# SYNTHESIS, CHARACTERIZATION, INSILICO STUDIES, AND BIOLOGICAL EVALUATION OF NOVEL HYBRID CHALCONE DERIVATIVES AS ANTIBACTERIAL AGENTS

A Dissertation submitted to

**THE TAMILNADU Dr. M.G.R MEDICAL UNIVERSITY**

**CHENNAI – 600032**

In partial fulfillment of the requirements for the award of the degree of

**MASTER OF PHARMACY**

**IN**

**BRANCH-II PHARMACEUTICAL CHEMISTRY**

Submitted by

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Under the Guidance of

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This is to certify that **A. SUBHASHINI (261915011)**, carried out the dissertation work on "**SYNTHESIS, CHARACTERIZATION, INSILICO STUDIES, AND BIOLOGICAL EVALUATION OF NOVEL HYBRID CHALCONE DERIVATIVES AS ANTIBACTERIAL AGENTS**'' for the award of degree of **MASTER OF PHARMACY** under **THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY**, **CHENNAI-600097** in the **academic year 2019-21** under my Supervision and Guidance in the Department of Pharmaceutical Chemistry, **C.L. BAID METHA COLLEGE OF PHARMACY, CHENNAI-600 097** during the academic year 2019-2021.

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This is to certify that the project entitled **"SYNTHESIS, CHARACTERIZATION, INSILICO STUDIES, AND BIOLOGICAL EVALUATION OF NOVEL HYBRID CHALCONE DERIVATIVES AS AN ANTIBACTERIAL AGENTS''** as submitted by **A. SUBHASHINI (261915011),** in partial fulfillment for the award of the degree of **Master of Pharmacy** in the **academic year 2019-21**. It was carried out at **C.L. BAID METHA COLLEGE OF PHARMACY, CHENNAI – 600097** under the guidance and supervision of **K. DHUNMATI, M. Pharm, Asst. Prof.,** in the Department of Pharmaceutical Chemistry during the academic year 2019 – 2021.

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## DECLARATION

I do hereby declare that the thesis entitled **"SYNTHESIS, CHARACTERIZATION, INSILICO STUDIES, AND BIOLOGICAL EVALUATION OF NOVEL HYBRID CHALCONE DERIVATIVES AS AN ANTIBACTERIAL AGENTS''** by **A. SUBHASHINI (261915011),** in partial fulfillment of the degree of **MASTER OF PHARMACY** was carried out at **C.L. BAID METHA COLLEGE OF PHARMACY, CHENNAI-600 097** under the guidance and Supervision of **K. DHUNMATI, M. Pharm, Asst. Prof.,** during the academic year 2019-2021. The work embodied in this thesis is original & is not submitted in part or full for any other degree of this or any other University.

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**(261915011)**

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**CONTENT**

**LIST OF ABBREVIATIONS:**

1. E. Coli - Escherichia Coli.
2. CFUs - Colony Forming Units.
3. GyrB – Gyrase Subunit B.
4. MDCK cell – Madin-Darby Canine kidney.
5. BBB – Blood Brain Barrier.
6. TPSA – Total polar surface area.
7. PSA – Polar surface area.
8. CNS – Central nervous system.
9. Rprop – Resilient back propagation.
10. GHS – Globally harmonized system.
11. FT-IR – Fourier Transform Infrared spectroscopy.
12. 1H NMR – Proton Nuclear magnetic resonance.
13. 13 C NMR – Carbon -13 Nuclear magnetic resonance.
14. IR – Infrared Spectroscopy.
15. Rf – Radio frequency.
16. DMSO – Dimethyl sulphoxide.
17. TM – Trimethylsilane.
18. MTCC – Medical transcription cetification commission.
19. Ibs – Irritation bowel syndrome.
20. SD – Standard Deviation.
21. ATP- Adenosine triphosphate.

# INTRODUCTION

# SYNTHESIS, CHARACTERIZATION, INSILICO STUDIES, AND BIOLOGICAL EVALUATION OF NOVEL HYBRID CHALCONE DERIVATIVES AS ANTIBACTERIAL AGENTS

## 1. INTRODUCTION:

### 1.1 CHALCONE:

The word “Chalcone” is derived from the Greek word “Chalcos”, meaning “Bronze” [1], Chalcone is a simple chemical scaffold of many naturally occurring compounds and has a widespread distribution in vegetables, fruits, teas, and other plants [2,3]. Chalcones are well-known intermediates for synthesizing various heterocyclic compounds it is used as scaffold or template in chemical synthesis and medicinal chemistry and the derivatives with the vertebrae of chalcones have been reported to possess various biological activities [4] such as antimicrobial [5], antibacterial [6], anti-inflammatory [7], antimalarial [8-9], anticancer [10], antifungal [11], antiproliferative [12], antitubercular [13], antidiabetic activities [14], etc. The presence of a reactive and unsaturated keto function in chalcones is found to be responsible for their antimicrobial activity, which may be altered depending on the type and position of substituent on the aromatic rings [15]. Chalcones are found as dimmers, oligomers, and conjugates of different kinds. Additionally, various groups of hydroxyl, methoxy, and alkenyl functionalities can be attached to the framework of chalcone and contribute to its structural diversity [16,17]. They are found as yellow pigments in flowers, roots, and leaves of species of genera Angelica, Artocarpus, and Dorstenia and are widely distributed in fruits, vegetables, spices, tea, and beer [18]. An increase in resistance to antimicrobial agents has been a major concern for public health over the last few decades. Thus, the need for new drugs/compounds for the new disease and resistant strains is an essential part of medical care. Several studies have shown that the introduction of different functional groups can improve the biological activity of chalcones.

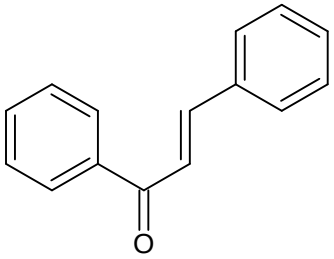
Chalcones is an open-chain flavonoid with α, β-unsaturated carbonyl group and is one of the important compound groups of flavonoid derived from nature and

synthetic compounds belonging to the flavonoid family which remain a fascination among researchers in the 21st century due to their simple chemistry, ease of synthesis, a large number of replaceable hydrogens to yield a variety of derivatives. Due to their abundance in plants and ease of synthesis, this class of compounds has generated great interest for possible therapeutic uses [19].

Chalcone is recognized as a privileged scaffold for the incorporation of different molecules or pharmacophores with various activities. In addition to the biological activities for multitargeting mechanisms, hybrid molecules are also selected for other reasons, such as improving solubility and oral bioavailability. Several chalcone-based compounds have been approved for clinical use. For example, metochalcone was once marketed as a choleretic drug, while sofalcone was previously used as an antiulcer and mucoprotective drug. However, the accurate mechanisms of action for the wide-ranging biological activities of chalcones are still not well understood.

**1.2 HYBRID CHALCONE:**

Chalcones, named so by Kostanecki and Tambor, are commonly known by different names such as benzylideneacetophenone, phenyl styryl ketone, β-phenylacrylophenone α-phenyl-β-benzoylethylene, etc. and constitute the central core of biologically active heterocyclic compounds. Chalcones constitute good synthons for a variety of novel heterocycles of the high therapeutic potential and good pharmaceutical profile [20,21]. Chalcones themselves are identified as interesting entities associated with several biological activities [22]. Chemically, they can be classified as open-chain molecules that present in their structure the fundamental nucleus 1,3-diarylprop-2-en-1-one, formed by two aromatic rings connected by a chain of three carbons, containing an α, β-unsaturated carbonyl [23]. It is believed that the presence of the double bond conjugated to the carbonyl function in an electron-π system such as benzene rings is responsible for the biological activity of the chalcones, although the presence of groups anchored in the aromatic rings contributes to improving the effectiveness of the activity [24,25].




(2*E*)-1,3-diphenylprop-2-en-1-one

Molecular Formula: $C_{15}H_{12}O$

Formula weight: 208.25518

Composition: C (86.51%) H (5.81%) O (7.68%)

Molar Refractivity: 67.10 ± 0.3 $cm^3$

Molar Volume: 189.8 ± 3.0 $cm^3$

Parachor: 490.9 ± 4.0 $cm^3$

Index of Refraction: 1.624 ± 0.02

Surface Tension: 44.7 ± 3.0 dyne/cm

Density: 1.097 ± 0.06 $g/cm^3$

Polarizability: 26.60 ± 0.5 $10^{-24}$ $cm^3$

RDBE: 10

Monoisotopic Mass: 208.088815 Da

Nominal mass: 208 Da

Average Mass: 208.2552 Da

M+: 208.088266 Da

M-: 208.089364 Da

[M+H] +: 209.096091 Da

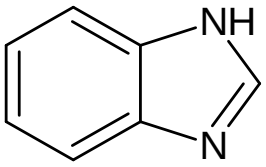
[M+H] - : 209.097189 Da

[M-H] + : 207.080441 Da

[M-H]-: 207.081539 Da

## 1.3 BENZIMIDAZOLE:

Benzimidazoles are found to be useful intermediates for the development of new molecules of biological or pharmaceutical interest. Substituted benzimidazole derivatives have been found to possess biological activities such as antitumor, antimicrobial, anthelmintic, antibacterial, analgesic, anti-inflammatory, etc. In recent times, new techniques have been adopted for the efficient synthesis of novel heterocycles by using heterogeneous, nano-catalysts and photocatalysis that are highly effective and eco-friendly [26].




1*H*-benzimidazole

Molecular Formula: $C_7H_6N_2$
Formula weight: 118.13594
Composition: C (71.17%) H (5.12%) N (23.71%)
Molar Refractivity: 36.61 ± 0.3 $cm^3$
Molar Volume: 95.0 ± 3.0 $cm^3$
Parachor: 264.8 ± 4.0 $cm^3$
Index of Refraction: 1.696 ± 0.02
Surface Tension: 60.1 ± 3.0 dyne/cm
Density: 1.242 ± 0.06 $g/cm^3$
Dielectric Constant; Not available
Polarizability: 14.51 ± 0.5 $10^{-24}cm^3$
RDBE: 6
Monoisotopic Mass: 118.053098 Da
Nominal Mass: 118 Da
Average Mass: 118.1359 Da
$M^+$: 118.05255 Da
$M^-$: 118.053647 Da

$[M+H]^+$: 119.060375 Da

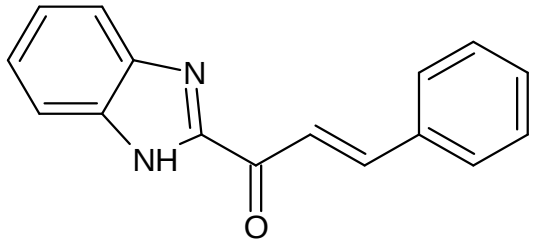
$[M+H]^-$: 119.061472 Da

$[M-H]^+$: 117.044725Da

$[M-H]^-$: 117.045822 Da

## 1.4 HYBRID BENZIMIDAZOLYL-CHALCONE:

Benzimidazole is one of the oldest known nitrogen heterocycles and was first synthesized by Hoebrecker and later by Ladenberg and Wundt during 1872-1878 [27]. The heterocyclic portion of the benzimidazole ring system has been referred to as glyoxaline [28], iminazole, 1,3-diazole, and imidazole [29]. Imidazole, the term used most frequently, indicates a five-membered heterocyclic ring system containing an imino group and tertiary nitrogen. This imidazole ring has been found in several naturally occurring products which include the α-amino acid histidine, a normal constituent of most proteins, histamine, purine, and biotin. The ring system in which the benzene ring is fused to the 4,5-positions of imidazole ring is designated as benzimidazole and is completely planar. The systematic numbering of the benzimidazole ring system. Although benzimidazole as possessing the proton at N1, there exists a rapid exchange between the –NH and =N- nitrogen atoms, and two tautomers, may be drawn for the benzimidazole molecule [30]. Benzimidazoles have most commonly been prepared from the reaction of 1,2- diaminobenzenes with carboxylic acids under harsh dehydrating reaction conditions, utilizing strong acids such as hydrochloric acid (Philips method), polyphosphoric acid, boric acid, or p-toluenesulfonic acid. However, the use of milder reagents, particularly Lewis acids, inorganic clays, or mineral acids, has improved both the yield and purity of this reaction. On the other hand, the synthesis of benzimidazoles via the condensation of 1,2-diaminobenzenes with aldehydes requires an oxidative reagent to generate the benzimidazole nucleus. Various oxidative reagents, such as nitrobenzene, benzoquinone, sodium metabisulfite, mercuric oxide, lead tetraacetate, iodine, copper(II) acetate, indium perfluorooctane sulfonates, ytterbium perfluorooctane sulfonates, and even air, have been employed for this purpose. Moreover, a variety of benzimidazoles could also be produced via coupling of 1,2- diaminobenzenes with carboxylic acid derivatives such as nitriles, imidates, orthoesters, anhydrides, or lactones [31].




Molecular Formula: $C_{16}H_{12}N_2O$

Formula Weight: 248.27928

Composition: C (77.40%) H (4.87%) N (11.28%) O (6.44%)

Molar Refractivity: 77.47 ± 0.3 $cm^3$

Molar Volume: 195.4 ± 3.0 $cm^3$

Parachor: 548.5 ± 4.0 $cm^3$

Index of Refraction: 1.723 ± 0.02

Surface Tension: 62.0 ± 3.0 dyne/cm

Density: 1.270 ± 0.06 $g/cm^3$

Polarizability: 30.71 ± 0.5 $10^{-24}cm^3$

RDBE: 12

Monoisotopic Mass: 248.094963 Da

Nominal Mass: 248 Da

Average Mass: 248.2793 Da

M+: 248.094414 Da

M-: 248.095512 Da

[M+H]+ : 249.102239 Da

[M+H]- : 249.103337 Da

[M-H]+ : 247.086589 Da

[M-H]- : 247.087687 Da

## 1.5 ANTIBACTERIAL ACTIVITY:

The societal burden of bacterial infections has increased in recent years, and from the moment we began to use antibiotics clinically, pathogenic bacteria began to resist them. These bacteria have spread across the globe and have now become a problem for healthcare systems, causing not only financial damage but serious harm to the health of patients with multidrug-resistant infections, with an orderly death rate corresponding to 6% of the total of these infections. Furthermore, the financial losses reach absurd heights. In the European Union, treatment costs caused by multidrug-resistant bacterial diseases reached up to 1.6 billion Euros, and days of prolonged hospitalization due to the same problem cost can reach up to 2.5 million Euros [32]. Chalcones are an important class of small aromatic compounds with various properties and biological applications. The treatment of bacterial infections remains a challenging therapeutic problem because of emerging infectious diseases and the increasing number of multidrug-resistant microbial pathogens. Despite the many antibiotics and chemotherapeutics available, the emergence of old and new antibiotic-resistant bacterial strains in the last decades constitutes a substantial need for new classes of antibacterial agents [33]. In addition, studies with chalcone derivatives showed that they have strong antibacterial activity against Gram-positive and Gram-negative strains [34-35].

Marketed drugs of chalcone used in clinical practice, selected drugs are Metochalcone, sofaclone, Hesperidin methyl chalcone, Licochalcone, Xanthohumol, and Isoliquirtigenin [36].

Marketed drugs of benzimidazole used in clinical practice, selected drugs are nocodazole, bendamustine, veliparib, glasdegib, crenolanib, abemaciclib, liarozole, and pracinostat [37].

## 1.6 DNA gyrase subunit B:

DNA gyrase is an essential bacterial enzyme that catalyzes the ATP-dependent negative super-coiling of double-stranded closed-circular DNA. Gyrase belongs to a class of enzymes known as topoisomerases that are involved in the control of topological transitions of DNA [38]. The DNA gyrase target is a known target for antibacterial agents since its blocking induces bacterial death.

DNA gyrase (topo II) is the only known topoisomerase that catalyzes the supercoiling of DNA. Subunit A is a homodimer of 105,000-dalton nalA protomers and subunit B consists of 95,000-dalton cou protomers (25) [39]. The mechanisms of action act as the drugs bind to the B subunit of gyrase and inhibit DNA supercoiling by blocking the ATPase activity [40].

The crystal structure of an n-terminal fragment of the Escherichia coli DNA gyrase B protein, complexed with a non-hydrolyzable ATP analogue, has been solved at 2.5 Å resolution. It consists of two domains, both containing novel protein folds. The protein fragment forms a dimer, whose N-terminal domains are responsible for ATP binding and hydrolysis. The C-terminal domains form the sides of a 20 Å hole through the protein dimer which may play a role in DNA strand passage during the supercoiling reaction.[41]

DNA gyrase, the only topoisomerase able to introduce negative supercoils into DNA, is essential for bacterial transcription and replication; absent from humans, it is a successful target for antibacterials. From biophysical experiments in solution, we report a structural model at 12–15 A˚ resolution of the full-length B subunit (GyrB). Analytical ultracentrifugation shows that GyrB is mainly a nonglobular monomer. Ab initio modeling of small-angle X-ray scattering data for GyrB consistently yields a ‘‘tadpole’’- like envelope. It allows us to propose an organization of GyrB into three domains—ATPase, Toprim, and Tail—based on their crystallographic and modeled structures. Our study reveals the modular organization of GyrB and points out its potential flexibility, needed during the gyrase catalytic cycle. It provides important insights into the supercoiling mechanism by gyrase and suggests new lines of research. [42]

During our studies on DNA gyrase we found a protein fraction from Escherichia coli that complemented the gyrase A protein to produce a DNA-relaxing (topoisomerase) activity. We have purified this protein to near homogeneity and shown that it has a molecular weight of 50,000 and is apparently a fragment of the gyrase B protein. [43]

**1.7 Escherichia coli:**

E. coli, a member of the bacterial family of Enterobacteriaceae, is the most prevalent commensal inhabitant of the gastrointestinal tracts of humans and warm-blooded animals, as well as one of the most important pathogens [44]. As a commensal, it lives in a mutually beneficial association with hosts and rarely causes disease. It is,

however, also one of the most common human and animal pathogens as it is responsible for a broad spectrum of diseases. The peculiar characteristics of the E. coli, such as ease of handling, availability of the complete genome sequence, and its ability to grow under both aerobic and anaerobic condition, makes it an important host organism in biotechnology. E. coli is used in a wide variety of applications both in the industrial and medical area and it is the most used microorganism in the field of recombinant DNA technology [45].

Escherichia coli (*E. coli*) is among the world's most well-studied organisms and is often found at the forefront of advancing technology. Not surprisingly, E. coli is on the leading edge of an ongoing shift in the field of genomics [46]. *E. coli* is often used as a representative microorganism in the research of novel water treatment and sterilization methods, including photocatalysis. By standard plate count methods, following sequential dilutions, and growth on agar gel plates, the concentration of viable organisms or CFUs (Colony Forming Units), in a known volume of treated water can be evaluated, allowing the comparative assessment of materials performance [47]. Due to the low cost and speed with which it can be grown and modified in laboratory settings, *E. coli* is a popular expression platform for the production of recombinant proteins used in therapeutics. One advantage to using *E. coli* over another expression platform is that *E. coli* naturally does not export many proteins into the periplasm, making it easier to recover a protein of interest without cross-contamination [48].

Escherichia coli is the predominant facultative anaerobe of the human colonic flora. The organism typically colonizes the infant gastrointestinal tract within hours of life, and, thereafter, E. coli and the host derive mutual benefit. E. coli usually remains harmlessly confined to the intestinal lumen; however, in the debilitated or immunosuppressed host, or when gastrointestinal barriers are violated, even normal "non-pathogenic" strains of E. coli can cause infection. Moreover, even the most robust members of our species may be susceptible to infection by one of several highly adapted E. coli clones which together have evolved the ability to cause a broad spectrum of human diseases. Infections due to pathogenic E. coli may be limited to the mucosal surfaces or can disseminate throughout the body [49].

Escherichia coli remains one of the most frequent causes of several common bacterial infections in humans and animals. E. coli is the prominent cause of enteritis, urinary tract infection, septicemia, and other clinical infections, such as

neonatal meningitis. E. coli is also prominently associated with diarrhea in pet and farm animals. The treatment of E. coli infections is threatened by the emergence of antimicrobial resistance. The prevalence of multidrug-resistant E. coli strains is increasing worldwide principally due to the spread of mobile genetic elements, such as plasmids. The rise of multidrug-resistant strains of E. coli also occurs in Europe. Therefore, the spread of resistance in E. coli is an increasing public health concern in European countries. This paper summarizes the current status of E. coli strains clinically relevant in European countries. Furthermore, therapeutic interventions and strategies to prevent and control infections are presented and discussed. The article also provides an overview of the current knowledge concerning promising alternative therapies against E. coli diseases [50].

E. coli strains are classified into pathogenic types (pathotypes are defined as a group of strains of the same species causing a common disease. Listed below (Table 1) [51].

**Table 1:** E. coli pathogenic types

| Pathotype (acronym) | Diseases |
|---|---|
| Enteric E.coli | |
| EnteroPathogenic E. coli (EPEC) | Diarrhoea in children |
| EnteroHaemorrhagic E. coli (EHEC) | Haemorrhagic colitis, HUS |
| EnteroToxigenic E. coli (ETEC) | Traveler's diarrhoea |
| EnteroAggregative E. coli (EAEC) | Diarrhoea in children |
| Diffusely Adherent E. coli (DAEC) | Acute diarrhoea in children |
| EnteroInvasive E. coli (EIEC) | Shigellosis-like |
| Adherent Invasive E. coli (AIEC) | Associated with Crohn disease |
| Extraintestinal E.coli (ExPEC) | |
| UroPathogenic E. coli (UPEC) | Lower UTI and systemic infections |
| Neonatal Meningitis E. coli (NMEC) | Neonatal meningitis |
| Avian Pathogenic E. coli (APEC) | Probable source of food-borne disease |

**1.8 DOCKING:**

Docking is a procedural method to predict the preferred orientation of one molecule to another when bound forming a stable complex.

Docking is important in Drug designing which is used for calculating the binding alignment of small molecular drugs or inhibitors to their protein targets and can predict affinity and activity of complex formed.

Molecular docking is an attractive scaffold to understand drug biomolecular interactions for the rational drug design and discovery, as well as in the mechanistic study by placing a molecule (ligand) into the preferred binding site of the target specific region of the DNA/protein (receptor) mainly in a non-covalent fashion to form a stable complex of potential efficacy and more specificity. The information obatained from the docking technique can be used to suggest the binding energy, free energy and stability of complexes. At, present, docking technique is utilized to predict the tentative binding parameters of ligand-receptor complex beforehand.
The main objective of molecular docking is to attain ligand-receptor complex with optimized conformation and with the intention of possessing less binding free energy.

Molecular docking can demonstrate the feasibility of any biochemical reaction as it is carried out before experimental part of any investigation. There are some areas, where molecular docking has revolutionized the findings. In particular, interaction between small molecules (ligand) and protein target (may be an enzyme) may predict the activation or inhibition of enzyme. Such type of information may provide a raw material for the rational drug designing. Some of the major applications of molecular docking are lead optimization, Hit identification, Drug-DNA interaction.

## 1.9 DRUG FILTERS

### ChemSketch:

ACD/ChemSketch Freeware is a drawing package that allows you to draw chemical structures including organics, organometallics, polymers and Markush structures. It also includes features such as calculation of molecular properties (eg. Molecular weight, density, molar refractivity, etc.), 2D and 3D structure cleaning and viewing, functionality for naming structures (fewer than 50 atoms and 3 rings), and prediction of log P.

### Swiss ADME:

A free webtool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. It allows to compute physicochemical descriptors as well as to predict ADME parameters, pharmacokinetic properties, druglike nature and medicinal chemistry friendliness of one or multiple small molecules to support drug discovery.

### ProTox II:

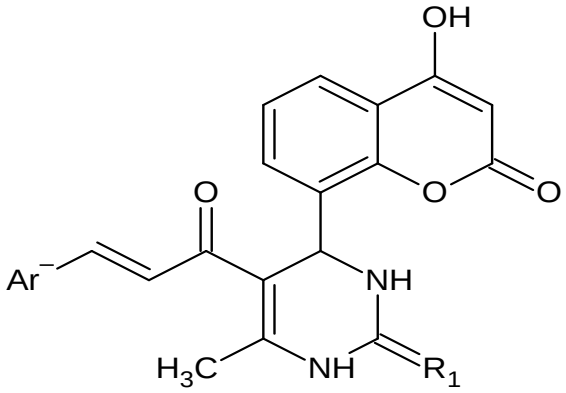
ProTox-II, a virtual lab for the prediction of toxicities of small molecules. The prediction of compound toxicities is an important part of the drug design development process. ProTox-II incorporates molecular similarity, fragment propensities, most frequent features and (fragment similarity based CLUSTER cross-validation) machine-learning, based a total of 33 model for the prediction of various toxicity endpoints such as acute toxicity, hepatotoxicity, cytotoxicity, carcinogenicity, mutagenicity, immunotoxicity, adverse outcomes(Tox21) pathways and toxicity targets.

