SYNTHESIS, CHARACTERIZATION, MOLECULAR DOCKING OF SOME NOVEL BENZOFURAN CHALCONE DERIVATIVES AND THEIR EVALUATION OF *INVITRO* ANTI CANCER ACTIVITY

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CERTIFICATE

This is to certify that the dissertation entitled **"SYNTHESIS, CHARACTERIZATION, MOLECULAR DOCKING OF SOME NOVEL BENZOFURAN CHALCONE DERIVATIVES AND THEIR EVALUATION OF *IN-VITRO* ANTI-CANCER ACTIVITY"** is a bonafied work done by **Mr. M.KATHIRAVAN, (261715753), DEPARTMENT OF PHARMACEUTICAL CHEMISTRY, COLLEGE OF PHARMACY, MADURAI MEDICAL COLLEGE, MADURAI-625020** in partial fulfillment of the Tamil Nadu Dr. M.G.R. Medical University rules and regulations for award of **MASTER OF PHARMACY IN PHARMACEUTICAL CHEMISTRY** under my guidance and supervision during the academic year 2018-2019.

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CONTENTS

DETAILS OF ABBREVIATIONS

%	Percentage
°C	Degree Centigrade
µg	Microgram
µM	Micro Mole
^{1}H-NMR	Proton Nuclear Magnatic Resonance
Ar	Aromatic
Comp.code	Compound code
DMSO	Dimethyl Sulfoxide
Gm	Gram
Hrs	Hour
IR	Infra-Red
m.p	Melting Point
m/z	Mass/charge
Mg	Milligram
Min	Minutes
Ml	Milliliter
Mol	Mole
MR	Molar Refractivity
nm	Nanometer
o, m, p	Ortho, Meta, Para
P_C	Critical pressure
Ppm	Parts per million
R_f	Retention factor
MTT	(4,5 Dimethyl thiazol-2yl)2,5-diphenyl tetrazolium bromide
p^H	Hydrogen ion Concentration
Str	Stretching
TLC	Thin Layer Chromatography
UV	Ultra violet
V_C	Critical volume
Δ	Delta

INTRODUCTION

MEDICINAL CHEMISTRY

Medicinal chemistry is defined as symbiotic mature science that is a combination of applied medicine and basic chemistry.

The earlier source of drugs were from, plants, animals and mineral sources, but due to the lack of potency and definitive cure there is need for the discovery of new drugs in the synthesised form with the values added to the already existing leading compound, so the synthetic medicinal chemistry plays a major role in drug discovery.

It is mainly focused on the discovery, design, synthesis and in analysis of novel medicinal molecules for the purpose of creating and developing drugs for pharmaceutical supply and uses.

The discovery of new drugs is one of the most exciting and rapidly developing fields in science because recently the difficulty and complexity of drug research has increased, so there is a need for number of launches of new medicine in the form of new molecular individuals.

The recent advancement like drug designing, combinatorial chemistry, molecular biology and genetic engineering makes the medicinal chemistry more curiosity.

The success of structurally based therapeutic evaluation clearly highlights that medicinal chemistry and pharmacy education are intimate in their origin as well as in future. **[1]**

HERETOCYCLES

Heterocylic chemistry is one of the most important and fascinating branches of organic chemistry. Heterocycles are present in a wide variety of drugs, vitamins, natural products, biomolecules and biologically active compounds such as antitumor, antibiotic, anti-inflammatory, antidepressant, antimalarial, anti-HIV, antmicrobial, antifungal antiviral, antidiabetic, herbicidal and insecticidal agents. Also, they have been frequently found as a key structural unitin synthetic pharmaceuticals and agrochemicals.[2]

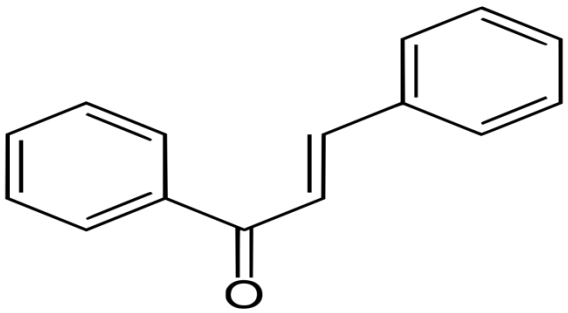
Among them, nitrogen, oxygen and sulphur hetero atoms containing six membered heterocyclic compounds are found to be of great importance in pharmaceutical applications.

CHALCONE[44]

Flavonoids comprise a large family of plant derived poly phenolic compounds classified as anthocyanidins, flavonols, chalcones, flavones, isoflavones. Chalcones an important intermediate of flavonoid synthetic pathway, has been shown to exhibit diverse biological and pharmacological activities.

Chalcones are unsaturated ketone containing the reactive keto ethylenic group –CO CH=CH. These are coloured compounds because of the presence of chromophore.

Chalcones are also called as benzalacetophenone or benzylidine acetophenone or phenyl styryl ketone.



Different methods are available for the preparation of chalcones. The most convenient Method is the claisen-schmidt condensation of equimolar quantities of aryl methylketone and

aryl aldehyde. This reaction is catalyzed by acids and bases under homogenous or heterogenous conditions.

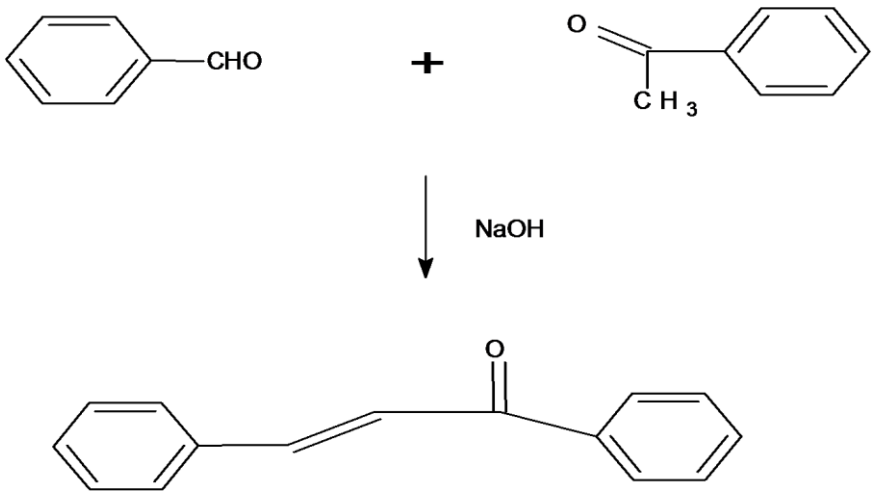
Chalcone derivative have wide variety of biological activities reported for these compounds include anti-inflammatory, anti-fungal, antibacterial, antimalarial and antitumor activity.

Chalcones with antioxidant activity (and compounds with such activity in general) have been demonstrated to have anticancer, anti-cardiovascular, anti-inflammatory and many other activities.

Claisen-schmidt Reaction:

This is the most convenient method for synthesis of chalcones. in this reaction equimolar quantities of substituted accetophenone condensed with substituted aldehydes in the presence of aqueous alcoholic alkali.

The condensation of aromatic aldehydes having no -hydrogen, with aliphatic aldehydes, ketones or esters, having active hydrogen, in the presence of 10% alkali solution to give - unsaturated aldehydes or ketones is known as claisen schmidt reaction.



Various condensing agents used in synthesis of chalcones:

Alkali:

It is most used condensing agents for synthesis of chalcones. It is used as an Aqueous solution of suitable concentraton 30%, 40%, 50%, 70%.

Hydrochloric acid:

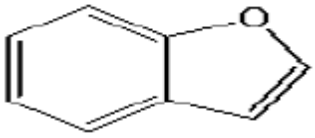
Dry hydrochloric acid gas in a suitable solvent like ethylacetate at 0^0C was used as a condensing agent in a few synthesis of chalcone from aromatic ketones.

Other condensing agents:

1. Amino acid
2. Perchloric acid

Chalcones, the compounds having 1,3-diaryl-2-propen- 1-one system, also have shown a broad spectrum of biological activities including anti-inflammatory [12], antimalarial [13], anti-invasive [14], **antibacterial** [15], and **anticancer** [16] activities. On the other hand, chalcones are capable of inducing apoptosis [17]. Consequently, these compounds are recognized as promising anticancer agents [18].

BENZOFURAN [66]



Benzofuran derivatives are an interesting class of heterocyclic compounds. Benzofuran derivatives are of great interest in medicinal chemistry and have drawn remarkable attention due to their biological activities with **chemotherapeutic properties**. Some benzofurans bearing various substituents at the C-2 position are greatly distributed in nature; for example, ailanthoidol, a neolignan derivative, has been reported to have antiviral, antioxidant, and antifungal activities. Furthermore, most of the compounds prepared from 2-

acetylbenzofuran have **antimicrobial**, anticancer, antitumor, anti-inflammatory, and antitubulin activities and are also used for treatment of cardiac arrhythmias.

Benzofuran scaffolds (oxygen heterocycles) have drawn considerable attention over the last few years due to their profound physiological and chemotherapeutic properties as well as their widespread occurrence in nature. Benzofuran derivatives are versatile biodynamic agents that can be used to design and develop new potentially useful therapeutic agents. These are of special interest to researchers for their wide range of biological activities and potential applications a pharmacological molecules.

Benzofuran derivatives display potent biological properties including antihyperglycemic, analgesic, antiparasitic, antimicrobial, antitumor and kinase inhibitor activities. In addition substituted benzofurans find application such as of fluorescent sensor, oxidant, antioxidants, brightening agents, a variety of drugs and in other field of chemistry and agriculture.

Moreover benzofurans occur in a great number of natural products. Many of the natural benzofurans have physiological, pharmacological and toxic properties. There are well known natural products having related benzofuran ring structures, which are particularly isolated from Machilus glaucescens, Ophryosporus charua, Ophryosporus lorentzii, Krameri ramosissima, and Zanthoxylum ailanthoidol.

The most recognized benzofurans are ailanthoidol, amiodarone and bufuralol compounds. Ailanthoidol, a neolignan with a 2-arylbenzofuran skeleton, has been reported to possess a variety of biological activities such as anticancer, antiviral, immunosuppressive, antioxidant, antifungal and antifeedant activities.

Amiodarone is a highly effective antiarrhythmic agent with class III activity. Bufuralol is a nonselective b-adrenoceptor antagonist developed by HoffmaneLa Roche. This compound is a good substrate of cytochrome P450 (CYP) and undergoes enantioselective and regioselective oxidations in liver. Benzofuran containing structures have been found among naturally occurring furocoumarins, such as psoralen and methoxalen isolated from the seed of Ammi majus L. and used for the treatment of psoriasis and other dermal diseases.

In order to explore diverse biological activities, investigating various methods for synthesis and structural modification of benzofuran ring have now become important goal of several research groups. Thus, benzofuran moiety can be taken as lead compound for the synthesis of novel derivatives with a variety of biological activities.

CHEMISTRY

Benzofuran is a heterocyclic compound consisting of fused benzene and furan ring (Fig). This colorless liquid is a component of coal tar. Benzofuran is the "parent" of many related compounds with more complex structures. These heterocyclic compounds show a wide range of pharmacological properties, and change of their structure offers a high degree of diversity that has proven useful for the search of new therapeutic agents. The broad spectrum of pharmacological activity in individual benzofuran indicates that this series of compounds is of an undoubted interest. From this point of view, synthetic methods may be of very useful aid in the production of specific structures characterized by given pharmacological qualities.

Moreover from a drug discovery perspective, synthesis of substituted benzofurans could be more interesting because they might constitute starting materials for the production of biologically active compounds. A diversity of synthetic routes can be applied to the synthesis of benzofurans. A convenient metal-free cyclization of ortho-hydroxystilbenes into 2-arylbenzofurans and 2- arylnaphthofurans is mediated by hypervalent iodine reagents.
Using stoichiometric (diacetoxyiodo) benzene in acetonitrile, desired products can be isolated in good yields.

