cells were centrifuged down at 300 rpm for 5 min to remove the supernatant. This process was repeated three to four times until the supernatant became colorless. The resultant pellet after the last centrifugation step was resuspended and quantified in an isotonic buffered solution (10 mM sodium phosphate buffer pH7.4) to yield a 2% (v/v) suspension.

The **Bis-AcPh** (250 µL) prepared in different concentrations, was added to the same volume of prepared erythrocyte suspension .The contents were then incubated for 1h at 37°C, then centrifuged for 10 min. The 250 µL of Ph (physiologic saline) consisting of different concentrations was added to the same amount (250 µL) of the made erythrocyte suspension. The mixture was then left to be incubated at 37°C over 1 h and followed by centrifugation at 10 min.

The absorbance of the supernatant was detected at 540 nm against a reagent blank, where Bis-AcPh was substituted by saline solution [11].

III.3.2.4. Evaluation of the antibacterial activity

The anti-bacterial activity of **Bis-AcPh** against four bacterial strains including two Gram-positive

(*Staphylococcus aureus* and *Bacillus cereus*) and two Gram-negative bacteria (*Escherichia coli* and *Salmonella enterica*) was examined. Two complementary procedures were done, disk diffusion assay to measure and evaluate inhibition zones and broth microdilution method to evaluate minimum inhibitory concentration (MIC).

III.3.2.4.1. Evaluation of the antibacterial activity using agar disc-difusion method

In this essay, we identified the bacteria as either sensitive or resistant using the agar disk-diffusion technique [12]. Each of the bacterial strains was cultivated at 37 °C in nutrient broth (BN) for 24 hours). To get a young culture, the various bacterial strains were subcultured and then used to develop a bacterial inoculum at an optical density of McFarlend 0.5 (10^8 Colony Forming Units/mL) in sterile sodium chloride (0.9%). A sterile cotton swab was used to inoculate dried plates (90 mm in diameter) with the bacterial inoculum. Sterilized paper disks of 6 mm in diameter (Wathman N°.3) were placed on the surface of each agar plate and were impregnated with 10 µL of Bis-AcPh prepared in DMSO solvent to get the concentration 100,50,25,12,5…mg/mL and Gentamicin (0.12 mg/mL) served as a control. After these preparations, Plates were incubated at the right condition of cultivating (24 h at 37°C), and then examined for any zones of growth inhibition.Finaly, a caliper was used to measure the diameter zone in millimeters.

III.3.2.4.2. Evaluation of the antibacterial activity using micro dilution method

The broth microdilution technique for determining the minimum inhibitory concentration (MIC) was performed as outlined by Merah et al [13]. Bacterial cultures grown overnight were diluted in sterile nutrient Mueller-Hinton (MH) broth to achieve a turbidity of 0.5 on the McFarland scale equivalent to the density 0.08 to 0.10 at 625 nm. 100 µL of the appropriate amount of Bis-AcPh (50 mg/ml) prepared in DMSO was mixed with 100 µL of MH broth, and then transferred serially from the 1^{st} well to the 12^{th} of a 96-well microplate, afterwards to each well 50 µL of bacterial suspension were added. The well plate were incubated for 24h at 37°C and the growth of the bacteria was evaluated by measuring the optical density at 625 nm. The antibacterial activity was described as the lowest absorbance that led to growth inhibition. MIC was the lowest concentration of compound that inhibited the visible growth of microorganisms.

III.4. Computational studies

III.4.1. Density Functional Theory

DFT (Density Functional Theory) is a quantum mechanical method used to study the

electronic properties of multi-particle systems such as atoms, molecules and condensed-matter systems, and offers simple ways of carrying out the calculation of the electronic properties of matter. The theory is based on a number of crucial theorems and principles, including two important theorems relating to the Hohenberg-Kohn relationship and the Kohn-Sham equations [14]. Using the density functional theory [15], the theoretical calculations of the optimization of the **Bis-AcPh** molecular structures, the mapping electrostatic potential, and the frontier molecular orbitals (FMOs) were carried out by the Gaussian 09 software Using B3LYP Beck's three-parameter hybrid exchange functional, Lee-Yang-Parr correlation functional [16], with a 6-31G (d, p) basis set [17,18].

From the FMOs' energies, some mathematic characteristic global quantum chemical descriptors (GQDs): Energy gap (E_{GAP}), ionization potential (I), electron affinities (A) [19], electronegativity (χ), chemical potential (μ) [20], global hardness (η) [21], global softness (σ), and Electrophilicity index (ω) [22] were computed according to the formulas (4) to (11) [23].

$$E_{GAP} = \Delta E = E_{LUMO} - E_{HOMO} \quad (4)$$

$$I = -E_{HOMO} \quad (5)$$

$$A = -E_{LUMO} \quad (6)$$

$$\chi = -[1/2(E_{LUMO} + E_{HOMO})] \quad (7)$$

$$\mu = [1/2(E_{LUMO} + E_{HOMO})] \quad (8)$$

$$\eta = 1/2(E_{LUMO} - E_{HOMO}) \quad (9)$$

$$\omega = \chi^2/2\eta \quad (10)$$

$$\sigma = 1/\eta \quad (11)$$

III.4.2. Molecular Docking Studies

Molecular docking studies were conducted to evaluate the binding interactions between the synthesized compound Bis-AcPh and (COX-2) enzyme, in support of its experimentally confirmed anti-inflammatory activity (both in vitro and in vivo). The X-ray crystal structure of COX-2 complexed with Diclofenac (PDB ID: 1PXX) was retrieved from the RCSB Protein Data Bank [24]. Three reference compounds were included for comparative purposes: Aspirin (used in in vitro assays), Indomethacin (used in in vivo assays), and Diclofenac (the co-crystallized ligand). Protein and ligand preparation steps were performed using UCSF Chimera 1.17.3 by removing water molecules, non-standard residues, and the

cocrystallized ligand, followed by the addition of polar hydrogens and Gasteiger charges [25]. Ligands were energy-minimized prior to docking. The simulations of molecular docking were conducted with AutoDock Vina 1.2.7, using the binding site defined around the active site of Diclofenac [26]. The grid box was centered at x = 25.19 Å, y = 23.65 Å, z = 16.69 Å, with dimensions of x = 21.45 Å, y = 15.75 Å, z = 14.80 Å, ensuring full coverage of the binding pocket and allowing sufficient space for flexible ligand accommodation. All docking runs were executed under identical parameters to enable consistent comparison. Binding affinities (in kcal/mol) and interaction modes were analyzed using Discovery Studio Visualizer and UCSF Chimera to assess the inhibitory potential of Bis-AcPh relative to the standard anti-inflammatory drugs. Binding affinities (in kcal/mol) and molecular interactions were analyzed and visualized using Discovery Studio Visualizer [27].

III.4.3. ADMET Study

To evaluate the pharmacokinetic and toxicity profiles of the synthesized compound Bis-AcPh in comparison with established anti-inflammatory agents, an in silico ADMET analysis was performed using SwissADME and ProTox-II web servers [28, 29]. The reference compounds selected for comparison were Aspirin, Indomethacin, and Diclofenac.

SwissADME was used to predict various absorption and distribution parameters, including gastrointestinal (GI) absorption, P-glycoprotein (P-gp) substrate identification, and cytochrome P450 (CYP) inhibition potential notably for isoenzymes such as CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4. Additionally, Lipinski's Rule of Five and other drug-likeness filters (such as Veber and Ghose filters) were applied to assess oral bioavailability potential and medicinal chemistry friendliness. All four compounds showed varying degrees of compliance with drug-likeness criteria, with Bis-AcPh exhibiting favorable physicochemical properties, including acceptable molecular weight, logP and oral bioavailability.

Toxicological parameters were predicted using the ProTox-II platform, which provided estimates for LD_{50} values (mg/kg), toxicity class (I–VI), and a set of potential organ-specific toxicities, including hepatotoxicity, carcinogenicity, mutagenicity, immunotoxicity, and cytotoxicity. The reference drugs showed varying toxicity profiles, with Diclofenac predicted to have higher hepatotoxic potential, in agreement with known clinical data. In contrast, Bis-AcPh demonstrated a lower predicted toxicity class, a higher LD_{50}, and no alerts for major organ toxicity, indicating a potentially safer profile.

Overall, the ADMET prediction results highlighted the promising pharmacokinetic behavior and low predicted toxicity of Bis-AcPh, supporting its potential as a safe and drug-like anti-inflammatory candidate for further in vivo investigation. These in silico findings also emphasized key differences in the metabolic and toxicological behavior of Bis-AcPh compared to conventional NSAIDs, reinforcing the rationale for its development.

III.4.4. Molecular Dynamics Simulation

To further evaluate the stability and dynamic behavior of the protein–ligand complexes obtained from molecular docking, molecular dynamics (MD) simulations were performed on the complex of the synthesized compound Bis-AcPh with (COX-2), using the reference Aspirin_COX-2 complex for comparison. The simulations were carried out using Gromacs with the CHARMM36 force field to assess the structural stability, flexibility, and interaction integrity of the systems [30]. Ligand topologies and parameters for Bis-AcPh and Aspirin were generated via SwissParam, ensuring compatibility with the force field. Each complex was solvated in a dodecahedral box using the TIP3P water model, neutralized with counterions, and subjected to energy minimization using the steepest descent algorithm until the maximum force dropped below 10.0 kJ/mol [31]. System equilibration was performed in two phases: NVT equilibration for 500 ps at 300 K using a V-rescale thermostat, followed by NPT equilibration for 100 ps with Berendsen pressure coupling at 1 bar. The production run was then conducted for 100 nanoseconds under periodic boundary conditions. Throughout the simulation, key structural parameters were monitored, including the root mean square deviation (RMSD) to track global conformational changes, root mean square fluctuation (RMSF) to assess residue flexibility, radius of gyration (Rg) to evaluate the compactness of the protein, and solvent accessible surface area (SASA) to estimate the degree of exposure to the solvent environment. This comprehensive MD analysis provided insight into the stability and binding performance of Bis-AcPh within the COX-2 active site in comparison to the standard anti-inflammatory drug Aspirin, reinforcing the potential of the synthesized compound as a stable and effective COX-2 inhibitor [32, 33].

III.4.5. MMPBSA Calculation

To quantitatively evaluate the binding affinity of the synthesized compound Bis-AcPh with COX-2, binding free energy calculations were performed using the Molecular Mechanics Poisson Boltzmann Surface Area (MM-PBSA) approach [34]. The MM-PBSA method provides a robust and widely accepted framework to estimate the binding free energy of

protein–ligand complexes based on trajectories extracted from molecular dynamics simulations. Calculations were conducted using the g_mmpbsa package, which enables the decomposition of the total binding free energy into key energetic components. The binding energy ($\Delta G^{binding}$) was computed using the following equation [35]:

$$\Delta G^{binding} = G^{complex} - (G^{protein} + G^{ligand}) \quad \text{.....(12).}$$

Where $G^{complex}$ represents the total free energy of the protein–ligand complex, while $G^{protein}$ and G^{ligand} correspond to the free energies of the isolated COX-2 receptor and ligand (Bis-AcPh or Aspirin), respectively. This analysis enabled a comparative assessment of the binding affinities between the synthesized compound and the reference drug, providing further insight into the stability and favorability of the interaction within the COX-2 active site.

III.5. Statistical analysis

Statistical analysis of the obtained graphs of the (Bis-AcPh) was carried out using GraphPad Prism program (version 5.03 for Windows).The data was analysed using one-way analysis of variance (ANOVA), and the finding were displayed as mean ± Standard Deviation (SD). Linear regression was used to calculate the IC50 values, and the results were considered to be statistically significant if $p < 0.05$. On the other hand, in vivo experimental results are expressed as the mean ± Standard Error of the Mean (SEM).

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Results and discussion

IV.1. Introduction

In this section of the thesis, we report the experimental finding related to the target compound bis. In the first part of this work, we focus on the synthesis strategy that allowed for the efficient construction of the desired bis ketone framework and its structural characterization with the help of various spectroscopic techniques. In the next part, the compound was subjected to some experimental assays for a comprehension of its biological activities. To support the experimental results, some theoretical studies were carried out by computational studies based on DFT and molecular docking approaches. 90°C /EtOH.

IV.2. Synthesis of Bis-AcPh

Under the conditions provided in **Figure 27**, the condensation of 2′-hydroxyacetophenone with 1,3-dichloropropanol was done in a 2:1 M ratio in accordance to Williamson ethers reaction to yield 1,3-Bis (2-acetylphenoxy)-2-propanol denoted as (**Bis-AcPh**).

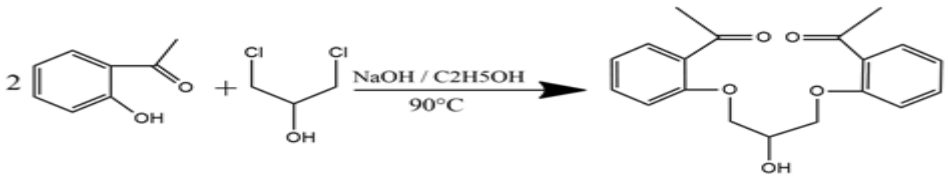

Figure 27. Synthesis of Bis-AcPh.

IV.3. Identification and characterizations

IV.3.1. Fourier Transform Infrared (FT-IR)

In order to analyse the structure of Bis-AcPh, the FTIR spectrum of the compound demonstrates the typical absorption bands of the functional groups existing in the synthesized product, as can be seen from **Figure 28**.

The band observed at around 3400 cm^{-1} represents the stretching vibration of O-H bond of the pendent arm (hydroxyl group) of the propane 2-ol moiety, whereas the series of weak bands at 2939, 2876, 2835 $cm^{-1}$are due to the vibrations of aliphatic methylene -(CH_2), in the propane spacer and (C-H) vibration of the aromatic ring. The two sharp peaks at 1660 cm^{-1}

and 1596 cm^{-1} are assigned respectively to the C=O stretching vibration of the acetyl groups, and the stretching vibration (C=C) of the aromatic ring, while the two asymmetric and symmetric intense bands characteristic of (Ar-O- CH_2) group are located at around 1240, 1047 cm^{-1} respectively [1].