### PreADMET:

PreADMET is a web-based application for predicting ADME data and building drug-like library using *in silico* method. It predicts permeability for Caco-2 cell, MDCK cell and BBB (blood-brain barrier), HIA(human intestinal absorption), skin permeability and plasma protein binding.

## 2. REVIEW OF LITERATURE

1. Phani Kumar Kola *et al.,* performed designed and synthesized 26 coumarins clubbed chalcone hybrids and screened *in vitro* antimicrobial activity and the derivatives were detected by agar well diffusion method and DPPH free radical assay. From the result, the compound with bearing phenyl substitution and 4-Cl phenyl substitution showed better antibacterial activity. [52]

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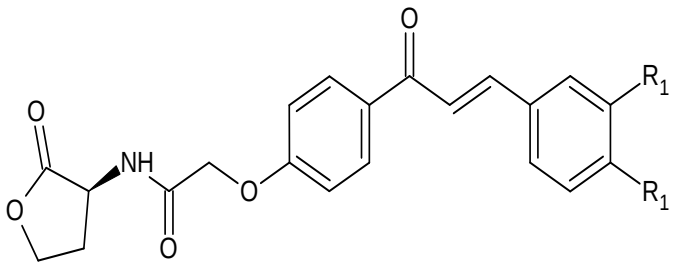

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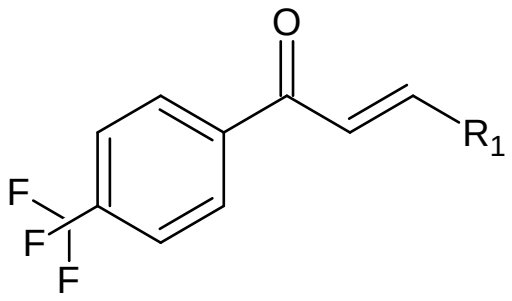


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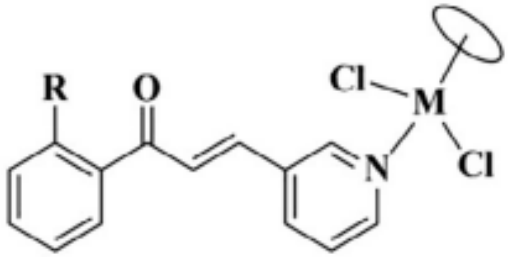


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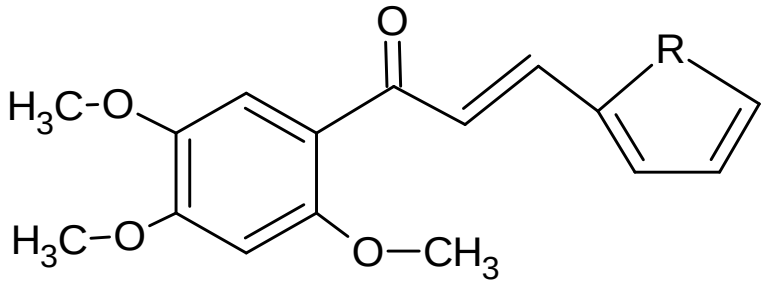

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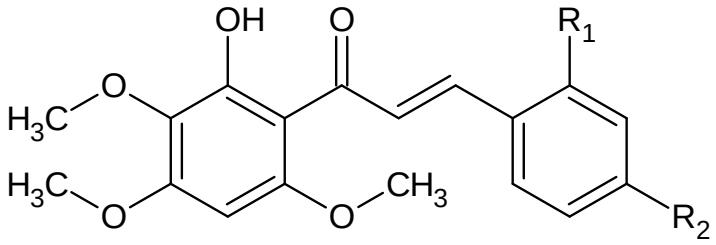


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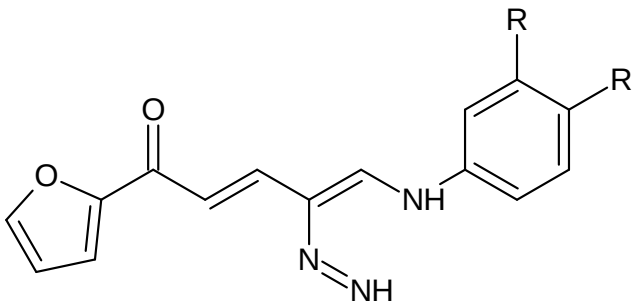

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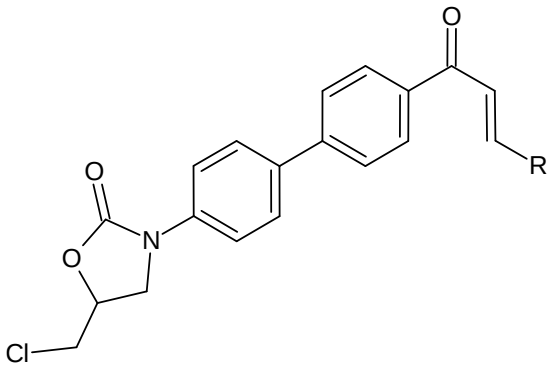


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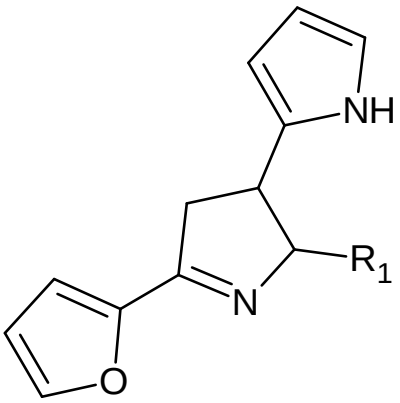


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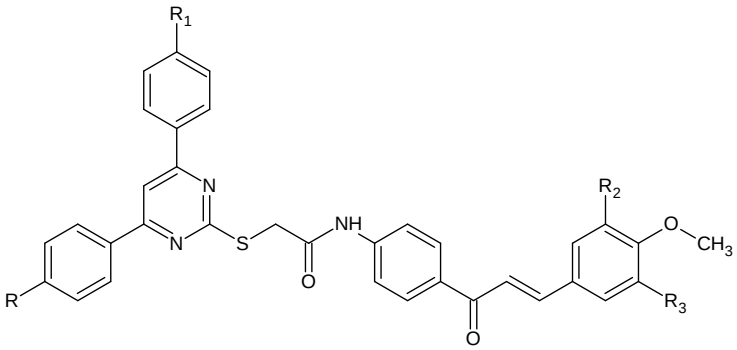


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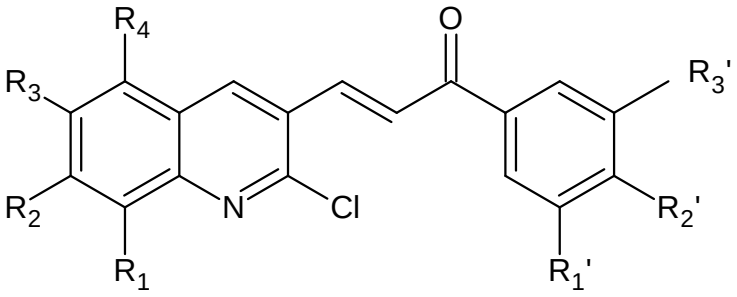


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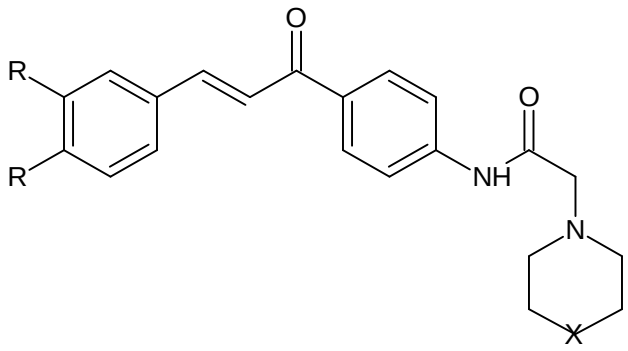


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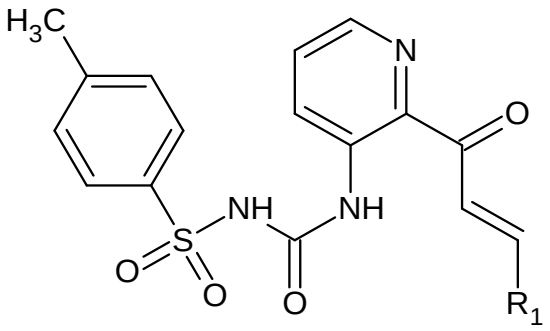


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neonatal meningitis. E. coli is also prominently associated with diarrhea in pet and farm animals. The treatment of E. coli infections is threatened by the emergence of antimicrobial resistance. The prevalence of multidrug-resistant E. coli strains is increasing worldwide principally due to the spread of mobile genetic elements, such as plasmids. The rise of multidrug-resistant strains of E. coli also occurs in Europe. Therefore, the spread of resistance in E. coli is an increasing public health concern in European countries. This paper summarizes the current status of E. coli strains clinically relevant in European countries. Furthermore, therapeutic interventions and strategies to prevent and control infections are presented and discussed. The article also provides an overview of the current knowledge concerning promising alternative therapies against E. coli diseases [50].

E. coli strains are classified into pathogenic types (pathotypes are defined as a group of strains of the same species causing a common disease. Listed below (Table 1) [51].

**Table 1:** E. coli pathogenic types

| Pathotype (acronym) | Diseases |
|---|---|
| Enteric E.coli | |
| EnteroPathogenic E. coli (EPEC) | Diarrhoea in children |
| EnteroHaemorrhagic E. coli (EHEC) | Haemorrhagic colitis, HUS |
| EnteroToxigenic E. coli (ETEC) | Traveler's diarrhoea |
| EnteroAggregative E. coli (EAEC) | Diarrhoea in children |
| Diffusely Adherent E. coli (DAEC) | Acute diarrhoea in children |
| EnteroInvasive E. coli (EIEC) | Shigellosis-like |
| Adherent Invasive E. coli (AIEC) | Associated with Crohn disease |
| Extraintestinal E.coli (ExPEC) | |
| UroPathogenic E. coli (UPEC) | Lower UTI and systemic infections |
| Neonatal Meningitis E. coli (NMEC) | Neonatal meningitis |
| Avian Pathogenic E. coli (APEC) | Probable source of food-borne disease |

**1.8 DOCKING:**

Docking is a procedural method to predict the preferred orientation of one molecule to another when bound forming a stable complex.

Docking is important in Drug designing which is used for calculating the binding alignment of small molecular drugs or inhibitors to their protein targets and can predict affinity and activity of complex formed.

Molecular docking is an attractive scaffold to understand drug biomolecular interactions for the rational drug design and discovery, as well as in the mechanistic study by placing a molecule (ligand) into the preferred binding site of the target specific region of the DNA/protein (receptor) mainly in a non-covalent fashion to form a stable complex of potential efficacy and more specificity. The information obatained from the docking technique can be used to suggest the binding energy, free energy and stability of complexes. At, present, docking technique is utilized to predict the tentative binding parameters of ligand-receptor complex beforehand.
The main objective of molecular docking is to attain ligand-receptor complex with optimized conformation and with the intention of possessing less binding free energy.

Molecular docking can demonstrate the feasibility of any biochemical reaction as it is carried out before experimental part of any investigation. There are some areas, where molecular docking has revolutionized the findings. In particular, interaction between small molecules (ligand) and protein target (may be an enzyme) may predict the activation or inhibition of enzyme. Such type of information may provide a raw material for the rational drug designing. Some of the major applications of molecular docking are lead optimization, Hit identification, Drug-DNA interaction.

## 1.9 DRUG FILTERS

### ChemSketch:

ACD/ChemSketch Freeware is a drawing package that allows you to draw chemical structures including organics, organometallics, polymers and Markush structures. It also includes features such as calculation of molecular properties (eg. Molecular weight, density, molar refractivity, etc.), 2D and 3D structure cleaning and viewing, functionality for naming structures (fewer than 50 atoms and 3 rings), and prediction of log P.

### Swiss ADME:

A free webtool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. It allows to compute physicochemical descriptors as well as to predict ADME parameters, pharmacokinetic properties, druglike nature and medicinal chemistry friendliness of one or multiple small molecules to support drug discovery.

### ProTox II:

ProTox-II, a virtual lab for the prediction of toxicities of small molecules. The prediction of compound toxicities is an important part of the drug design development process. ProTox-II incorporates molecular similarity, fragment propensities, most frequent features and (fragment similarity based CLUSTER cross-validation) machine-learning, based a total of 33 model for the prediction of various toxicity endpoints such as acute toxicity, hepatotoxicity, cytotoxicity, carcinogenicity, mutagenicity, immunotoxicity, adverse outcomes(Tox21) pathways and toxicity targets.

### PreADMET:

PreADMET is a web-based application for predicting ADME data and building drug-like library using *in silico* method. It predicts permeability for Caco-2 cell, MDCK cell and BBB (blood-brain barrier), HIA(human intestinal absorption), skin permeability and plasma protein binding.

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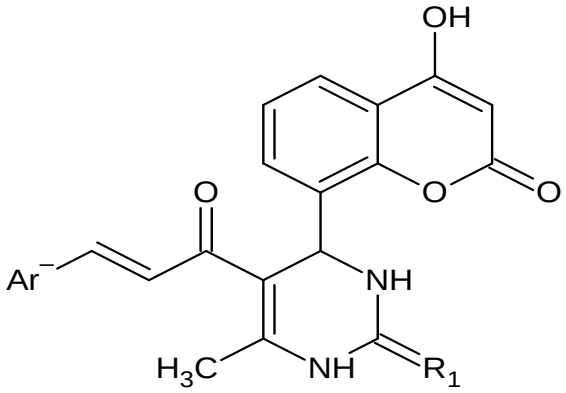


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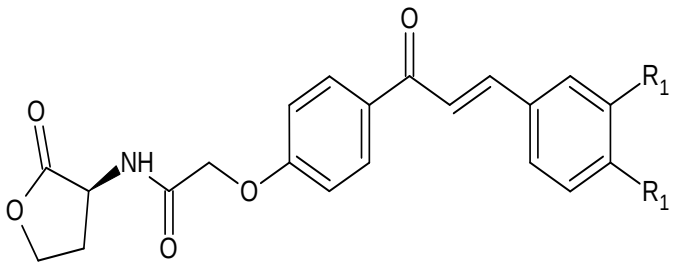

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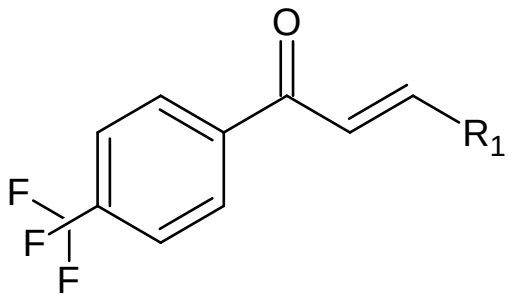


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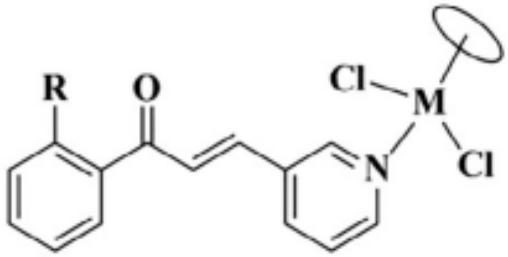


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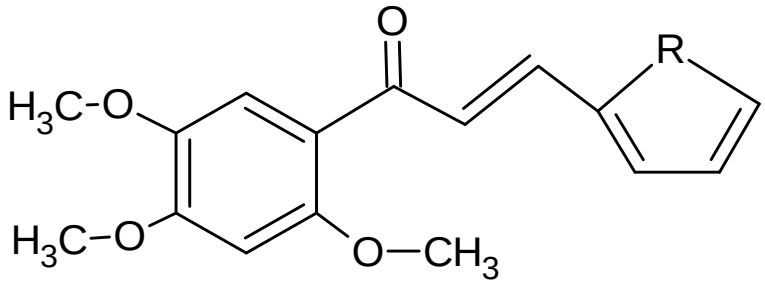

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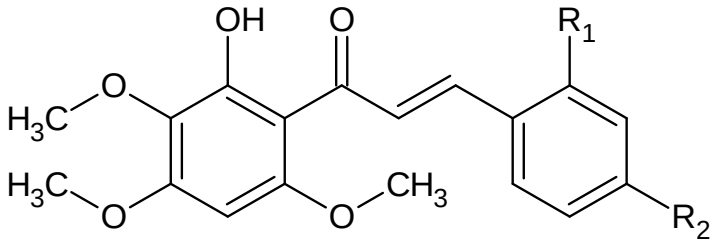


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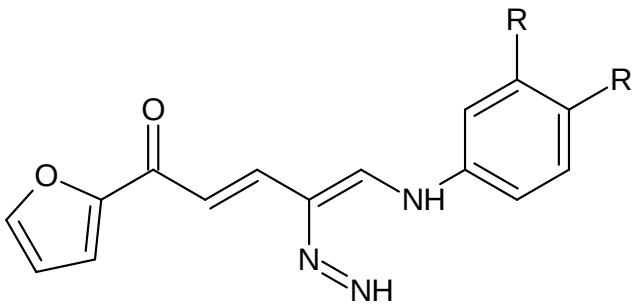

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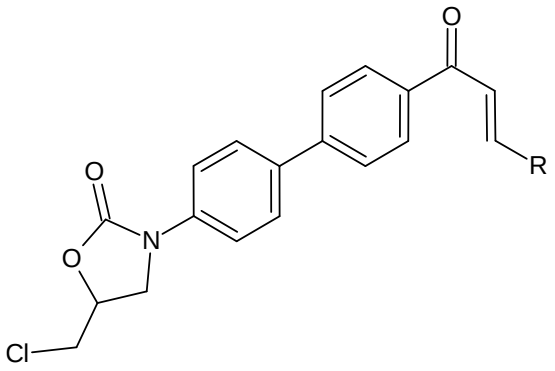


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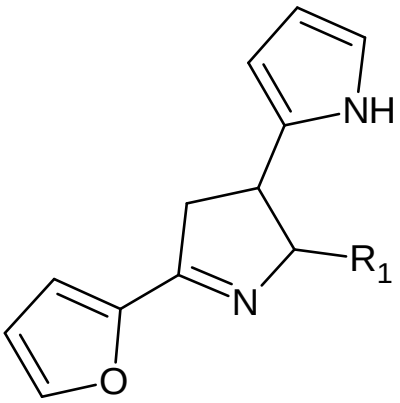


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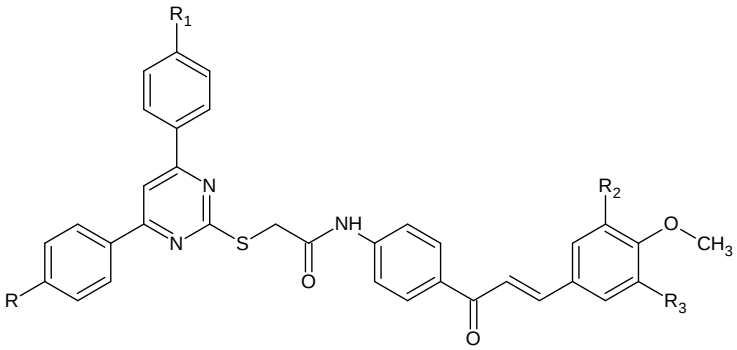


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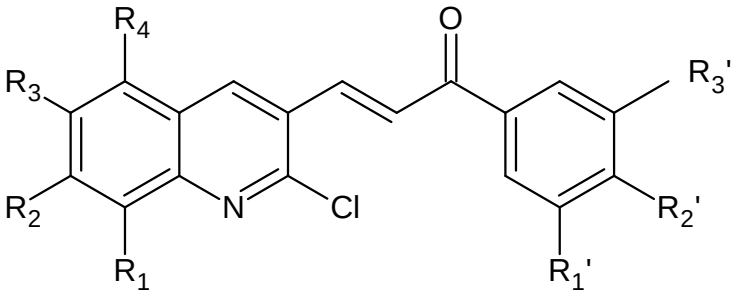


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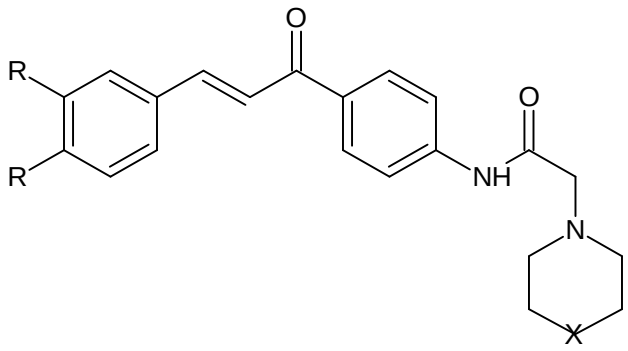


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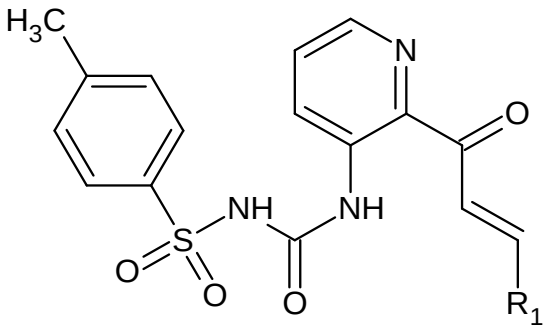


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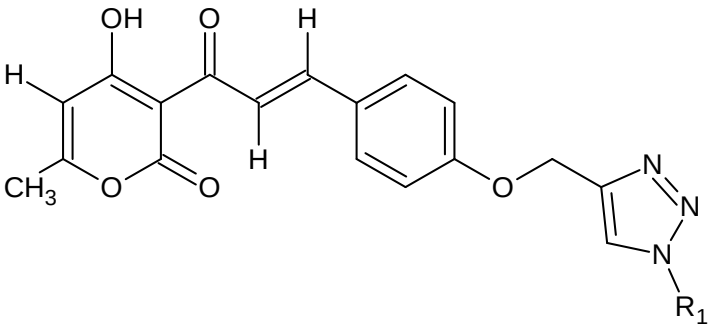


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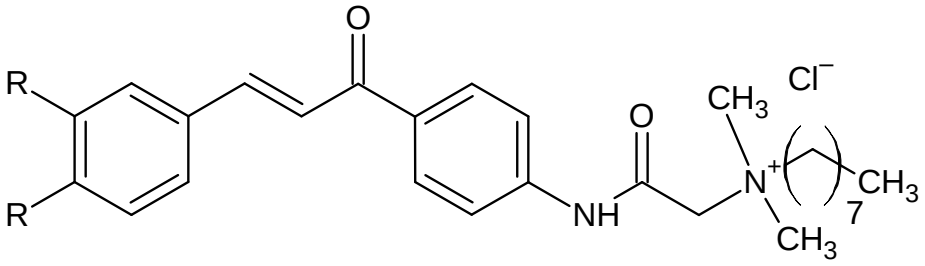


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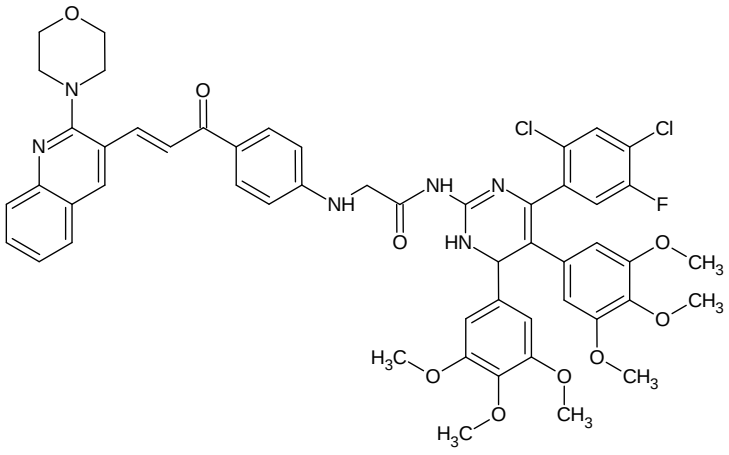