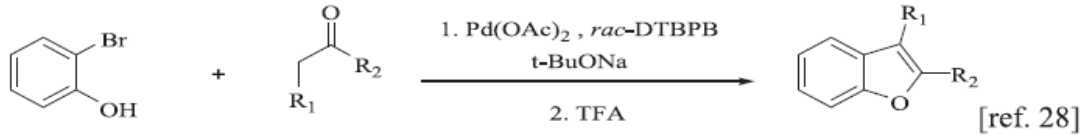
A one-pot synthesis of benzofurans which utilizes a palladium-catalyzed enolate arylation demonstrates broad substrate scope and provides differentially substituted benzofurans in moderate yields. The utility of the method is further demonstrated by the synthesis of the natural product eupomatenoid in three steps.

Substituted benzofurans can be synthesized from their corresponding substituted 1-allyl-2-allyloxybenzenes using ruthenium-catalyzed C- and O-allyl isomerization followed by ring-closing metathesis.

An effective, Ru-catalyzed cycloisomerization of benzannulated homo- and bis homopropargylic alcohols affords benzofurans and isochromenes chemo- and regioselectively (5-, and 6-endo cyclizations). The presence of an amine/ammonium baseeacid pair is crucial for the catalytic cycle .

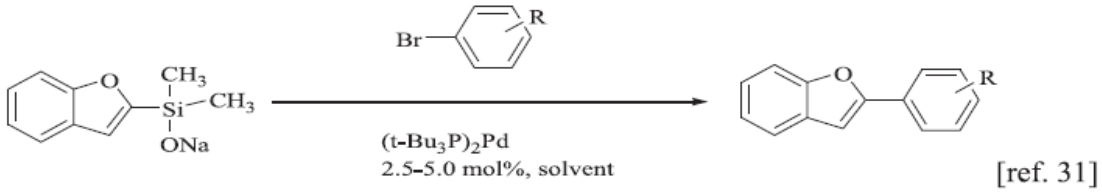
An effective and mild microwaveassisted route to 2-substituted benzofurans directly from carboxylic acids allows the preparation of a-alkyl-2- benzofuranmethanamines from N-protected a-amino acids without racemization in good yields. 2,3-Disubstituted benzo [b]furans are readily prepared under very mild reaction conditions by the Sonogashira

coupling of various O-iodoanisoles and terminal alkynes, followed by an electrophilic cyclization. Aryl- and vinylicsubstituted alkynes give cyclization products in excellent yields



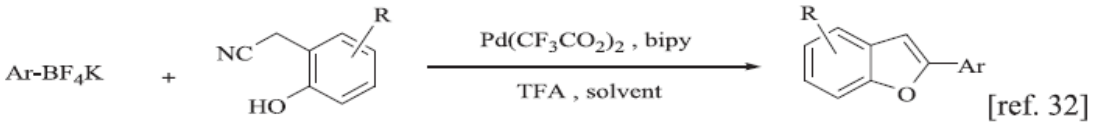
[ref. 28]

(R_1 = Me, H, Et; R_2 = Ph, 3,5-diOMe-C_6H_3, etc.)



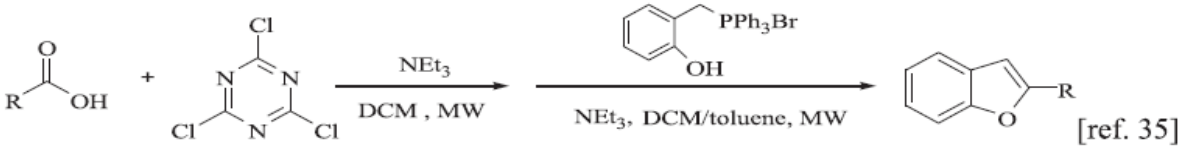
[ref. 31]

(R = 4-OCH_3, 4-OTBS, 3-CH_2OTBS, 2-CH_3, 4-NO_2, 4-CN)



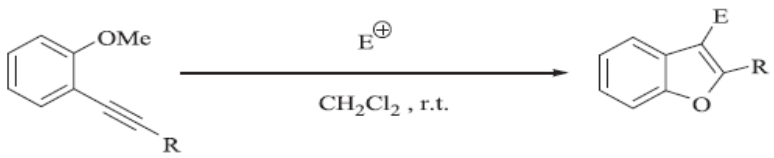
[ref. 32]

(R = OMe, Me, Cl, H, Br; Ar = Ph, 4-Br-C_6H_4, 4-OMe-C_6H_4 , etc..)



[ref. 35]

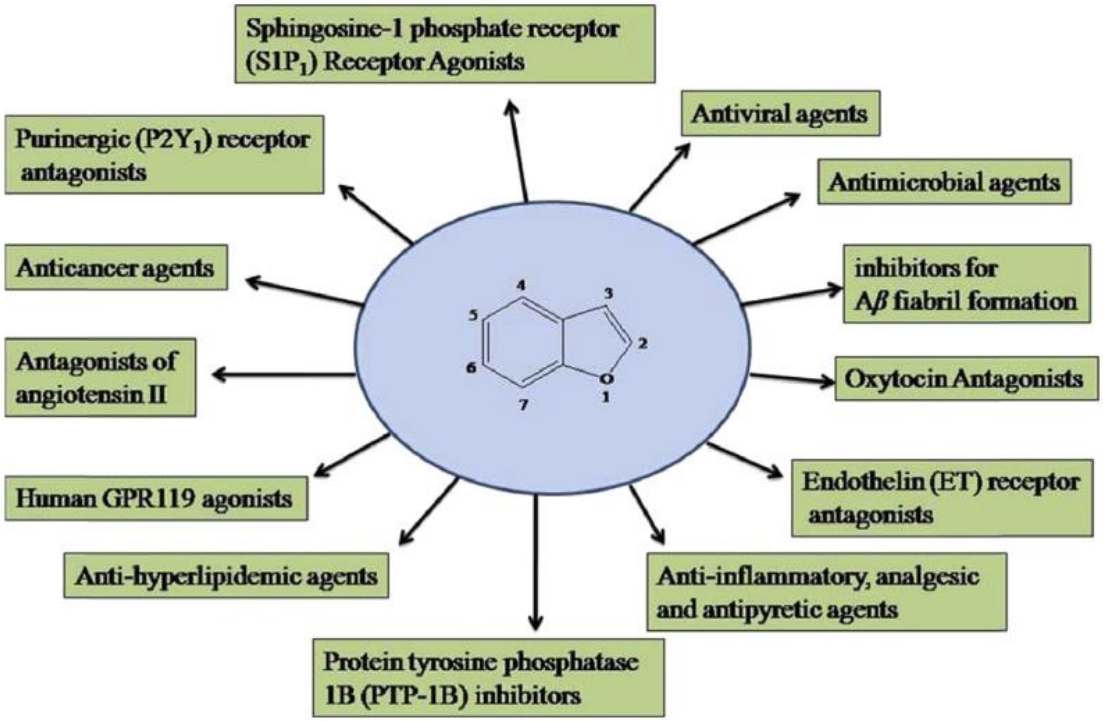
(R = Ph, t-Bu, etc.)



⊕E = ICl, I_2 , PhSeCl, or *p*-$O_2NC_6H_4SCl$ [ref. 36]

(R = Ph, 4-OMe-C_6H_4, 2-AcO-C_6H_4, cyclohexenyl)

Scheme 1. Synthetic routes for benzofuran derivatives.



MOLECULAR DESIGN

Molecular design is the process of finding new medicines based on the knowledge of a biological target, it enabled the chemist to predict the structure and the value of certain properties of various compounds

It also allows the medicinal chemist to evaluate the interaction between a compound and its target site before synthesising a compound so as to increase the ability by reducing the side effects.[22]

Various software used:

- Chem sketch
- Chemdoodle

- Mol inspiration
- Swiss target prediction
- A log P 2.1 Program
- J log p

DEFINITIONS:

Molecular weight:

It is defined as the total of each atomic weight of the atom present in the molecule. For a desirable drug it should be less than 500.

Molar refractivity:

It is defined as a total polarizability of a mole of a substance dependant on temperature and pressure. It is related to Drug-receptor interaction and volume of the molecule

.

Hydrogen bond donar:

This is the group providing the hydrogen. When hydrogen attached to a highly Nitrogen or an Oxygen atom.

Hydrogen bond acceptor:

An electronegative atom with lone pair of electrons and able to provide electron density via the hydrogen bond. It receives the hydrogen atom

Rotatable bonds:

It is a single, non-rigid bond attached to non-hydrogen atom. This is related to the bioavailablity of the compound. For a desirable compound, the number of rotatable bonds should be less than 10.

Total polar surface area:

It is defined as a sum of surfacezs of polar atoms in a molecule. It is a very useful parameter for prediction of drug transport properties. This parameter shown to correlate very well with human intestinal absorption.

Log p:

It is the ratio of concentration of the substance in the mixture of two immimisible solvents. It is used to measure the hydrophilic or lipophilic character of the chemical substances.

Log s:

Aqueous solubility of a compound significantly affects its absorption and distribution characteristics. Low solubility leads to bad absorption. For a desirable drug. Log s value should be -4 and above.

Mutagenic:

The chemical or toxins which cause a generic mutation within living cells leads to the formation of cancerous cells.

Tumorigenic:

The chemicals or substances those are capable of forming tumors.

Irritant:

The chemicalor biological substances tend to make skin itching or an inflammatory response.

LIPINSKI'S RULE OF FIVE

It helps to predict the poor absorption and permeability of the potential drug. The desirable drug must obey the following rules states by lipinskis.

1. Molecular weight should be less than 500
2. Log p value should be less than 5
3. Number of H-bond donors should be less than 5
4. Number of H-bond acceptor should be less than 10
5. Molar refractivity should be less than 150

CHARACTERIZATION TECHNIQUES

MELTING POINT DETERMINATION

Melting points were determined with open capillary by using melting point apparatus,

SPECTROSCOPY

Spectroscopy is the branch of science that deals with the study of interaction of electromagnetic radiation with matter.

Spectroscopy is one of the most powerful tools available for the study of atomic and molecular structure of organic compounds.[**27**]

I.R spectroscopy:

IR spectrum were recorded by absorption of infrared radiation it causes changes in vibrational energy in the ground state of the molecule. It is used to identify of functional group of the organic compound. [28]

NMR spectroscopy:

Nuclear magnetic resonance (NMR) spectroscopy is a technique that permits the transition of a molecule at the level of the individual atom and giving information about the environment of that atom. Structural determination and identity of organic compounds, molecular conformation.

Mass spectroscopy:

Molecules are bombarded with electrons of sufficient energy, loss of an electron and formation of positive ion. It is used to determine the molecular weight of the compounds.

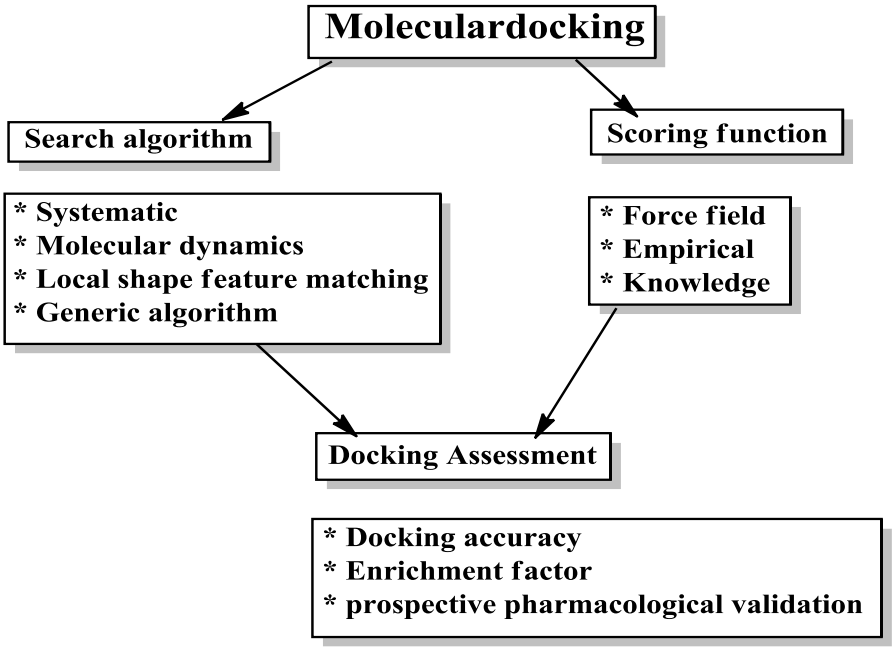
MOLECULAR DOCKING

Molecular docking provides useful information about drug receptor interactions. It analyzes the binding orientation of small molecule drug candidates to their protein targets in order to predict the affinity and activity of the small molecule.