The emergence bond of (OH), together with (C=O) and the two asymmetric and symmetric intense bands characteristic of (Ar-O-CH2) group prove the formation of the organic compound **Bis-AcPh**.

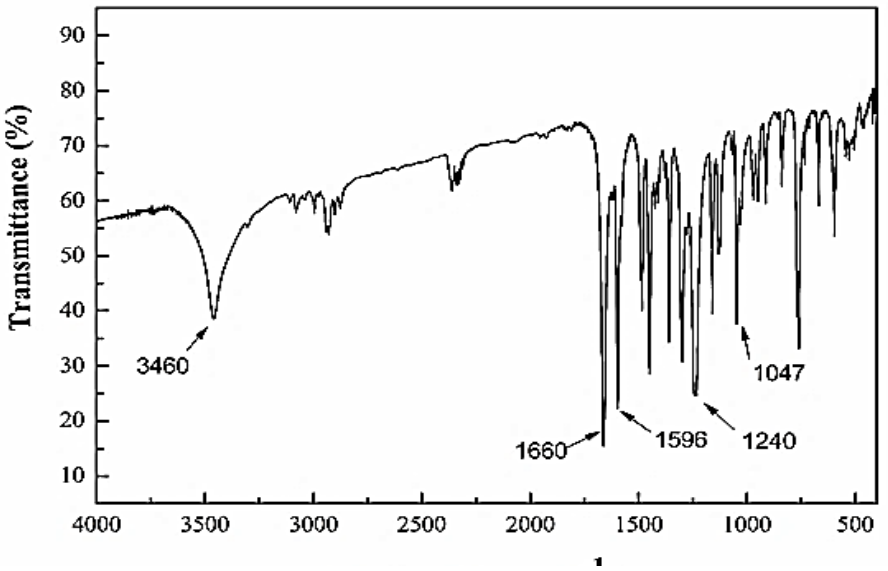

Figure 28. The IR spectrum of Bis-AcPh.

IV.3.2. Electronic spectroscopy spectrum

The UV-VIS spectrum of the compound **Bis-AcPh** (**Figure 29**) recorded in ethanol shows three bands; the first band that centered at 212 nm can be ascribed to n-σ* transition of the simple bond (C-O). The second band situated at 248 nm can be attributed to π-π* electronic transitions of the (C=C), and the (C-C) bonds within the aromatic ring. While the third bond located at 293 nm represent the n-π* transition of (C=O) bond [2,3].

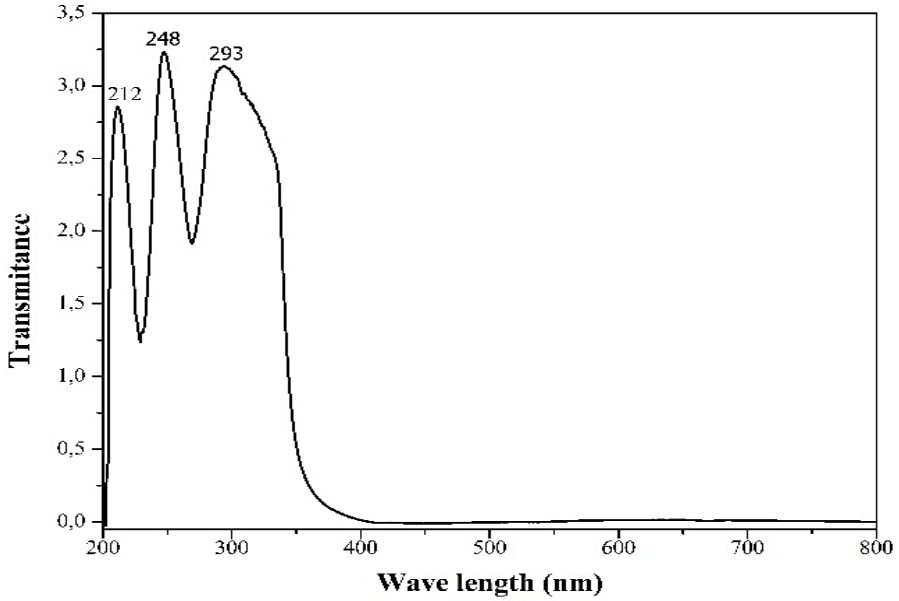

Figure 29. Electronic Spectrum of Bis-AcPh.

IV.3.3. Nuclear Magnetic Resonance (NMR) spectrum

The ^{1}H-NMR spectrum of Bis-AcPh (**Figures 30 and Figure 31**) recorded in DMSO-d_6 is in agreement with the symmetricity of designed synthesized bis-ketone. The ^{1}H-NMR spectrum shows a singlet at 2.57 ppm attributed to the 3 proton (H-10, H-10') of the methyl group of the acetyl group, indicates the existence of methyl ketone in the structure. The quartet at 4.34 ppm (j=5.0 Hz), and the doublet at 5.54 ppm (j=5.0 Hz) are related to the proton (H7, H7') of the carbon linked to the phenoxy groups, and the proton (H8,) of the carbon linked to the hydroxyl group. Four doublets with integration of two protons at (7.01-7.59) ppm region coresponding to the aromatic ring proton (H-3 to H-6 and H-3′ to H-6′), this finding confirming symmetrical structure of the molecule.

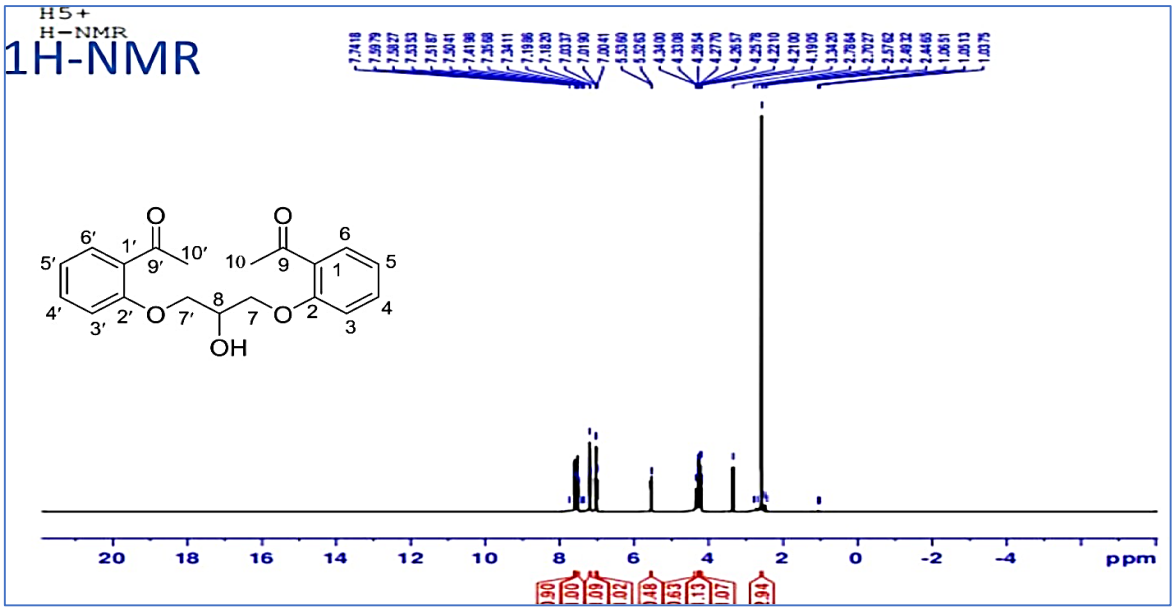

Figure 30. [1]H-NMR spectrum of Bis-AcPh in DMSO-d6.

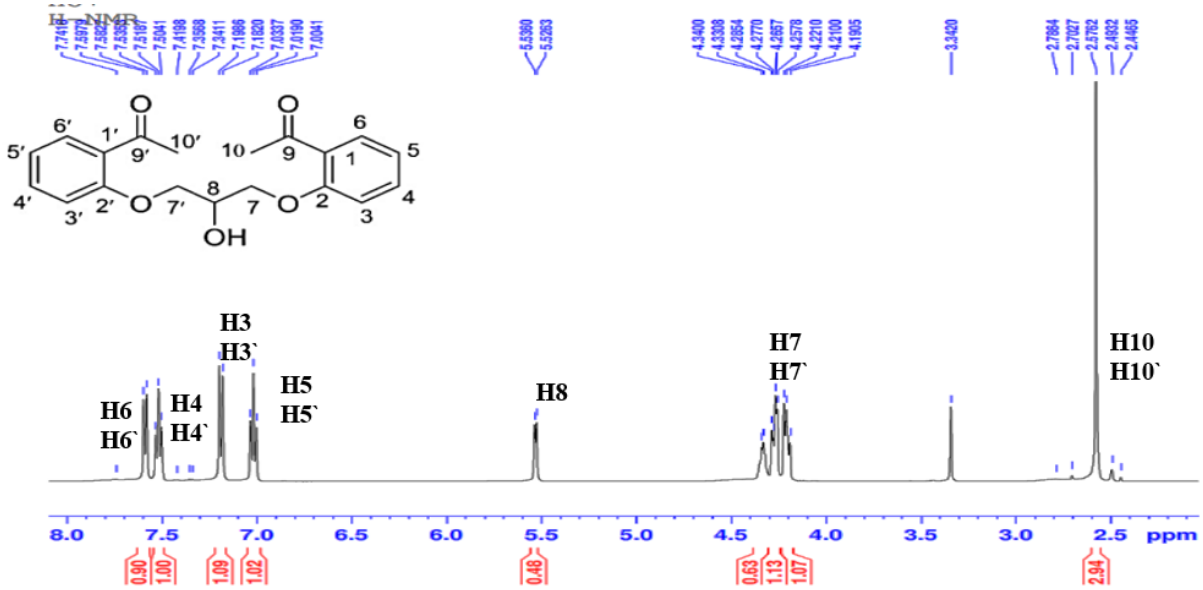

Figure 31. [1]H-NMR spectrum of Bis-AcPh (2.5-7.5 ppm) (DMSO-d6).

The suggested structure is further supported by the [13]C-NMR spectrum (**Figure 32**) which displays signals with good symmetry. The [13]C-NMR chemical shifts of of Bis-AcPh are given in **table 1.** Bold values are of DEPT 135 (C-H) (**Figure 33**).

Table 1. ^{13}C-NMR chemical shifts of of Bis-AcPh.

δ (ppm)	Attribution
198.3	The carbonyl (C-9, C-9′) of the acetyl group
158.2	Aromatic carbons (C-2, C-2′) attached to oxygen atoms
134.4	**The aromatic carbons (C-4, C-4′)**
132.2	**The aromatic carbons (C-6, C-6′)**
128.4	The aromatic carbons (C-1, C-1′)
121.0	**The aromatic carbons (C-5, C-5′)**
112.7	**The aromatic carbons (C-3, C-3′)**
70.6	**The two carbon (C-7, C-7′) of the methoxy segment**
67.7	**The central carbon (C-8) of the propanol bridge**
32.2	**The methyl carbons (C-10, C-10′) of the acetyl group**

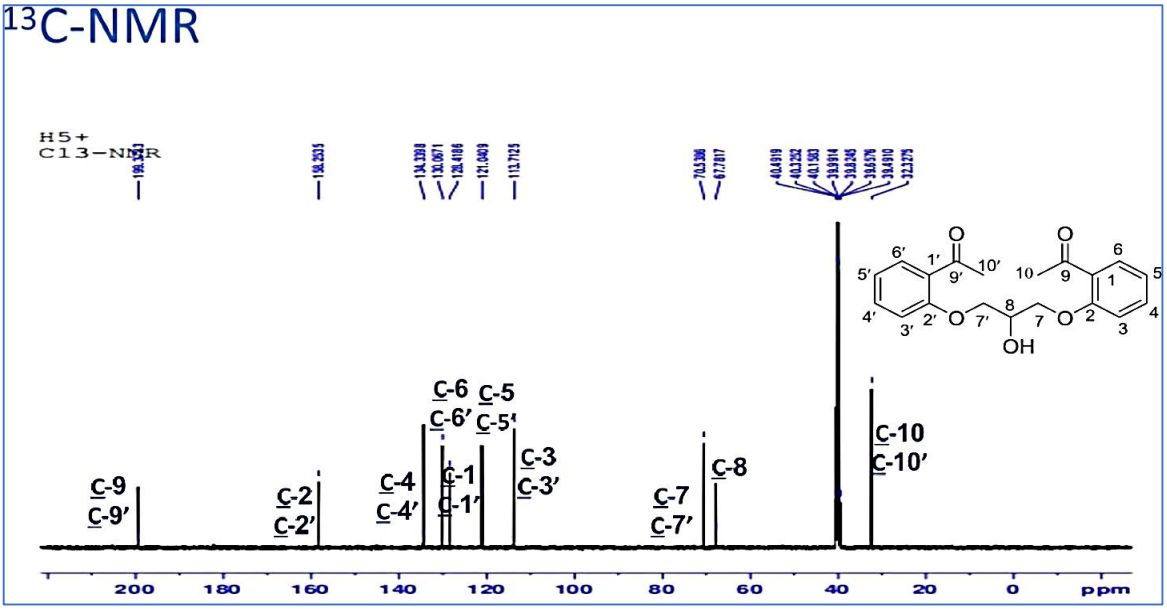

Figure 32. ^{13}C-NMR spectrum of Bis-AcPh (DMSO-d6)

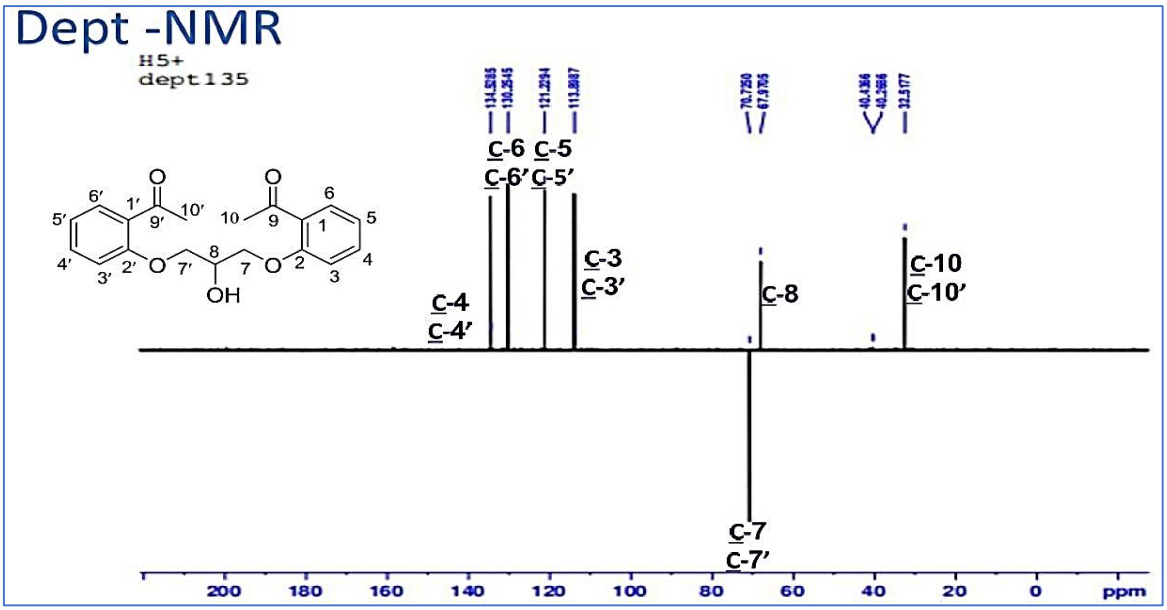

Figure 33. DEPT 135 spectrum of Bis-AcPh (DMSO-d6).