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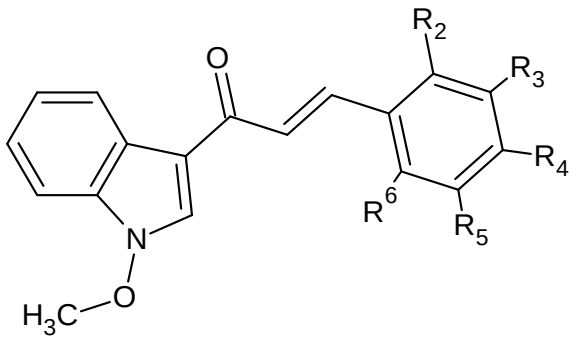
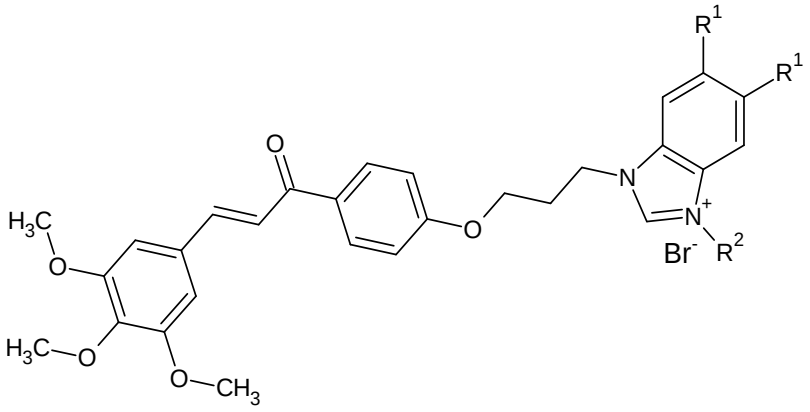
19. Mahammadali Khanusiya *et al*., performed a series of Novel chalcone-sulphonamide hybrids undergoes through the Claisen-Schmidt condensation of a substituted aldehyde with para amino acetophenone and screened antibacterial activity. From the result, showed the compound have more potent growth inhibitory activity against bacterial strain also used as a sulpha drug. [70]

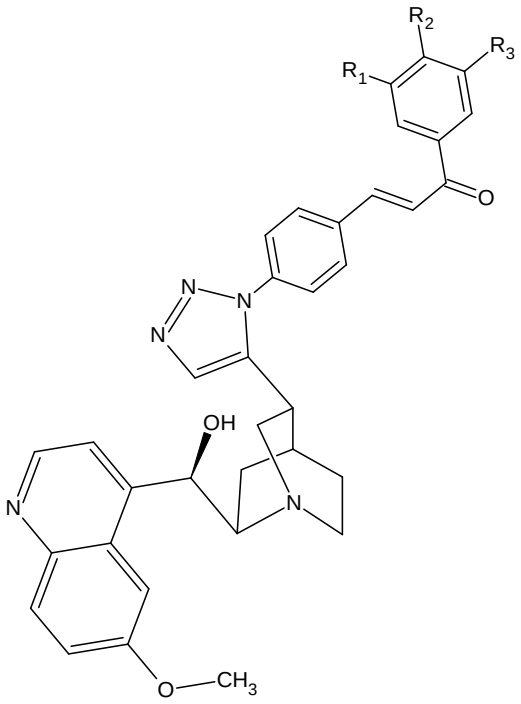
20. Zuzana Kudlickova *et al*., performed designed and synthesized a new 1-methoxyindole and 2-alkoxyindole chalcone hybrids acted as an antiproliferative activity. From the result, the significant derivative performed the best balance on high antiproliferative potency against colorectal carcinoma cell line HCT116. [71]



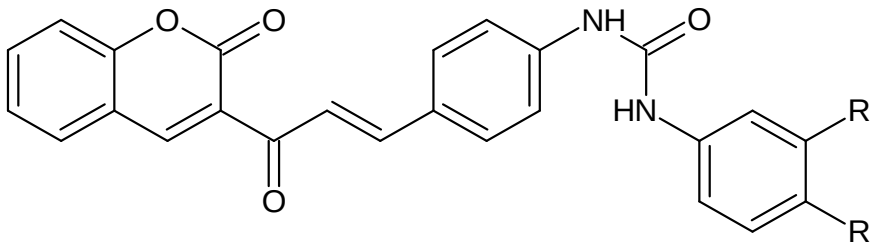

21. Jun-Li yang *et al*., performed the series of novel Trimethoxyphenyl-Derived chalcone –benzimidazolium salts were designed, synthesized and evaluated for their anticancer activity. Screened *in vitro* against five different human tumor cell lines. From the result, the compound with 5,6-dimethylbenzimidazole substituent at position-3 of the benzimidazole ring showed the most potent inhibitory activity. [72]



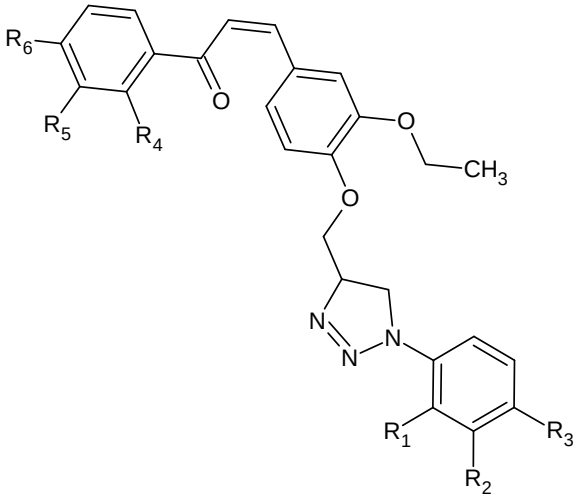

22. Tamas Jernei *et al.,* performed synthesized a systematic series of novel cinchona-chalcone hybrid compounds, containing 1,4-disubstituted- and 1,5-disubstituted 1,2,3-triazole linkers used by means of copper (I) and ruthenium (II)- catalyzed click reaction of quinine- and quinidine-derived alkynes with azide-substituted chalcones and screened by anticancer activity. [73]



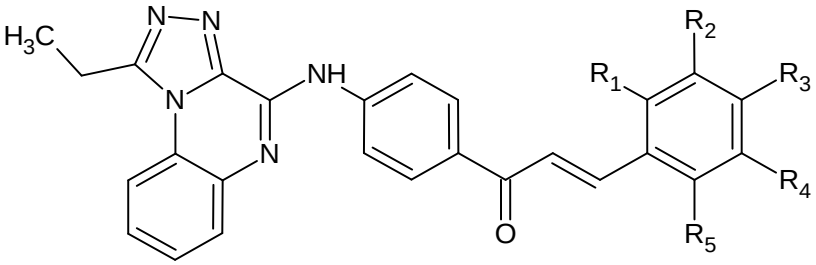

23. Belma Zengin Kurt *et al.,* performed the synthesized and series of novel coumarin-chalcone compound containing urea moiety and screened anticancer agents. From the result, the coumarin derivatives beared diversely substituted chalcone-urea moieties showed better inhibitory activity. [74]



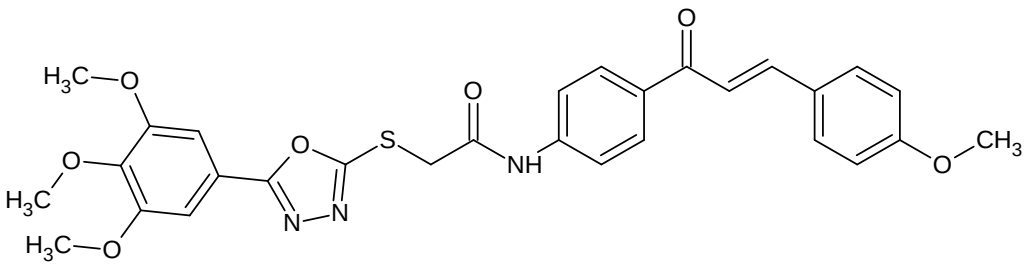

24. N.J.P. Subhashini *et al.,* performed synthesized and studied the molecular docking in novel 1,2,3-triazole tethered chalcone hybrids and evaluated as their anticancer activity. From the result, the compound meta chloro substituent attached to the triazole ring and meta hydroxyl substituted attached to the chalcone ring of compound performed molecular docking values showed the highest dock score of -8.102 and -83.05 Kcal/mol and delta G bind. [75]



25. Mohamed Alswah *et al.,* performed the designed and synthesized a series of hybrid of triazoloquinoxalin –chalcone. From the result, the compound was evaluated as their potent anticancer activity against a set of cancer lines. [76]

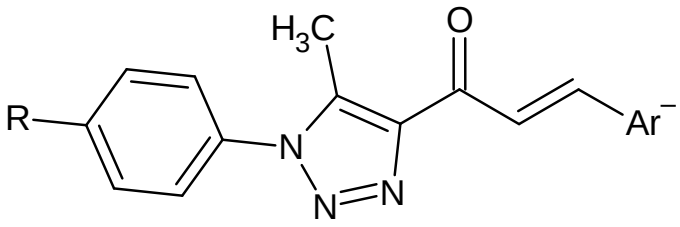


26. Samar H. Abbas *et al.,* performed the synthesized a new series of 1,3,4-oxadiazole/ chalcone hybrids were designed and screened anticancer activity. From the result, the compound showed the strongest cytotoxic activity against K-562, jurkat and KG-1a leukemia cell lines.
[77]

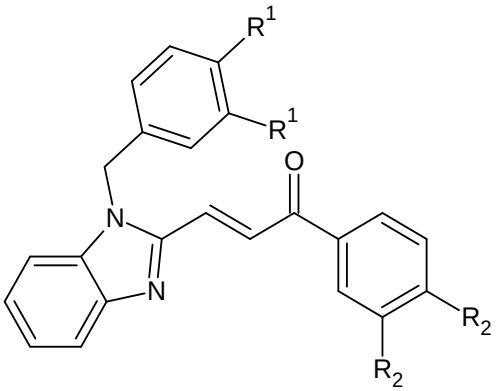


27. Khalid B. Selim *et al.,* performed the synthesized a new series of 1,2,3-triazole-chalcone hybrids *in vitro* cytotoxicity activity were screened against a

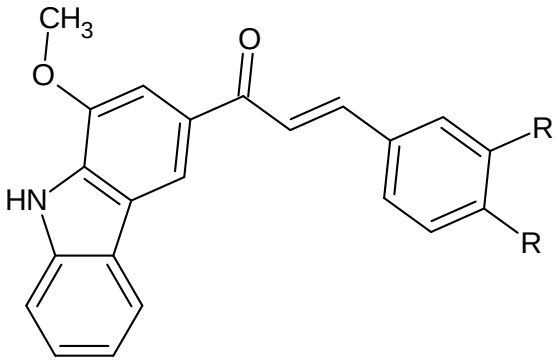
panel of 60 human cancer cell lines. From the result, the compound with 3,4-dimethoxyphenyl chalcone moiety obtained excellent potent anticancer. [78]



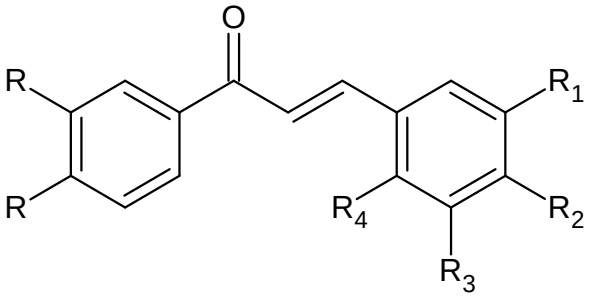

28. Wei Zhou *et al.,* performed designed, synthesized and introduced a novel series of Benzimidazole - Chalcone (BCHs) Hybrids as Non-Intercalative Topoisomerase II catalytic inhibitors and acted as antitumor activity. From the result, the compound showed good inhibitory effect on the Topo II mediated DNA relaxed assay. [79]



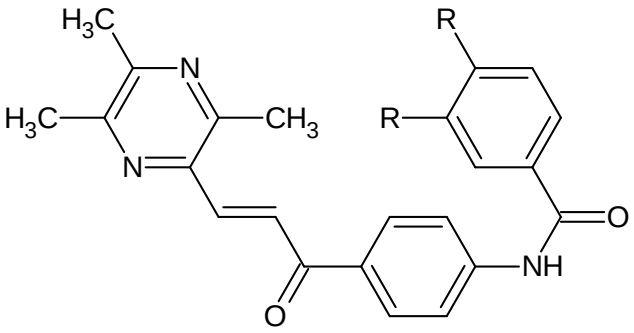

29. Debarshi Kar Mahapatra *et al.,* performed the development of Murrayanine-Chalcone hybrids involves an effort to combine two privilege scaffolds for enhancing hypoglycemic activity which is represented all the novel murranyanine-chalcone hybrids. From the result, the compound with two lipophilic substituents at meta positions in the b-ring showed the highest anti-hyperglycemic activity. [80]



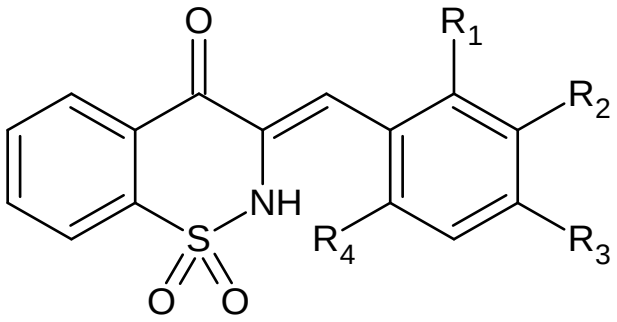

30. Jufrizal Syahri *et al.,* performed studied QSAR analysis of Chalcone derivatives designed and screened antimalarial activity. From the result, the compound showed the best antimalarial activity and the electronic and molecular descriptors for 31 chalcone derivatives were calculated and used by DFT/B3LYP. [81]



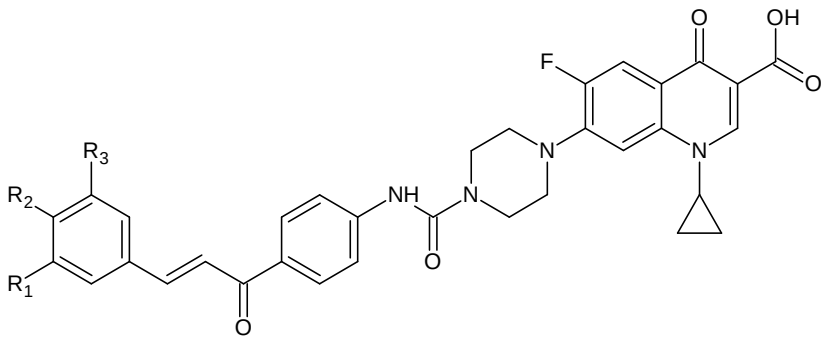

31. Yingqi Luo *et al.,* performed the synthesized a series of novel ligustrazine-chalcone hybrids derivatives and evaluated *invitro* and *invivo* antitumor activity. From the result, the compound showed *invitro* and *invivo* proliferation inhibition potency against breast cancer with good therapeutic potential and here promised candidates for the treatment of TNBC. [82]



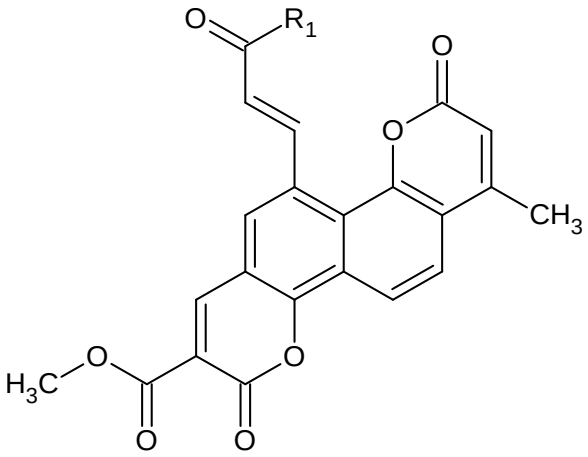
32. Muhammad Hanif *et al*., performed synthesised a two important bioactive compounds such as μ chalcones and 1,2 benzothiazines was evaluated for alkaline phosphatase inhibitory activity against the standard reference compound Levomisole. Molecular docking studies were performed. From the result, the compound have their combination derivatives found to be most potent inhibitor done for h-TNAP. [83]




33. Gamal El-Din A. Abuo-Rahma *et al.,* performed novel urea linked ciprofloxacin-Chalcone Hybrids had antiproliferative Topoisomerases I and II Inhibitory Activities and Capases-Mediated Apoptosis. From the result, the compound with 3,4,5-trimethoxy substituted at $R_1$, $R_2$ and $R_3$ showed the highest antiproliferative activity among the tested compounds. [84]



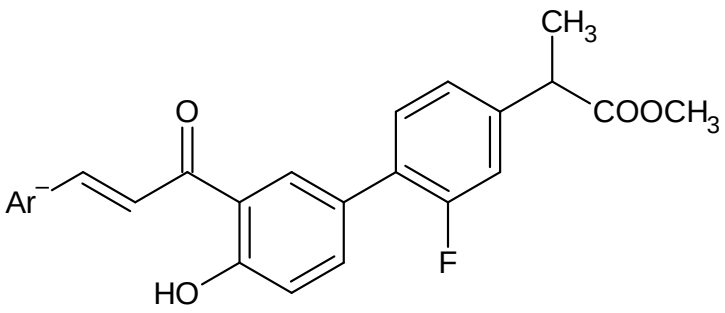

34. Koneni V. Sashidhara *et al.,* perforemd synthesized a series of novel biscoumarin-chalcone hybrids and evaluated for their anti-inflammatory activity. From the result, the compound showed the best active potent activity to inhibit TNF-α inhibition used by whole blood assay. [85]



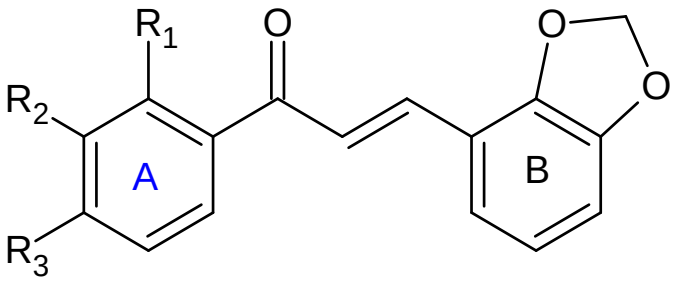
35. Biswaranjan Das *et al.,* performed designed and molecular docking studies on 36 designed novel hybrid peptide analog were used by Flex X v2.1.3 programs and screened anti-inflammatory activity. Suggested performance on a majority of the compounds docking pose analyze and many of the compounds having the flex X docking with highest scoring were used for analysis higher than the co-crystalized ligand IH6(-26.78). [86]

36. Pichjira Sooknual *et al.,* performed synthesized and neuroprotective effects of novel chalcone–triazole hybrid designed and were by replacing the 4-hydroxy group with 1,2,3-triazole ring containing 4-substitutes. [87]

37. Yong Deng *et al*., performed a series of 4'-OH-flurbiprofen-chalcone hybrids were designed, synthesized and screened potential multifunctional agents had used for the treatment of Alzheimer's Disease. From the result, the compound exhibited better inhibitory activity. *In vitro* blood-brain barrier were performed that on early phase of drug discovery and also penetrate brain successfully got a therapeutic effect on CNS drugs respectively. [88]



38. Arwa Hammuda *et al*., perfomed designed and synthesized of novel series of substituted chalcones as potent selective monoamine oxidase-B (MAO-B) inhibitors and ten novel chalcone were prepared by Claisen-Schmidt condensation reaction and From the result, the compound to be more active,in which suggested that the chalcone scaffold bearing a benzodioxol ring binds to human MAO-B with higher affinity than the dimethoxyphenyl moiety. [89]

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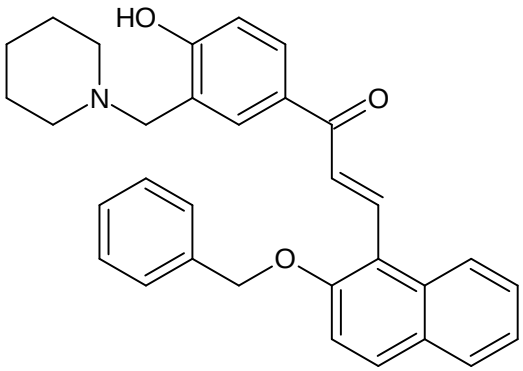

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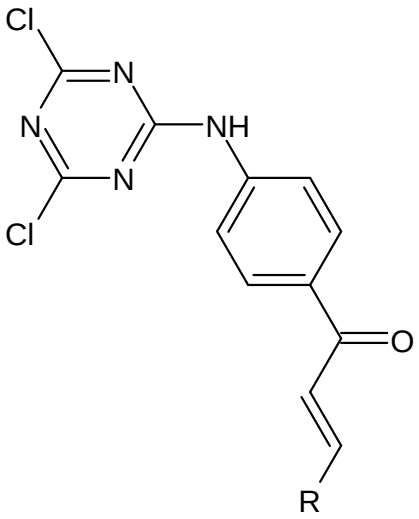


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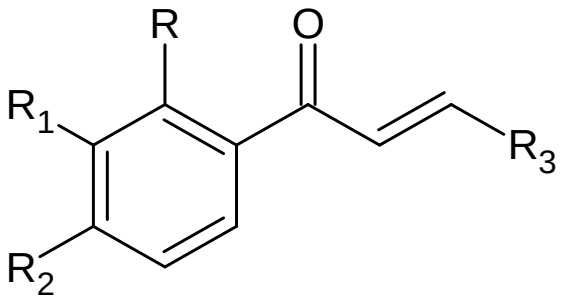
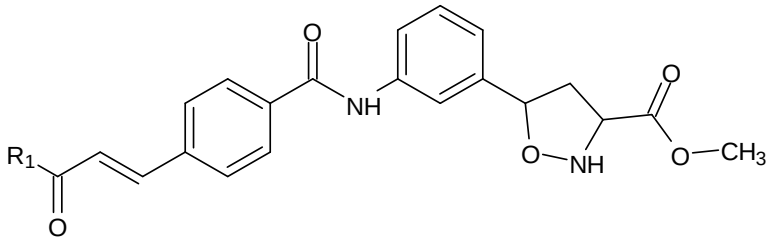


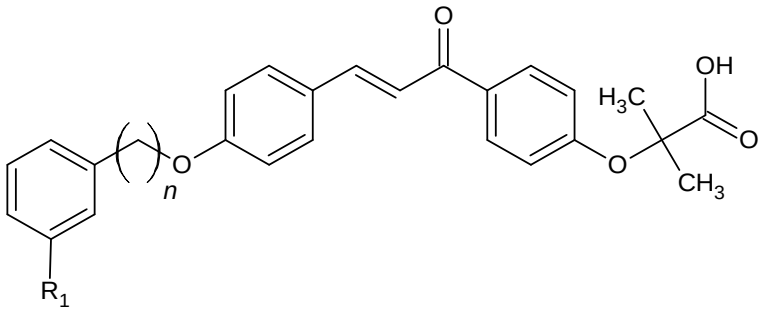

42. Harpreet Kaur *et al.,* reported were synthesized triazole and chalcone analogues and screened antitubercular activity. From the result, the compound showed the best potent and studied molecular docking of bioactive compounds were in good congruence with *in-vitro* studies.