Docking is considered to be a powerful simulation of the molecular recognition process. It is used to illustrate the probable molecular interaction of a designed ligand with the protein of interest, predict the affinity and activity of the ligand, and identify the energy of the interaction between the ligand and protein.

DEFINITION

"Molecular docking may be defined as an optimization problem, which would describe the 'best-fit' orientation of a ligand that binds to a particular protein of interest. However, since both the ligand and the protein are flexible, a 'hand-in-glove' analogy is more appropriate than 'lock-and-key'."



It is an invaluable tool in the field of molecular biology, computational structural biology, computer-aided drug designing, and pharmacogenomics.

Aims of docking studies:

- Accurate structural modeling
- Correct prediction of activity.

Steps of ligand docking:

- Preparation of ligands
- Preparation of proteins
- Setup ligand protein docking calculations
- Evaluation of results

Classification of docking:

Based on the types of ligand, docking can be classified as:

- Protein-small molecule (ligand) docking
- Protein-nucleic acid docking
- Protein-protein docking.

Advantages of docking:

- The application of docking in a targeted drug-delivery system is a huge benefit. One can study the size, shape, charge distribution, polarity, hydrogen-bonding, and hydrophobic interactions of both ligand (drug) and receptor (target site).
- It helps in the identification of target sites of the ligand and the receptor molecule.
- It also helps in the understanding of different enzymes and their mechanism of action.
- The "scoring" feature in docking helps in selecting the best-fit or the best drug from an array of options.
- It has huge advantage when it comes to the study of protein interactions.
- There are a millions of compounds, ligands, drugs, and receptors, the 3D structure of which has been crystallized. Virtual screening of these compounds can be made.

Limitations of docking:

- In protein-small molecule docking, there can be problems in the receptor structure. A reliable resolution value for small- molecule docking is below 1.2 A, while most crystallographic structures have a resolution between 1.5 and 2.5 A increasing the use of homology models in docking should be locked at with care as they have even poorer resolution. Most applications accept and yield good results for structures below 2.2A. All the same, care should be taken while picking a structure.
- The scoring functions used in docking, almost all of them, do not take into account the role played by covalently bound inhibitors or ions.
- The methodology and research in protein-protein docking have to be greatly increased as the success in this field is greatly hampered by many false positives and false negatives.

CANCER[77]

Cancer is one of the leading causes of death worldwide, accounting for death of 82 lakh people in the year 2012. Top three death-causing cancers are lung cancer, liver cancer, and stomach cancer which killed 15.9 lakh, 7.45 lakh, and 7.23 lakh people, respectively, in the same year. On gender-wise, lung cancer is the leading cause of deaths in males while breast cancer in females, killing 10 lakh males and 5.21 lakh females in single year, respectively.

In India, 6.82 lakh people died because of various types of cancer during the same period, of which 48,697 males and 70,218 females died because of lung cancer and breast cancer, respectively. [1] Therefore, one can say that worldwide, lung cancer and breast cancer are the leading cause of cancer-related deaths in males and females, respectively. Worldwide, lung cancer is the foremost cause of cancer-related deaths, whereas in India, breast cancer is the topmost cause.

Higher incidence of lung cancer can be accrued to increased air pollution or smoking while the reasons for higher incidence of breast cancer in India are late diagnosis due mainly to lack of awareness on early detection, barriers to health services or change in lifestyle such as obesity, late pregnancy, hormone replacement therapy, and lower lifetime duration of breastfeeding.

Cancer, also known as malignant tumor or neoplasm, is a broad term used for a large collection of diseases that can affect any organ or tissue of the body. One of the defining features of cancer is the rapid generation of undifferentiated cells that grow outside their natural boundaries, and which can also invade adjacent or distant tissues or organs of the body (metastasis).

Normally, when cells become old or damaged, they undergo programmed cell death, i.e. apoptosis and new cells replace them to fulfill the need of the body. Whereas in cancer case, this orderly process is disrupted and as the cells become old or damaged, instead of dying, they survive. These cells can divide into less specialized cells (tumor) and are able to ignore the signals which stop division or by which apoptosis is started in normal cells.

Depending on the potential clinical behavior, a tumor can be divided into two categories: Benign and malignant. Benign tumor, termed by attaching the suffix "-oma" to the type of cells in which the tumor arises, for example, fibroma, adenoma, and papilloma, will remain localized and the patient generally survives to local surgical procedures. Malignant tumors of solid mesenchymal tissues are called sarcomas; for example, cancer of fibrous tissue is known as fibrosarcoma while those ascending from the mesenchymal cells of the blood are leukemias or lymphomas. However, malignant tumors of epithelial cells are termed as carcinomas irrespective of the origin of tissue (as the epithelial cells are originated from three germ cell layers).

Therefore, malignant tumors arising in the renal tubular epithelium (mesoderm), skin (ectoderm), and lining epithelium of the gut (endoderm) are all carcinomas. Carcinomas are

subdivided further. Carcinomas with glandular pattern, squamous cells, and undifferentiated cells are called adenocarcinomas, squamous cell carcinomas, and undifferentiated carcinoma, respectively

As cancer was primarily considered a disease of uncontrolled cell division, by measuring the regression in tumor size, identification of a cytotoxic or an antiproliferative compound was considered as the main objective endpoint of efficacy of a compound in preclinical and clinical anticancer drug development for decades. For rapid screening of new anticancer compounds, murine models of rapidly growing cancer were developed, for example, sarcoma 180, carcinoma 755, and L1210 mouse leukemia model which were later replaced by the P388 murine leukemia model.

Several clinically important anticancer agents such as methotrexate, actinomycin D, 6-marcaptopurine, 5-fluorouracil were identified using these murine models; however, successes were achieved mainly in the cases of rapidly growing cancers, e.g. lymphomas, childhood leukemia, and germline tumors while relatively limited successes were seen in the treatment of the slow-growing common solid tumors of the adults, e.g. lung, breast, and colorectal cancers.

Cancer is one of the most important clinical problems worldwide. Among the wide range of compounds approved as potential anticancer agents, derivatives with functionalities as α, β-unsaturated Michael acceptor have attracted great interest

Previous reported studies have proposed that anticancer compounds such as alkylating agents bind directly to various cellular nucleophiles, thus lacking selectivity. However, Michael acceptors can be structurally modified so that they can react selectively with target nucleophiles

Breast cancer is one of the leading causes of death in females in both developing and developed countries globally [5].

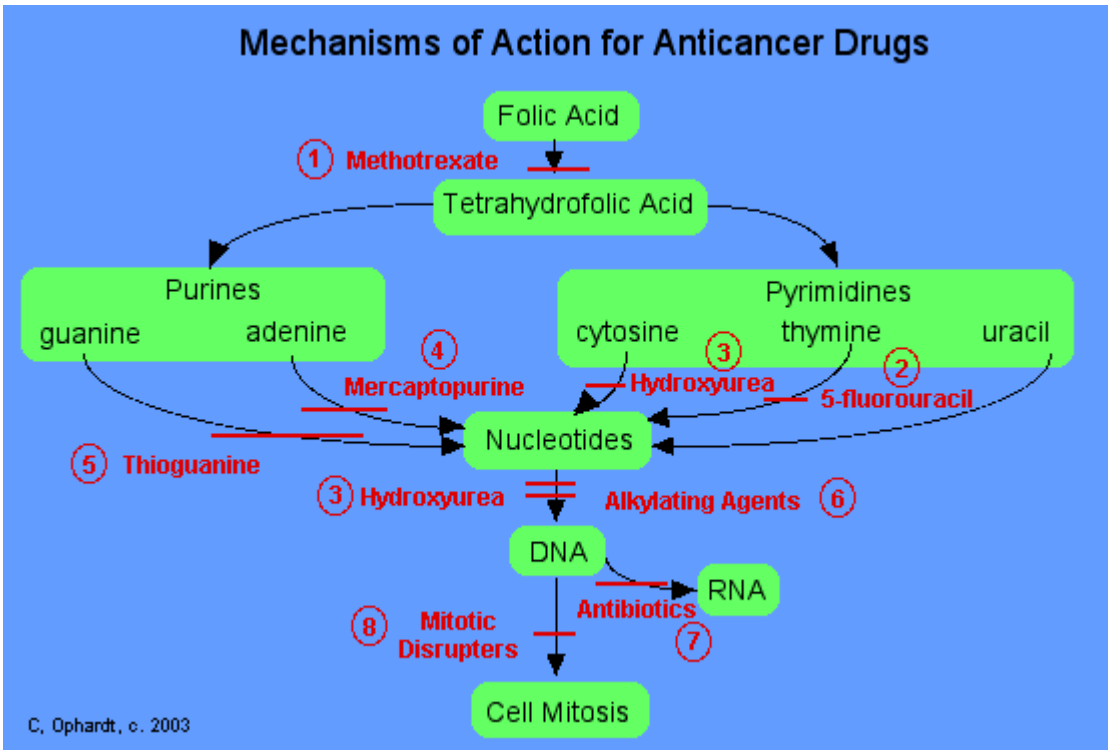
Among the common chemotherapeutic agents currently used in the treatment of metastatic breast cancer are the antimitotic drugs, which bind primarily to tubulin [6].

Chalcones are the precursors of flavonoids and isoflavonoids that exhibit a wide range of biological properties including anticancer activity [7,8].

Several mechanisms of action in chalcone-based compounds, such as anticancer agents, have been identified and these include the induction of apoptosis, DNA and mitochondrial damage, inhibition of angiogenesis, tubulin inhibition, kinase inhibition, and drug efflux protein activities, or a combination of some of these mechanisms [9]

Previous investigations suggest that the replacement of one or both phenyl rings of chalcones with a heteroaryl moiety results in heterocycle-appended chalcone hybrids with enhanced and synergistic anticancer properties [10,11].

Mechanism of anti-cancer drugs:



Invitro-anticancer activity:

By Various Methods:

Cell Growth Determination [77]

Cell growth can be determined by various accepted methods that utilize the exclusion of certain dyes by live cell membranes. Selection of a particular method depends on factors such as minimum number of cells required, sensitivity, speed, and ease of handling. The various preferable methods for cytotoxicity studies are 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay, sulforhodamine B (SRB) assay, propidium iodide (PI) assay, and luciferase assay.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Assay

It is the earliest of all the methods and was developed by Mosmann in the year 1983, in which a colorless tetrazolium salt is metabolized into colored insoluble formazan in proportion to viable cells. The formazan can be solubilized in dimethyl sulfoxide or acidic isopropanol and quantified spectrophotometrically.[18] The tetrazolium salt is an electron acceptor which is reduced to a colored formazan by accepting electrons from NADH, NADPH, and other oxidized substrates or appropriate coenzymes. The reduction of MTT occurs at multiple cellular sites including mitochondria. MTT assay is simple, rapid, and convenient, but the endpoint of assay is influenced by various factors such as concentration of D-glucose in the culture medium at the time of spectrophotometric assessment. Furthermore, the kinetics of MTT formazan production vary in a cell line-specific manner and quantitation of drug cytotoxicity is influenced by the length of exposure to MTT. It is, therefore, needed to standardize the assay conditions for each cell line as to minimize their effects on assay results. This would include optimization of cell inoculation densities and assay length in such way that these do not result in exhaustion of nutrients from the medium, and the concentration as well as exposure duration of MTT should also be standardized.