IV.4. Density functional theory study

IV.4.1. Optimized geometries

The molecular structure of **Bis-AcPh** was theoretical optimized with DFT/B3LYP method with 6-31G (d,p) basis set simulations. The **Figure 34. (a)** indicates it 3D geometrical form, and It clearly shows how the molecule configuration deviate greatly from a planar geometry. The molecule is deformed from the planar configuration; the angle between the support planes of the aromatic rings has the value 46.48° (Figure 3.b). Moreover, the C-C and C=C lengths bonds are within the normal ranges and around 120° ,which denotes the sp2 hybridization of all C atoms, whereas the low difference can be explained by the VSEPR theory [4]. The outcome of the DFT calculations correlates well with similar earlier molecular structures [5,6].

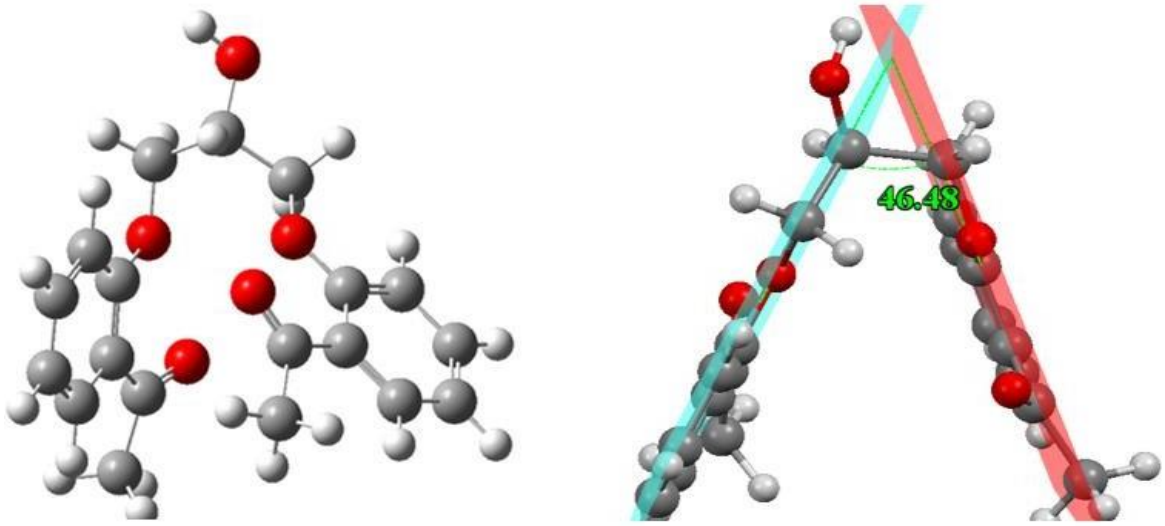

Figure 34. (a) DFT-Optimized molecular structures: BZT organic part, (b) distortion of the molecular structure.

IV.4.2. Mapping electrostatic potential

The **Figure 35** shows the surface of total electron density mapped with the electrostatic potentials of **Bis-AcPh**. The surface reveals a positive potential around hydrogen and alkyl carbon, whereas the most negative potential highlighted by a red color is located around the central oxygen atoms of the molecule. This last site can attract electrophilic compounds. Moreover, a weak negative potential surrounding the cycle as shown by a yellow color can be observed and is related to the π-conjugated electrons. The non-homogeneity of the potential distributions generates a dipolar moment of 1.583Debye.

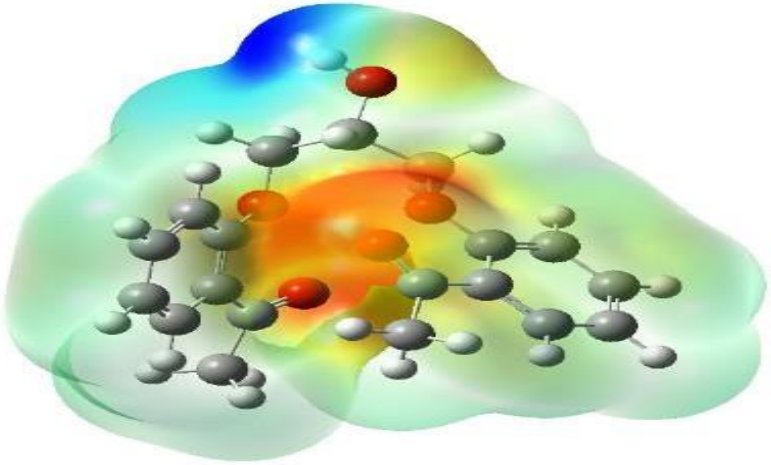

Figure 35. The total electron density mapped with the electrostatic potential of the Bis-AcPh.

IV.4.3. Frontier Molecular Orbitals (FOMs)

FMOs (HOMO: Highest Occupied Molecular and LUMO: Lowest Unoccupied Molecular Orbitals) are terms describing the electron transfer process within the surrounding medium; since the HOMO can give electrons, and the LUMO has the tendency to receive them [7]. The **Figure 36** displays the plotted HOMO and LUMO of the titled compound. As can be observed, the LUMO is located on the aromatic cycle and its surroundings, while the HOMO is distributed throughout the molecule except the sections of methyl groups. These finding demostrate that the most actives sites for transferring electrons to the surrounding medium are essentially the aromatic cycle. Along with that, the LUMO and HOMO energies, as well as some Global quantum physic-chemical descriptors (GQCDs) are an important tool for estimating the electrons transfer, the reactivity and the stability of the **Bis-AcPh.**

From these (GQCDs) we can list the gap energy (ΔE), the ionization energy (I) that represents the amount of energy necessary to remove an electron out of a molecule or atom. The electron affinity (A) that is the energy change associated with the addition of an electron to a neutral atom or molecule. The global hardness (η) is the measure of the resistance to the polarization or deforming of an electron cloud, the global softness (σ) is the reverse side of it. The electronegativity (χ) is the tendency of an atom in a molecule to attract electrons, that the capacity of electrons to exit an equilibrium system is represented by the quantity of chemical potential (μ). Finally, the global electrophilicity index (ω) is defined as the energy change on migration of the electrons of the donor HOMO of the molecule to the acceptor LUMO [8].

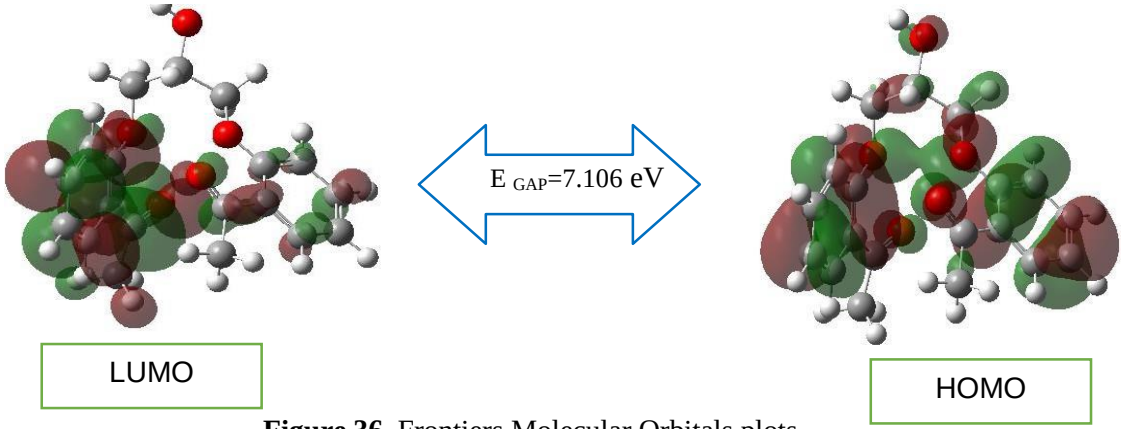

Figure 36. Frontiers Molecular Orbitals plots.

The LUMO and HOMO energies are 5.971Ev and-1.135 respectively (**Table 2**), the low value of the HOMO's energy indicates the electron donating character.

Table 2. LUMO and HOMO energies and the some corresponding Global Quantum Physic-Chemical Descriptors (GQCDs).

Quantum parameters	
E_{LUMO} (eV)	5.971
E_{HOMO} (eV)	-1.135
Ionization potential (I, eV)	1.135
Electron affinity (A, eV)	-5.971
ΔE gap (eV)	7.106
Dipolar moment (D)	1.583
Global hardness (η, eV)	3.553
Global softness (σ, eV^{-1})	0.281
Electronegativity (χ, eV)	-2.418
Chemical potential (μ, eV)	2.418
Global electrophlicity (ω, eV)	0.823

IV.4.4. Chemical reactivity and stability

The chemical stability of Bis-AcPh can be explained through the gap energy EGAP with the value of 7.106 eV. Hence, the large value reflects the low reactivity and high stability and vice versa. Moreover, the hardness, softness, and chemical potential having the values 3.553 eV, 0.281 eV^{-1}, and 2.418 eV, respectively, are other parameters that can discuss the reactivity and stability. Soft molecules have less energy of excitation compared to hard molecule as the Bis-AcPh, which requires high energy of excitation [9]. The calculation of the GQCDs reveals that the μ was found to be 2.418 eV, this value suggests according to chelation therapy, that charges transfer with the external medium may occur [10]. In order To assess the molecule's efficiency to donate electrons to the surrounding medium, the Q_{max} was estimated according to the Formula:

$$Qmax = -\mu/\eta$$

The total electronic charge Q_{max} proves again the donating character of the Bis-AcPh molecule [11].

IV.5. Biological activities

IV.5.1. Antioxidant activity

IV.5.1.1. Scavenging effect on DPPH radical

The chemical compounds that can delay or inhibit the oxidation process are called antioxidants, and are usually in smallest concentrations [12, 13].The Antioxidant defense has multiple strategies to defend the body. It is capable of preventing or inhibiting formation of free radicals, or neutralizing radicals or converting them to less hazardous molecules. It also inhibits toxic byproducts, halts chain reactions, inactivates toxic metals, and enhances the body natural antioxidant process [14]. Molecules designated as antioxidants are substances that generously donate electrons to offset and neutralize harmful oxidants [15].

DPPH assay is commonly employed for determining the potential of radical scavenging activity because of its stability in the radical form along with the simplicity of the test [16]. DPPH is a relatively stable free radical because it can gain either an electron or a proton in order to produce a stable molecule. Due to its odd electron, it exhibits an intense absorption band in the visible

spectrum at 517 nm. When an antioxidant donates electron or a proton to the DPPH radical, the absorbance of DPPH solution diminishes [15]. This activity has been used to determine the ability of compounds and plants extracts to scavenge free radicals [17].

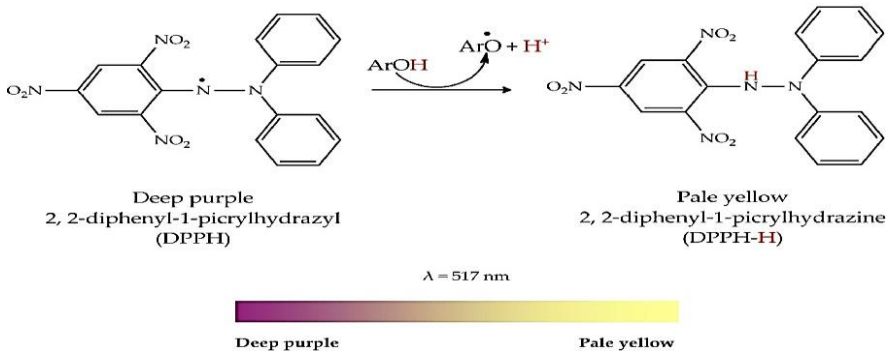

Figure 37. 2-diphenyl-1-picrylhydrazyl (DPPH) reaction mechanism [18].

In this study, the antioxidant potential of our compound Bis-AcPh against DPPH radical was investigated in respect to the 50% inhibitory concentration (IC_{50}) values. It was shown by

the results (**Figure 38**) that moderate scavenging activity was exhibited by Bis-AcPh with IC_{50} values 459.81±0.09µg/ml, whereas much stronger activity was displayed by the standard antioxidant BHT that has an IC_{50} of 87.26±0.01 µg/ml. As a lower IC_{50} value indicate a greater antioxidant capacity, these results imply that Bis-AcPh is less effective than BHT in neutralizing DPPH radicals.