[93]

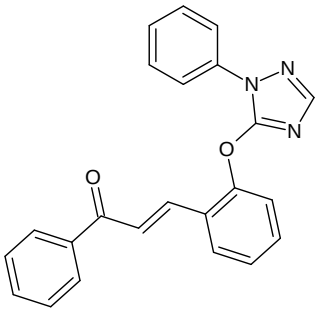
43. Venkata Madhavi Yaddanapudi *et al.,* performed synthesized new chalcone linked 5-phenyl-3-isoxazole carboxylic acid methyl esters-chalcone hybrids compound potentially active against drug resistant *mycobacterium tuberculosis*. Finally concluded that, 35 synthesized derivatives described the significant most potent in derivatives each against Mtb H37Rv. [94]



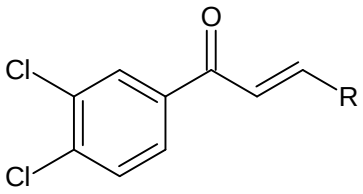

44. Rasha M. Hassan *et al.,* were synthesized a novel 4-(2-phenylethoxy) chalcone fibrate hybrids and screened antihyperlipidemic activity. From the result, the compound obtained good agonistic activity on PPARα compared to fenofibric acid showed the better anti-hyperlipidemic activity. [95]



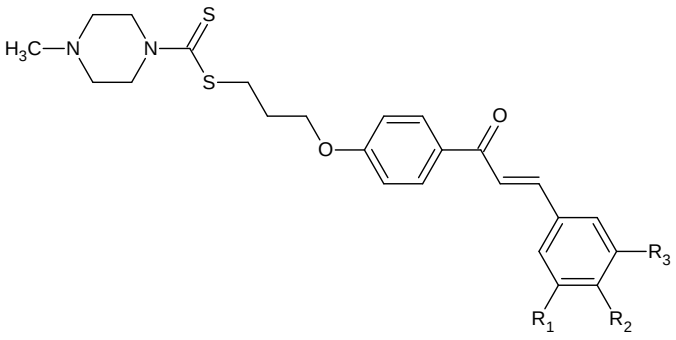

45. Synthesized and performed nineteen chalcone-tetrazole hybrids and their cyclic pyrazoline-tetrazole structures and screened *in vitro* antiproliferative activity were previously reported by Heidi S. Abd EIMonaem et al., and Based on MTT-Assay. From the result, the compound found to be the excellent potent antiproliferative activity against colon HCT-116 and prostate PC-3 cell lines. [96]




46. Synthesized Dichloro Substituted chalcones and Dihydropyrazole derivatives evaluated for their antiproliferative activity previously were explained by Afzal b. Shaik *et al*., and From the result, the compound containing 2-thienyl moiety in the chalcone series exhibited highest antiproliferative activity. [97]




47. Hong-Min Liu *et al.,* performed synthesized a series of novel dithiocarbamate-chalcone derivates and screened antiproliferative activity and From the result, the compound found to be best activity growth inhibition against SK-N-SH investigated arresting the cell cycle at GO/G1 phase. [98]



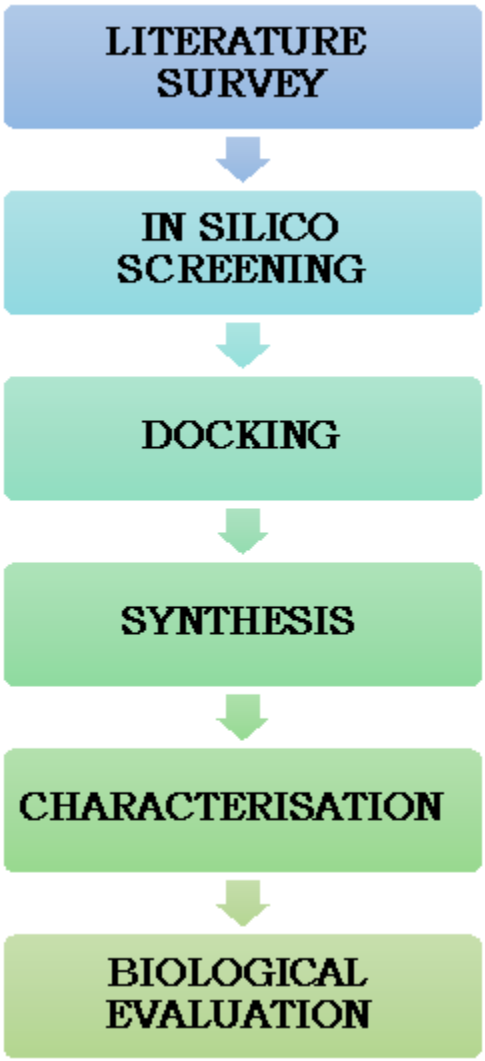
## 3. AIM AND OBJECTIVE

- Bacterial resistance is undoubtedly recognised as a major medical challenge in most healthcare systems.
- Many governmental and agency reports have been published regarding the agricultural use of antibacterials, advising less use of antibacterials, appropriate choice of antibacterials and regimens, prevention of cross-infection and development of new antibacterials.
- The main objectives of this study is to develop effective antibacterial compounds with a broad spectrum of antibacterial activity and less potential to develop resistance.

The work is aimed at

- To design hybrid benzimidazolyl chalcone derivatives using ACD/ChemSketch FREEWARE software.
- To perform docking studies for all designed compounds with DNA gyrase subunit B using Autodock vina (Version 1.5.6) in Pyrx.
- To synthesize hybrid benzimidazolyl chalcone derivatives based on docking score.
- To characterize the synthesized compounds using IR, NMR, Mass spectrometry.
- To evaluate the antibacterial activity of the synthesized compounds.

## 4. PLAN OF THE STUDY:



# 5. MATERIALS AND METHODS

## 5.1 DESIGN OF COMPOUNDS:

20 compounds were designed using Chemsketch software. Physicochemical properties were calculated for all the compounds.

**Molar refractivity:**

Molar refractivity, A, is a measure of the total polarizability of a mole of a substance and is dependent on the temperature, the index of refraction, and the pressure.

The molar refractivity is defined as

$$A = \frac{4}{3}\pi N_A \alpha$$

Where $N_A \approx 6.02210^{23}$ is the Avogadro constant and $\alpha$ is the mean polarizability of the molecule.

**Molar Volume:**

The molar volume, symbol $V_m$, is the volume occupied by one mole of a substance (chemical element or chemical compound) at a given temperature and pressure. It is equal to the molar mass (M) divided by the mass density (ρ). It has the SI unit cubic meters per mole (m3/mol), although it is more practical to use the units cubic decimeters per mole (dm3/mol) for gases and cubic centimeters per mole (cm3/mol) for liquids and solids. The molar volume of a substance can be found by measuring its molar mass and density then applying the relation

$$Vm = M/p$$

**Index of refraction:**

The refractive index or index of refraction of a material is a dimensionless number that describes how light propagates through that medium.

It is defined as

$$n = c/v$$

Where, c is the speed of light in vacuum and v is the phase velocity of light in the medium.

**Surface Tension:**

Surface tension is the elastic force of a fluid surface which makes it acquire the least surface area possible. Surface tension has the dimension of force per unit length, or of energy per unit area.

**Polarizability:**

Polarizability is the ability to form instantaneous dipoles. It is a property of matter. Polarizabilities determine the dynamical response of a bound system to external fields and provide insight into a molecule's internal structure. In a solid, polarizability is defined as the dipole moment per unit volume of the crystal cell.

**Parachor:**

It is an empirical constant for a liquid that relates the surface tension to the molecular volume and that may be used for a comparison of molecular volumes under conditions such that the liquids have the same surface tension and for determinations of partial structure of compounds by adding values obtained for constituent atoms and structural features called also molar parachor, molecular parachor.

Parachor is a quantity defined according to the formula:

$$P = Y^{1/4} M/d$$

Where: $Y^{1/4}$ is the fourth root of surface tension

M is the molar mass

D is the density.

**Density:**

The density, or more precisely, the volumetric mass density, of a substance is its mass per unit volume.

$$\rho = m / V$$

**Dielectric constant**

Substances have capacity to produce dipoles in another molecule. Dielectric constant is a measure of this capacity and it is a physical property. It is affected by both the attractive forces that exist between atoms and also molecules. It is denoted by E.

## 5.2 *IN SILICO* SCREENING OF DESIGNED COMPOUNDS

*In silico* is an expression meaning "performed on computer or via computer simulation" in reference to biological experiments. When lead molecules have been identified, they have to be optimized in terms of potency, selectivity, pharmacokinetics (i.e.) absorption, distribution, metabolism and excretion (ADME) and toxicology before they can become candidates for drug development. *In silico* approaches to predict pharmacokinetic parameters (ADME) were pioneered by Lipinski *et al*. By studying the physicochemical properties of >2000 drugs from the WDI (World Drug Index, Derwent Information, London), which can be assumed to have entered Phase II human clinical trials (and therefore must possess drug-like properties), the so-called ‘rule-of five’ was derived to predict oral bioavailability (intestinal absorption) of a compound that can be considered as the major goal of drug development.

The ADME properties of the designed compounds were evaluated using Swiss ADME and PreADMET online softwares. Toxicity of all the designed compounds was evaluated by using ProTox II software.

**SwissADME:**

**Lipinski's rule of five**

Lipinski's rule of five also known as the Pfizer's rule of five or simply the rule of five (RO5) is a rule of thumb to evaluate drug likeness or determine if a chemical compound with a certain pharmacological or biological activity has chemical

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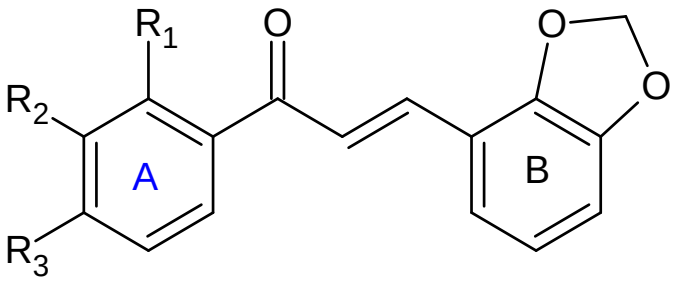


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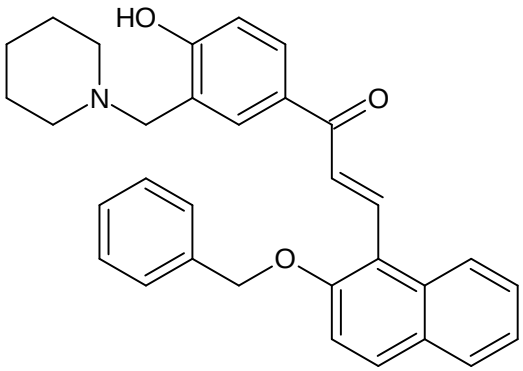

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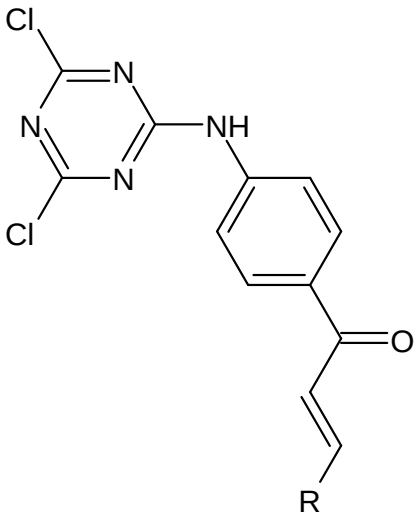


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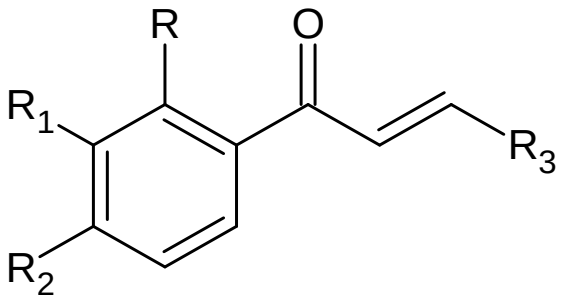


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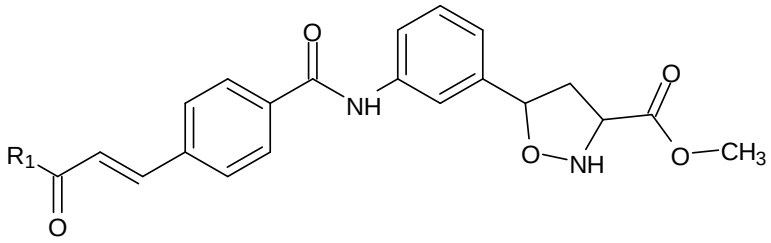
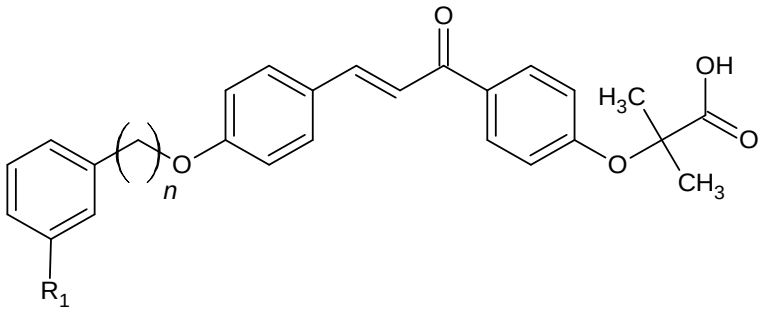
[93]

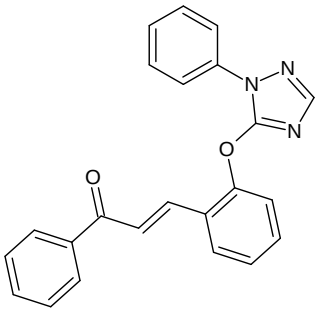
43. Venkata Madhavi Yaddanapudi *et al.,* performed synthesized new chalcone linked 5-phenyl-3-isoxazole carboxylic acid methyl esters-chalcone hybrids compound potentially active against drug resistant *mycobacterium tuberculosis*. Finally concluded that, 35 synthesized derivatives described the significant most potent in derivatives each against Mtb H37Rv. [94]



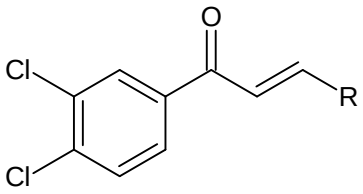

44. Rasha M. Hassan *et al.,* were synthesized a novel 4-(2-phenylethoxy) chalcone fibrate hybrids and screened antihyperlipidemic activity. From the result, the compound obtained good agonistic activity on PPARα compared to fenofibric acid showed the better anti-hyperlipidemic activity. [95]



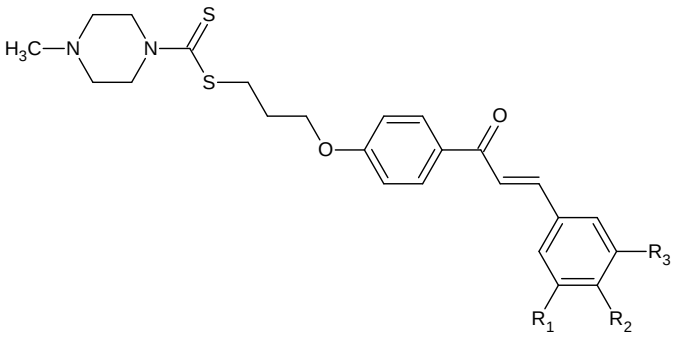

45. Synthesized and performed nineteen chalcone-tetrazole hybrids and their cyclic pyrazoline-tetrazole structures and screened *in vitro* antiproliferative activity were previously reported by Heidi S. Abd EIMonaem et al., and Based on MTT-Assay. From the result, the compound found to be the excellent potent antiproliferative activity against colon HCT-116 and prostate PC-3 cell lines. [96]




46. Synthesized Dichloro Substituted chalcones and Dihydropyrazole derivatives evaluated for their antiproliferative activity previously were explained by Afzal b. Shaik *et al*., and From the result, the compound containing 2-thienyl moiety in the chalcone series exhibited highest antiproliferative activity. [97]




47. Hong-Min Liu *et al.,* performed synthesized a series of novel dithiocarbamate-chalcone derivates and screened antiproliferative activity and From the result, the compound found to be best activity growth inhibition against SK-N-SH investigated arresting the cell cycle at GO/G1 phase. [98]



# AIM AND OBJECTIVE

## 3. AIM AND OBJECTIVE

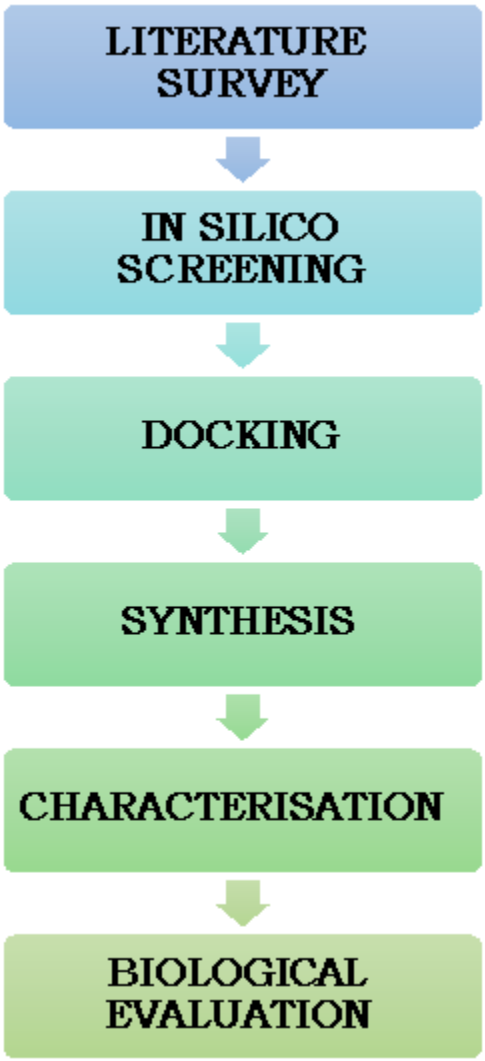
- Bacterial resistance is undoubtedly recognised as a major medical challenge in most healthcare systems.
- Many governmental and agency reports have been published regarding the agricultural use of antibacterials, advising less use of antibacterials, appropriate choice of antibacterials and regimens, prevention of cross-infection and development of new antibacterials.
- The main objectives of this study is to develop effective antibacterial compounds with a broad spectrum of antibacterial activity and less potential to develop resistance.

The work is aimed at

- To design hybrid benzimidazolyl chalcone derivatives using ACD/ChemSketch FREEWARE software.
- To perform docking studies for all designed compounds with DNA gyrase subunit B using Autodock vina (Version 1.5.6) in Pyrx.
- To synthesize hybrid benzimidazolyl chalcone derivatives based on docking score.
- To characterize the synthesized compounds using IR, NMR, Mass spectrometry.
- To evaluate the antibacterial activity of the synthesized compounds.

# PLAN OF STUDY

## 4. PLAN OF THE STUDY:



# MATERIALS AND METHODS

# 5. MATERIALS AND METHODS

## 5.1 DESIGN OF COMPOUNDS:

20 compounds were designed using Chemsketch software. Physicochemical properties were calculated for all the compounds.

**Molar refractivity:**

Molar refractivity, A, is a measure of the total polarizability of a mole of a substance and is dependent on the temperature, the index of refraction, and the pressure.

The molar refractivity is defined as

$$A = \frac{4}{3}\pi N_A \alpha$$

Where $N_A \approx 6.02210^{23}$ is the Avogadro constant and $\alpha$ is the mean polarizability of the molecule.

**Molar Volume:**

The molar volume, symbol $V_m$, is the volume occupied by one mole of a substance (chemical element or chemical compound) at a given temperature and pressure. It is equal to the molar mass (M) divided by the mass density (ρ). It has the SI unit cubic meters per mole (m3/mol), although it is more practical to use the units cubic decimeters per mole (dm3/mol) for gases and cubic centimeters per mole (cm3/mol) for liquids and solids. The molar volume of a substance can be found by measuring its molar mass and density then applying the relation

$$Vm = M/p$$

**Index of refraction:**

The refractive index or index of refraction of a material is a dimensionless number that describes how light propagates through that medium.

It is defined as

$$n = c/v$$

Where, c is the speed of light in vacuum and v is the phase velocity of light in the medium.

**Surface Tension:**

Surface tension is the elastic force of a fluid surface which makes it acquire the least surface area possible. Surface tension has the dimension of force per unit length, or of energy per unit area.

**Polarizability:**

Polarizability is the ability to form instantaneous dipoles. It is a property of matter. Polarizabilities determine the dynamical response of a bound system to external fields and provide insight into a molecule's internal structure. In a solid, polarizability is defined as the dipole moment per unit volume of the crystal cell.

**Parachor:**

It is an empirical constant for a liquid that relates the surface tension to the molecular volume and that may be used for a comparison of molecular volumes under conditions such that the liquids have the same surface tension and for determinations of partial structure of compounds by adding values obtained for constituent atoms and structural features called also molar parachor, molecular parachor.

Parachor is a quantity defined according to the formula:

$$P = Y^{1/4} M/d$$

Where: $Y^{1/4}$ is the fourth root of surface tension

M is the molar mass

D is the density.

**Density:**

The density, or more precisely, the volumetric mass density, of a substance is its mass per unit volume.

$$\rho = m / V$$

**Dielectric constant**

Substances have capacity to produce dipoles in another molecule. Dielectric constant is a measure of this capacity and it is a physical property. It is affected by both the attractive forces that exist between atoms and also molecules. It is denoted by E.

## 5.2 *IN SILICO* SCREENING OF DESIGNED COMPOUNDS

*In silico* is an expression meaning "performed on computer or via computer simulation" in reference to biological experiments. When lead molecules have been identified, they have to be optimized in terms of potency, selectivity, pharmacokinetics (i.e.) absorption, distribution, metabolism and excretion (ADME) and toxicology before they can become candidates for drug development. *In silico* approaches to predict pharmacokinetic parameters (ADME) were pioneered by Lipinski *et al*. By studying the physicochemical properties of >2000 drugs from the WDI (World Drug Index, Derwent Information, London), which can be assumed to have entered Phase II human clinical trials (and therefore must possess drug-like properties), the so-called ‘rule-of five’ was derived to predict oral bioavailability (intestinal absorption) of a compound that can be considered as the major goal of drug development.

The ADME properties of the designed compounds were evaluated using Swiss ADME and PreADMET online softwares. Toxicity of all the designed compounds was evaluated by using ProTox II software.

**SwissADME:**

**Lipinski's rule of five**

Lipinski's rule of five also known as the Pfizer's rule of five or simply the rule of five (RO5) is a rule of thumb to evaluate drug likeness or determine if a chemical compound with a certain pharmacological or biological activity has chemical

properties and physical properties that would make it a likely orally active drug in humans.

**The rule:**

Lipinski's rule states that, in general, an orally active drug has no more than one violation of the following criteria:

- ✓ No more than 5 hydrogen bond donors (the total number of nitrogen–hydrogen and oxygen–hydrogen bonds)
- ✓ No more than 10 hydrogen bond acceptors (all nitrogen or oxygen atoms)
- ✓ A molecular mass less than 500 daltons
- ✓ An octanol-water partition coefficient log P not greater than 5

**Ghose Filter:**

This filter defines drug-likeness constraints as follows:

- ✓ Calculated log P is between -0.4 and5.6
- ✓ Molecular weight is between 160 and480
- ✓ Molar refractivity is between 40 and130
- ✓ The total number of atoms is between 20 and70.

**Veber Filter:**

The molecules fitting to these two properties have a high probability of good oral bioavailability.

- ✓ Rotatable bond: max. **12**
- ✓ Polar Surface Area: max. **140A²**

**Egan Rule**

Predicts good or bad oral bioavailability.

- ✓ **0 ≥ TPSA ≤132**
- ✓ **-1 ≥ logP ≤6.**

**Molar Refractivity:**

It is a measure of the total polarizability of a mole of a substance and is dependent on the temperature, the index of refraction, and the pressure.

The molar refractivity is defined as

$$A = 4PI/3\ N_A\alpha$$

Where $N_A = 6.022\times10^{23}$ is the Avogadro constant and α is the mean polarizability of a molecule

**Polar surface area (PSA) or topological polar surface area (TPSA):**

It is a measure of apparent polarity of a molecule is defined as the surface sum overall polar atoms, primarily oxygen and nitrogen, also including their attached hydrogen atoms. PSA is a commonly used for the optimization of a drug's ability to permeate cells. Molecules with a polar surface area of greater than 140 angstroms squared tend to be poor at permeating cell membranes.

For molecules to penetrate the blood–brain barrier (and thus act on receptors in the central nervous system), a PSA less than 90 angstroms squared is usually needed.

Topological PSA (TPSA, fast 2D calculation).

**ADME Guideline:**

- ✓ TPSA < 140 Å2 good intestinal absorption.
- ✓ TPSA < 70 Å2 good brain penetration.

**Lipophilicity:**

Lipophilicity is the ability of a molecule to mix with an oily phase rather than with water, is usually measured as partition coefficient, *P*, between the two phases and is often expressed as log *P*. Lipophilicity has also been found to affect a number of pharmacokinetic parameters: higher lipophilicity (log*P*>5) gives, in general, lower solubility, higher permeability in the gastrointestinal tract, across the blood–brain barrier and other tissue membranes, higher affinity to metabolizing enzymes and efflux pumps, and higher protein binding. Low lipophilicity can also negatively impact permeability and potency and thus results in low BA and efficacy.

**Partition coefficient, P:**

It is defined as a particular ratio of the concentrations of a solute between the two solvents (a biphase of liquid phases), specifically for un- ionized solutes, and the logarithm of the ratio is thus **log *P***. When one of the solvents is water and the other is a non-polar solvent, then the log *P* value is a measure of lipophilicity or hydrophobicity.

- ✓ log $P_{oct/wat}$= log[solute]unionized octanol / [solute] unionized water
- ✓ log $P_{oct/wat}$=log $C_O/C_W$

Lipophilicity not only impacts solubility but also influences permeability, potency, selectivity, absorption, distribution, metabolism, and excretion (ADME) properties and toxicity. A desired logP value (octanol-water partition coefficient) is no more than 5.

**Water Solubility:**

Water solubility is a measure of the amount of chemical substance that can dissolve in water at a specific temperature. Solubility is common physicochemical

parameter for drug discovery compounds. Determination of the aqueous solubility of the drug candidate is an important analysis as it reflects the bioavailability of the compound.