Sulforhodamine B Assay

SRB assay is a rapid, sensitive, and inexpensive method, which utilizes a bright pink anionic dye, that binds electrostatically to the basic amino acids of trichloroacetic acid fixed cells. The protein-bound dye is extracted with Tris base (tris (hydroxymethyl) aminomethane), after washing off the unbound dye, and thus, protein content can be

quantified indirectly spectrophotometrically. This method is suitable for an ordinary laboratory as well as for a very large-scale antitumor screening. The endpoint of SRB assay is nondestructive, not time critical (stable) and comparable with other fluorescence assays. Although this labor intensive method (several washing steps) offers practical advantage of high flux screening of anticancer drugs, results obtained with SRB assay are not significantly different from the results obtained with the MTT assay.

Propidium Iodide Assay

Ethidium bromide and PI are two cationic fluorescent dyes, known to pass only through the membranes of dead or dying cells and intercalate with DNA. Binding of these dyes with the DNA increases their fluorescence (more intensely by PI); therefore, fluorescence is seen only in nuclei of dead cells. These dyes are stable after uptake, and viability can be determined even after several days and therefore are more accurate and reliable. Cells are incubated with PI, and the number of nonviable cells is assessed by the subsequent fluorescence detection (first measurement). The second measurement will be taken after freezing the cells for 24 hours at −20°C. Due to freezing, PI will be intercalated into the DNA of all the cells and the difference in the two measurements will give the number of viable cells. The assay is a simple, rapid without any washing step and only 150–500 cells per well are sufficient for drug testing. One major drawback of this assay is that PI also binds with double-stranded RNA which might be present in the cytoplasm, but this can be overcome using RNAase enzyme during the assay.

Luciferase Assay

The nucleotide adenosine triphosphate (ATP) is the principal donor of free energy as it is needed by all cells to remain alive and perform their specialized functions and levels of cytoplasmic ATP decreases in case of any injury or hypoxia. Therefore, by measuring the amount ATP, one can determine the living status of a cell. Cellular ATP, after cell lysis, is free to react with luciferin and luciferase and which results in the generation of high-quantum chemiluminescence. Intensity of emitted light is linearly related to ATP concentration with optimum conditions. Luciferase assay showed better sensitivity and reproducibility over several days when compared to MTT assay and was able to detect the viability of cells when cell count was as low as 2000 cells/well, while in case of the MTT assay, minimum 25,000

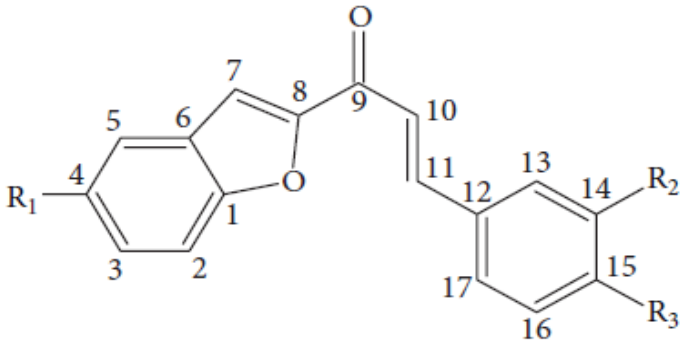
cells/well are required for above background readings. The shortcoming of this method is that quenching of the sample can influence the luminescence readout.

Other methods for the determination of cell viability are also available, but their usefulness is limited by several problems occurring with them, for example, in case of trypan blue dye exclusion assay, cells must be counted within 3–5 min as the number of dead cells increases with time and in case of lactate dehydrogenase assay, results could be misleading if the agent under investigation affects only intracellular activities.

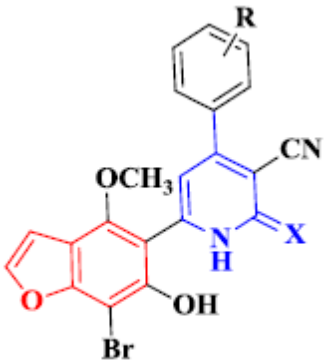
Monolayer cellular screens are the most convenient and frequently applied methods for cytotoxic studies but have certain disadvantages as these do not mimic heterogeneity of three-dimensional *in vivo* growth. Drugs such as signal transduction inhibitors, antibodies, bioreductive drugs, antiangiogenic peptides or small molecules, and anti-telomerase cannot be evaluated properly by monolayer cellular screens. Techniques such as growing cells in two-dimensions on matrices or in three-dimensions by encapsulation which mimic physical and biological properties of *in vivo* environment more appropriately are gradually replacing the monolayer cell screens. However, these techniques are still in their nascent phase and until fully available either specially designed cell screens or biochemical assays are best suited for above mentioned classes of drugs

LITERATURE REVIEW

1. **Demet Coskun et al; (2016)** A novel series of chalcones, 3-aryl-1-(5-bromo-1-benzofuran-2-yl)-2-propanones propenones (**3a**–**f**), were designed, synthesized, and characterized. *invitro* antitumor activities of the newly synthesized (**3a**–**f**) and previously synthesized (**3g**–**j**) chalcone compounds were determined by using human breast (MCF-7) and prostate (PC-3) cancer cell lines. Antitumor properties of all compounds were determined by 3-(4,5-dimethylthiazol- 2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay method[23]

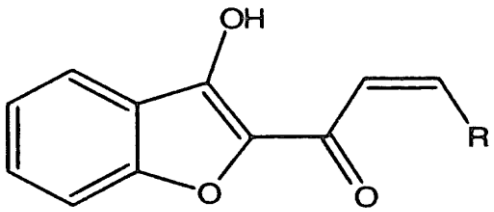


2. **Kamelia M. Amin et al; (2017)** Synthesed a new set of benzofuran and 5H-furo[3,2-g]chromone linked various heterocyclic functionalities using concise synthetic approaches aiming to gain new antiproliferative candidates against MCF-7 breast cancer cells of p38a MAP kinase inhibiting activity compared with Doxirubicin. Cell cycle analysis and apoptosis detection data demonstrated that compound VIa induced G2/M phase arrest and apoptosis in MCF-7 cancer cells, in addition to its activation of the caspases-9 and -3. Gold molecular docking the highly acceptable correlation between the calculated docking scores of fitness and the biological data of p38a MAP kinase inhibition. benzofuran and 5H-furo[3,2-g]chromone derivatives was considered as promising nuclei for breast cancer.[46]

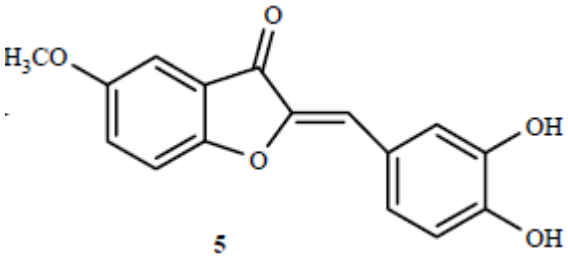


Va,b, VIa,b

3. **P.M.Gurubasavaraja Swamy et.al (2008);** synthesised some novel chalcones containing 3-Hydroxy Benzofuran and screened the anti-microbial activity. 2-acetyl-3-hydroxy benzofuran were allowed to react separately with different aromatic aldehydes in presence of 50% alkaline medium to yield 3-hydroxy benzofuran substituted chalcones and these were identified by spectral data and screened for antimicrobial activity.[50]

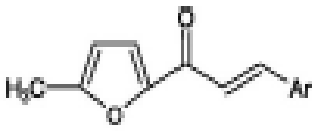


4. **Mahmoud Reza Heidari et.al; (2009)** The anti-inflammatory and analgesic effects of novel rigid benzofuran-3, 4 dihydroxy chalcone by formalin, hot-plate and carrageenan tests in mice was studied. The structure activity relationship (SAR) shows that benzofuran-3-one derivatives may be more effective in this respect. In this study, a new (Z)-2-(3,4-dihydroxybenzylidene)-5-methoxybenzofuran-3(2*H*)-one (compound 5) was synthesized and its analgesic and anti-inflammatory effects were evaluated by formalin, carrageenan and hot-plate method in mice. It results showed compound 5 induced significant anti-inflammatory effect ($P<0.01$). Maximum analgesia (42.6 %) was obtained at dose of 25mg/kg in the first phase of formalin test. it seems that compound 5 has potential for discovery of a compound with potent anti-inflammatory and analgesic effects and its scaffold could be use for further structural modifications[47]

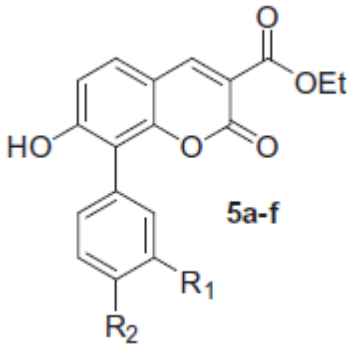


5

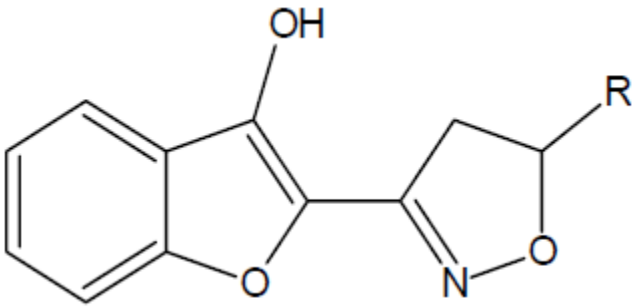
5. **Mohammed Rayees Ahmad et.al;(2011)** Chalcones were synthesized by conventional and microwave assisted synthesis methods. By microwave assisted synthesis, a considerable increase in the reaction rate has been observed and that too, with better yields. The compounds screened for their cytotoxic activity and antioxidant activity[48]



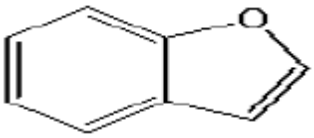
6. **Lucas C.C et.al;(2009)** Synthesed the biphenyl chalcone and coumarin derivatives was successfully accomplished via Suzuki coupling by using PEG-400 as a solvent under microwave irradiation. Salient feature of this methodology includes: short reaction time, good to excellent yields, and prominent tolerance of different functional groups.[49]



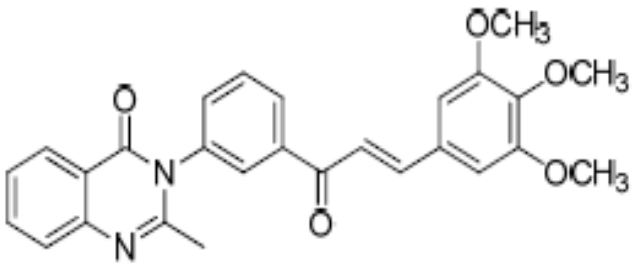
7. **P.M.Gurubasavaraja Swamy et.al (2008)** The substituted bensofuran chalcone derivative of 3-Hydroxy Bezofuran Chalcones (2a-g) prepared by the reaction of 2-acetyl-3-hydroxybenzofuran(1) with different aromatic aldehydes in the presence of a strong base, cyclocondensation of 3-Hydroxy benzofuran with hydroxylamine hydrochloride resulted in the formation of various Isoxazolines bearing hydroxyl benzofuran (3a-f). The structures of all the compounds have been established on the basis of analytical and spectral data. All the compounds have screened for antibacterial, while compounds 2a,2c,3a,3b, showed only moderate activity against staphylococcus at 500 mg/ml, compounds 2d, 2f, 3d, 3e, showed promising activity against Candida albicans at 500mg/ml concentration.[45]