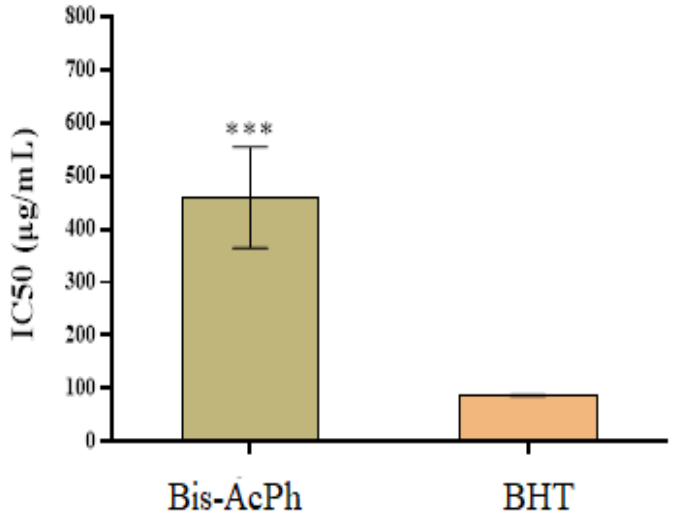

Figure 38. DPPH radical scavenging effect of Bis-AcPh. Outcomes are given as mean ± SD, (n = 3), ***: $p < 0.001$ in comparison with BHT.

IV.5.1.2. Result of reducing power assay

The electron donating capacity of bioactive compounds or food components is reflected in their antioxidant activity, which can be measured by reducing power. Bioactive compounds with antioxidant effects can either reduce or inactivate oxidants [19].

The reducing capacity of a bioactive compound can be quantified by the direct reduction of Fe^{3+} ferricyanide complexes $Fe[(CN)_6]_3$ to the ferrous (Fe^{2+}) form $Fe[(CN)_6]_2$. In the reducing power procedure, the existence of antioxidants in the sample reduce Fe^{3+} to Fe^{2+} by giving an electron, the proportion of Fe^{2+} complex in the samples can then be detected at 700 nm using Perl's Prussian blue by adding $FeCl_3$ to the ferrous (Fe^{2+}) form. Higher absorbance value at the final reaction mixture at 700 nm signifies that the compounds tested have higher reducing capacity [20].

$$4FeCl_3 + 3K_4[Fe(CN)_6]^{3-} \rightarrow Fe_4[Fe(CN)_6]_3^{4-} + 12KCl$$

Figure 39. Reducing power reaction mechanism [20].

Our finding showed in (**Figure 40**.) revealed that the IC_{50} value of the reference drug compound **VitC** was noted as 21.91 ± 0.48 µg/ml, however the **Bis-AcPh** gave 577.55±0.01 µg/ml. In this test also the synthesized compound, display a poor reducing power in comparison to Vit C.

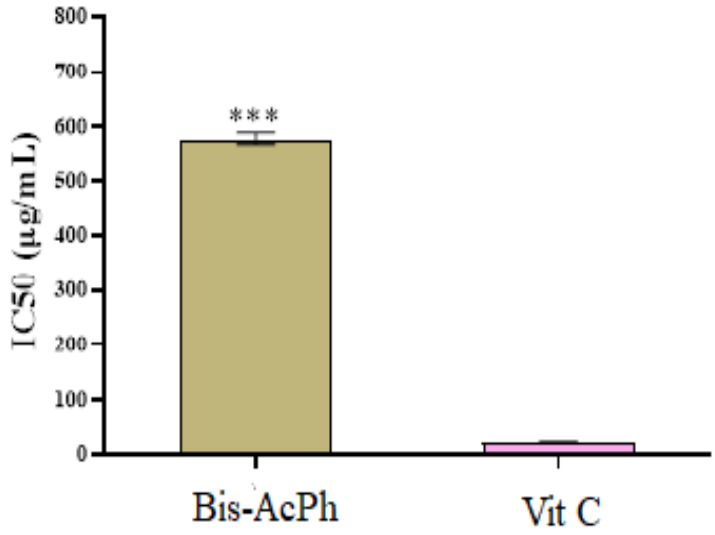

Figure 40. Reducing power activity of Bis-AcPh. Outcomes are given as mean ± SD, (n = 3), ***: $p < 0.001$ in comparison with Vit C.

From the DPPH scavenging ability and reducing power essays, the activities of the **Bis-AcPh** are much lower than the references compounds BHT and Vit C. this result due to it chemical structure that is poor in hydroxyl group that directly influences its hydrogen-donating capacity.

Antioxidant activity is often related to the functional groups that can release or neutralise free radicals, mainly the hydroxyl (OH) often present on phenolic compounds, including polyphenols in plants extracts [21]. The reducing properties are generally linked to the presence of reductones, which have been shown to exhibit antioxidant activity by interrupting the free radical chain through the donation of a hydrogen atom. In 1,3-bis(2-Acetylphenoxy)-2-propanol, the two aromatic rings contain an acetyl groups at the ortho position, the active hydrogen that ordinarily functions in antioxidant reactions as the phenols is replaced by the acetyl group ($COCH_3$). The presence of acetyl groups on the two phenoxy groups in **Bis-AcPh** disrupt this sort of resonance stability and hence, our compound has poor antioxidants properties.

Furthermore the starting material 2'-hydroxyacetophenone have a hydroxyl in the ortho position of the aromatic ring, this hydroxyl group was replaced by ether linkages ($-OCH_2$)

through the Williamson ether synthesis. So, the active hydrogen that ordinarily functions in antioxidant reactions as the phenols is replaced by the ether group (-OCH_2) .This modification slows down the possibility of the molecule to donate a hydrogen atom or electrons, which are crucial in neutralizing reactive oxygen species. Highly active antioxidant compounds often have double bounds and resonance structures that stabilize the radicals produced after donating electrons or hydrogen atoms.

Generally, synthetic antioxidants are compounds containing phenolic moieties with various degree of alkyl substitution [22]. These phenolic antioxidants transform Peroxy radicals into hydroperoxides and during oxidation becoming phenoxy radicals themselves. The phenoxy radicals can intereact in different manners with another peroxy radical leading to a nonradical products [23].

In order to understand the role of phenolic -OH groups in the antioxidant property of curcumin, Priyadarsini et al [24] carried out a comparative study between the curcumin (1,7-bis[4-hydroxy-3-methoxyphenyl]-1,6-heptadiene-3,5-dione) and dimethoxy curcumin (1,7-bis[3,4-dimethoxyphenyl]-1,6-heptadiene-3,5-dione). The antioxidant effect was determined by examination of the radiation induced lipid peroxidation of rat liver microsomes. The finding indicated that with the same concentrations, curcumin was able to inhibit lipid peroxidation by 82%, whereas dimethoxy curcumin only inhibited 24%. Kinetic studies made using the DPPH radical test pointed out that the bimolecular rate constant of curcumin was around 1800 times greater than that of dimethoxy curcumin, further confirming its stronger hydrogen-donating capacity. Cyclic voltammetry and pulse radiolysis experiments revealed that the presence of phenolic hydroxyl groups is essential to electron transfer mediated free radical scavenging, because dimethoxy curcumin displayed much weaker radical generation. Taken together, these results confirm that the phenolic hydroxyl group is the dominant factor in curcumin’s antioxidant mechanism.

An instructive comparative study realized by Chen et al [25] showed that although methoxyl and phenolic hydroxyl could augment the antioxidant activity of phenolic acids, but the phenolic -OH had a greater efficacy. DFT calculations supported this view by showing that phenolic -OH groups have lower bond dissociation energies and more favorable electron/proton transfer energetics, greatly increasing activity whereas replacing the -OH with a methoxy group (an ether-like substituent) dramatically decreases antioxidant potential.

IV.5.2. Anti-inflammatory activity

Inflammation constitutes a nonspecific immunological defence mechanism, which is triggered by mechanical injuries, microbial infections, burns, allergens and other noxious stimuli. When nociceptors are activated by harmful stimuli, a range of chemical mediators are released, including excitatory amino acids, vasoactive amines (histamine, Arachidonic acids (prostaglandins E2, leukotrienes), proteins, peptides, nitric oxide (NO), serotonin, and cytokines [TNF-α and interleukin-1], which act on particular receptors and ion channels, are some of the substances that cause pain and inflammation [26]. A series of biological activities, such as enzyme activation, mediator release, cell movement, fluid extravasation, protein denaturation, and membrane changes, are set off by pro-inflammatory mediators. Many inflammatory-related disorders, including rheumatoid arthritis, asthma, atherosclerosis, Alzheimer's disease, cancer, and diabetes, are mostly caused by increased vascular permeability brought on by these mediators. Targeting these mediators is therefore an essential tactic for reducing inflammation and averting related diseases [27].

IV.5.2.1. In-vitro anti-inflammatory activity

A protein denaturation essay is one of the in vitro tests that have been used to determine whether a natural or synthetic compound has anti-inflammatory effects because inflammation is one of the principal causes of protein denaturation [28]. Hence, the capacity of a compound to avoid protein denaturation implies that the compound could possess anti-inflammatory properties [29].

The main goal of the egg albumin denaturation analysis is to find out whether specific agents or other compounds can inhibit or slow down the egg albumin denaturation or not under certain conditions. Denaturation is the process in which protein changes its structure and becomes incapable of performing its biological role. [30]. Egg albumin is a model protein whose denaturation can take place when it is exposed to extreme temperatures, pH levels, or other denaturing substances. Through this, the original structure of the egg albumin is disturbed, changing its physical properties and rendering it to lose its functional ability. The egg albumin denaturation assay is a method used to determine the ability of a drug or a compound to inhibit or diminish the denaturation of egg albumin to help determine its anti-inflammatory properties [31]. The egg albumin denaturation technical is promised on the concept that anti-inflammatory-active substances have the ability to stabilize proteins and prevent the denaturation process, which is usually linked to inflammation, and tissue

destruction.thus agents or compounds that have a strong ability to lower the denaturation of egg albumin may have anti-inflammatory properties [32].

The results of anti-inflammatory screening of the **Bis-AcPh** and the standard Aspirin drug by employing egg albumin denaturation test are presented in **Figure 41**. Our finding showed that the inhibition rate of denaturation increase with increasing the concentration of both the **Bis-AcPh** and the Aspirin from 0.3125 mg/mL to 2.5 mg/ml. At the concentrations (0.3125and 0.625 mg/ml) Bis-AcPh showed a relatively weaker anti-inflammatory activity with inhibition values almost two-fold less than aspirin. This indicates that the compound at lower concentrations is less affinity or less efficacious in stabilizing proteins against thermal denaturation. However, with the increase of concentration, the compound exhibited a significant enhancement of activity; the inhibition reached 50% at 1.25mg/ml, and at the maximum test concentration (2.5mg/ml) Bis-AcPh gave an inhibition rate of 95.16±4.47% which was statistically similar ($p > 0.05$) to the value 99.58±0.06% given by aspirin.

These finding suggested that Bis-AcPh has an anti-inflammatory effect that depends on it concentration, and at higher concentrations it is as effective as Aspirin one of the strongest standard anti-inflammatory drugs. The experiments were performed in quadruplicate determinations and the results are depicted as mean ± standard error of the mean (SEM).

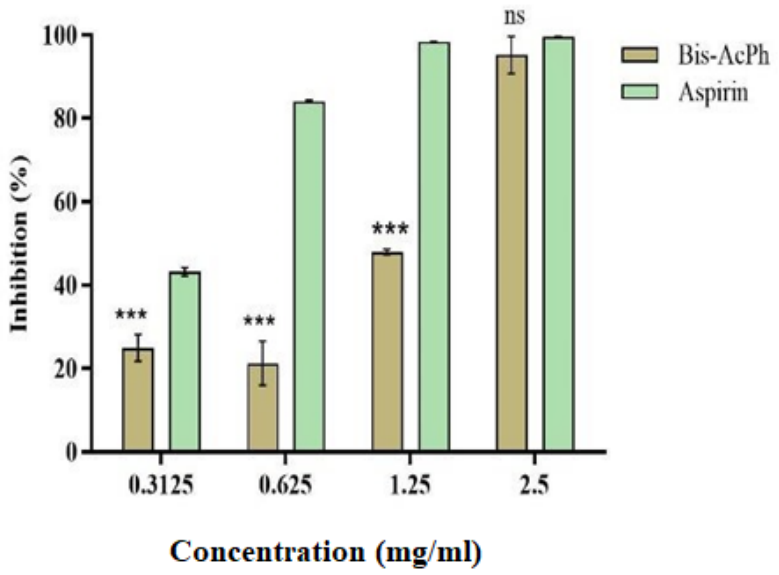

Figure 41. Protein denaturation assay of Bis-AcPh and the Aspirin drug, based on the compounds concentrations. Data are stated as the mean ±SD (n=4), ns: no significant difference $p > 0.05$, ***: $p< 0.001$ in comparison with Aspirin.

IV.5.2.2. In vivo anti-inflammatory activity

c) Xylene-induced ear edema

Xylene-induced ear edema in mice is a commonly used experimental model for acute inflammation. It is reproducible and provides good predictive results for the screening of anti-inflammatory agents [33].This model can trigger vasodilation, increase blood vessel permeability, and ultimately lead to edema. The way xylene causes inflammation is tied to sensory neurons that are sensitive to capsaicin. When these neurons are activated, they release various mediators that kickstart the inflammatory response, a process referred to as neurogenic inflammation [34].

The percentage inhibition of ear edema in mice treated with **Bis-AcPh** and indomethacin is shown in **Figure 42**. It was indicated by the results that a higher anti-inflammatory effect was exhibited by Bis-AcPh as the rate of inhibition was 86.15 ± 2.66% compared to indomethacin that showed a slight lower effect with an inhibition rate of 81.54 ± 6.52%.This significant finding suggests that Bis-AcPh has the potential to be an effective anti-inflammatory agent.