**Log S:**

The aqueous solubility of a compound significantly affects its absorption and distribution characteristics. Typically, a low solubility goes along with a bad absorption and therefore the general aim is to avoid poorly soluble compounds.

Log S value is a unit stripped logarithm (base10) of the solubility measured in mol/liter. Log S value should be greater than -4.

**Rotatable Bonds:**

The bioavailability of a drug like molecule is related with it rotatable bond number. Less than seven rotatable bonds are essential for good bioavailability. Many highly potent molecules carried more than 10 rotatable bonds and still administered through oral route.

**Hydrogen bond acceptors and donors:**

12 or fewer H-bond donors and acceptors will have a high probability of good oral bioavailability.

**PreADMET Drug-Likeliness:**

Drug likeness is a qualitative concept used in drug design for how "druglike" a substance is with respect to factors like bioavailability. It is estimated from the molecular structure before the substance is even synthesized and tested. The most well-known rule relating the chemical structures to their biological activities is Lipinski's rule and it is called the 'rule of five'. Another well-known rule is the Lead-like rule. PreADMET contains drug- likeness prediction module based on these rules.

**ADME Prediction:**

Numerous *in vitro* methods have been used in the drug selection process for assessing the intestinal absorption of drug candidates. Among them, Caco2-cell model and MDCK (Madin-Darby canine kidney) cell model has been recommended

as a reliable in vitro model for the prediction of oral drug absorption. In absorption, this module provides prediction models for in vitro Caco2-cell and MDCK cell assay. Additionally, *in silico* HIA (human intestinal absorption) model and skin permeability model can predict and identify potential drug for oral delivery and transdermal delivery.

In distribution, BBB (blood brain barrier) penetration can give information of therapeutic drug in the central nervous system (CNS), plasma protein binding model in its disposition and efficacy. In order to build these QSAR models, genetic functional approximation is used to select relevant descriptors from all 2D descriptors that calculated by Topomol module, followed by Resilient back- propagation (Rprop) neural network to develop successful nonlinear model.

**Toxicity prediction:**

*In silico* toxicity prediction will have more and more importance in early drug discovery since 30% of drug candidates fail owing to these issues.

**ProTox II :**

ProTox II, a virtual lab for the prediction of toxicities of small molecules.The prediction of compound toxicities is an important part of the drug design development process. Computational toxicity estimations are not only faster than the determination of toxic doses in animals, but can also help to reduce the amount of animal experiments.

ProTox II incorporates molecular similarity, fragment propensities, most frequent features and (fragment similarity based CLUSTER cross- validation) machine-learning, based a total of 33 models for the prediction of various toxicity endpoints such as acute toxicity, hepatotoxicity, cytotoxicity, carcinogenicity, mutagenicity, immunotoxicity, adverse outcomes (Tox21) pathways and toxicity targets.

**Toxic doses and Toxicity classes:**

Toxic doses are often given as LD50 values in mg/kg body weight. The LD50 is the median lethal dose meaning the dose at which 50% of test subjects die upon exposure to a compound.x

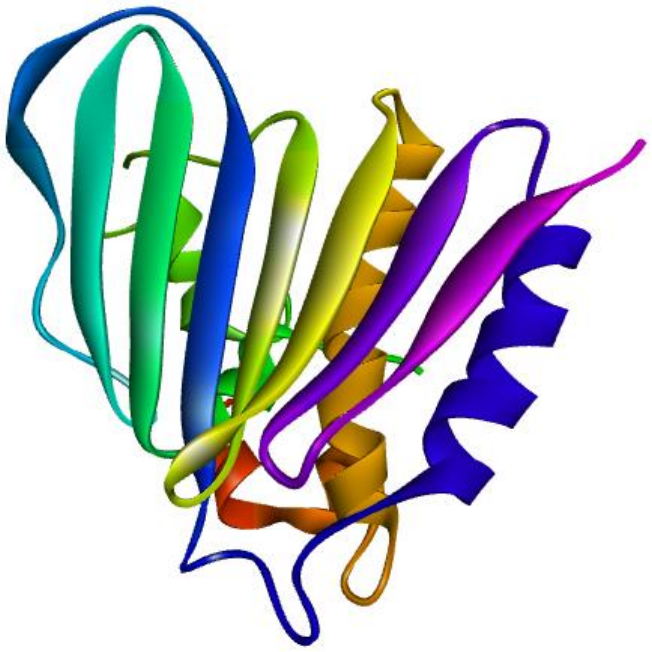
Toxicity classes are defined according to the globally harmonized system of classification of labelling of chemicals (GHS). LD50 values are given in [mg/kg]:

- Class I: fatal if swallowed ($LD50 \leq 5$)
- Class II: fatal if swallowed ($5 < LD50 \leq 50$)
- Class III: toxic if swallowed ($50 < LD50 \leq 300$)
- Class IV: harmful if swallowed ($300 < LD50 \leq 2000$)
- Class V: may be harmful if swallowed ($2000 < LD50 \leq 5000$)
- Class VI: non-toxic ($LD50 > 5000$)

## 5.3 DOCKING

Docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using, for example, scoring functions.

In this study, Autodock vina (Version 1.5.6) in PyrX is used to perform docking studies. Docking studies were performed with DNA gyrase subunit B (PDB ID: 1KZN). Protein was downloaded from RCSB and used for docking analysis.



**3D View of 1KZN**

**5.4 SYNTHESIS:**

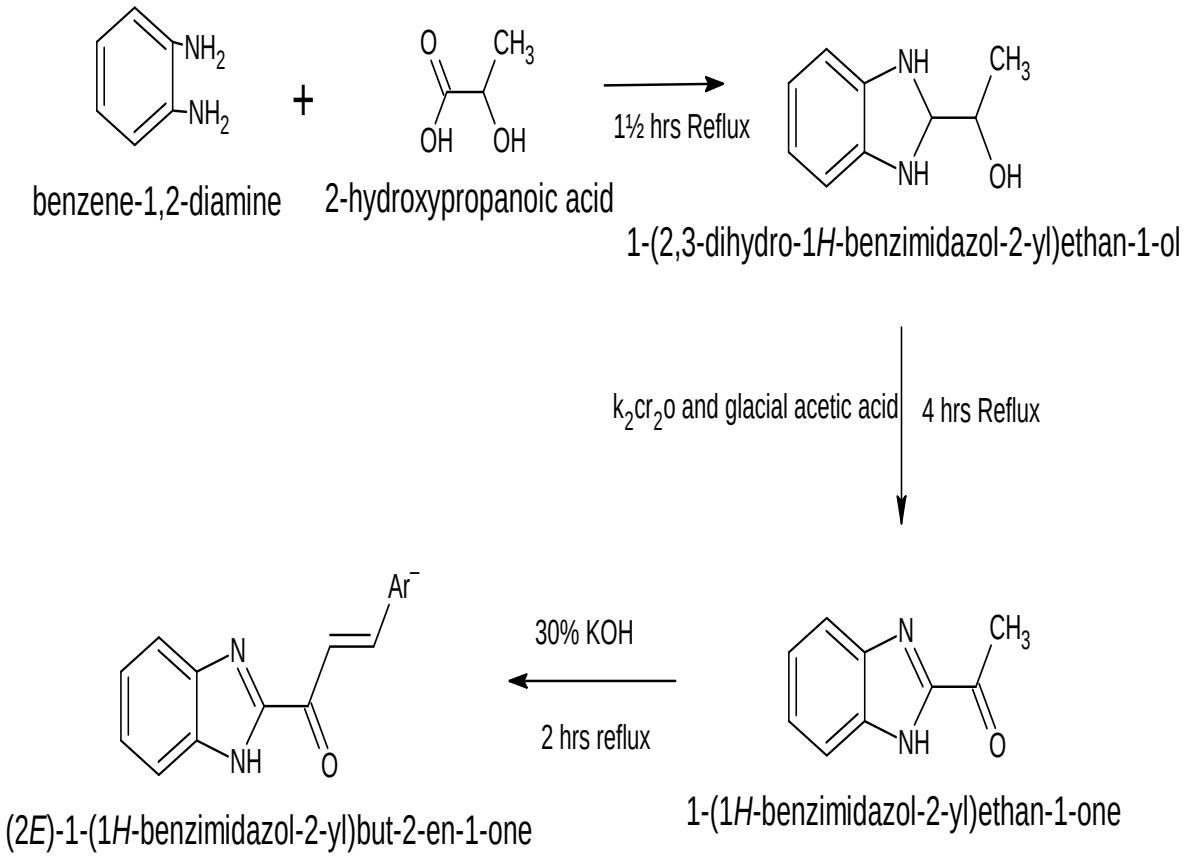



**Figure 1: Synthetic Scheme**

**General Procedure for Synthesis:**

**STEP 1:**

**Synthesis of 2-hydroxy ethyl Benzimidazole:**

The Lactic acid (0.01 mol) and Ortho-Phenylenediamine (0.01 mol) were refluxed for one and half an hour. The mixture was cooled, 25% KOH was added until the product was just alkaline to litmus.

**STEP 2:**

**Synthesis of 2-acetyl Benzimidazole:**

The 2-hydroxy ethyl benzimidazole (0.01 mol) was taken and it was oxidized in presence of potassium dichromate (0.01 mol) and refluxed for 4 h with glacial acetic acid. After refluxing the product, it was cooled and neutralized by adding ammonia solution. Then the product dried, recrystallized and the melting point was determined.

**STEP 3:**

**Synthesis of benzimidazolyl chalcones:**

The 2-acetyl benzimidazole (0.0l mol) in 10 ml 30% potassium hydroxide with 0.012 molar of various aromatic aldehydes are added separately, the refluxed for 2 hours with water bath, allow cooling to the room temperature and pouring the mixture into the beaker containing ice cold water, with stirring to get a product and the above products were collected by filtration with ice cold water, dried and recrystallized from 90% ethanol.

## 5.5. CHARACTERIZATION:

All the synthesized compounds were characterized by using FT-IR, $^{1}$H- NMR, $^{13}$C-NMR, MASS Spectroscopy.

### Infrared Spectroscopy:

The infrared spectroscopy is one of the most powerful analytical techniques, this offers the possibility of chemical identification. The most important advantages of infrared spectroscopy over the other usual methods of structural analysis are that it provides useful information about the functional groups present in the molecule quickly. The technique is based upon the simple fact that a chemical substance shows marked selectable absorption in the infrared region. After absorbing IR radiations the molecules of a chemical compound exhibit small vibrations, giving rise to closely packed absorption bands called as IR absorption spectrum which may extend over a wide wavelength range. Various bands will be present in IR spectrum which corresponds to the characteristic functional groups and bonds present in a chemical substance. Thus an IR spectrum of a chemical compound is a fingerprint for its identification.

### Nuclear Magnetic Resonance Spectroscopy:

It is the branch of spectroscopy in which radiofrequency waves induces transitions between magnetic energy levels of nuclei of a molecule. The magnetic energy levels are created by keeping nuclei in a magnetic field. Without the magnetic field the spin states of nuclei are degenerated i.e., possess the same energy and the energy level transition is not possible. The energy level transition is possible with the application of external magnetic field which requires different Rf radiation to put them into resonance. This is a measurable phenomenon. It is a powerful tool for the investigation of nuclei structure. $^{1}$HNMR and $^{13}$CNMR Spectras of the prepared derivatives were done by using 400-MHz and 500-MHz Bruker spectrometer using internal standard as tetra methyl silane. $^{1}$H and $^{13}$C NMR Spectral were taken with dimethyl sulphoxide (DMSO) as a solvent and the data of chemical shift were shown as delta values related to trimethylsilane (TM) in ppm.

**Mass spectroscopy:**

Mass spectrometer performs three essential functions. First, it subjects molecules to bombardment by a stream of more amounts of energy electrons, converting some of the molecules to ions, which are then accelerated in a field of electric. Second, the ions which are accelerated are divided according to their ratios of mass to charge in an electric or magnetic field. Finally, the ions that have particular mass-to-charge ratio are detected by a device which can count the number of ions striking it. The detector's output is amplified and fed to a recorder. The trace from the recorder is a mass spectrum a graph of particles detected as a function of mass-to- charge ratio. The Mass spectra of the synthesized compounds were taken using Agilent spectrometer.

**Synthesis of (2*E*)-1-(1*H*-benzimidazol-2-yl)-3-(3-hydroxy-4-methoxyphenyl) prop-2-en-1-one (3b):**

The 2-acetyl benzimidazole (0.0l mol) in 10 ml 30% potassium hydroxide with 0.012 molar of Vanillin are added separately, the refluxed for 2 hours with water bath, allow cooling to the room temperature and pouring the mixture into the beaker containing ice cold water, with stirring to get a product and the above products were collected by filtration with ice cold water, dried and recrystallized from 90% ethanol.
Dark brown solid; Yield:78.8% ; Solubility: DMSO; mp: 135-145 °**C**; FT-IR(KBr, $cm^{-1}$): 1026.95 (C-C), 1504.31 (C=C), 3417.63 (NH), 1582.21 (CH=CH), 3731.14 (C-OH), 1454.34 (C-$CH_3O$). Elemental analysis for C17H14N2O3 Calculated C (69.38%), H(4.79%), N(9.52%), O(16.31%) found C (68.38%), H(4.19%), N(9.72%), O(15.31%).

**Synthesis of (2*Z*)-1-(1*H*-benzimidazol-2-yl)-3-(2-bromo-4,5-dimethoxyphenyl)prop-2-en-1-one (3d) :**

The 2-acetyl benzimidazole (0.0l mol) in 10 ml 30% potassium hydroxide with 0.012 molar of 2,Bromo-4,5-dimethoxybenzaldehyde are added separately, the refluxed for 2 hours with water bath, allow cooling to the room temperature and pouring the mixture into the beaker containing ice cold water, with stirring to get a product and

the above products were collected by filtration with ice cold water, dried and recrystallized from 90% ethanol.

Yellowish brown solid; yield:89.7%; Solubility: DMSO; mp: 205-210 °C; FT-IR(KBr, $cm^{-1}$): 1188.98 (C-C), 1589.35 (C=C), 3417.32 (NH), 1669.98 (C=N), 539.72 (C-Br), 1445.80 (C-$CH_3O$), ), Elemental analysis for Calculated $C_{18}H_{15}BrN_2O_3$ C(55.83%), H(3.9%), N(7.23%), Br(20.63%), O(12.39%) found C(54.83%), H(2.9%), N(7.33%), Br(20.73%), O(12.89%). $M^+$ calculated for $C_{18}H_{15}BrN_2O_3$ is 387.22 found: 387.22.

**Synthesis of (2*Z*)-1-(1*H*-benzimidazol-2-yl)-3-(2,4-dimethoxyphenyl)prop-2-en-1-one (3f) :**

The 2-acetyl benzimidazole (0.0l mol) in 10 ml 30% potassium hydroxide with 0.012 molar of 2,4-dimethoxybenzaldehyde are added separately, the refluxed for 2 hours with water bath, allow cooling to the room temperature and pouring the mixture into the beaker containing ice cold water, with stirring to get a product and the above products were collected by filtration with ice cold water, dried and recrystallized from 90% ethanol.

Yellowish brown solid; yield:87.8% ; Solubility: DMSO; mp: 100-105 **°C;** FT-IR(KBr, $cm^{-1}$): 828.85 (C-C), 1504.30 (C=C), 3417.51 (NH), 1731.29 (C=O), 1436.05 (C-$OCH_3$). Elemental analysis for Calculated $C_{18}H_{16}N_2O_3$ C(67.9%), H(8.23%), N(8.8%), O(15.07%) found C(67.8%), H(8.13%), N(8.5%), O(15.02%).

**Synthesis of (2*Z*)-1-(1*H*-benzimidazol-2-yl)-3-(4-hydroxy-3,5-dimethoxyphenyl)prop-2-en-1-one (3j) :**

The 2-acetyl benzimidazole (0.0l mol) in 10 ml 30% potassium hydroxide with 0.012 molar of syringaldehyde are added separately, the refluxed for 2 hours with water bath, allow cooling to the room temperature and pouring the mixture into the beaker containing ice cold water, with stirring to get a product and the above products were collected by filtration with ice cold water, dried and recrystallized from 90% ethanol.

Pinkish brown solid; yield: 90.5% : Solubility: DMSO; mp: 330-335 **°C;** FT-IR(KBr, $cm^{-1}$): 843.51 (C-C), 1557.24 (C=C), 3361.63 (N-H), 1643.61 (C=N), 1495.21 (C-$OCH_3$), Elemental analysis for Calculated $C_{18}H_{16}N_2O_4$ C(66.66%), H(4.97%), N(8.64%),

O(19.73%) found C(65.66%), H(4.77%), N(7.64%), O(19.53%). $M^+$ calculated for C18H16N2O4 is 324.33 found: 324.33.

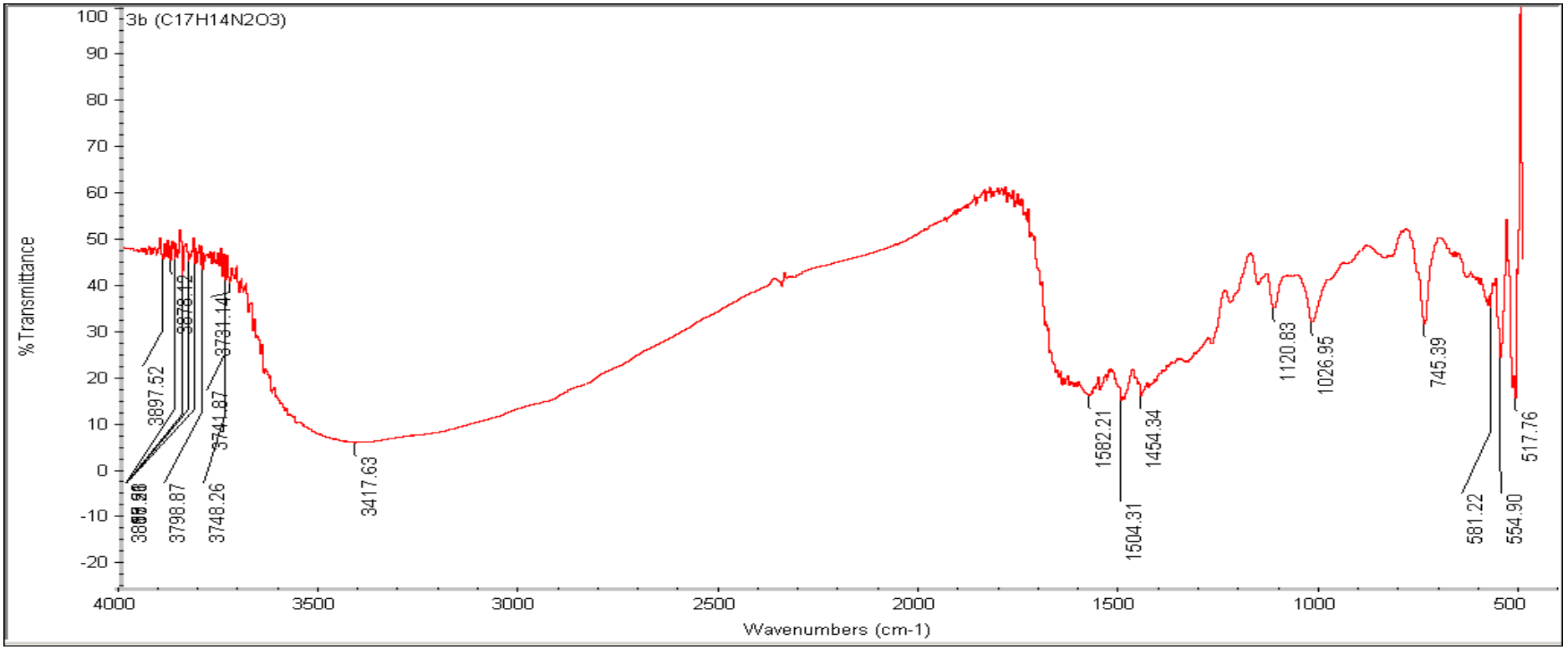
**Synthesis of (2*E*)-1-(1*H*-benzimidazol-2-yl)-3-[4-(dimethylamino)phenyl]prop-2-en-1-one (3r) :**

The 2-acetyl benzimidazole (0.0l mol) in 10 ml 30% potassium hydroxide with 0.012 molar of Para Dimethyl amino benzaldehyde are added separately, the refluxed for 2 hours with water bath, allow cooling to the room temperature and pouring the mixture into the beaker containing ice cold water, with stirring to get a product and the above products were collected by filtration with ice cold water, dried and recrystallized from 90% ethanol.

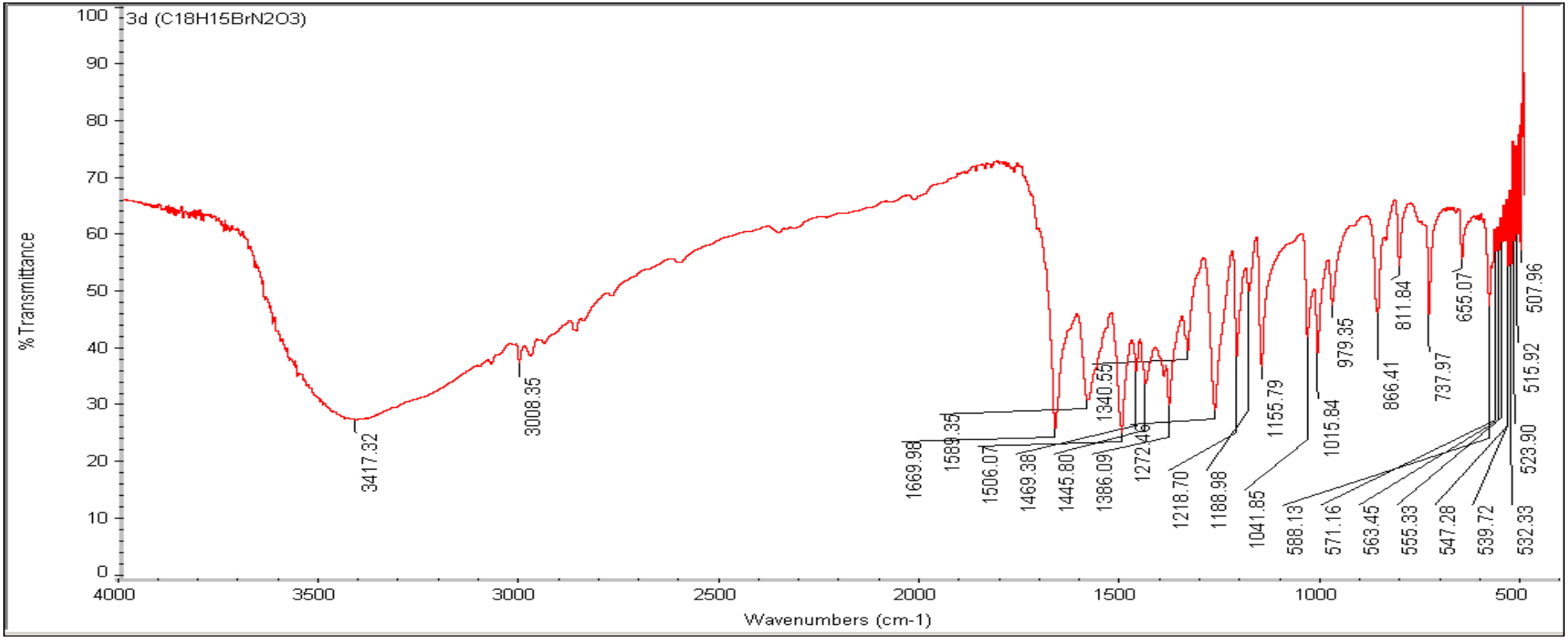
Reddish brown solid; yield:86.9%; Solubility: DMSO; mp: 200-205 **°C;** FT-IR(KBr, $cm^{-1}$): 812.73 (C-C), 1550.79 (C=C), 3428.61 (N-H), 1600.82 (C=N), 1000.00 (C-N), 824.88 (N-$CH_3$). Elemental analysis for Calculated C18H17N3O C(74.2%), H(5.88%), N(14.42%), O(5.49%) found C(73.4%), H(5.78%), N(13.42%), O(5.69%).