8. **Ajit.K.Nangare et.al;(2015)** The purpose of this review was to provide an overview of different property of Benzofuran and some of its application in synthesis of pharmacologically active drug. Literature indicates that compounds having Benzofuran nucleus have wide range of therapeutic uses that include antibacterial, antifungal, anti-inflammatory, analgesic, antidepressant, anticonvulsant, antitumor, imaging, Anti-HIV, antidiabetic, antitubercular, antioxidant and miscellaneous activity.[51]

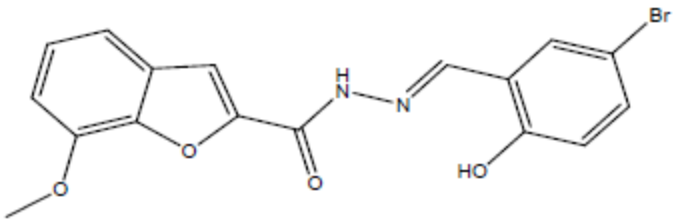


9. **Zahoor A. Wani et.al; (2015)** Synthesized the novel quinazolinone-chalcone derivative and evaluated its anticancer potential. Anticancer potential of A novel quinazolinone-chalcone derivative 2-Methyl-3-(3-((E)-3-(3,4,5-trimethoxyphenyl)- 2-propenoyl)phenyl)-3,4-dihydro-4-quinazolinone (8b) was determined through MTT assay, colony formation assay, Wound healing assay, Cell cycle and Western Blot Analysis in Mia paca-2 cells treated with 8b. The cytotoxicity studies showed a concentration dependent decrease in cell viability of HCT-116, HL-60, PC-3, A-549, Mia pacca-2 and MCF-7 cell lines with IC50 values ranging from 5.5 to 8.5 μM.[52]

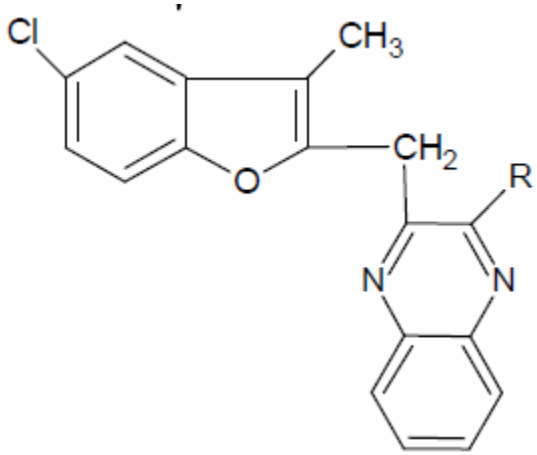


8b

10. **Mayank J. Mamtora et.al;(2015)** this study explains O,N,O-donating ligand was prepared from the condensation of 2-aminobenzohydrazide and 5- bromo-2-hydroxybenzaldehyde to give the Schiff base. The structure of the compounds was confirmed by MASS, IR, NMR, 13C NMR, ESI MASS. The synthesized compounds were screened for their antibacterial and antifungal activities.[53]



11. **Raga Basawaraj et.al;(2008)** Some new series of benzofuran quinoxaline derivatives (12-16) were prepared from benzofuran analogues of dibromo chalcones (7-11) by treatment with orthophenylene diamine in dry toluene. These compounds were screened for *in vitro* antibacterial and antifungal activities.[54]



(12-16)

12. **KrishnakumarLohidashan et.al; (2018)** Checked the realibility of new series of pyrazoline spacer compounds on other PASS online bioactive, Swiss ADME predictor. The PASS, Swiss ADME assisted docking approach and the use of combo heterocyclic ring with pyrazoline scaffold to derive and synthesize effective antimalarial agents.[55]

13. **S.N.Mokale et al; (2015)** Synthesis, invitro screening, and docking analysis of novel pyrrolidine and piperidine-substituted ethoxy chalcone as anticancer agents[56]

14. **N. Ashokkumar et al: (2018)** study results Green synthesis, characterization, *in silico* molecular docking and *in vitro* anticancer activity of 1,2,3-triazolyl dihydropyrimidine-2-thione hybrids[57]

15. **Koneni V. Sashidhara et.al. (2014);** benzofuran chalcone hybrids as potential multifunctional agents against Alzheimer disease: synthesis and invivo studies with transgenic caenorhabditis elegans.[58]

16. **Shaik KA.et al: (2014);** developed the chem doodle- drawing of structures, 3D Representation of molecule, predicting 1H and 13C NMR, Searching of molecule along with the same. in drawing tool, cyclic rings like cyclopropane, cyclobutane, benzene ring, are made available for ease of chemist. [59]

17. **Jacek.Kujawskiet. et al; (2012)** determined molecules hydrophobicity (lipophilicity) usally quantified as log P where P is the partition co-efficient using computational methods. An interesting tool for calculation of log P Co efficient is presented: the virtual computational chemistry laboratory (VCCLAB) package. The package includes the A LOG P.S suitable for log P calculations.[60]

18. **Paulo A.Netz.J et al; (2009**) Docking studies on DNA-ligand interactions, Building and applications of a protocol to identify the binding.[63]

19. **Li.Zhang et.al; (2003)** Flavonoids: Promising Anti-cancer agents.[64]

20. **M. Maggiolini et al;(2002)** Explains Estrogenic and antiproliferative activities of isoliquiritigenin in MCF7 breast cancer cells.[17]

21. **T. Sakai et al; (2012**) study of chalcone, which induces apoptosis in synovial sarcoma cell lines by MTT Assay.[69]

22. **S. K. Kumar et al;(2003)** Design, synthesis, and evaluation of novel boronic-chalcone derivatives as antitumor agents[70]

23. **B. Srinivasan et al; (2009)** Structure activity relationship studies of chalcone leading to 3-hydroxy- 4,3!,4!,5!-tetramethoxy chalcone and its analogues as potent nuclear factor B inhibitors and their anticancer activities[71]

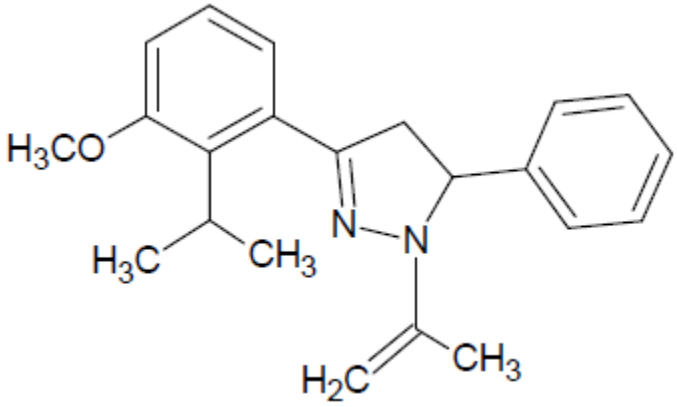
24. **D. Kumar et al;(2010)** Synthesis and biological evaluation of indolyl chalcones as antitumor agents.[72]

25. **H. Baek et al; (2013)** Chalcones, inhibitors for topoisomerase i and cathepsin B and L, as potential anti-cancer agents.[73]

26. **N. S. Hari Narayana Moorthy et al; (2011)** Structural feature study of benzofuran derivatives as farnesyltransferase inhibitors Farnesylation is a critical step for membrane

binding and the biological function of G-proteins. In the present investigation, we have studied the structural features of some molecules that are acting on the farnesyltransferase (FTase) enzyme for the inhibition of the farnesylation step in G-proteins. The benzofuran derivatives have activity against FTase inhibition and antiproliferative activity on QG56 cell lines.[74]

27. **S.D.Tala *et al.,*** reported on synthesis of some new chalcone and pyrazole derivatives with antimicrobial activity, 2013[77]



28. **Jaya seema et al; (2018)** designing of the anticancer Nano composite sustained release properties by using grahene oxide nanocarrier with phenethyl Isothiocyanate as Anticancer agent.[66]

29. **Sheng Tian et al; (2015)** Used the concept of drug likeness, established from the analyses of the physiochemical properties or/and structural features of the existing small organic drugs or drug candidates, to filterout compounds with undesirable properties, especially poor ADMET properties.[68]

30. **Ayaz mohmood Dar et al; (2017**) developed molecular docking which is a kind of bioinformatics modelling which involves the intersction of two or more molecules give the stable adduct. Depending upon binding properties of ligand and target. It predicts the three dimensional structure of anycomplex. Molecular docking generates different possible sdduct structures that are ranked and grouped together using scoring function in the software[67]

31. **Demet Coskun1 et al. (2017);** Chalcone and its derivatives exhibit anticancer potential in different cancer cells. A new series of benzofuran substituted chalcone derivatives was synthesized by the base-catalyzed Claisen-Schmidt reaction of the 1-(7-ethoxy-1-benzofuran-2-yl) ethanone with different aromatic aldehydes to yield 1-(7- ethoxy-1-

benzofuran-2-yl) substituted chalcone derivatives **3a-j**. The derivatives were characterized by elemental analysis, FT-IR, 1H-NMR and 13C-NMR spectroscopy techniques. The anti-growth effect of chalcone compounds was tested in breast cancer (MCF-7), non-small cell lung cancer (A549) and prostate cancer (PC-3) cell lines by the SRB and ATP cellviability assays[75]

32. **Krishnamoorthy et al (2017);** subjected eight pyrimidine derivatives containing substituted imidazole moity to molecular docking studies for inhibition of the vascular endothelial growth factor receptor 2 (VIGFR2) PDB ID 1VR2 and good affinity towards the active pocket as good inhibitor of vascular endo thelial growth factor receptor 2.[76]

AIM AND OBJECTIVES

In my project, primary goals are designing, synthesizing new compounds (**5a**–**e**) with both benzofuran and chalcone units optimized with docking studies then examining anticancer activity bearing no substituent in the benzofuran ring as different series against human breast cancer cell lines (MCF-7) and human Liver cancer cells (HepG2)

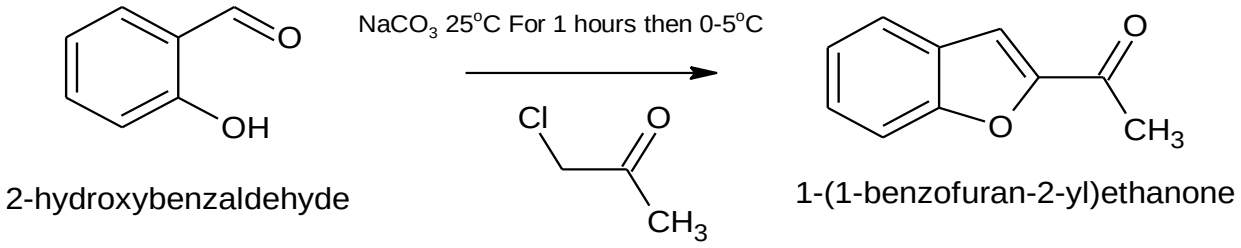
From the literature survey benzofuran and chalcone derivatives are known for various pharmacological activities such as **antimicrobial**, antifungal, cytotoxic, antileishmanial, anti-inflammatory, **anticancer**, tyrosine kinase inhibitors, and antimalarial etc.,

Objectives of the study:

- ✓ To select the appropriate nucleus or lead molecule and their derivatives
- ✓ To optimize the method of synthesis for the proposed compounds.
- ✓ To synthesize the various Novel Benzofuran chalcone derivatives(5a-e)
- ✓ To optimize with *in-silico* docking studies
- ✓ To characterize the synthesized compound by FT-IR, H1, C13 NMR and Mass spectroscopy and various analytical studies.
- ✓ To evaluate the anticancer activitY MTT Assay method.