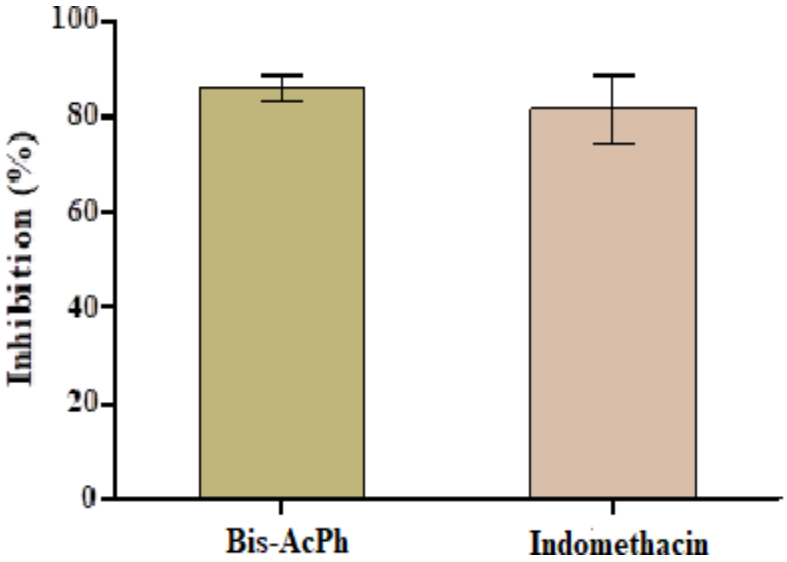

Figure 42. Inhibition percent of xylene-induced ear edema of Bis-AcPh and Indomethacin in mice after of treatment. Values are illustrated at the mean ± SEM (n = 6).

d) Croton oil-induced ear edema

Croton oil induction of ear edema is an in vivo assay commonly applied in the study of topical acute anti-inflammatory effect. The test is easy, effective, sensitive and fast and requires only few compounds in order to conduct assays. This model also has been widely utilized in screening and

testing of new candidate compounds with anti-inflammatory properties and may be applicable in treating inflammatory skin-related infections [35].

The major irritant in croton oil, 12-O-tetradecanoylphorbol-13-acetate (TPA), activates protein kinase C. This, in turn, induces the release of pro-inflammatory cytokines and other mediators, including arachidonic acid and phospholipase A2 [36], which leads to the release of platelet activation factor. This series of events triggers an increase in vascular permeability and vasodilation, the migration of polymorphonuclear leukocytes and the release of histamine and serotonin, as well as moderate synthesis of inflammatory eicosanoids in response to the irritation produced by the chemical [37].

Similarly, under the croton oil induced model (**Figure 43**), **Bis-AcPh** at a dose of 2mg/ml exhibited an inhibition rate of 71.28% ± 5.47, whereas indomethacin the positive control at a concentration of 0.5 mg had a less rate of inhibition (61.02% ± 3.42). The findings obtained suggest that Bis-AcPh exhibits potent, reproducible anti-inflammatory effect that is more effective than the reference drug indomethacin in the acute and irritant-induced ear edema.

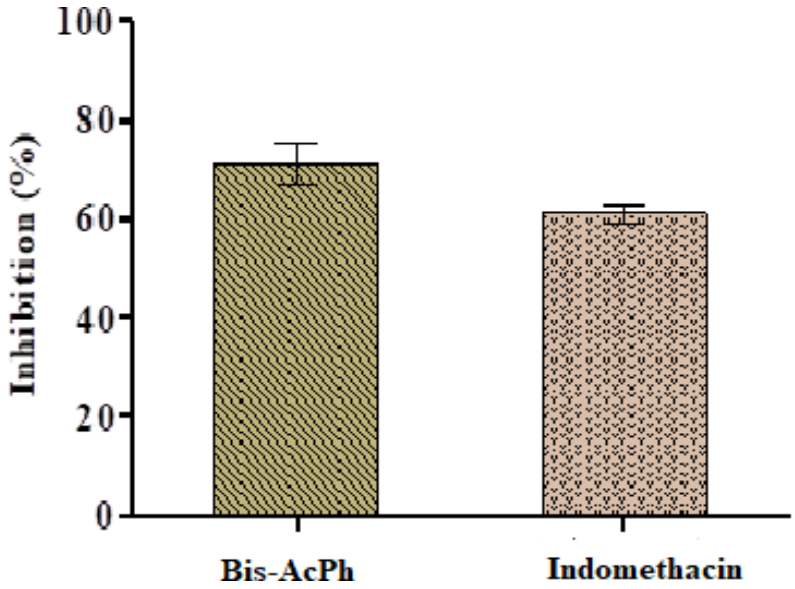

Figure 43. Inhibition percent of croton oil-induced ear edema of Bis-AcPh and Indometacin after 6 h of treatment. Data are presented at the mean ± SEM (n = 6).

Based on *in vitro* and *in vivo* anti-inflammatory studies, **Bis-AcPh** exhibited good anti-inflammatory activity, which may be caused by the structural features of the compound that allows it to interact with the biological mechanism responsible for the inflammation.

The phenoxy groups in the Bis-AcPh molecule may interact with the enzymes and proteins responsible for regulating inflammation. Enyu R. et al. [38] indicated that the phenoxy moiety

plays a major role in the anti-inflammatory activity of the compounds. They explored the molecule [2-(4-acetylphenoxy)-9,10-dimethoxy-6,7-dihydropyrimido[6,1-a]isoquinolin-4-one, assigned as EI-03], which was found to decrease levels of TNF-α, a molecule that usually fuels inflammation, while boosting levels of IL-10, a molecule that reduces inflammation. This suggests that EI-03 could be an effective treatment for autoimmune diseases, as it reduces inflammation and restores immune system balance. Various studies have analysed several bis-ketone compounds, particularly α,β-unsaturated carbonyl groups, in relation to their anti-inflammatory potential. These compounds, such as curcumin, chalcones and other β-diketones, exhibit strong biological activity, including anti-inflammatory properties, and the carbonyl group is evidently crucial to these activities [39].

IV.5.3. Cytotoxicity against red blood cells

Erythrocytes, the simplest structure of red blood cells (RBCs) makes them an ideal subject for studying the effects of drugs on cell membranes. These cells don't have any special parts, and the cytoskeleton is basically just a spectrin net underneath the lipid bilayer. They are also be easily isolated in large amounts as a pure monoculture. Hemolysis may be caused by certain concentrations of surfactants, like saponins, and other drugs that have that have been shown membrane active drugs. The major proposed mechanisms of this are hemolysis via lipid solubilization and hemolysis following the development of pore and large membrane defects. Nevertheless, the predominant mechanism of hemolysis is determined by the type and concentration of the applied surfactant [40].

When cells are treated with a cytotoxic compound, it can lead to a range of health issues, including a loss of membrane integrity and rapid cell death due to hemolysis [41]. Riaz et al. highlight that a good way to assess the effects of different compounds in vitro is by examining the mechanical stability of RBC membranes during cytotoxicity screening. It's also well-known that a compound's hemolytic activity often reflects its overall cytotoxicity towards normal cells. Conversely, the mechanical stability of RBC membranes used as a reliable criterion for evaluating the cytotoxic effects of different compounds in vitro. In this regard, many healthy individuals may experience adverse effects at sufficiently high concentrations of hemolytic drugs. Thus, it is essential to explore how toxic substances affects the hemolytic activity of RBCs [42].

The cytotoxicity and the biocompatibility of the synthetic molecule Bis-AcPh was investigated by the hemolytic test against RBCs. The rate of hemolysis was calculated by

comparing the absorbance of the molecule to the absorbance of the reference surfactant TritonX-100 which showed 100% hemolysis.

The figure 44 shows how the hemolysis of human red blood cells (RBCs) is affected by varying doses of Bis-AcPh (0.25, 0.5, 1.0, and 2.0 mg/mL), compared to the positive control Triton X-100. According to the findings, the rate at which the red blood cells were hemolysed by Bis-AcPh was between 0.42%, and 1.00% for all the tested concentrations compared to the TritonX-100. The results clearly demonstrate that Triton X-100 induced complete hemolysis (approximately 100%), which is consistent with its strong amphiphilic nature, enabling it to integrate into and disrupt erythrocyte membranes. In contrast, Bis-AcPh caused negligible hemolysis at all concentrations tested, with levels consistently below 2%, which is statistically significant; the p value was less than 0.001 in comparison with Triton X-100.

All drug delivery systems that enter into the bloodstreams react with red blood cells (RBCs). The hemolysis caused by the in vitro erythrocytes is considered a simple and reliable procedure to estimate the compatibility of the Bis-AcPh with the blood. The results of the hemolytic assay experience show that at relatively high (up to 2 mg/ml) concentrations, our compound did not show useful hemolytic action on human electrolytes (RBCs). In in vitro investigations, the stage of hemolysis might be classified as 'not significant' when it varies within the range of 5 to 25% [43]. In our investigations, we found that the hemolytic degree of Bis-AcPh was less than 2% within the examined concentration range. Hence, the Bis-AcPh did not have any visible hemolytic effect on human red blood. Moreover, this is a good indicator of biocompatibility of this molecule.

It is clear from the negligible hemolytic activity of Bis-AcPh that there is no significant interaction or destabilization of RBC membranes. The reason for this is that our compound has limited water solubility and low amphiphilicity, which means it cannot penetrate and perturb the phospholipid bilayer as well as Triton X-100 can. The partition coefficient (Log P) is a measure of a molecule's affinity for either the lipid or aqueous phase. The theoretical prediction in the next part, show that Bis-AcPh has a Log P value of 2.73, which means it is moderately lipophilic, and log S value was -3.51 which mean that it has very low aqueous solubility. According to these physicochemical characteristics, Bis-AcPh lacks the ideal amphiphilic ratio required to break through and damage lipid membranes. Specifically, its moderate lipophilicity prevents it from entirely integrating into membrane lipid bi-layers, while its low hydrophilicity restricts its capacity to diffuse in aqueous settings.

Hemolytic compounds have a propensity to be lipophilic and amphiphilic, a property which serves to enhance their capacity to disrupt cell membranes [44]. Triton X-100 is amphiphilic, enabling it to dissolve well in water-based solutions, as well as in aqueous and hydrophobic solutions. However, our compound Bis-AcPh is less soluble in water and does not reach the level of amphiphilicity needed to break membranes. It may lack the appropriate hydrophilic balance required to break cell membranes in the same way as Triton X-100.

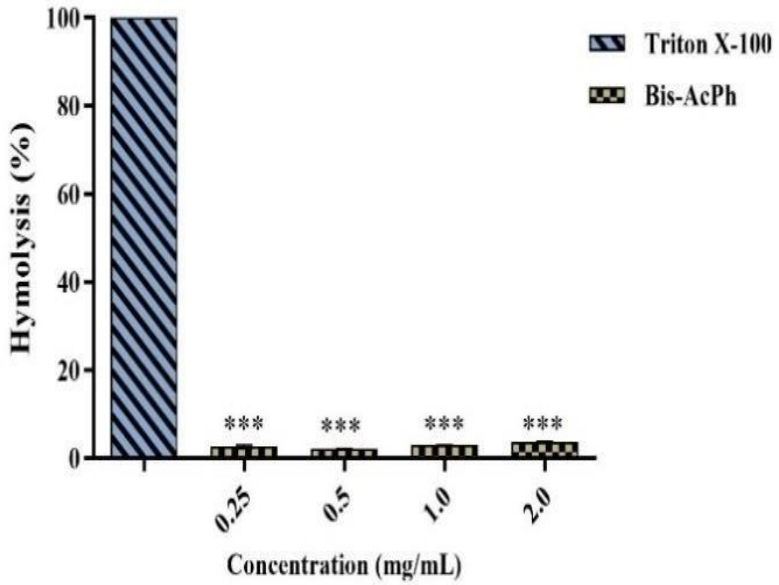

Figure 44. The hemolysis rate induced on humain red blood cells by various dose of Bis-AcPh. The values are represented as the mean ± SD, based on (n = 3), *** indicates $p < 0.001$ in comparison to Triton X-100.

Some examples of bis-ketones, such as curcumin and its derivatives, have been studied because of their low cytotoxicity. Research has assessed the difference in hemolytic activity between curcumin and nanocurcumin, demonstrating that nanocurcumin evidently has a slightly higher affinity for red blood cells (RBCs) than curcumin. However, even in this state, the level of hemolysis remains extremely low and there is no significant difference in the hemolytic levels of nanocurcumin and parent curcumin [45].

IV.5.4. Antibacterial activity

IV.5.4.1. Results of Disk diffusion assay

The disk diffusion test is the most commonly used method in most institutions and hospitals for assessing the antimicrobial properties of substances [46]. It has several advantages over other methods: it is simple, cost-effective, can handle a large number of microorganisms and

antimicrobial agents, and the results are easy to interpret [47].The sensitivity or resistance of the bacterial strains to the tested compound can be determined by measuring the diameter of the inhibition zones. Table below summarizes the results obtained.

Based on the classification suggested by Billerbeck [48], the antimicrobial activity may be explained as follows:

- Strong: When the diameter of the inhibition zone exceeds 13 mm (sensitive strain);
- Moderate: When the range of the diameter is between 6mm and 13mm (intermediate strain);
- Weak or no activity: When the diameter of the inhibition zone is less than or equal to 6 mm (resistant strain).

Antibacterial effect of 1,3-Bis(2-acetylphenoxy)-2-propanol was initially tested using the disk diffusion technique using representative standard strains of Gram-positive and Gram-negative bacteria on nutrient agar media, as listed on table . The tested compound was solubilized in dimethylsulfoxide, and the data are compared with the standard antibiotic Gentamicin. The compound showed quantifiable zones of inhibition against all the strains tested with significant variation between Gram (+) and Gram (-) bacteria (**Table 3**).

Table 3. Diameters of inhibition zones (mm) of bacteria growth induced by Bis-AcPh and by the antibiotic Gentamicin.