## IR Spectral data

### 3b:



**3d:**


3d (C18H15BrN2O3)
% Transmittance
Wavenumbers (cm-1)
100
90
80
70
60
50
40
30
20
10
0
4000
3500
3000
2500
2000
1500
1000
500
3417.32
3008.35
1669.98
1589.35
1506.07
1469.38
1445.80
1386.09
1340.55
1272.46
1218.70
1188.98
1155.79
1041.85
1015.84
979.35
866.41
811.84
737.97
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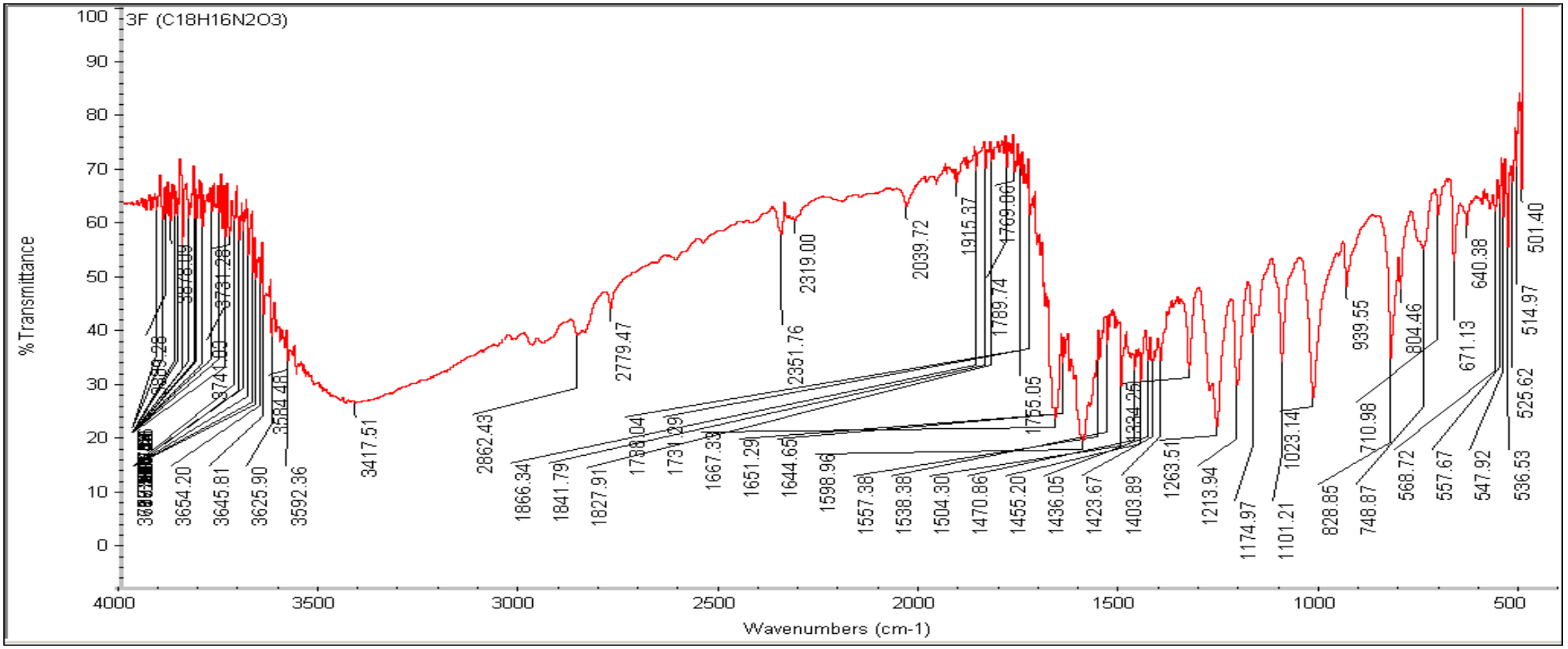
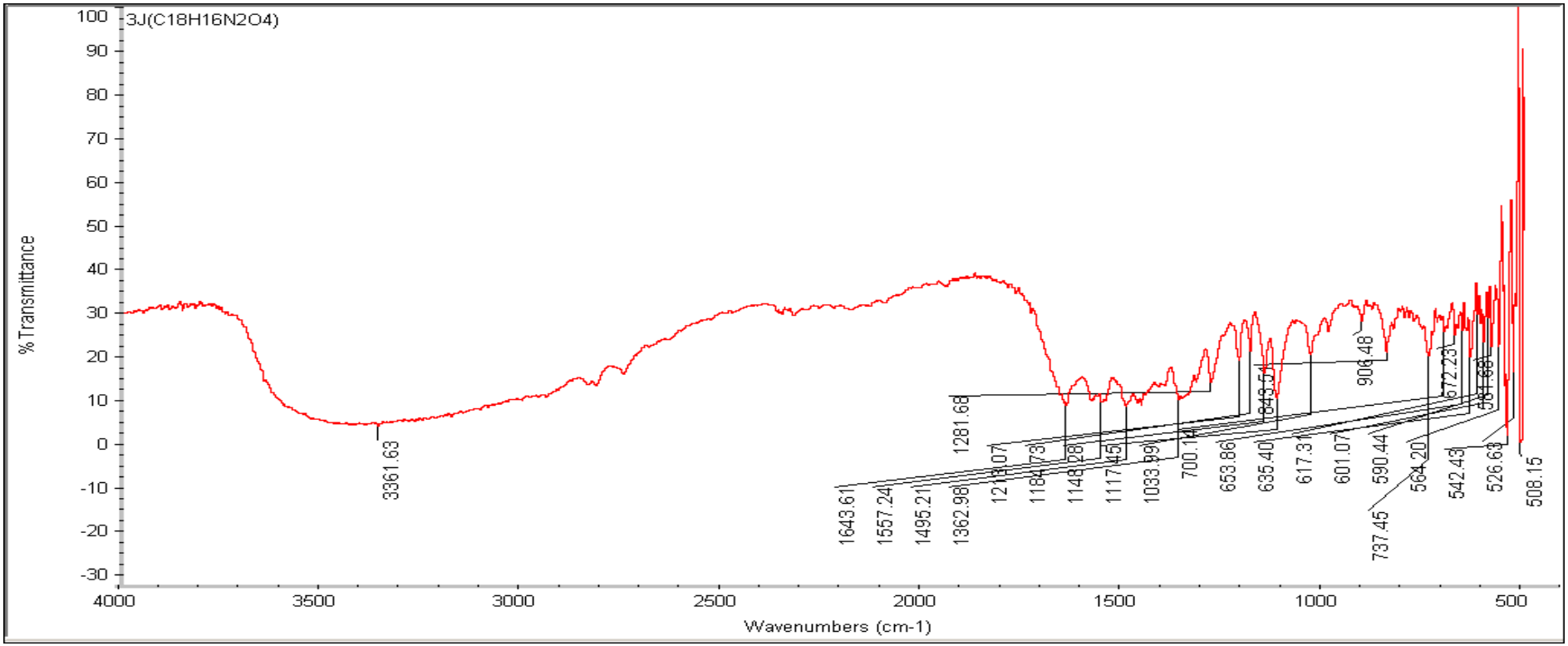
555.33
547.28
539.72
532.33
523.90
515.92
507.96

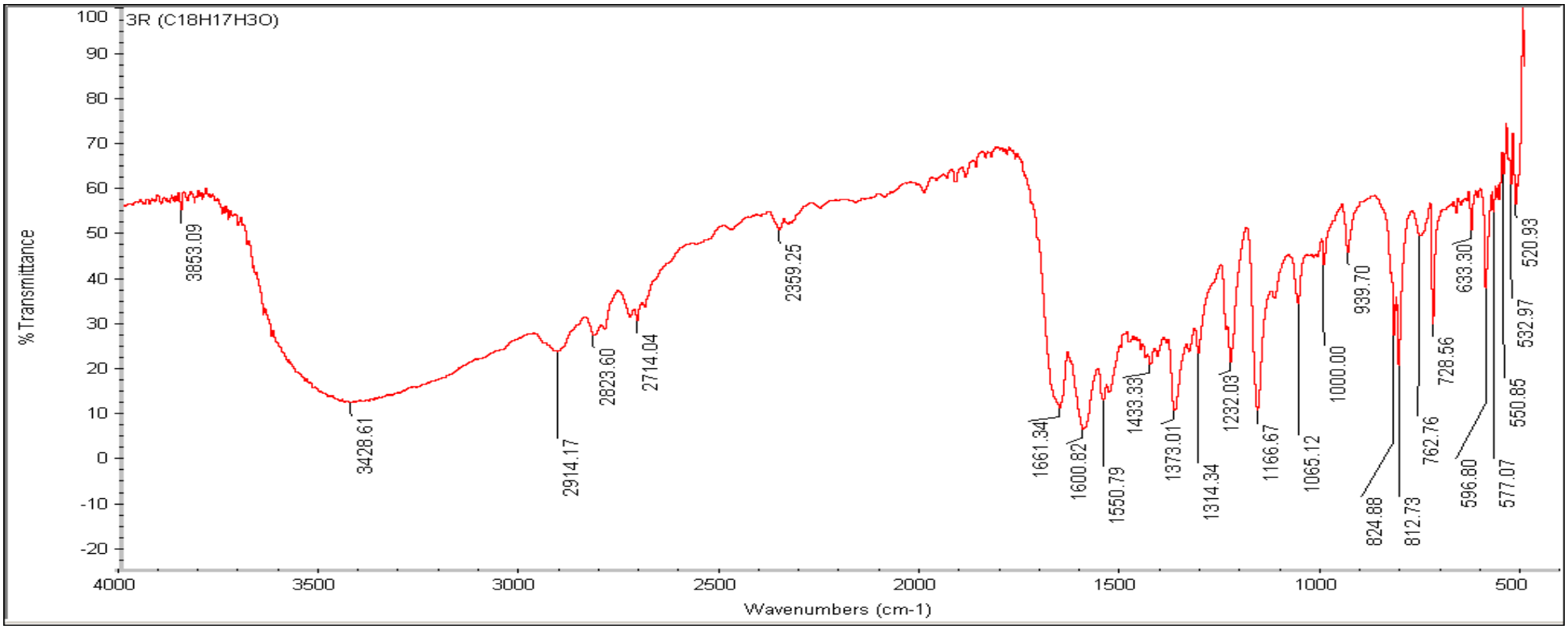
**3f:**


3F (C18H16N2O3)
% Transmittance
Wavenumbers (cm-1)
100
90
80
70
60
50
40
30
20
10
0
4000
3500
3000
2500
2000
1500
1000
500
3654.20
3645.81
3625.90
3592.36
3417.51
2862.43
2779.47
2351.76
2319.00
2039.72
1915.37
1866.34
1841.79
1827.91
1667.39
1651.29
1644.65
1598.96
1557.38
1538.38
1504.30
1470.86
1455.20
1436.05
1423.67
1403.89
1263.51
1213.94
1174.97
1101.21
1023.14
939.55
828.85
748.87
710.98
671.13
640.38
568.72
557.67
547.92
536.53
525.62
514.97
501.40

**3j:**

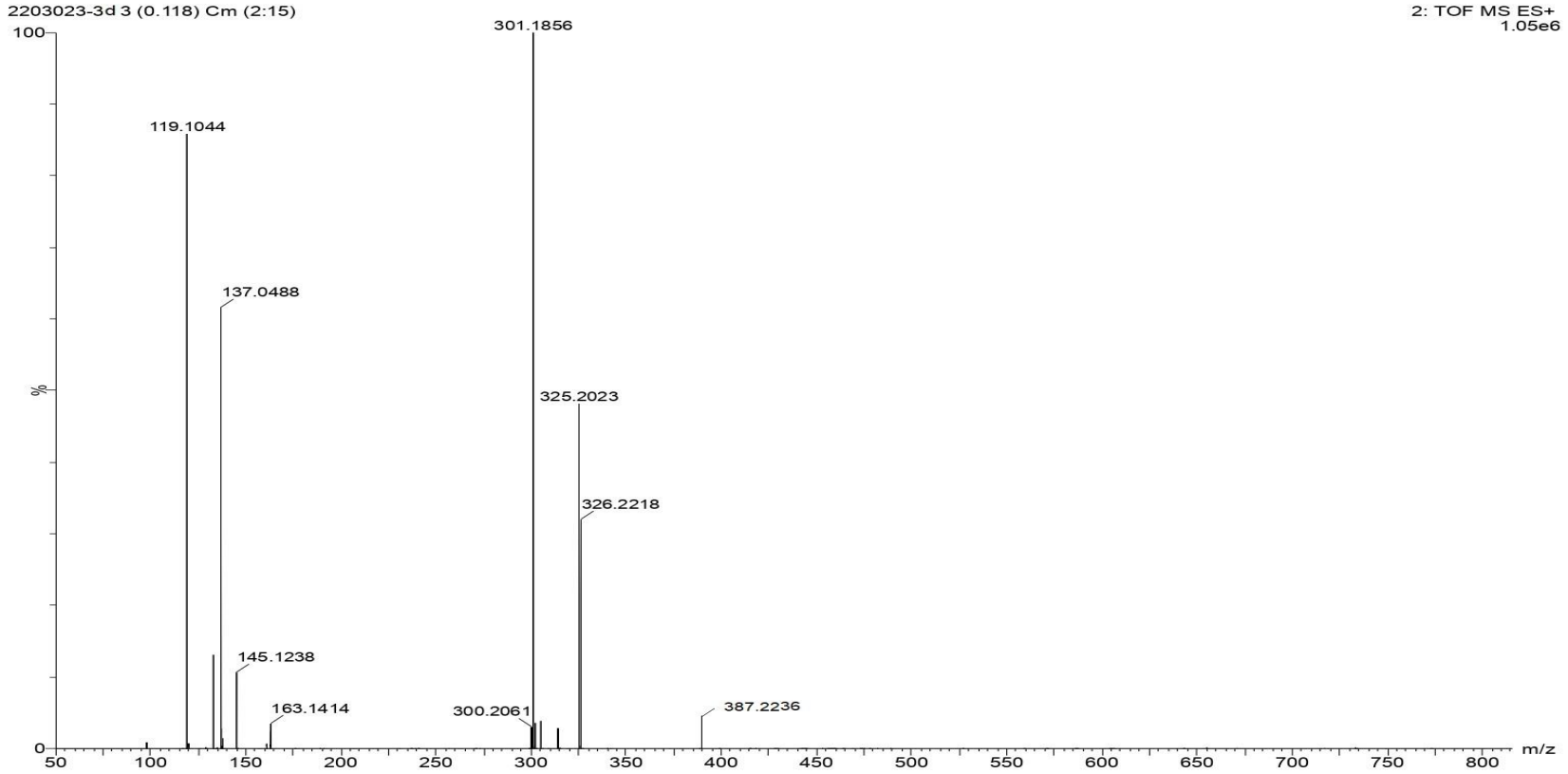

3J(C18H16N2O4)
% Transmittance
Wavenumbers (cm-1)
3361.63
1643.61
1557.24
1495.21
1362.98
1281.68
1184.73
1148.28
1117.45
1033.99
700.12
653.86
635.40
617.31
601.07
590.44
564.20
542.43
526.63
508.15
737.45
904.48
672.23

**3r:**

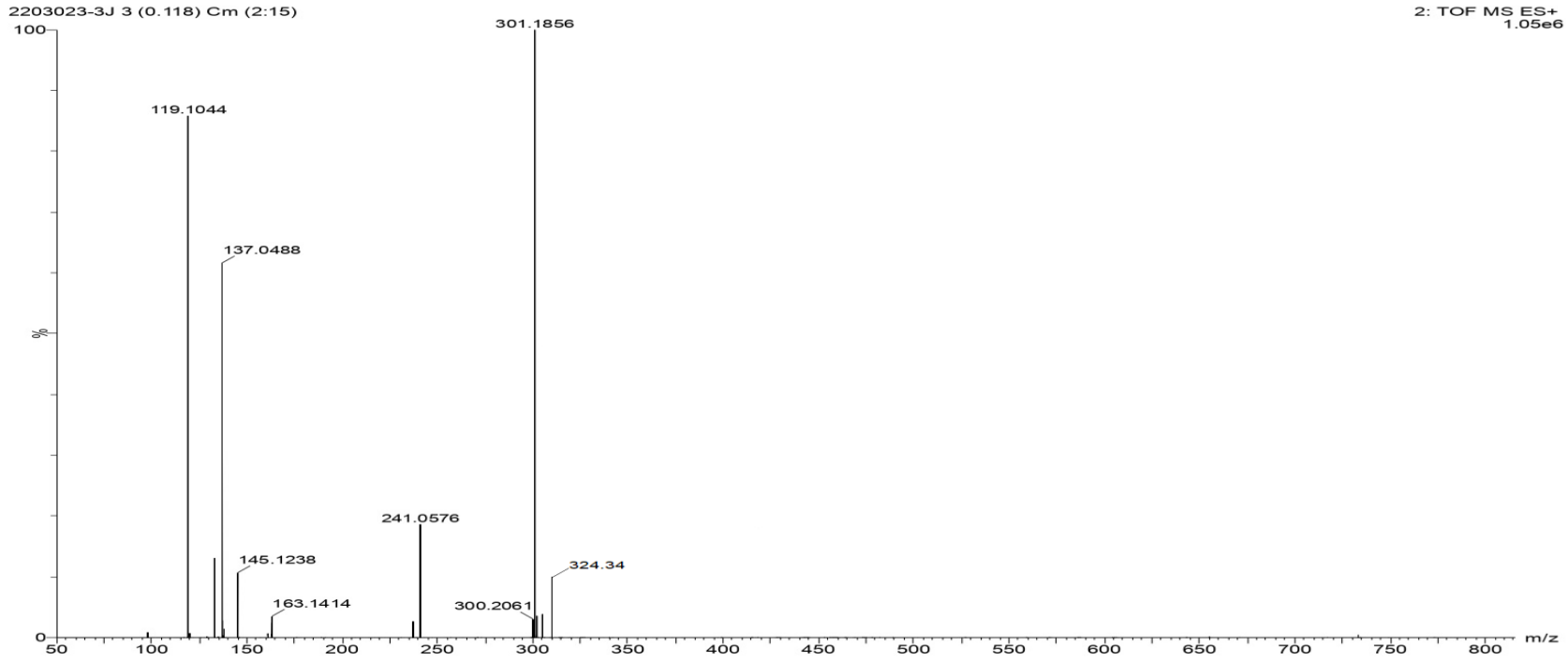

3R (C18H17H3O)
% Transmittance
Wavenumbers (cm-1)
3853.09
3428.61
2914.17
2823.60
2714.04
2359.25
1661.34
1600.82
1550.79
1433.33
1373.01
1314.34
1232.03
1166.67
1065.12
1000.00
939.70
824.88
812.73
762.76
728.56
633.30
596.80
577.07
550.85
532.97
520.93

**MASS SPECTRA:**

**(3d):**



**(3j):**


2203023-3J 3 (0.118) Cm (2:15)
2: TOF MS ES+
1.05e6
100
%
0
301.1856
119.1044
137.0488
241.0576
145.1238
324.34
163.1414
300.2061
50
100
150
200
250
300
350
400
450
500
550
600
650
700
750
800
m/z

## 5.6. ANTIBACTERIAL ACTIVITY:

## AGAR WELL DIFFUSION METHOD:

## PRINCIPLE:

The antimicrobials present in the given sample were allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimeters.

## MATERIALS REQUIRED:

(*E..coli-* 443*)* was purchased from MTCC, Chandihar, India. Nutrient Agar medium, Nutrient broth, Gentamicin antibiotic solution was purchased from Himedia, India. Test samples, petri-plates, test tubes, beakers conical flasks were from Borosil, India. Spirit lamp, double distilled water.

## 1. AGAR- WELL DIFFUSION METHOD:

### a. Nutrient Agar Medium

The medium was prepared by dissolving 2.8 g of the commercially available Nutrient Agar Medium (HiMedia) in 100ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petriplates (25-30ml/plate) while still molten.

### b. Nutrient broth

Nutrient broth was prepared by dissolving 2.8 g of commercially available nutrient medium (HiMedia) in 100ml distilled water and boiled to dissolve the medium completely. The medium was dispensed as desired and sterilized by autoclaving at 15 lbs pressure (121ºC) for 15 minutes.

**PROCEDURE:**

Petri plates containing 20 ml nutrient agar medium were seeded with 24 hr culture of bacterial strains were adjusted to 0.5 OD value according to McFarland standard, (*E.coli*- 443) Wells were cut and concentration of sample 3b, 3r, 3f, 3d and 3j (500, 250, 100 and 50 µg/ml) was added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the wells. Gentamicin antibiotic was used as a positive control. The values were calculated using Graph Pad Prism 6.0 software (USA).

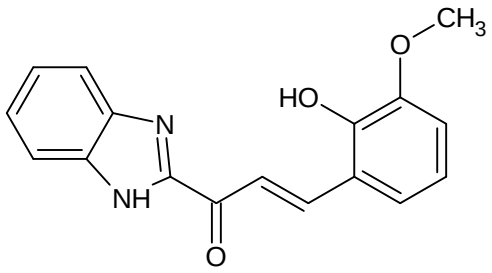
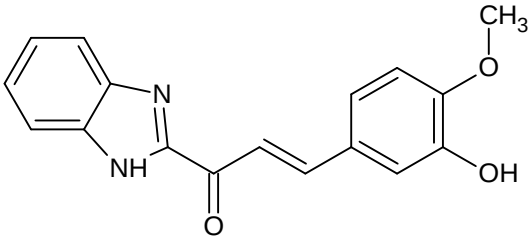
# RESULTS AND DISCUSSION

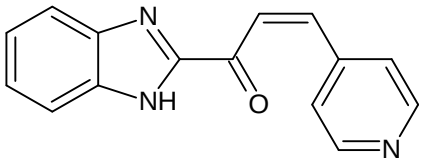
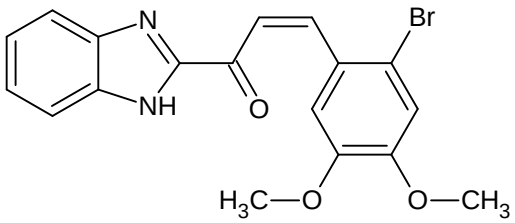
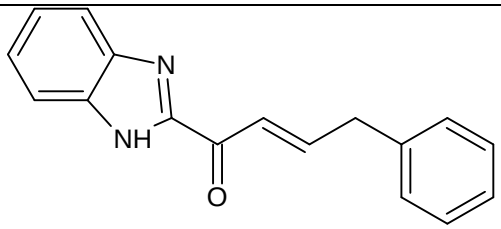
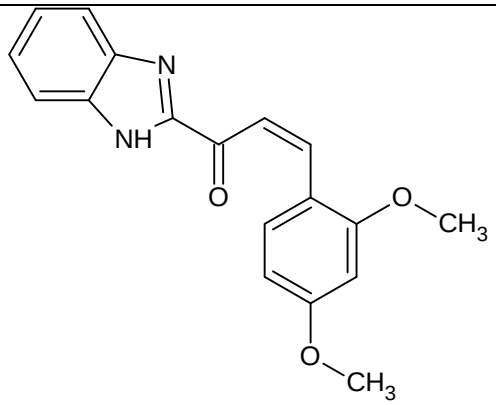
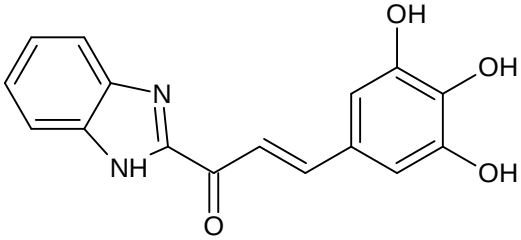
## 6. Results and discussion

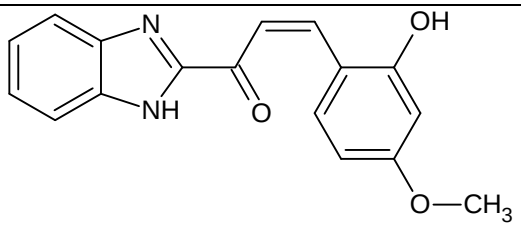
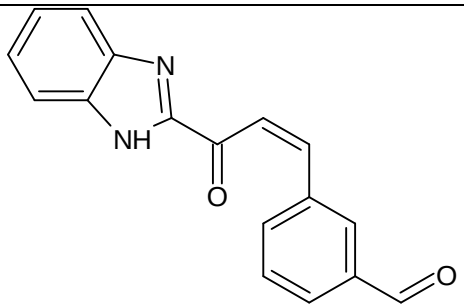
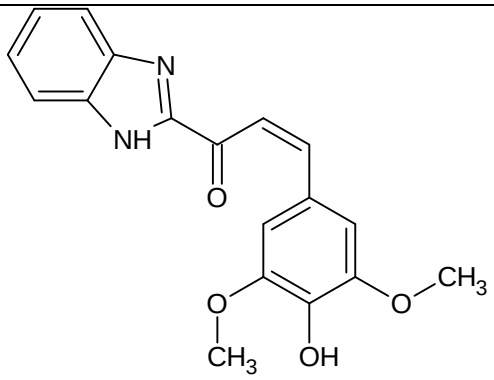
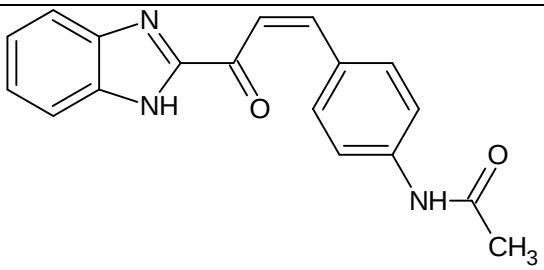
### 6.1. DESIGN OF COMPOUNDS

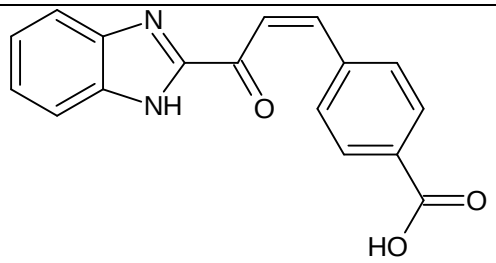
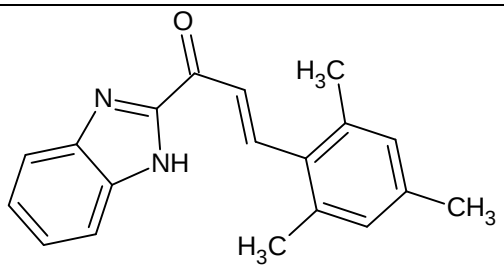
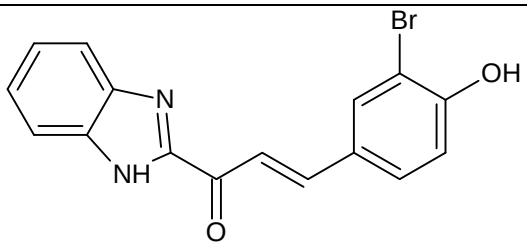
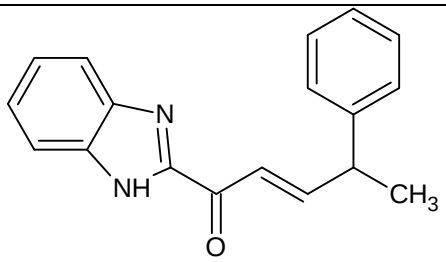
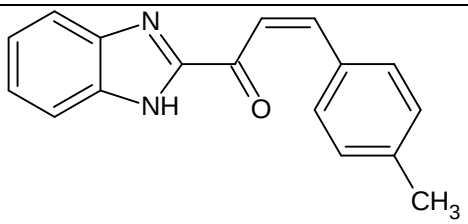
20 Compounds were designed by using Chemsketch and their structures were tabulated in Table No. 2 & 3. Their physicochemical properties were calculated.