4.1 SCHEME OF SYHTHESIS

STEP 1:



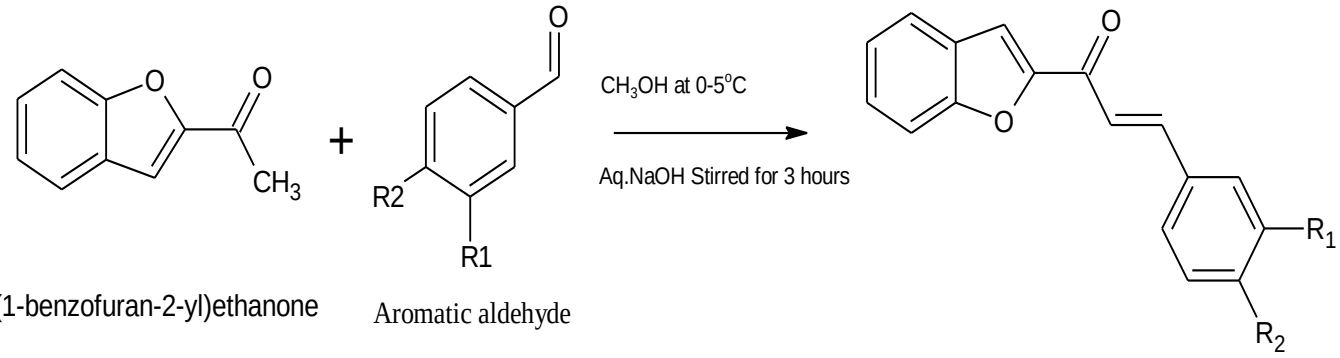
2-hydroxybenzaldehyde

1-chloropropan-2-one

1-(1-benzofuran-2-yl)ethanone

(1) (2) (3)

STEP-2:



1-(1-benzofuran-2-yl)ethanone

Aromatic aldehyde

(3) (4) (5a-e)

SUBSTITUTIONS:

COMPOUND	R_1	R_2
3a	H	H
3b	H	Cl
3c	H	OCH_3
3d	H	NO_2
3e	CH_3	OH

4.2 MOLECULAR DESIGN

The Software tools like Chemdoodle, Molinspiration, Chemsketch were used to design the molecule for synthesis.

A) Chemdoodle:

It is used to assess the *LIPINSKI'S RULE*. It is the rule of five used by *LIPINSKI* to improve the bioavailability of the drug. Lipinski rule states that the orally active drugs have:

- Molecular weight ≤ 500
- logP ≤ 5
- hydrogen bond acceptors ≤ 10
- hydrogen bond donors ≤ 5

The molecules violating any one of the above rule will not have proper bio-availability.

B) Molinspiration:

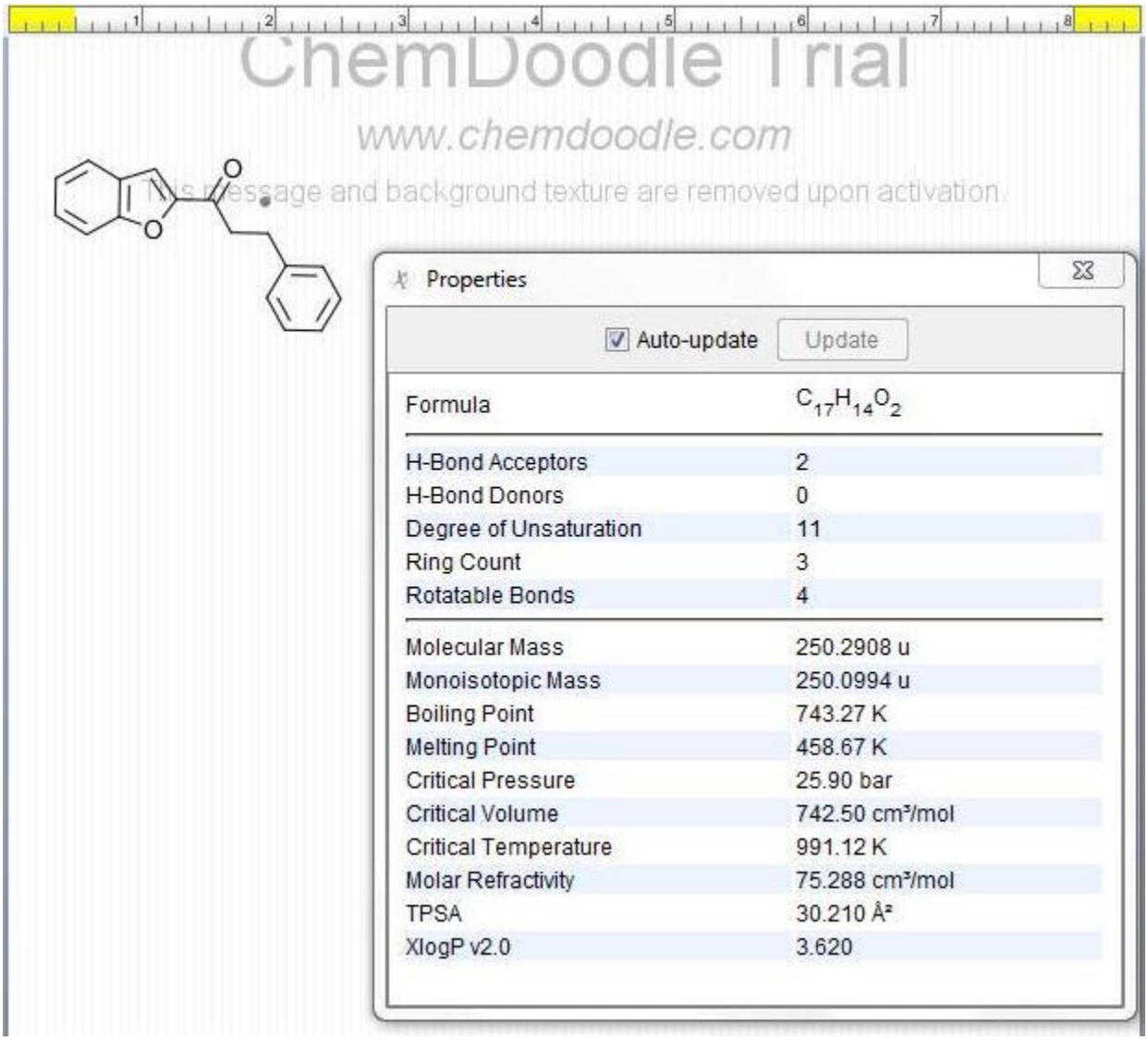
Virtual Screening is the computational chemistry technique to assess the large drug databases to identify the new drug molecules. It screens the molecules and provides the bioactivity score between -3 and 3. Molecules with highest bioactivity score will be more biologically active and produces better activity.

C) Chemsketch:

It is a software tool used for the prediction of molecular properties such as molecular mass, LogP, molar refractivity, parachor, molar volume, surface tension, polarizability and elemental composition

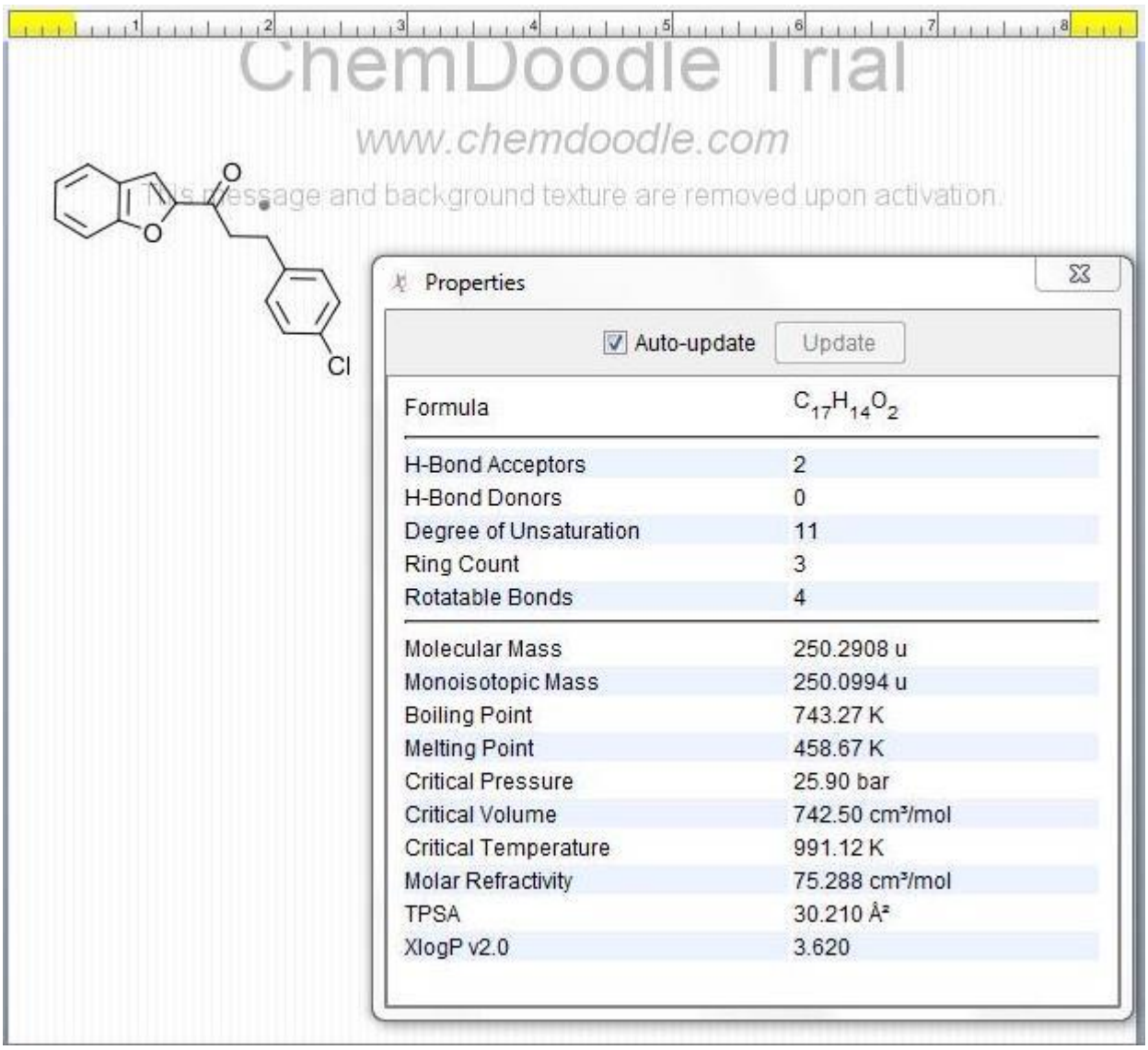
CHEMDOODLE:

COMPOUND -5a



Formula	$C_{17}H_{14}O_2$
H-Bond Acceptors	2
H-Bond Donors	0
Degree of Unsaturation	11
Ring Count	3
Rotatable Bonds	4
Molecular Mass	250.2908 u
Monoisotopic Mass	250.0994 u
Boiling Point	743.27 K
Melting Point	458.67 K
Critical Pressure	25.90 bar
Critical Volume	742.50 cm³/mol
Critical Temperature	991.12 K
Molar Refractivity	75.288 cm³/mol
TPSA	30.210 Å²
XlogP v2.0	3.620