Diameter of inhibition (mm)					
Strain	Replicate 1	Replicate 1	Replicate 1	Mean SD (mm)	Gentamicin
Staphylococcus aureus	18	18	19	18.3 ± 0.6	25
Bacillus cereus	8	11	11	10.0 ± 1.7	26
Escherichia coli	9	7	6	7.3 ± 1.5	24
Salmonella Enteritidis	7	8	6	7.0 ± 1.0	24

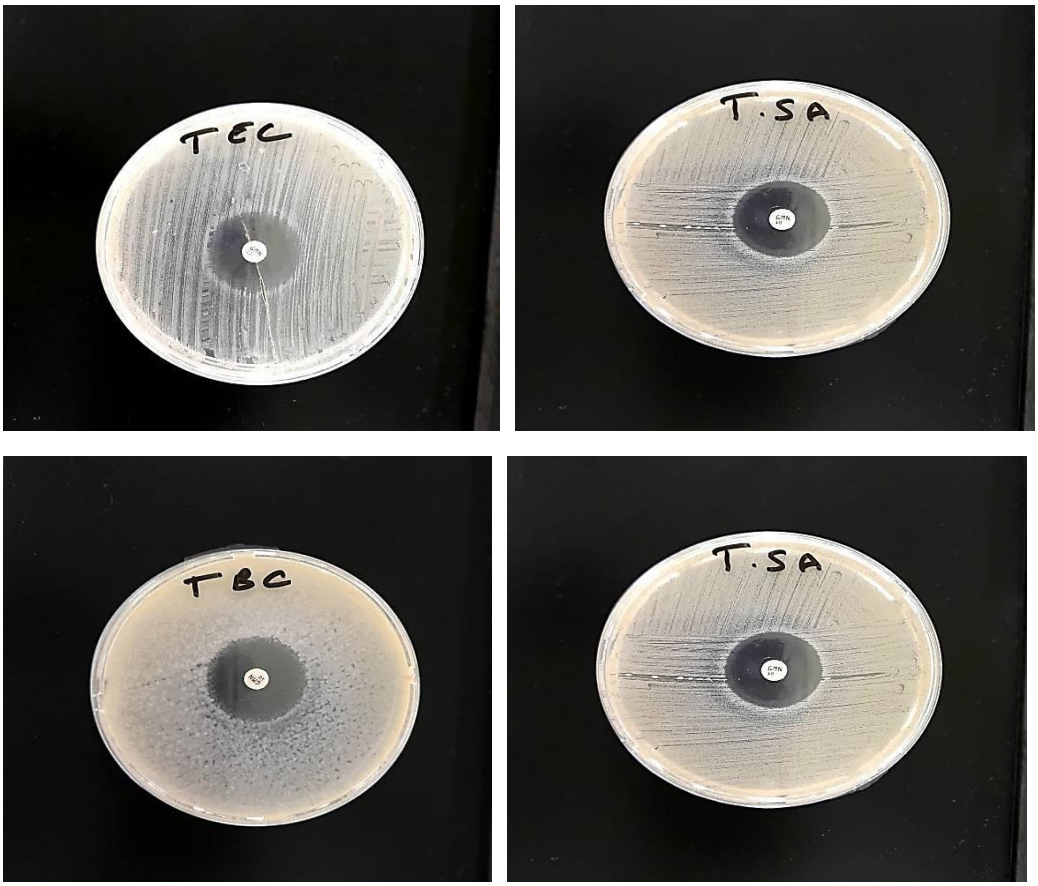

Figure 45. Results of the antibacterial test of the positive control Gentamicin against Escherichia coli (TEC), Salmonella Enteritidis (TSE), Bacillus cereus (TBC), and Staphylococcus aureus (TSE).

The results in the **table 3** revealed that the most extensive inhibition zone was observed for *Staphylococcus aureus* (18.3 mm) followed by B. cereus (10.0 mm). Conversely, *Escherichia coli* and *Salmonella Enteritidis* exhibited lower sensitivity, with inhibition zones measuring 7.3 mm and 7.0 mm, respectively.

The finding revealed that Bis-AcPh exhibited variable antibacterial effects. These effects depended on the bacterial species tested. *Staphylococcus aureus* showed the biggest inhibition zone, which suggest a high degree of sensitivity. Such intense activity can be attributed to the Gram-positive nature of *S. aureus* that does not have the outer membrane barrier as Gram-negative bacteria have. Consequently, **Bis-AcPh**, is able to penetrate the thick yet permeable peptidoglycan layer more effectively. *Bacillus cereus* was the second most sensitive strain indicating a moderate antibacterial action. Even though also Gram-positive, *B. cereus* is reported to have sporing capacity and structural modifications that could decrease permeability to compounds or target availability, which could contribute to its decreased sensitivity relative to *S. aureus*. Conversely, Gram-negative bacteria such as *Escherichia coli* and *Salmonella enterica* had much smaller inhibition. The weak

activity against these strains is in line with the intrinsic resistance mechanisms of Gram-negative organisms.

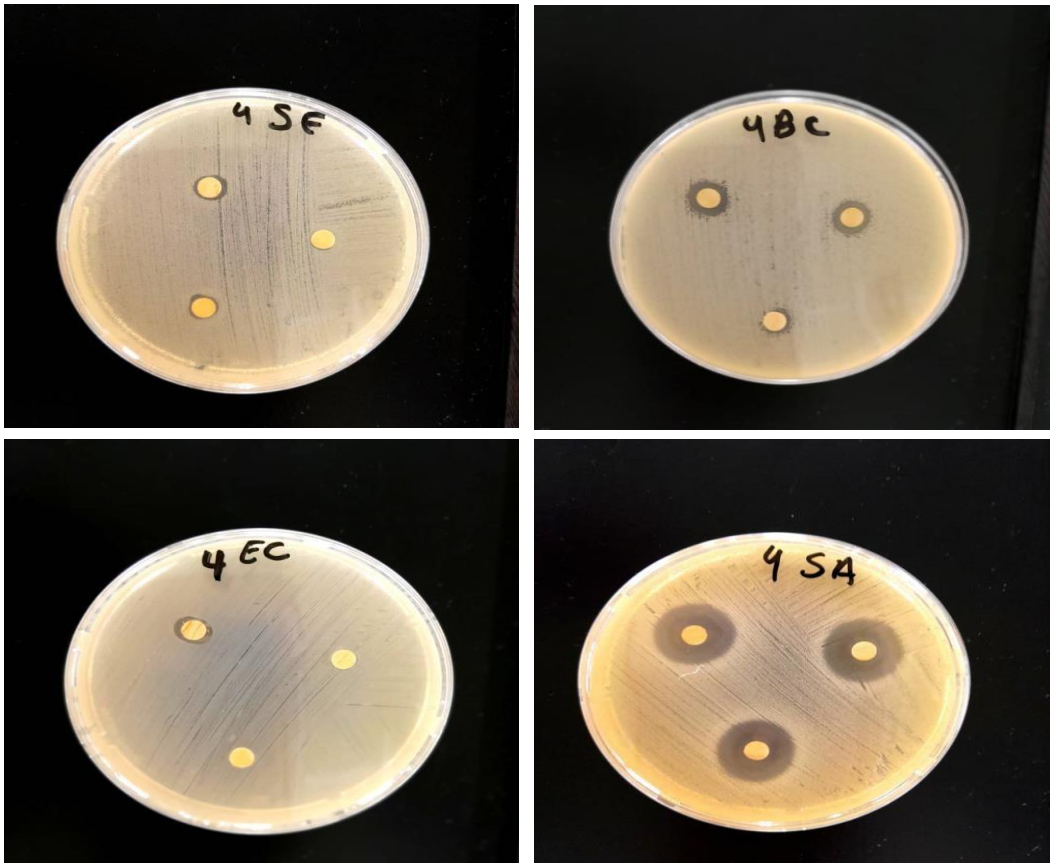

Figure 46. Results of the antibacterial test of Bis-AcPh against Escherichia coli (EC), Salmonella Enteritidis (SE), Bacillus cereus (BC), and Staphylococcus aureus (SA).

IV.5.4.2. Results of Minimum Inhibitory Concentration (MIC) assay

To give a quantitative assessment of antibacterial potency, broth micro dilution was used to determine MIC values of **Bis-AcPh**; the values are presented in the **table 4.**

Our compound significantly inhibits Gram-positive bacteria, as evidenced by the fact that it had the lowest values of MIC on *Bacillus cereus* (0.3125mg/mL) and *Staphylococcus* aureus (0,625mg/mL). Conversely, the highest MICs were observed in *Salmonella enterica* (5 mg/mL) and *Escherichia coli* (1,25 mg/mL), which indicates a reduced susceptibility of Gram-negative organisms. Most of studies on the minimal inhibitory concentration (MIC) estimated that MIC values range for antibiotic is 0.01-10 µg/ml, whereas plant extracts are considered antimicrobials if their MICs are between 100-1000 µg/ml [49].

Table 4. MIC values of Bis-AcPh.

Bacterial strain	Replicate 1 (mg/ml)	Replicate 2 (mg/ml)	**MIC of Bis-AcPh (mg/ml)**
Staphylococcus aureus	0,625	0,625	0,625
Bacillus cereus	0,3125	0,3125	0,3125
Escherichia coli	1,25	1,25	1,25
Salmonella Enteritidis	5	5	5

The resulting of gentamicin assay given in **table 5** are references points to know the antibacterial activity of our compound. From the table, gentamicin was found to be highly active against all the strains tested with MICs varied from 0.01 mg/mL against *Staphylococcus aureus* the gram (+) strain to 0,05 mg/mL against *Escherichia coli* the gram (-) strain. Our compound **Bis-AcPh** marked higher MICs than gentamicin, indicating that it is generally less powerful against the tested bacterial strain compared to the positive control gentamicin. However, it still shows significant antibacterial activity, especially against Gram-positive strains. This makes it a promising candidate for developing future antibacterial drugs.

DFT computations showed that **Bis-AcPh** has a large HOMO LUMO energy gap (7.11 eV), high global hardness (3.55 eV) and low electrophilicity (0.82 eV), implying a stable and low-reactivity molecule. A moderate value of the dipole moment (1.58 D) indicates balance polarity, which contributes to the activity because of the ability to interact with lipid-rich membrane of Gram- positive bacteria that is why the strong activity is achieved with *Staphylococcus aureus* and *Bacillus cereus*. Conversely, its limited polarity and comparatively high hardness may diminish penetration into the outer membrane of Gram-negative bacteria, causing less powerful inhibition of *Escherichia coli* and *Salmonella enterica*. According to these results, the antibacterial effect of the compound is primarily due to non-covalent interactions including hydrophobic contacts and hydrogen bonding and not due to covalent bond with bacterial enzyme

Table 5. MIC values of Gentamicin [50].

Bacterial strain	Replicate 1 (mg/ml)	Replicate 2 (mg/ml)	MIC of Gentamicin (mg/ml)
Staphylococcus aureus	0.01	0.01	0.01
Bacillus cereus	0.02	0.02	0.02
Escherichia coli	0,05	0,05	0,05
Salmonella Enteritidis	-	-	-

Serafim et al. [51] have studied the antibacterial properties of a family of synthetic 1,3-bis(aryloxy)propan-2-amines against a number of Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA). The compounds were found to have potent antibacterial properties with MICs of 2.5-10 µg/mL and MBCs near MICs. Molecular docking experiments found that these molecules are structurally similar to inhibitors of essential bacterial targets, including FtsZ, NorA, and FabI. These findings indicate that these derivatives have potential to develop new antibacterial agents especially against multidrug resistant gram-positive strains.

Sivakumar et al [52] tested the antibacterial activity of 20 acetophenone compounds against Gram-positive (*Bacillus subtilis*, *Staphylococcus aureus*) and Gram-negative (Salmonella typhi, Enterobacter aerogenes, Proteus vulgaris) bacteria. They have found that some compounds, especially those with a 4-methyl, 2-hydroxy, 3-bromo, 4-ethoxy, 3- nitro or 4-nitro substituent were the most active. In order to gain a clearer idea of the reasons behind the differences in activity between different compounds, the authors applied an approach called Quantitative Structure-Activity Relationship (QSAR). They discovered that antibacterial activity was highly dependent on the shape of the molecules, on their electronic characteristics and on their chemical structure.

IV.6. Molecular Docking Studies

The molecular docking analysis provided insight into the binding behavior of the synthesized compound Bis-AcPh compared to three reference anti-inflammatory drugs Aspirin, Indomethacin and Diclofenac within the active site of the COX-2 enzyme (PDB ID: 1PXX). Bis-AcPh exhibited the strongest binding affinity with a docking score of -10.568 kcal/mol, followed closely by Indomethacin (-10.102 kcal/mol) and Diclofenac (-10.059 kcal/mol),

while Aspirin showed the lowest affinity (-6.839 kcal/mol) (**Table 6**). This higher affinity of Bis-AcPh is supported by its formation of strong hydrogen bonds with catalytically critical residues Tyr355, Tyr385, and Ser350 (**Figure 47**) at short distances ranging from 1.3 to 1.8 Å, indicating high specificity and optimal orientation within the binding pocket. These interactions are particularly significant, as Tyr355 and Tyr385 are known to play key roles in ligand stabilization and COX-2 enzymatic function. Additionally, Bis-AcPh established several hydrophobic interactions with Val349, Leu359, Trp387, and Leu531 within distances of 2.3 to 3.8 Å, enhancing the compound's stabilization deep within the hydrophobic core of the active site. In comparison, Aspirin formed fewer and weaker interactions, both hydrogen bonds and hydrophobic contacts, which were located at relatively longer distances (up to 5.2 Å), suggesting a looser and less effective binding mode. Although Indomethacin and Diclofenac displayed similar binding energies to Bis-AcPh, their interaction patterns involved slightly longer hydrogen bond distances and broader, less compact hydrophobic interactions, which may result in lower specificity. The interaction profile of Bis-AcPh, particularly its engagement with residues like Trp387 and Leu531 that contribute to COX-2 selectivity, indicates a strong potential for selective COX-2 inhibition. This observation aligns well with the compound’s confirmed anti-inflammatory activity observed in both *in vitro* and *in vivo* assays, where it outperformed Aspirin and matched or surpassed the effects of Indomethacin. Overall, Bis-AcPh’s favorable combination of strong binding energy, critical hydrogen bonding, and optimal hydrophobic interactions supports its potential as a novel and effective COX-2 inhibitor, offering a promising therapeutic profile with potentially improved selectivity and safety.