**Table 2: Structures of designed compounds**

| Compound No. | Structure | IUPAC name |
|---|---|---|
| **3a** |  | (2*E*)-1-(1*H*-benzimidazol-2-yl)-3-(2-hydroxy-3-methoxyphenyl)prop-2-en-1-one |
| **3b** |  | (2*E*)-1-(1*H*-benzimidazol-2-yl)-3-(3-hydroxy-4-methoxyphenyl)prop-2-en-1-one |

| | | |
|---|---|---|
| **3c** |  | (2*Z*)-1-(1*H*-benzimidazol-2-yl)-3-(pyridin-4-yl)prop-2-en-1-one |
| **3d** |  | (2*Z*)-1-(1*H*-benzimidazol-2-yl)-3-(2-bromo-4,5-dimethoxyphenyl)prop-2-en-1-one |
| **3e** |  | (2*E*)-1-(1*H*-benzimidazol-2-yl)-4-phenylbut-2-en-1-one |
| **3f** |  | (2*Z*)-1-(1*H*-benzimidazol-2-yl)-3-(2,4-dimethoxyphenyl)prop-2-en-1-one |
| **3g** |  | (2*E*)-1-(1*H*-benzimidazol-2-yl)-3-(3,4,5-trihydroxyphenyl)prop-2-en-1-one |

| | | |
|---|---|---|
| **3h** |  | (2*Z*)-1-(1*H*-benzimidazol-2-yl)-3-(2-hydroxy-4-methoxyphenyl)prop-2-en-1-one |
| **3i** |  | 3-[(1*Z*)-3-(1*H*-benzimidazol-2-yl)-3-oxoprop-1-en-1-yl]benzaldehyde |
| **3j** |  | (2*Z*)-1-(1*H*-benzimidazol-2-yl)-3-(4-hydroxy-3,5-dimethoxyphenyl)prop-2-en-1-one |
| **3k** |  | *N*-{4-[(1*Z*)-3-(1*H*-benzimidazol-2-yl)-3-oxoprop-1-en-1-yl]phenyl}acetamide |

| | | |
|---|---|---|
| **3l** |  | 4-[(1*Z*)-3-(1*H*-benzimidazol-2-yl)-3-oxoprop-1-en-1-yl]benzoic acid |
| **3m** |  | (2*E*)-1-(1*H*-benzimidazol-2-yl)-3-(2,4,6-trimethylphenyl)prop-2-en-1-one |
| **3n** |  | (2*E*)-1-(1*H*-benzimidazol-2-yl)-3-(3-bromo-4-hydroxyphenyl)prop-2-en-1-one |
| **3o** |  | (2*E*)-1-(1*H*-benzimidazol-2-yl)-4-phenylpent-2-en-1-one |
| **3p** |  | (2*Z*)-1-(1*H*-benzimidazol-2-yl)-3-(4-methylphenyl)prop-2-en-1-one |

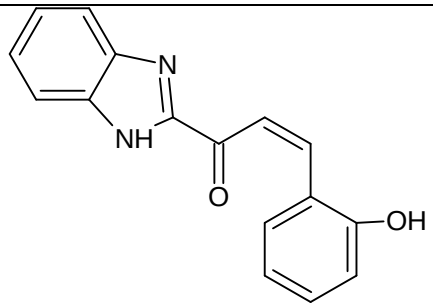
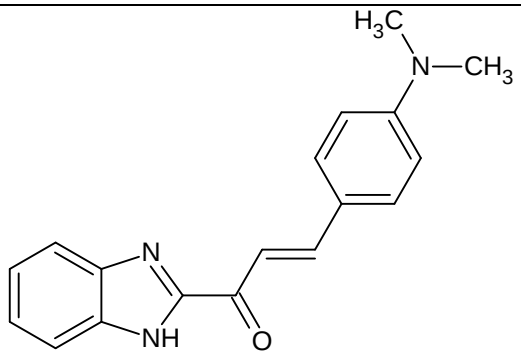
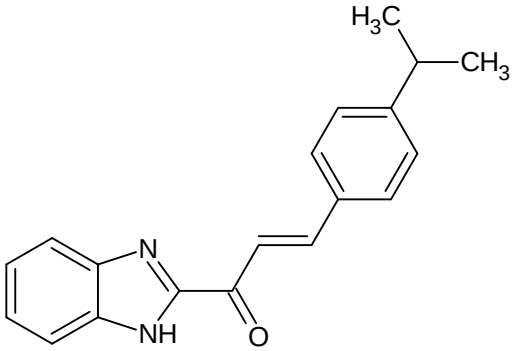
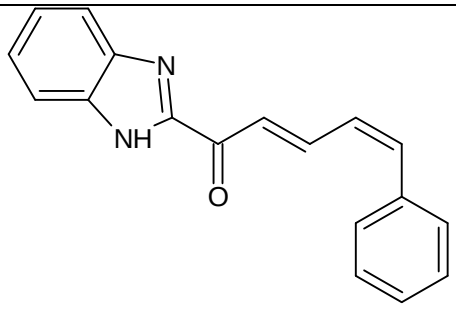
| | | |
|---|---|---|
| **3q** |  | (2*Z*)-1-(1*H*-benzimidazol-2-yl)-3-(2-hydroxyphenyl)prop-2-en-1-one |
| **3r** |  | (2*E*)-1-(1*H*-benzimidazol-2-yl)-3-[4-(dimethylamino)phenyl]prop-2-en-1-one |
| **3s** |  | (2*E*)-1-(1*H*-benzimidazol-2-yl)-3-[4-(propan-2-yl)phenyl]prop-2-en-1-one |
| **3t** |  | (2*E*,4*Z*)-1-(1*H*-benzimidazol-2-yl)-5-phenylpenta-2,4-dien-1-one |

**Table 3: Chemsketch Results for designed compounds**

| Compd. Code. | Formula | Molecular weight (g/mol) | Fraction Csp3 | Number rotatable bonds | Number H bond acceptors | Number H bond donors | Molar refractivity | TPSA (Å) |
|---|---|---|---|---|---|---|---|---|
| **3a** | C17H14N2O3 | 294.30 | 0.06 | 4 | 4 | 2 | 84.42 | 75.21 |
| **3b** | C17H14N2O3 | 294.30 | 0.06 | 4 | 4 | 2 | 84.42 | 75.21 |
| **3c** | C15H11N3O | 249.27 | 0.00 | 3 | 3 | 1 | 73.70 | 58.64 |
| **3d** | C18H15BRN2O3 | 387.23 | 0.11 | 5 | 4 | 1 | 96.58 | 64.21 |
| **3e** | C17H14N2O | 262.31 | 0.06 | 4 | 2 | 1 | 79.92 | 45.75 |
| **3f** | C18H16N2O3 | 308.33 | 0.11 | 5 | 4 | 1 | 2.77 | 64.21 |
| **3g** | C16H12N2O4 | 296.28 | 0.00 | 3 | 5 | 4 | 81.97 | 106.44 |
| **3h** | C17H14N2O3 | 294.30 | 0.06 | 4 | 4 | 2 | 84.42 | 75.21 |
| **3i** | C17H12N2O2 | 276.29 | 0.00 | 4 | 3 | 1 | 81.29 | 62.82 |
| **3j** | C18H16N2O4 | 324.33 | 0.11 | 5 | 5 | 2 | 90.91 | 84.44 |
| **3k** | C18H15N3O2 | 305.33 | 0.06 | 5 | 3 | 2 | 90.21 | 74.85 |
| **3l** | C17H12N2O3 | 292.29 | 0.00 | 4 | 4 | 2 | 82.86 | 83.05 |
| **3m** | C19H18N2O | 290.36 | 0.16 | 3 | 2 | 1 | 90.80 | 45.75 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **3n** | C16H11BRN2O2 | 343.17 | 0.00 | 3 | 3 | 2 | 85.62 | 65.98 |
| **3o** | C18H16N2O | 276.33 | 0.11 | 4 | 2 | 1 | 84.72 | 45.75 |
| **3p** | C17H14N2O | 262.31 | 0.06 | 3 | 2 | 1 | 80.87 | 45.75 |
| **3q** | C16H12N2O2 | 264.28 | 0.00 | 3 | 3 | 2 | 77.92 | 65.98 |
| **3r** | C18H17N3O | 291.35 | 0.11 | 4 | 2 | 1 | 90.11 | 48.99 |
| **3s** | C19H18N2O | 290.36 | 0.16 | 4 | 2 | 1 | 90.48 | 45.75 |
| **3t** | C18H14N2O | 274.32 | 0.00 | 4 | 2 | 1 | 85.04 | 45.75 |

## Table 4: MOLINSPIRATION RESULTS

| S.NO. | Compound | MilogP | TPSA | N atoms | Molecular weight | Number of violations | Num. rotable bonds |
|---|---|---|---|---|---|---|---|
| 1 | 3a | 2.77 | 75.21 | 22 | 294.30 | 0 | 4 |
| 2 | 3b | 2.75 | 75.21 | 22 | 294.30 | 0 | 4 |
| 3 | 3c | 2.12 | 58.64 | 19 | 249.27 | 0 | 3 |
| 4 | 3d | 3.61 | 64.21 | 24 | 387.23 | 0 | 5 |
| 5 | 3e | 3.38 | 45.75 | 20 | 262.31 | 0 | 4 |
| 6 | 3f | 3.27 | 64.21 | 23 | 308.33 | 0 | 5 |
| 7 | 3g | 2.15 | 106.44 | 22 | 296.28 | 0 | 3 |
| 8 | 3h | 3.20 | 75.21 | 22 | 294.30 | 0 | 4 |
| 9 | 3i | 3.17 | 62.82 | 21 | 276.29 | 0 | 4 |
| 10 | 3j | 2.76 | 84.44 | 24 | 324.33 | 0 | 5 |
| 11 | 3k | 2.63 | 74.85 | 23 | 305.33 | 0 | 5 |
| 12 | 3l | 3.32 | 83.05 | 22 | 292.29 | 0 | 4 |
| 13 | 3m | 4.43 | 45.75 | 22 | 290.36 | 0 | 3 |
| 14 | 3n | 3.93 | 65.98 | 21 | 343.17 | 0 | 3 |
| 15 | 3o | 4.18 | 45.75 | 21 | 276.33 | 0 | 4 |
| 16 | 3p | 3.86 | 45.75 | 20 | 262.31 | 0 | 3 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **17** | **3q** | 3.17 | 65.98 | 20 | 264.28 | 0 | 3 |
| **18** | **3r** | 3.51 | 48.99 | 22 | 291.35 | 0 | 4 |
| **19** | **3s** | 4.92 | 45.75 | 22 | 290.36 | 0 | 4 |
| **20** | **3t** | 3.93 | 45.75 | 21 | 274.32 | 0 | 4 |

## 6.2. IN SILICO SCREENING OF DESIGNED COMPOUNDS

- The ADME properties of the designed compounds were evaluated using SWISS ADME software, their results were tabulated (Table no: 6)
- Toxicity of all the designed compounds were evaluated by using ProTox II software and the results were tabulated (Table No :5). The designed compounds are inactive for mutagenicity and cytotoxicity.
- Antibacterial agents generally possess greater number of hydrogen bond acceptors. All the designed compounds have less than 5 hydrogen bond donors and 6-10 hydrogen bond acceptors.This obeys Lipinski rule of rule. Molecular weight of all the designed compounds was around 500 daltons.
- All our designed compounds shows log P value between 1-5. It was found that lipophilicity plays a major role in determining where drugs are distributed within the body after adsorption and, as a consequence, how rapidly they are metabolized and excreted. In the biological system drug disposition depends on the ability to cross membranes, so there is a strong relationship with measures of lipophilicity. So there is a strong lipophilic character of the molecule plays a major role in producing the antimicrobial effect.
- TPSA has been used as descriptor for characterizing absorption and passive transportation properties through biological membranes, allowing a good prediction of transport of candidate drugs in the intestines and through the blood-brain barrier. Compounds with TPSA values within the range 140 $A°^2$ have good intestinal absorption. TPSA (Total Polar Surface Area) of our designed compounds were found to be in the range of 88-140 $A°^2$.Therefore it is expected that our compounds might possess good intestinal absorption.
- The designed compounds passed Lipinski rule of five, that the drug is suitable for oral administration.

**Table 5: PROTOX II RESULTS**

| Compound no | Predicted LD50 mg/kg | Predicted toxicity class | Hepatotoxicity | Immunotoxicity | Mutagenicity | Cytotoxicity | Carcinogenicity |
|---|---|---|---|---|---|---|---|
| 1 | 2000 | 4 | Inactive | Active | Active | Inactive | Active |
| 2 | 5000 | 5 | Inactive | Active | Active | Inactive | Active |
| 3 | 1000 | 4 | Active | Inactive | Inactive | Inactive | Inactive |
| 4 | 4000 | 5 | Inactive | Inactive | active | Inactive | Inactive |
| 5 | 2000 | 4 | Active | Inactive | Inactive | Inactive | Inactive |
| 6 | 2000 | 4 | Inactive | Active | Active | Inactive | Active |
| 7 | 2000 | 4 | Inactive | Active | Active | Inactive | Active |
| 8 | 5000 | 5 | Inactive | Active | Active | Inactive | Active |
| 9 | 2000 | 4 | Active | Active | Inactive | Inactive | Inactive |
| 10 | 2000 | 4 | Inactive | Active | Active | Inactive | Active |
| 11 | 3200 | 5 | Active | Inactive | Active | Inactive | Active |
| 12 | 3000 | 5 | Active | Inactive | Inactive | Inactive | Inactive |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 13 | 4000 | 4 | Inactive | Inactive | Active | Inactive | Inactive |
| 14 | 5000 | 4 | Active | Inactive | Active | Inactive | Inactive |
| 15 | 3000 | 5 | Inactive | Inactive | Inactive | Inactive | Inactive |
| 16 | 3000 | 4 | Inactive | Inactive | Inactive | Inactive | Inactive |
| 17 | 1000 | 4 | Active | Inactive | Active | Inactive | Inactive |
| 18 | 3200 | 5 | Inactive | Active | Inactive | Inactive | Active |
| 19 | 3500 | 4 | Inactive | Inactive | Active | Inactive | Inactive |
| 20 | 4000 | 5 | Inactive | Active | Active | Inactive | Inactive |

**Table 6: Swiss adme results**

| Compound no | Lipinski | Ghose | Veber | Egan | Muegge | Bioavailability score | GI absorption | BBB permeation | P-gp substrate | Log $K_p$ cm/s | Synthetic accessibility score |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Yes | Yes | Yes | Yes | Yes | 0.55 | High | Yes | No | -5.82 | 3.35 |
| 2 | Yes | Yes | Yes | Yes | Yes | 0.56 | High | Yes | No | -5.82 | 3.28 |
| 3 | Yes | Yes | Yes | Yes | Yes | 0.55 | High | Yes | No | -6.03 | 3.17 |
| 4 | Yes | Yes | Yes | Yes | Yes | 0.55 | High | Yes | No | -5.67 | 3.47 |
| 5 | Yes | Yes | Yes | Yes | Yes | 0.55 | High | Yes | No | -5.15 | 3.27 |
| 6 | Yes | Yes | Yes | Yes | Yes | 0.55 | High | Yes | No | -5.67 | 3.43 |
| 7 | Yes | Yes | Yes | Yes | Yes | 0.55 | High | No | No | -6.32 | 3.26 |
| 8 | Yes | Yes | Yes | Yes | Yes | 0.55 | High | Yes | No | -5.82 | 3.34 |
| 9 | Yes | Yes | Yes | Yes | Yes | 0.55 | High | Yes | No | -5.82 | 3.28 |
| 10 | Yes | Yes | Yes | Yes | Yes | 0.55 | High | No | No | -6.02 | 3.44 |
| 11 | Yes | Yes | Yes | Yes | Yes | 0.55 | High | Yes | No | -6.20 | 3.41 |
| 12 | Yes | Yes | Yes | Yes | Yes | 0.55 | High | No | No | -5.87 | 3.23 |
| 13 | Yes | Yes | Yes | Yes | Yes | 0.55 | High | Yes | No | -4.75 | 3.56 |
| 14 | Yes | Yes | Yes | Yes | Yes | 0.55 | High | Yes | No | -5.61 | 3.21 |
| 15 | Yes | Yes | Yes | Yes | Yes | 0.55 | High | Yes | No | -5.00 | 3.82 |
| 16 | Yes | Yes | Yes | Yes | Yes | 0.55 | High | Yes | No | -5.10 | 3.34 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 17 | Yes | Yes | Yes | Yes | Yes | 0.55 | High | Yes | No | -5.62 | 3.24 |
| 18 | Yes | Yes | Yes | Yes | Yes | 0.55 | High | Yes | No | -5.44 | 3.50 |
| 19 | Yes | Yes | Yes | Yes | Yes | 0.55 | High | Yes | No | -4.72 | 3.48 |
| 20 | Yes | Yes | Yes | Yes | Yes | 0.55 | High | Yes | No | -4.97 | 3.49 |

## 6.3. DOCKING:

Docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using, for example, scoring functions.

In this study, Autodock vina (Version 1.5.6) in PyrX is used to perform docking studies. Docking studies were performed with DNA gyrase subunit B (PDB ID: 1KZN). Protein was downloaded from RCSB and used for docking analysis.

This molecular docking study was performed using the selected protein from the protein data bank (PDB ID: 1KZN). Protein was prepared prepared by removing the ligand group, nucleic acid groups, heteroatoms, water molecules and then adding polar hydrogens. Prepared protein was saved in .pdb (protein data bank) format. The structure of the ligand was drawn using ACD/Chemsketch FREEWARE and saved in.mol format. The energy minimization of the ligands was performed using PyRx.Ink and converted to .pdbqt format.

Auto dock vina is used to estimate the affinities and interactions of DNA gyrase subunit B.

The docking studies for five designed compounds having high predicted activities were done along with ciprofloxacin, which has high experimental biological activity. Compounds 3b, 3d, 3f, 3j and 3r exhibited binding energies of -7.8, -8, -8.2, -8.2 and -7.5 kcal/mol respectively.

Compound 3b showed interaction by forming hydrogen bond interaction with two amino acids A:ASN46 and A:ARG13 with carbonyl group. Hydrophobic interaction of A: THR16 with Aryl group. Compound 3d showed interaction by forming hydrogen bond interaction with three amino acid A: ARG76, A: ARG76 and A: ARG76. Hydrophobic interaction of two amino acid are A: ILE78 and A: THR16 respectively. Compound 3f showed interaction by forming hydrogen bond interaction with two amino acid A: ASN46 with carbonyl group and A: ARG76 with methoxyl group.

Compound 3j showed interaction by forming hydrogen bond interaction with amino acid is A:ARG76 with hydroxyl group. The hydrophobic interaction of amino acid A:ILE78 with imidazole ring. And finally the compound r showed interaction by forming hydrogen bond interaction with two amino acid A:ASN46 and A:ARG13 with carbonyl group.Hydrophobic interaction of amino acid A:THR16 with Aryl group respectively. In comparison to ciprofloxacin, as a reference, there is interaction by forming hydrophobic interaction of amino acid A:ASN46 with aryl group respectively. Some of the amino acids which showed interactions with standard drug ciprofloxacin was also similar with the designed compounds. These results indicate that ligands can act as antibacterial agents against DNA gyrase subunit B.

3D molecular interactions visualizations of standard compound ciprofloxacin with the active site of 1KZN.

**Table 7: Docking score**

| COMPOUND CODE | BINDING SCORE |
|---|---|
| 3a | -8.3 |
| 3b | -7.8 |
| 3c | -7.8 |
| 3d | -8 |
| 3e | -7.3 |
| 3f | -8.2 |
| 3g | -7.6 |
| 3h | -7.6 |
| 3i | -8.1 |
| 3j | -8.2 |
| 3k | -8.3 |

| | |
|---|---|
| 3l | -8.1 |
| 3m | -8.3 |
| 3n | -8 |
| 3o | -7.9 |
| 3p | -7.8 |
| 3q | -7.9 |
| 3r | -7.5 |
| 3s | -7.6 |
| 3t | -7.9 |

From the docking score, docked five compound which good binding affinity were selected for synthesis.

The interaction of ligand ciprofloxacin, 3b, 3d, 3f, 3j and 3r with protein 1KZN is shown in figure 2-7.