COMPOUND-5b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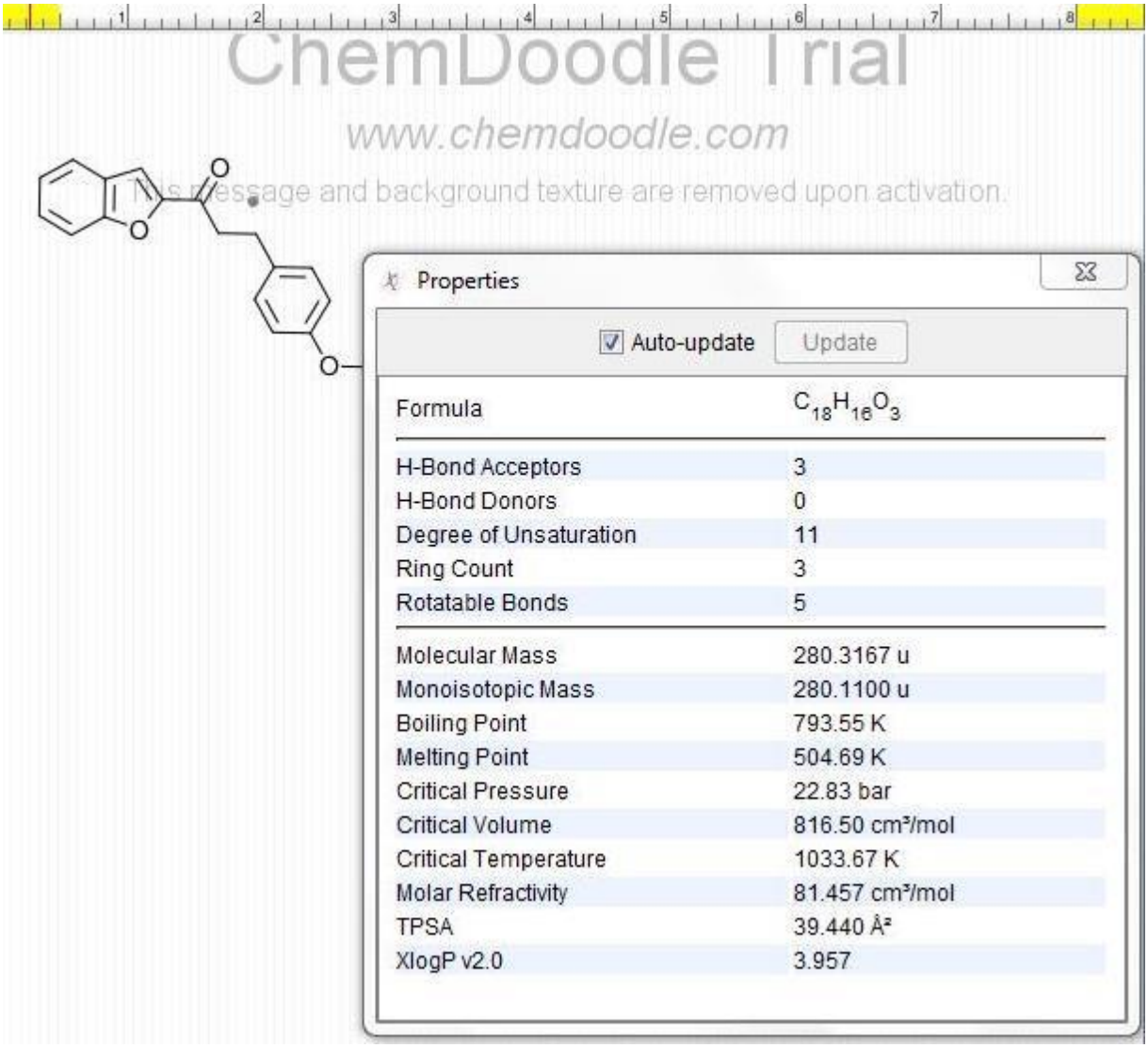
Formula
$C_{17}H_{14}O_2$
H-Bond Acceptors 2
H-Bond Donors 0
Degree of Unsaturation 11
Ring Count 3
Rotatable Bonds 4
Molecular Mass 250.2908 u
Monoisotopic Mass 250.0994 u
Boiling Point 743.27 K
Melting Point 458.67 K
Critical Pressure 25.90 bar
Critical Volume 742.50 cm³/mol
Critical Temperature 991.12 K
Molar Refractivity 75.288 cm³/mol
TPSA 30.210 Å²
XlogP v2.0 3.620

COMPOUND-5c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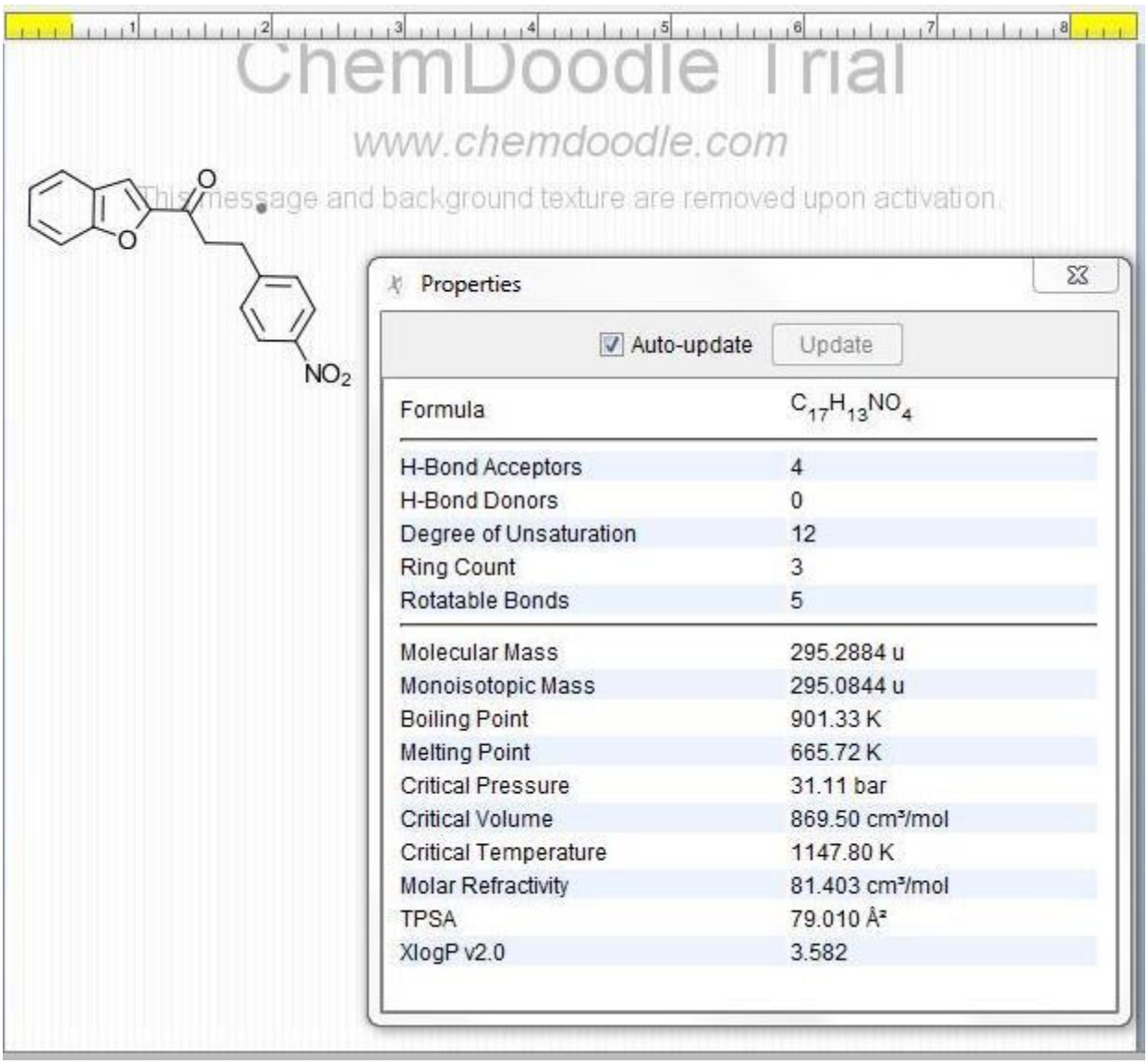
Formula
$C_{18}H_{16}O_3$
H-Bond Acceptors 3
H-Bond Donors 0
Degree of Unsaturation 11
Ring Count 3
Rotatable Bonds 5
Molecular Mass 280.3167 u
Monoisotopic Mass 280.1100 u
Boiling Point 793.55 K
Melting Point 504.69 K
Critical Pressure 22.83 bar
Critical Volume 816.50 cm³/mol
Critical Temperature 1033.67 K
Molar Refractivity 81.457 cm³/mol
TPSA 39.440 Å²
XlogP v2.0 3.957

COMPOUND-5d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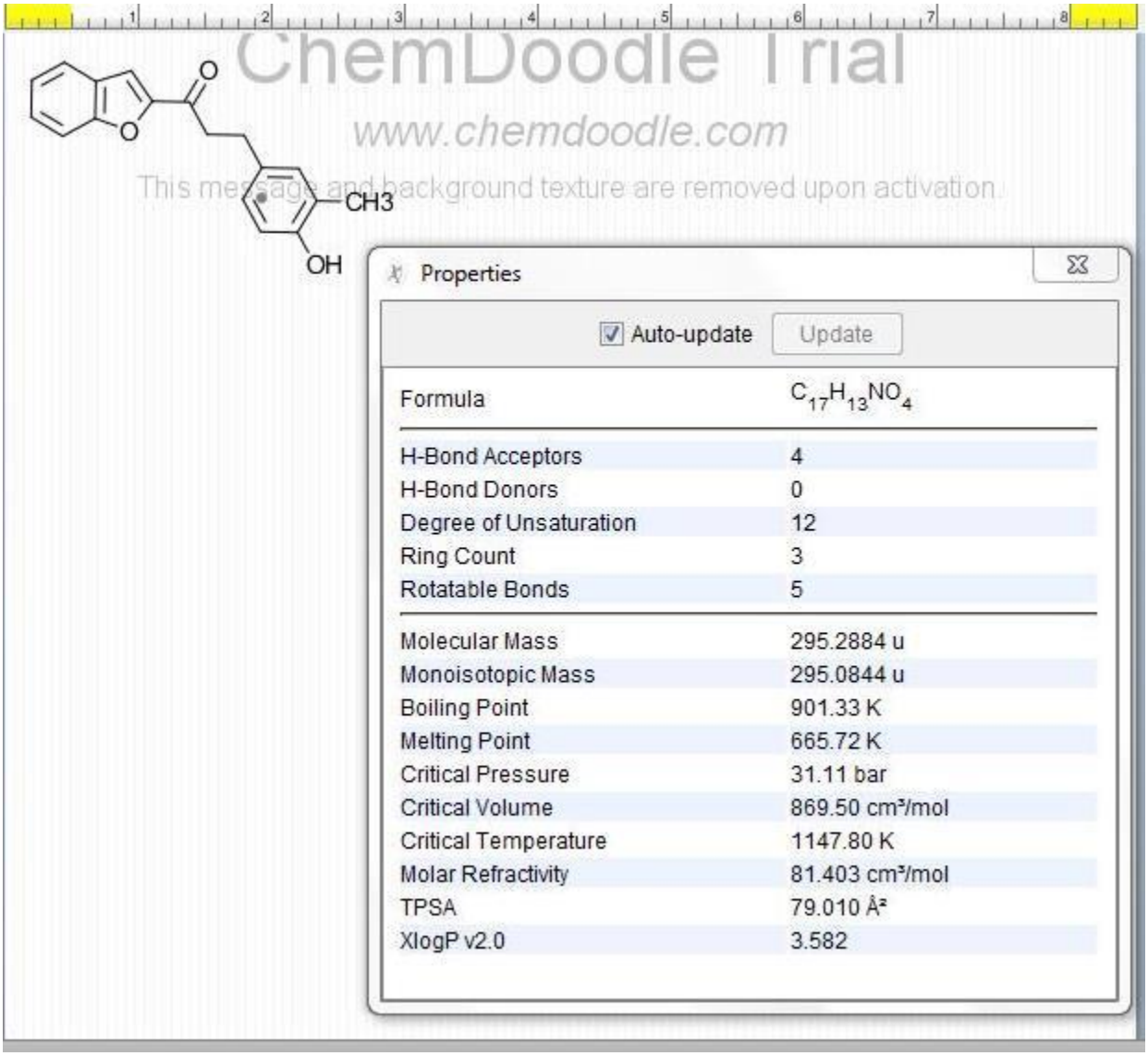
Formula C17H13NO4
H-Bond Acceptors 4
H-Bond Donors 0
Degree of Unsaturation 12
Ring Count 3
Rotatable Bonds 5
Molecular Mass 295.2884 u
Monoisotopic Mass 295.0844 u
Boiling Point 901.33 K
Melting Point 665.72 K
Critical Pressure 31.11 bar
Critical Volume 869.50 cm³/mol
Critical Temperature 1147.80 K
Molar Refractivity 81.403 cm³/mol
TPSA 79.010 Å²
XlogP v2.0 3.582