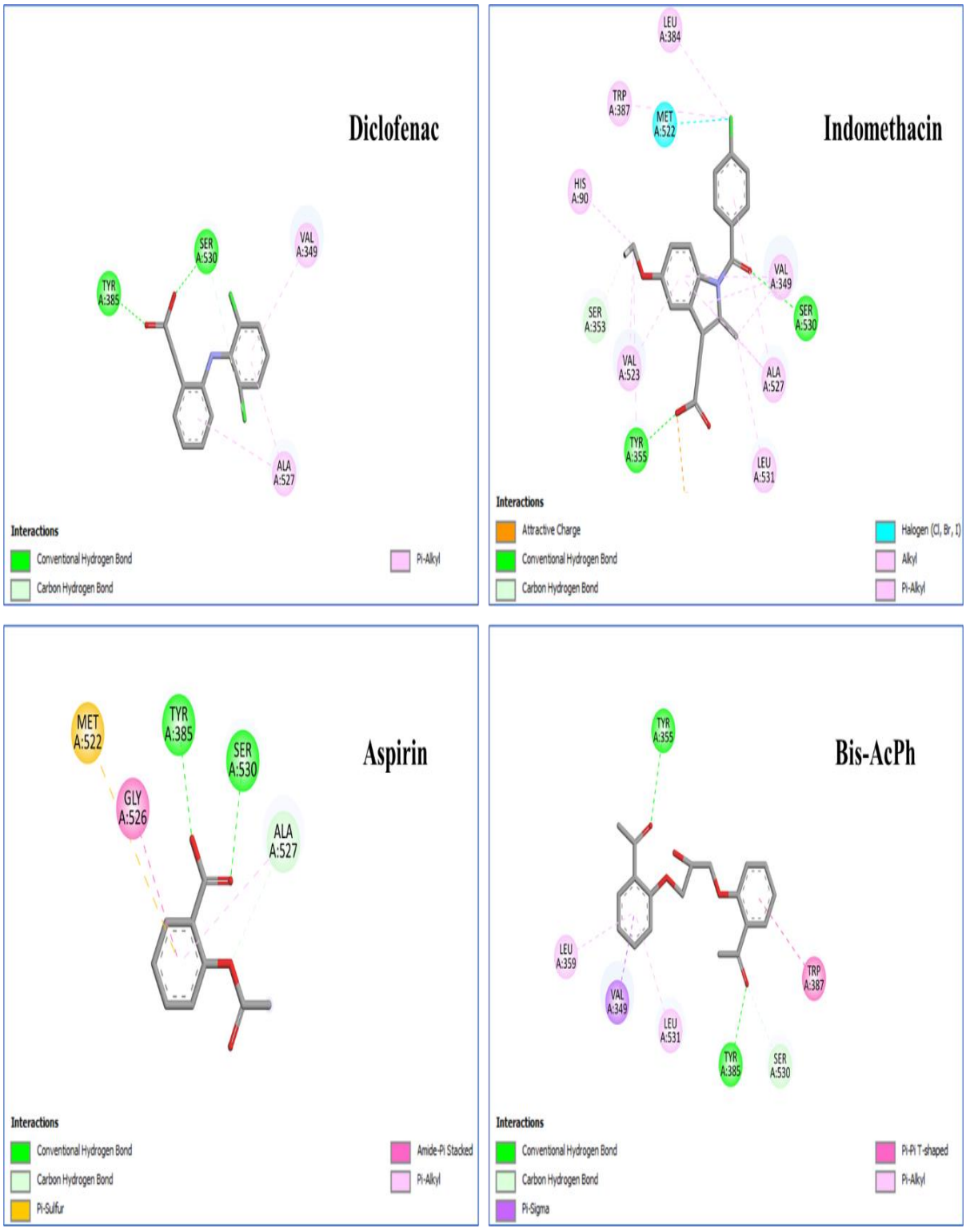

Figure 47. Binding interactions of Bis-AcPh and reference compounds (Aspirin, Indomethacin, and Diclofenac) within the active site of COX-2 (PDB ID: 1PXX).

Table 6. Molecular docking results of Bis-AcPh and reference compounds (Aspirin, Indomethacin, Diclofenac) against COX-2, showing binding affinities, hydrogen bonding, and hydrophobic interactions.

Molecules	ΔG (kcal/mol)	Category			
		H-bonds	Distance (Å)	Hydrophobic	Distance (Å)
Bis-AcPh	-10.568	Tyr355, Tyr385, Ser350	[1.3 – 1.8]	Val349, Leu359 Trp387, Leu531	[2.3 – 3.8]
Aspirin	-6.839	Tyr385, Ser350, Ala527	[1.8 – 2.8]	Gly526, Ala527	[4.0 – 5.2]
Indomethacin	-10.102	Tyr355, Ser530, Ser353	[2.0 – 2.6]	His90, Val349, Ser353, Val532, Ala527, Leu531	[4.2 – 5.9]
Diclofenac	-10.059	Tyr385, Ser350	[2.0 – 2.5]	Val349, Ala527	[4.3 – 5.5]

IV.7. ADMET Study

The ADME (Absorption, Distribution, Metabolism, and Excretion) profiling of the synthesized compound Bis-AcPh compared to the reference anti-inflammatory drugs Aspirin, Indomethacin, and Diclofenac reveals several pharmacokinetic characteristics relevant to drug-likeness and oral bioavailability. All the four compounds demonstrated high gastrointestinal (GI) absorption, and are not substrates for P-glycoprotein (P-gp); indicating favorable passive absorption and limited active efflux, which are advantageous for oral drugs. Bis-AcPh showed a moderate lipophilicity value with a Log P of 2.73, which is within the optimal range for oral bioavailability and membrane permeability. This value is higher than Aspirin (1.28) but lower than Indomethacin (3.63) and Diclofenac (3.66), suggesting a balanced hydrophilic-lipophilic profile. In terms of aqueous solubility (Log S, ESOL model), Bis-AcPh demonstrated moderate solubility (-3.51), which is lower than Aspirin (-1.85) but significantly better than Indomethacin (-4.86) and Diclofenac (-4.65), indicating acceptable solubility for formulation development. Regarding metabolism, Bis-AcPh and Aspirin were predicted to be substrates or inhibitors of cytochrome P450 (CYP450) enzymes, whereas

Indomethacin and Diclofenac were not. This may imply a potential for drug–drug interactions in the case of Bis-AcPh, which warrants further metabolic profiling. All molecules fulfilled Lipinski's rule of five, confirming their drug-like properties. However, Bis-AcPh had a lower predicted bioavailability score (0.55) compared to the three references (0.85) (**Table 7**), possibly due to its molecular structure or interaction with metabolic enzymes. Notably, Bis-AcPh showed exceptional synthetic accessibility (score = 0.23), indicating it is considerably easier to synthesize than Aspirin (2.51), Indomethacin (1.52), and Diclofenac (3.20), which is advantageous from a drug development and production standpoint. Altogether, Bis-AcPh displays a favorable ADME profile with good oral absorption, drug-likeness, and manufacturability, supporting its potential as a viable anti-inflammatory drug candidate.

Table 7. Comparative ADME properties of Bis-AcPh and reference anti-inflammatory drugs (Aspirin, Indomethacin, and Diclofenac) predicted using SwissADME.

	Bis-AcPh	Aspirin	Indomethacin	Diclofenac
Log *P*	2.73	1.28	3.63	3.66
Log S (ESOL)	-3.51	-1.85	-4.86	-4.65
GI absorption	High	High	High	High
P-gp substrate	No	No	No	No
CYT P450	Yes	Yes	No	No
Lipinski	Yes	Yes	Yes	Yes
Bioavailability Score	0.55	0.85	0.85	0.85
Synthetic accessibility	0.23	2.51	1.52	3.20

The toxicity profile of Bis-AcPh and the reference drugs (Aspirin, Indomethacin, and Diclofenac) was predicted using the ProTox-III platform, focusing on various toxicological endpoints including hepatotoxicity, carcinogenicity, mutagenicity, immunotoxicity, cytotoxicity, as well as the predicted LD_{50} and toxicity class. Notably, Bis-AcPh was

predicted to be inactive for all tested toxicological endpoints, including hepatotoxicity, cytotoxicity, mutagenicity, carcinogenicity, and immunotoxicity, suggesting a favorable safety profile. In contrast, Diclofenac was predicted to be hepatotoxic and cytotoxic, aligning with its known adverse effects in clinical settings. All compounds except Diclofenac were classified as non-hepatotoxic, and none of the molecules were predicted to be mutagenic, carcinogenic, or immunotoxic. Regarding acute oral toxicity, Bis-AcPh showed a significantly higher LD_{50} value of 10,000 mg/kg, indicating very low acute toxicity and placing it in toxicity class 6 (the safest category). In comparison, Aspirin, Diclofenac, and Indomethacin showed much lower LD_{50} values of 250, 53, and 12 mg/kg, corresponding to toxicity classes 3, 3, and 2, respectively. These results highlight the superior predicted safety of Bis-AcPh, making it a promising candidate for further preclinical development as a safer alternative to conventional NSAIDs (**Table 8**).

In summary, Bis-AcPh exhibited the strongest binding affinity to COX-2 among all tested compounds, with favorable interactions at the active site. ADME predictions confirmed its good oral absorption, drug-likeness, and synthetic accessibility. Moreover, toxicity profiling revealed a safe profile with no hepatotoxicity or cytotoxicity and a high LD_{50} value. These findings suggest that Bis-AcPh is a promising and safe anti-inflammatory candidate for further investigation.

Table 8. In silico toxicity prediction of Bis-AcPh and reference compounds (Aspirin, Indomethacin, and Diclofenac) using ProTox-III.

	Bis-AcPh	Aspirin	Indomethacin	Diclofenac
Hepatotoxicity	Inactive	Inactive	Inactive	Active
Carcinogenicity	Inactive	Inactive	Inactive	Inactive
Mutagenicity	Inactive	Inactive	Inactive	Inactive
immunotoxicity	Inactive	Inactive	Inactive	Inactive
Cytotoxicity	Inactive	Inactive	Inactive	Active
LD_{50} (mg/kg)	10000	250	12	53
Class	6	3	2	3

IV.8. Molecular Dynamics Simulation (MDS)

The RMSD profile revealed that the Bis-AcPh_COX-2 complex (average RMSD = 0.197 nm) exhibited greater structural stability throughout the simulation compared to the Aspirin_COX-2 complex (average RMSD = 0.253 nm) (**Table 9)**. The lower RMSD value for Bis-AcPh indicates smaller deviations from the initial structure, reflecting a more rigid and well-maintained binding conformation. Moreover, the RMSD trajectory for Bis-AcPh displayed a rapid equilibration phase followed by stable fluctuations within a narrow range, whereas Aspirin showed slightly larger oscillations, suggesting higher conformational flexibility. These findings imply that Bis-AcPh achieves a more consistent and stable interaction with the COX-2 binding site, potentially enhancing its inhibitory performance.

The RMSF analysis showed that both complexes exhibited relatively low residue-level fluctuations, indicating overall structural stability. However, the Bis-AcPh_COX-2 complex displayed a slightly higher average RMSF value (0.147 nm) compared to the Aspirin_COX-2 complex (0.130 nm), suggesting modestly increased flexibility in certain localized regions of the protein. These fluctuations were primarily observed in loop segments near the binding site, which may reflect an induced-fit adaptation upon Bis-AcPh binding. In contrast, the lower RMSF values in the Aspirin complex indicate a more rigid binding environment. The slight increase in flexibility with Bis-AcPh could facilitate better conformational accommodation and potentially optimize ligand–protein interactions without compromising global stability.

The radius of gyration (Rg) analysis indicated that the COX-2 protein maintained a compact and stable conformation in both ligand-bound systems throughout the simulation. The Bis-AcPh_COX-2 complex exhibited an average Rg value of 2.434 nm, which was slightly lower than that of the Aspirin_COX-2 complex (2.453 nm). This subtle decrease suggests a marginally more compact protein structure upon Bis-AcPh binding, potentially resulting from tighter ligand-induced packing within the binding pocket. The stable Rg trajectories for both complexes confirm the absence of significant unfolding events, while the slightly reduced Rg for Bis-AcPh implies that its binding may promote a more consolidated protein structure, which is consistent with the lower RMSD values observed.

Average SASA for the COX-2_Bis-AcPh complex (250.380 nm^2) was slightly lower than for the COX-2_Aspirin complex (255.214 nm^2). This reduction in solvent-exposed surface area (4.8 nm^2) indicates that Bis-AcPh binding produces marginally greater burial of protein

surface consistent with tighter ligand packing or partial closure of the binding pocket. When interpreted alongside the lower RMSD and slightly reduced Rg observed for Bis-AcPh, the SASA decrease supports a model in which Bis-AcPh promotes a more compact and less solvent-exposed active-site environment, which can favor hydrophobic contacts and entropically favorable desolvation contributions to binding.

Molecular dynamics analyses revealed that the Bis-AcPh_COX-2 complex exhibited superior overall stability compared to the Aspirin_COX-2 complex, as evidenced by its lower RMSD value (0.197 nm vs. 0.253 nm). RMSF profiles showed slightly higher local flexibility for Bis-AcPh (0.147 nm) than for Aspirin (0.130 nm) (**Figure 48**), mainly in loop regions near the active site, suggesting a potential induced-fit adaptation. The Rg analysis indicated a marginally more compact protein conformation upon Bis-AcPh binding (2.434 nm vs. 2.453 nm), consistent with tighter ligand-induced packing. This was further supported by SASA results, where Bis-AcPh binding reduced the solvent-exposed surface area (250.380 nm^2, 255.214 nm^2), implying a more buried and hydrophobically stabilized binding pocket. Collectively, these findings suggest that Bis-AcPh binding promotes a stable, compact, and well-adapted COX-2 conformation, which may enhance binding affinity compared to Aspirin based on RMSD, RMSF, Rg and SASA.