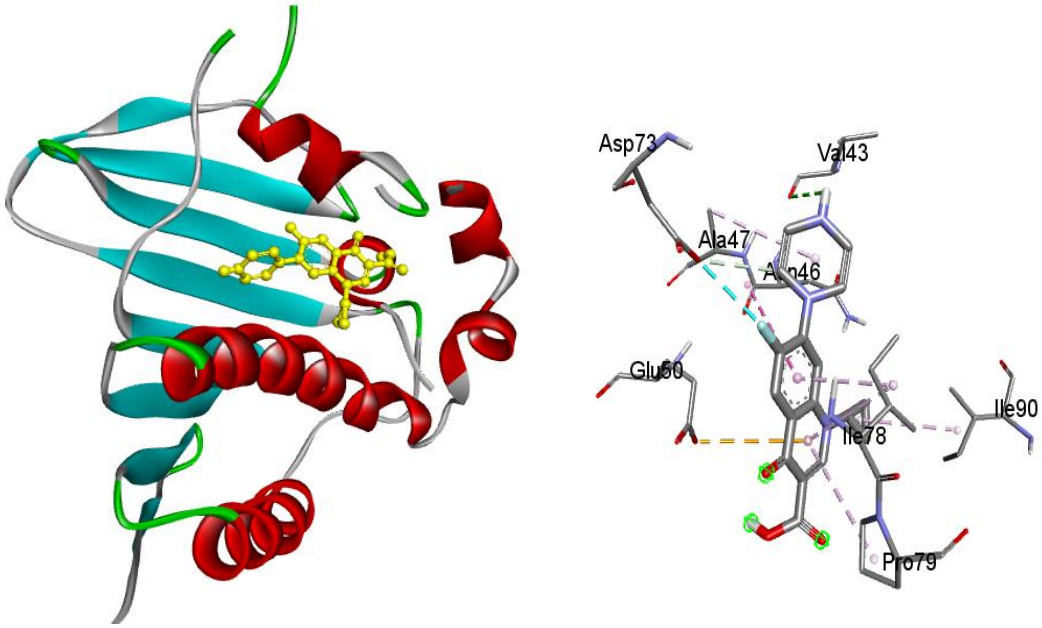



**Figure 2:** 3D molecular interaction visualization of ciprofloxacin with the active site of 1KZN.

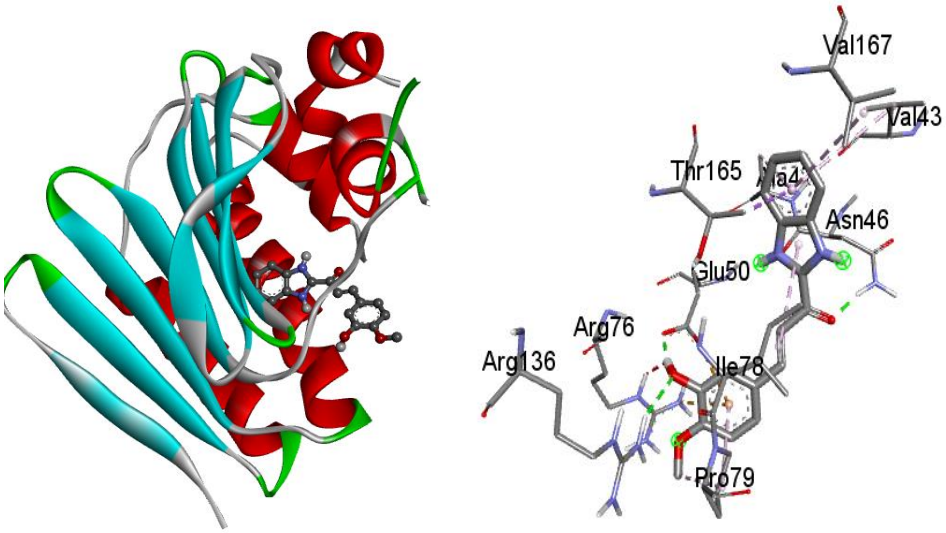



**Figure 3**: 3D molecular interaction visualizations of ligand 3b with the active site of 1KZN.

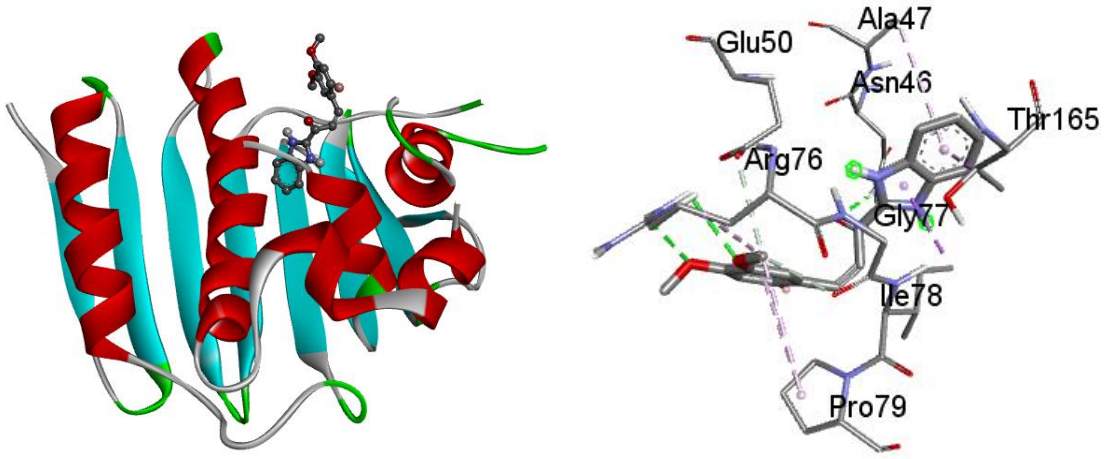



**Figure 4**: 3D molecular interaction visualizations of ligand 3d with the active site of 1KZN.

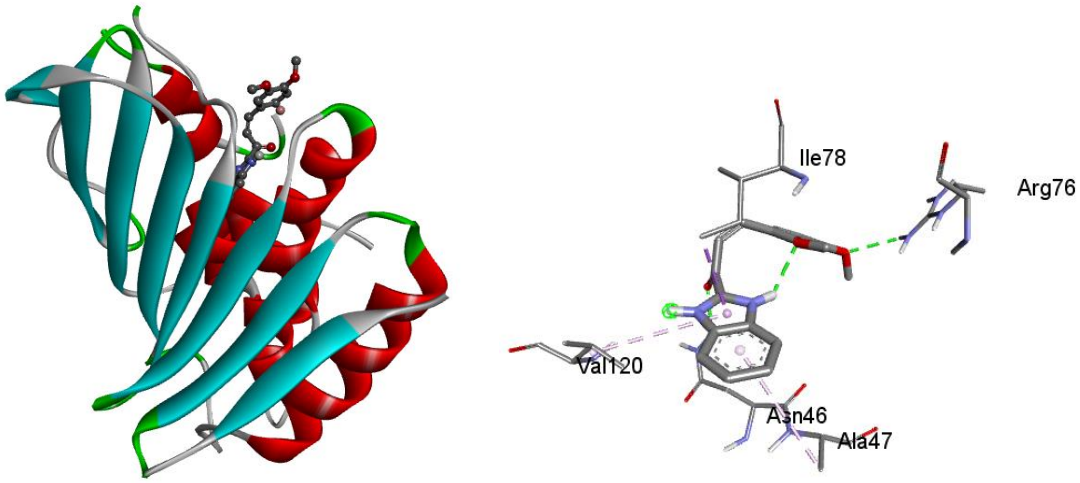



**Figure 5:** 3D molecular interaction visualizations of ligand 3f with the active site of 1KZN.

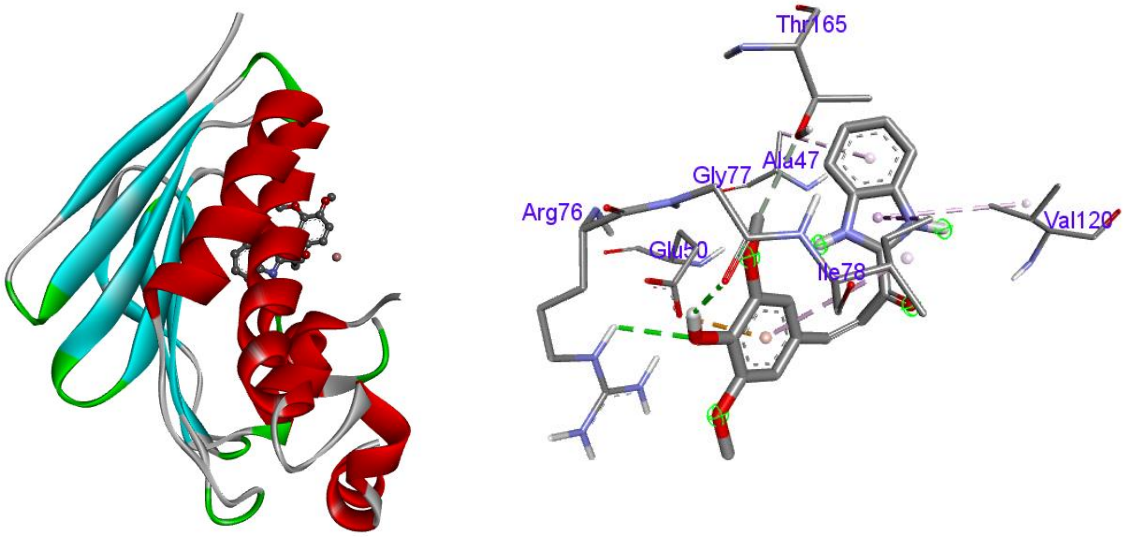



**Figure 6:** 3D molecular interaction visualizations of ligand 3j with the active site of 1KZN

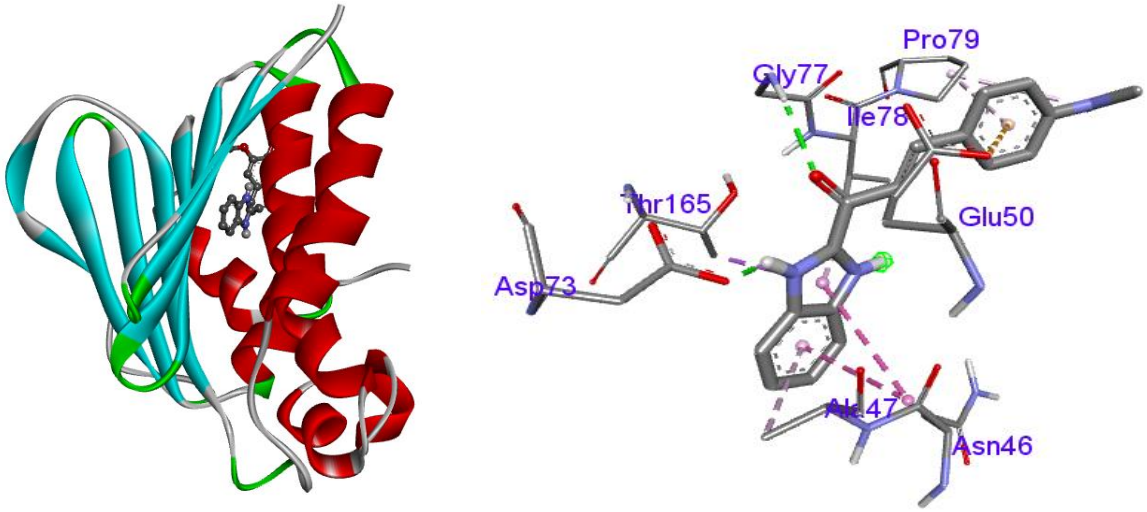



**Figure 7:** 3D molecular interaction visualizations of ligand 3r with the active site of 1KZN.

| S.No. | Compound No. | Docking score | Amino acid interaction with distance |
|---|---|---|---|
| 1. | **3b** | -7.8 | ASN A:46 - 1.98<br>ARG A:13 - 2.72<br>THR A:16 – 3.89 |
| 2. | **3d** | -8 | ASN A:46 - 2.11<br>ARG A:76 - 2.86<br>ARG A:76 - 2.67<br>THR A:16 - 3.93 |
| 3. | **3f** | -8.2 | ASN A:46 - 2.12<br>ARG A:76 - 2.70<br>ILE A:78 - 3.58 |
| 4. | **3j** | -8.2 | ARG A:76 - 2.90<br>ILE A:78 - 3.81 |
| 5. | **3r** | -7.5 | GLY A:77 - 2.30<br>GLU A:50 - 4.84<br>THR A:16 - 3.82 |
| 6. | **Ciprofloxacin** | -7.3 | ASN A:46 - 5.52 |

**Table 8:** Docking interaction of ligands

## 6.4. SYNTHESIS

The schematic respresentation of the synthesis of (2E)-1-(1h-benzimidazol-2-yl)but-2-en-1-one derivatives is respresented in scheme 1. Synthesized by reaction of the 2-acetyl benzimidazole (0.0l mol) in 10 ml 30% potassium hydroxide with 0.012 molar of various aromatic aldehydes are added separately, the refluxed for 2 hours with water bath, allow cooling to the room temperature and pouring the mixture into the beaker containing ice cold water, with stirring to get a product and the above products were collected by filtration with ice cold water, dried and recrystallized from 90% ethanol.The yield of the synthesized compounds ranges from 78 -90%.

All the compounds were characterized by IR, MS, and elemental analyses. The IR spectra of synthesized compounds showed absorption bands due to stretching vibrations C-C, C=C, N-H, C-N at 812 – 1188 $cm^{-1}$, 1504 – 1589 $cm^{-1}$, 3361 – 3428 $cm^{-1}$, 1600 – 1669 $cm^{-1}$ respectively. The mass spectrum showed molecular ion peak which was in agreement with molecular mass of compound.

## 6.5. Biological Studies:

The synthesized compounds have been subjected to antibacterial activity through agar well diffusion method using Gentamicin antibiotic was used as a positive control for *E. coli*.

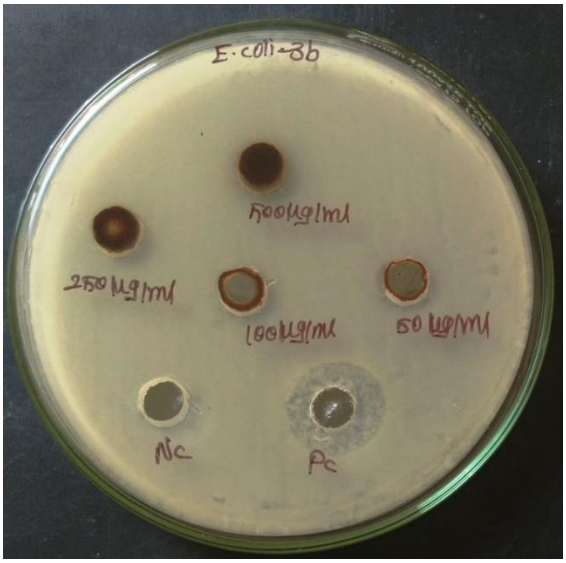
Among tested compounds, compound **3d** with 2, Bromo-4,5-dimethoxy benzaldehyde derivative, compound **3j** with syringaldehyde derivative and compound **3r** with para dimethyl amino benzaldehyde derivative showed good activity compared to standard gentamicin against E. coli with zone of inhibition 10.75±1.06, 14.5±0.7, 12.5±0.7 respectively. Compound **3b** with vanillin derivative and compound **3f** with 2,4-dimethoxybenzaldehyde resulted lesser activity than standard gentamicin antibiotic against E. coli with zone of inhibition 10.25±0.35 and 11.5±0.7. The results of antibacterial activity are given in Table 8.

**Table 9. SD± Means of zone of inhibition obtained by sample 3b, 3d, 3f, 3j and 3r against *E. coli.***

| S. No | Name of the test organism | Name of the test sample | Zone of inhibition (mm) SD ± Mean | | | | |
|---|---|---|---|---|---|---|---|
| | | | 500 µg/ml | 250 µg/ml | 100 µg/ml | 50 µg/ml | PC |
| 1. | *E. coli* | 3b | 7.5±0.7 | 4.25±0.35 | 3.25±0.35 | 3.2±0.28 | 10.25±0.35 |
| 2. | | 3d | 9.5±0.7 | 5.25±0.35 | 0 | 0 | 10.75±1.06 |
| 3 | | 3f | 6.5±0.7 | 4.25±0.35 | 3.25±0.35 | 0 | 11.5±0.7 |
| 4. | | 3j | 13.5±0.7 | 10.25±0.35 | 5.25±0.35 | 3.25±0.35 | 14.5±0.7 |
| 5. | | 3r | 11.25±0.35 | 7.25±0.35 | 5.25±0.35 | 4.25±0.35 | 12.5±0.7 |

SD – Standard Deviation, *Significance - p< 0.0

**Fig 8: Effect of sample 3b against *E. coli.***



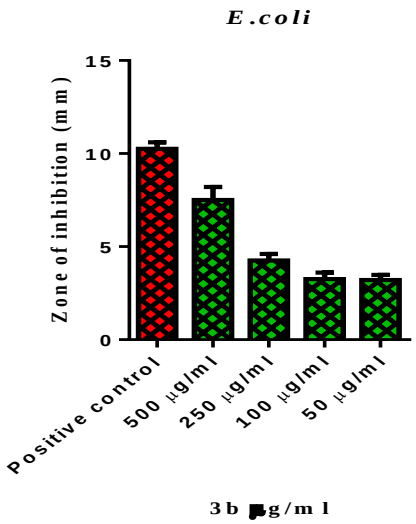




**Fig 9: Effect of sample 3d against *E. coli.***

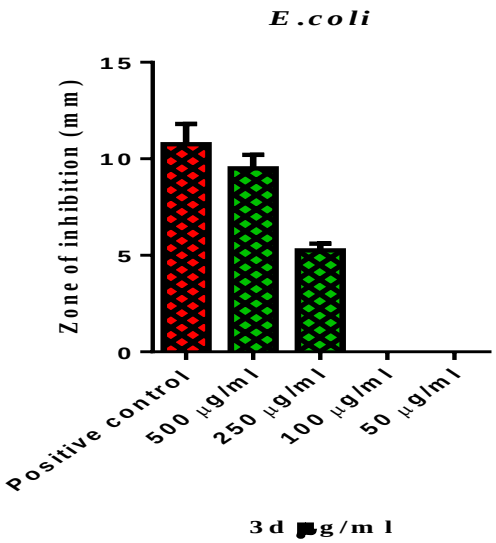
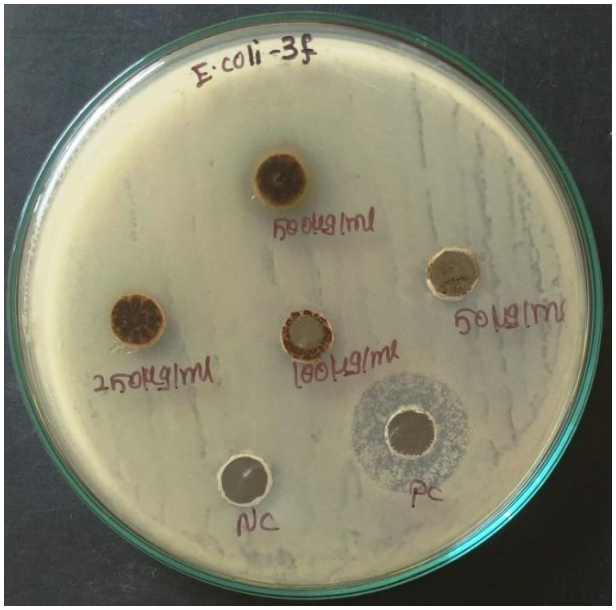



**Fig 10: Effect of sample 3f against *E. coli.***



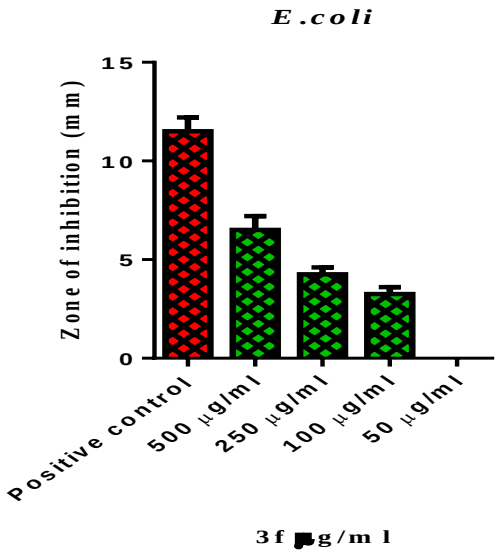
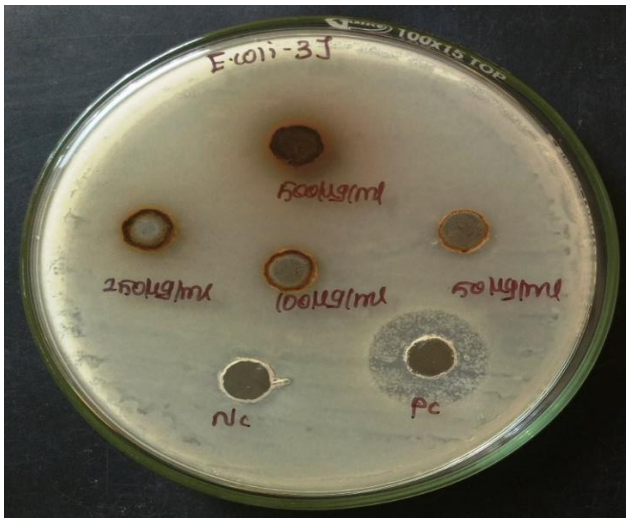




**Fig 11: Effect of sample 3j against *E. coli.***



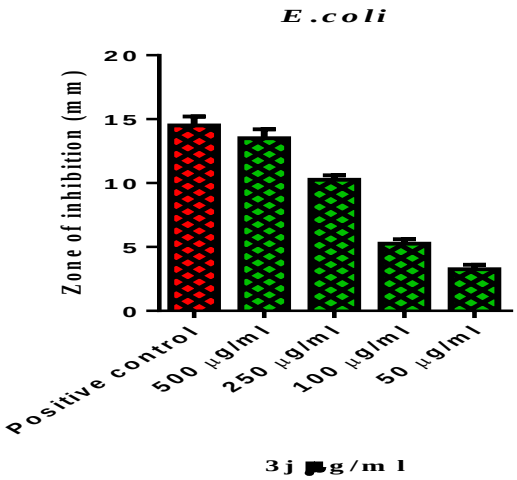
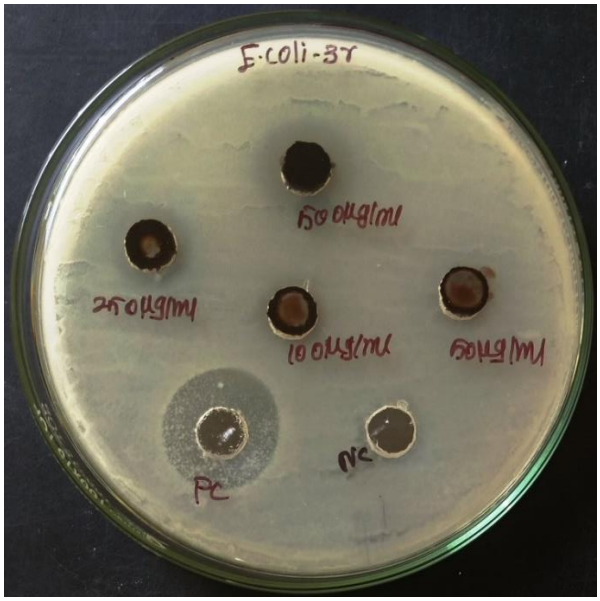
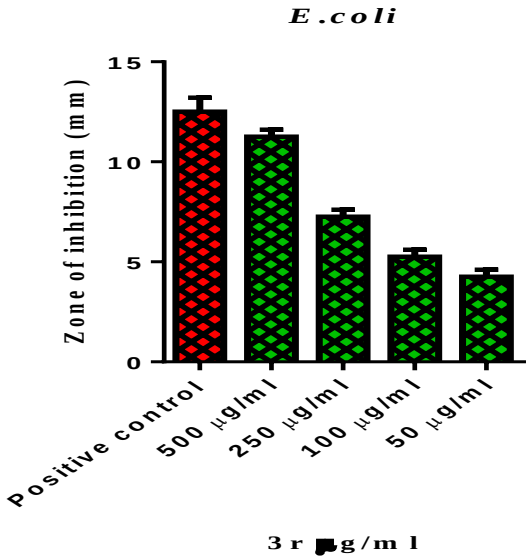



**Fig 12: Effect of sample 3r against *E. coli.***



# CONCLUSION

**CONCLUSION:**

All the designed compounds were subjected to molecular docking studies for structural and interaction information. Ciprofloxacin and all designed compound 3a-t were docked in the active site of DNA gyrase subunit B and Binding affinities was calculated. Compound 3b, 3d, 3f, 3j and 3r had good binding affinities values of -7.8, -8, -8.2, -8.2 and -7.5 respectively than ciprofloxacin. Molecular docking studies revealed that the designed compounds interacted with DNA gyrase subunit B mainly through hydrogen bond and hydrophobic interaction.

In silico studies were performed for all the compounds using Swiss ADME, PreADMET, Molinspiration, and Protox II. Based on docking and insilico studies, five compounds with good docking score and passing Lipinski's rule were selected for synthesis.

Chemical structures of the synthesized compounds were ascertained on the basic of their spectral data (IR and Mass).

Invitro antibacterial activity for the synthesized compounds was performed by Agar-well diffusion method. The analysis of antibacterial activity results indicated that the compound **3d** with 2, Bromo-4,5-dimethoxy benzaldehyde derivative, compound **3j** with syringaldehyde derivative and compound **3r** with para dimethyl aminobenzaldehyde derivative showed good activity and Compound **3b** with vanillin derivative and compound **3f** with 2,4-dimethoxybenzaldehyde exhibited lesser activity than standard gentamicin antibiotic against E.coli.

In this view, the above work its basic concluded that the novel hybrid benzimidazolyl chalcone with a promising target and it can show effective antimicrobial agent.

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# INTERNATIONAL CONFERENCE ON DRUG DISCOVERY

Feb 29th - Mar 2nd, 2020, BITS-Pilani, Hyderabad

# Certificate

*This is to certify that* **Subhashini A** *has successfully participated in International Conference on Drug Discovery held at BITS-Pilani Hyderabad Campus from 29th Feb to 2nd Mar 2020*

**R. RAGHU**
Vice President

SCHRÖDINGER.

**Prof. P. YOGEESWARI**
Head, CIIE, BITS Pilani

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# Certificate

**This is to certify that Dr./ Mr. / Ms.** A. SUBASHINI
**of** C.L.Baid Metha College Of Pharmacy, Chennai.

**actively participated in "International Pharma Webinar on Molecular Docking with Autodock - An Easier Approach for Beginners " organised by MNR College of Pharmacy on 10th June, 2020.**

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