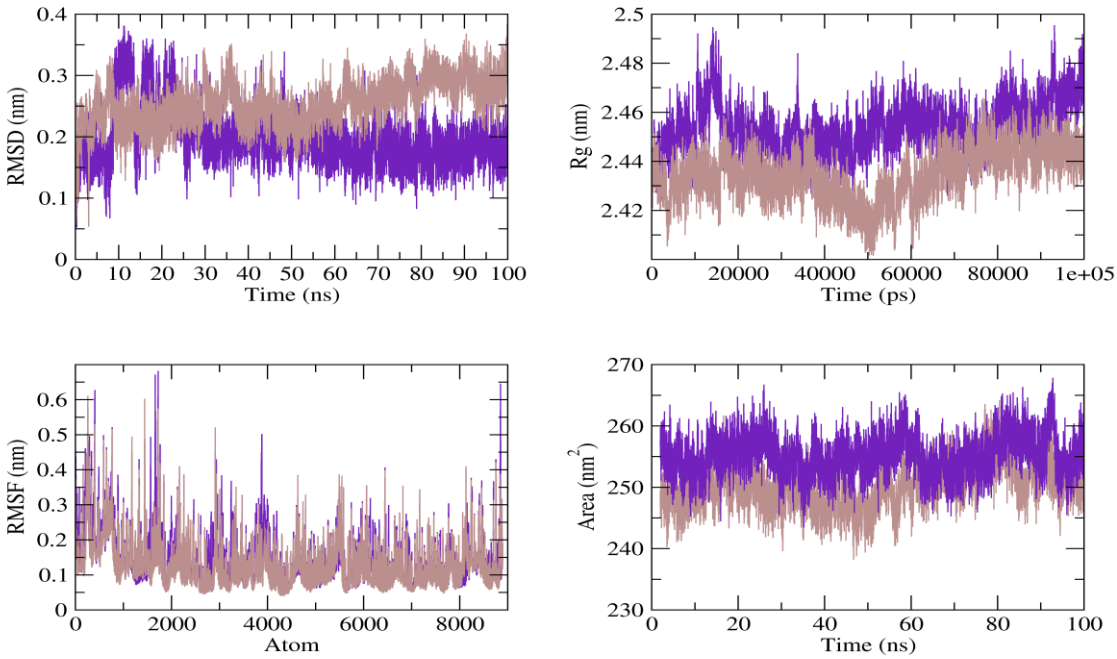

Figure 48. Superimposed molecular dynamics trajectories of COX-2 complexes with Bis-AcPh (brown) and Aspirin (indigo) showing structural stability over the simulation period.

Table 9. Comparative molecular dynamics analysis of COX-2 complexes with aspirin and Bis-AcPh based on RMSD, RMSF, Rg and SASA.

	Aspirin_COX-2	Bis-AcPh_COX-2
RMSD (nm)	0.253	0.197
RMSF (nm)	0.130	0.147
Rg (nm)	2.453	0.434
SASA (nm^2)	255.214	250.380

IV.9. MMPBSA Calculation

The MMPBSA calculations reveal that the Bis-AcPh_COX-2 complex exhibits a more favorable binding free energy compared to the Aspirin_COX-2 complex, as indicated by a more negative ΔTOTAL value (-20.51 kcal/mol, -8.07 kcal/mol). This suggests that Bis-AcPh binds more strongly to COX-2 (**Table 10**).

In the gas-phase interaction terms, both the van der Waals ($\Delta^{VDWAALS}$) and electrostatic energy (Δ^{EEL}) contributions were substantially more favorable for Bis-AcPh, with $\Delta^{VDWAALS}$ being nearly twice as strong (-43.27 kcal/mol) compared to Aspirin (-22.82 kcal/mol). This highlights the dominant role of hydrophobic and shape-complementarity interactions in stabilizing the Bis-AcPh complex. The electrostatic component (Δ^{EEL}) also favored Bis-AcPh (-15.85 kcal/mol) over Aspirin (-13.62 kcal/mol), albeit to a smaller extent.

Regarding solvation energies, the polar solvation term (Δ^{EPB}) was unfavorable for both ligands, but the penalty was greater for Bis-AcPh (42.75 kcal/mol) than Aspirin (30.93 kcal/mol), possibly due to larger desolvation effects upon binding. Similarly, the non-polar solvation contribution (ΔE^{NPOLAR}) was slightly more favorable for Bis-AcPh (-4.14 kcal/mol) compared to Aspirin (-2.55 kcal/mol).

The net binding free energy (Δ^{TOTAL}) results from the balance between the favorable gas-phase interactions and the unfavorable polar solvation penalties. The notably more negative ΔTOTAL for Bis-AcPh indicates that its stronger van der Waals and electrostatic

interactions more than compensate for the higher polar solvation cost, leading to overall enhanced binding affinity compared to Aspirin.

Table 10. Binding free energy components (MMPBSA, kcal/mol) for COX-2 complexes.

	Aspirin_COX-2	Bis-AcPh_COX-2
$\Delta^{VDWAALS}$	-22.82	-43.27
Δ^{EEL}	-13.62	-15.85
ΔE^{PB}	30.93	42.75
ΔE^{NPOLAR}	-2.55	-4.14
ΔG^{GAS}	-36.44	-59.13
ΔG^{SOLV}	28.37	38.61
Δ^{TOTAL}	-8.07	-20.51

IV.10. References

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Conclusion

The current doctoral project was devoted to designing, synthesizing, and testing the biological and theoretical investications of the bis-ketone analog, 1,3-Bis(2-acetylphenoxy)-2-propanol (Bis-AcPh), which was synthesized by the Williamson ether reaction. IR, UV, ^{1}H-NMR and ^{13}C-NMR analyses of structure were used to elucidate the successful preparation of the target compound.

On one hand, its antioxidant activity measured using the DPPH radical scavenging and reducing power assays was moderate compared to the standard antioxidants the BHT and ascorbic acid.

Conversely, the compound had strong anti-inflammatory properties. In vitro egg albumin-denaturation based assays demonstrated its capacity to prevent protein denaturation with significant effect and in vivo experiments in xylene-induced and croton oil-induced ear edema models further confirmed high-topical anti-inflammatory potential.

Toxicity screening indicated that hemolytic activity of the Bis-AcPh is very low (<2%), which proves that it is biocompatible and safe at the concentrations utilized.

Notably, antibacterial activity was also evident with the compound, with selective activity against Gram-positive strains, in particular Staphylococcus aureus, indicating potential uses as an integrated anti-inflammatory and antimicrobial.

The experimental results were supported and complemented by computational analyses. DFT calculations were used to give insight into the electronic structure of Bis-AcPh, molecular docking studies showed that the binding affinity to the COX-2 enzyme was incredibly high, higher than reference compounds like aspirin.

Predictions of ADMET confirmed that it was a suitable drug-like candidate, with good oral absorption, good pharmacokinetics and no hepatotoxicity or cytotoxicity, which further highlights its safety profile.

Furthermore, molecular dynamics simulations (MDS) revealed that the Bis-AcPh-COX-2 complex had better structural stability than the Aspirin-COX-2 complex and this has indicated that there is consistency between the computational and experimental anti-inflammatory findings.

This study shows that Bis-AcPh does not possess a strong antioxidant activity but rather appears as a powerful, safe and selective anti-inflammatory agent with added antibacterial effects.

Perspectives

The work in this thesis reached various results, and makes many observations to analyze that could be classified for the continuation of the work in short and long term perspectives.

In the short term, we continue the study of some pharmacology properties of this synthesized compound and its derivatives, by focusing on the modification of it structure for example, to improve antioxidant potential.

Prepare a Schiff bases and metals complexes from this molecule as this compound has the ability for the complexion of metals.

In the long term, we use this molecule as a ligand connected to polymeric material for studding its chelating properties against metals for water treatment, and we can use the prepared polymeric support bearing this molecule or its derivatives for the proteins purifications.

ABSTRACT

In this investigation, the bis-ketone 1,3-Bis(2-acetylphenoxy)-2-propanol (Bis-AcPh) was synthesized via the reaction between 1,3-dichloropropanol and 2'-hydroxyacetophenone, and different spectroscopic models, including IR, UV, ^{1}H-NMR, and ^{13}C-NMR, were used to characterize its structure. This bis-ketone was screened for its antioxidant effect by performing DPPH radical scavenging and reducing power methods. In vitro anti-inflammatory activity was estimated using the egg albumin denaturation test, while the topical anti-inflammatory effect was investigated through xylene and croton oil-induced ear edema in mice model. Its antibacterial activity and its cytotoxicity were also tested. On the other hand, some theoretical studies were performed through DFT, molecular docking, ADMET, and MDS predictions. The experimental results demonstrate that the molecule exhibited poor antioxidant activity. Whereas the molecule displayed a potent *in vitro and in vivo* anti-inflammatory activity. It is noteworthy that the hemolytic degree was less than 2%, this indicates a very low cytotoxicity. Also, the compound showed good antibacterial activity against gram (+) bacteria, especially *Staphylococcus aureus*. Regarding the computational study results, the Bis-AcPh exhibited the strongest binding affinity to COX-2 among all tested compounds, with favorable interactions within the active site. This observation aligns well with the compound's confirmed anti-inflammatory activity observed in both *in vitro* and *in vivo* assays. ADMET predictions confirmed its good oral absorption, drug-likeness, and synthetic accessibility. Moreover, the toxicity profiling revealed a safe profile with no hepatotoxicity or cytotoxicity. Finally, Molecular dynamics analyses (MDS) revealed that the Bis-AcPh_COX-2 complex exhibited superior overall stability compared to the Aspirin_COX-2 complex.

Keywords: bis-ketone, antioxidant, anti-inflammatory, DFT calculation, molecular docking, ADMET prediction, Molecular dynamics analyses (MDS).

الـــمـــلـــخـــص:

في هذا البحث، تم تحضير المركب (Bis-AcPh) ثنائي الكيتون 1,3 ثنائي (2-أستيل فينوكسي)-2 بروبانول ,عن طريق التفاعل بين المركب 1,3 ثنائي كلورو بروبانول, والمركب 2-هيدروكسي أسيتوفينون. في حين تم تشخيص بنيته الكيميائية باستخدام تقنيات طيفية مختلفة: IR,UV,NMR(^{1}H,^{13}C). تم تقييم هذا المركب من حيث نشاطه كمضاد للأكسدة باستخدام طريقتي تثبيط جذر DPPH ومن حيث قوة الإختزال. في حين تم تقييم نشاطه المضاد للإلتهاب عن طريق تثبيط تشوه ألبومين البيض, و باستخدام نموذج وذمة الأذن المحفزة بالزيلين وزيت الكروتون في الفئران .إضافة الى ذلك ,تم إختبار نشاطه السمي ونشاطه المضاد للبكتيريا. من ناحية أخرى تم إجراء بعض الدراسات النظرية من خلال النظرية الوظيفية للكثافة, الإلتحام الجزيئي ,والتحليلات الديناميكية الجزيئية. أظهرت النتائج التجريبية أن هذا الجزيء يمتلك نشاطا مضادا للأكسدة أقل بكثير من مضادات الأكسدة المرجعية. عكس ذلك أظهر المركب نشاطا قويا ضد الالتهاب. وتجدر الإشارة إلى أن نسبة التحلل الدموي كانت أقل من 2%,ما يعني سمية منخفضة جدا لهذا المركب. كما أظهر هذا الجزيء نشاطا جيدا ضد بكتيريا المكورات العنقودية الذهبية. فيما يتعلق بنتائج الدراسات الحسابية , أظهر المركب أقوى ألفة ارتباط مع إنزيم COX-2 من بين جميع المركبات المختبرة, مع تفاعلات إيجابية في الموقع النشط للإنزيم. تتوافق هذه النتائج تماما مع النشاط المضاد للإلتهابات المؤكد للمركب، والذي لوحظ في كل من التجارب المخبرية والحيوية. وأكدت تنبؤات ADMET امتصاصه الجيد وتشابهه مع الدواء وسهولة استخدامه في التركيبات الصناعية. علاوة على ذلك، كشف تحليل السمية عن سلامة المركب دون أي سمية كبدية أوخلوية, وأخيرا كشفت تحليلات الديناميكية الجزيئية أن معقد Bis-AcPh_COX-2 أظهر استقرارا كليا مقارنة بمعقد Aspirin_COX-2.

الكلمات المفتاحية: ثنائي الكيتون, مضاد الأكسدة, مضاد الإلتهاب , النظرية الوظيفية للكثافة, الإلتحام الجزيئي, التحليلات الديناميكية الجزيئية.

Résumé

Dans cette étude, le bis-cétone 1,3-Bis (2-acétylphénoxy)-2-propanol a été synthétisé par la réaction de condensation entre le 1,3-dichloropropanol et le 2'hydroxyacetophenone, et l'élucidation structurale du produit a été réalisée à l'aide des méthodes spectroscopiques, notamment IR, UV, RMN (^{1}H, ^{13}C). Ce composé a fait l'objet d'une évaluation de ses propriétés antioxydantes, par les méthodes de piégeage du radical DPPH et par le pouvoir réducteur. L'activité anti-inflammatoire in vitro a été estimée à l'aide du test de dénaturation de l'albumine du blanc d'œuf, tandis que l'effet anti-inflammatoire topique a été étudié à travers le modèle d'œdème de l'oreille induit par le xylène et l'huile de croton chez la souris. Son activité antibactérienne a été évaluée par des bactéries Gram (+) et Gram (-), et sa cytotoxicité a également été examinée. Certaines études théoriques ont été réalisées via DFT, l'amarrage moléculaire, ADMET et les prédictions MDS**.** Les résultats expérimentaux obtenus indiquent que la molécule présente une activité antioxydante inférieure à celle des antioxydants de référence. En revanche, elle a montré une activité anti-inflammatoire in *vitro et in vivo* remarquable. Par ailleurs, le degré d'hémolyse observé était inférieur à 2,00 %**,** ce qui signifie que le composé n'a démontré aucun effet toxique sur les globules rouges. Le composé a présenté une bonne activité antibactérienne vis-à-vis de *Staphylococcus aureus.* Concernant les résultats de l'étude computationnelle, le Bis-AcPh a montré la plus forte affinité de liaison à COX-2 parmi tous les composés testés. Cette observation concorde avec l'activité anti-inflammatoire confirmée du composé observée lors d'essais in vitro et in vivo. Les prédictions ADMET ont confirmé sa bonne absorption orale, sa similarité médicamenteuse et son accessibilité synthétique. Enfin, les analyses de dynamique moléculaire (MDS) ont révélé que le complexe Bis-AcPh_COX-2 présentait une stabilité globale supérieure à celle du complexe Aspirine_COX-2.

Mots-clés : bis-cétone, antioxydant, anti-inflammatoire, calculs DFT, amarrage moléculaire, Prédiction ADMET, analyses de dynamique moléculaire (MDS).