COMPOUND-5e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Formula
$C_{17}H_{13}NO_4$
H-Bond Acceptors 4
H-Bond Donors 0
Degree of Unsaturation 12
Ring Count 3
Rotatable Bonds 5
Molecular Mass 295.2884 u
Monoisotopic Mass 295.0844 u
Boiling Point 901.33 K
Melting Point 665.72 K
Critical Pressure 31.11 bar
Critical Volume 869.50 cm³/mol
Critical Temperature 1147.80 K
Molar Refractivity 81.403 cm³/mol
TPSA 79.010 Å²
XlogP v2.0 3.582

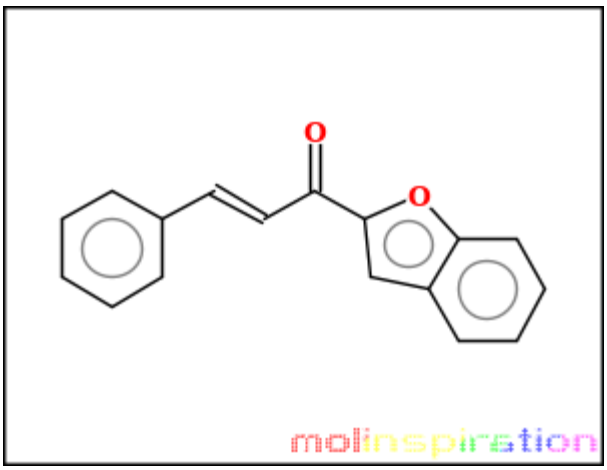
Molinspiration:

This software is used to calculate the following properties

- Log P
- Molecular Weight
- Number of H-bond donor
- Number of H-bond acceptor
- Number of rotatable bonds

Bioactive score by molinspiration:

Compound 5a:



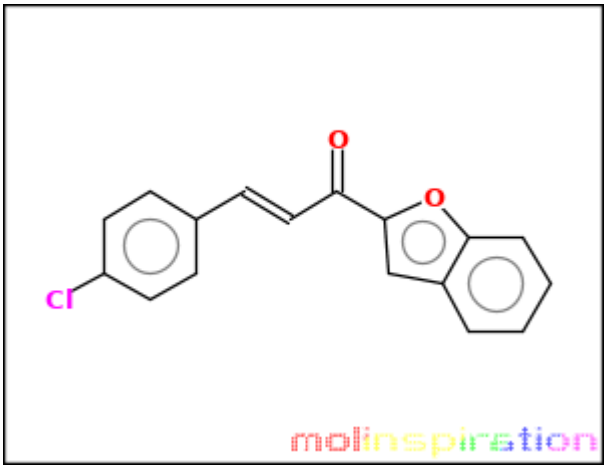
Molinspiration property engine v2018.10

miLogP	4.38
TPSA	30.21
natoms	19
MW	248.28
nON	2
nOHNH	0
nviolations	0
nrotb	3
volume	227.41

Molinspiration bioactivity score v2018.03

GPCR ligand	-0.37
Ion channel modulator	-0.34
Kinase inhibitor	-0.60
Nuclear receptor ligand	-0.40
Protease inhibitor	-0.39
Enzyme inhibitor	-0.07

Compound 5b:



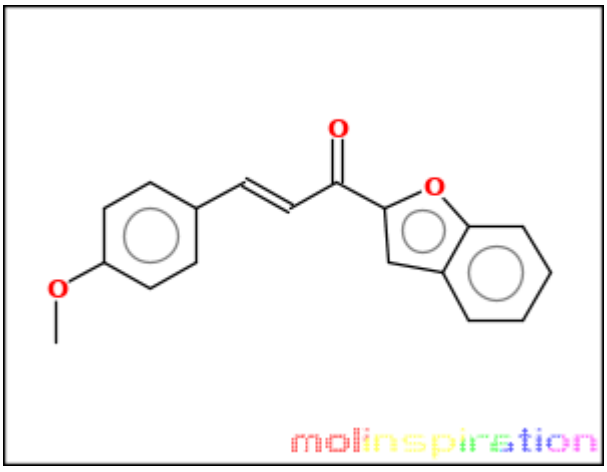
Molinspiration property engine v2018.10

miLogP	5.05
TPSA	30.21
natoms	20
MW	282.73
nON	2
nOHNH	0
nviolations	1
nrotb	3
volume	240.95

Molinspiration bioactivity score v2018.03

GPCR ligand	-0.31
Ion channel modulator	-0.33
Kinase inhibitor	-0.55
Nuclear receptor ligand	-0.36
Protease inhibitor	-0.37
Enzyme inhibitor	-0.10

Compound 5c:



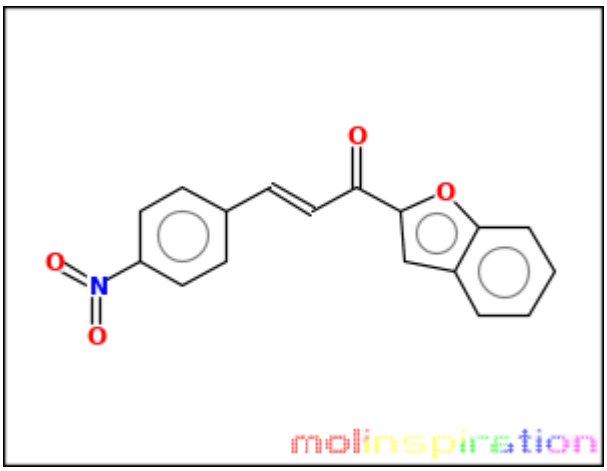
Molinspiration property engine v2018.10

miLogP	4.43
TPSA	39.45
natoms	21
MW	278.31
nON	3
nOHNH	0
nviolations	0
nrotb	4
volume	252.96

Molinspiration bioactivity score v2018.03

GPCR ligand	-0.32
Ion channel modulator	-0.40
Kinase inhibitor	-0.52
Nuclear receptor ligand	-0.29
Protease inhibitor	-0.33
Enzyme inhibitor	-0.11

Compound 5d:



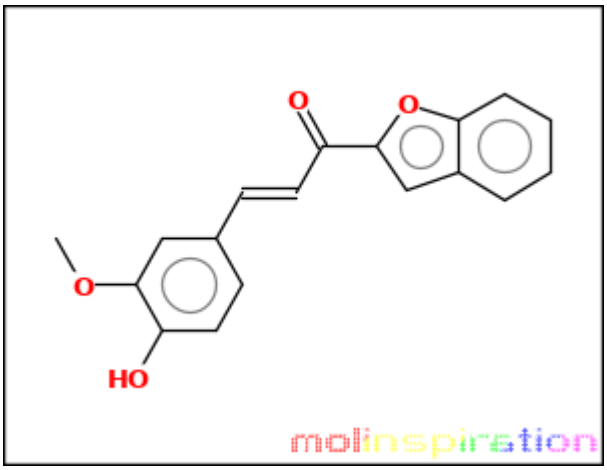
Molinspiration property engine v2018.10

miLogP	4.33
TPSA	76.03
natoms	22
MW	293.28
nON	5
nOHNH	0
nviolations	0
nrotb	4
volume	250.75

Molinspiration bioactivity score v2018.03

GPCR ligand	-0.40
Ion channel modulator	-0.36
Kinase inhibitor	-0.59
Nuclear receptor ligand	-0.36
Protease inhibitor	-0.39
Enzyme inhibitor	-0.19

Compound 5e:



Molinspiration property engine v2018.10

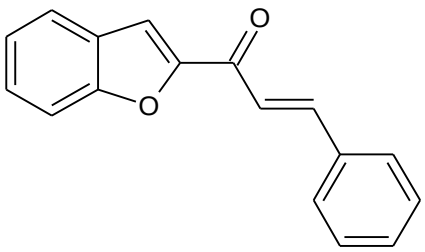
miLogP	3.71
TPSA	59.67
natoms	22
MW	294.31
nON	4
nOHNH	1
nviolations	0
nrotb	4
volume	260.98

Molinspiration bioactivity score v2018.03

GPCR ligand	-0.25
Ion channel modulator	-0.35
Kinase inhibitor	-0.43
Nuclear receptor ligand	-0.20
Protease inhibitor	-0.32
Enzyme inhibitor	-0.05

CHEMSKETCH

COMPOUND 5a:



(2*E*)-1-(1-benzofuran-2-yl)-3-phenylprop-2-en-1-one

PROPERTIES:

Molecular Formula = $C_{17}H_{12}O_2$

Formula Weight = 248.27598

Composition = C(82.24%) H(4.87%) O(12.89%)

Molar Refractivity = 77.25 ± 0.3 cm^3

Molar Volume = 206.7 ± 3.0 cm^3

Parachor = 548.7 ± 4.0 cm^3

Index of Refraction = 1.670 ± 0.02

Surface Tension = 49.6 ± 3.0 dyne/cm

Density = 1.200 ± 0.06 g/cm^3

Dielectric Constant = Not available

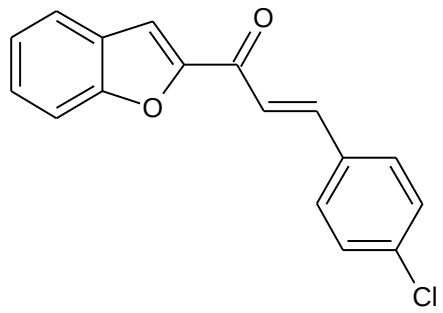
Polarizability = 30.62 ± 0.5 $10^{-24}cm^3$

Monoisotopic Mass = 248.08373 Da

Nominal Mass = 248 Da

Average Mass = 248.276 Da

COMPOUND 5b:

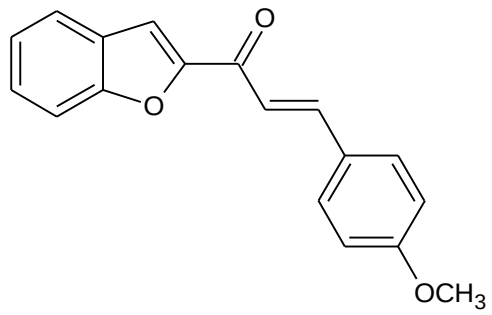


(2*E*)-1-(1-benzofuran-2-yl)-3-(4-chlorophenyl)prop-2-en-1-one

PROPERTIES:

Molecular Formula	= $C_{17}H_{11}ClO_2$
Formula Weight	= 282.72104
Composition	= C(72.22%) H(3.92%) Cl(12.54%) O(11.32%)
Molar Refractivity	= 82.14 ± 0.3 cm^3
Molar Volume	= 218.6 ± 3.0 cm^3
Parachor	= 584.6 ± 4.0 cm^3
Index of Refraction	= 1.674 ± 0.02
Surface Tension	= 51.0 ± 3.0 dyne/cm
Density	= 1.292 ± 0.06 g/cm^3
Dielectric Constant	= Not available
Polarizability	= 32.56 ± 0.5 $10^{-24}cm^3$
Monoisotopic Mass	= 282.044757 Da
Nominal Mass	= 282 Da
Average Mass	= 282.721 Da

COMPOUND 5c:



(2*E*)-1-(1-benzofuran-2-yl)-3-(4-nitrophenyl)prop-2-en-1-one

PROPERTIES:

Molecular Formula = $C_{18}H_{14}O_3$

Formula Weight = 278.30196

Composition = C(77.68%) H(5.07%) O(17.25%)

Molar Refractivity = 83.92 ± 0.3 cm^3

Molar Volume = 230.7 ± 3.0 cm^3

Parachor = 605.4 ± 4.0 cm^3

Index of Refraction = 1.647 ± 0.02

Surface Tension = 47.4 ± 3.0 dyne/cm

Density = 1.206 ± 0.06 g/cm^3

Dielectric Constant = Not available

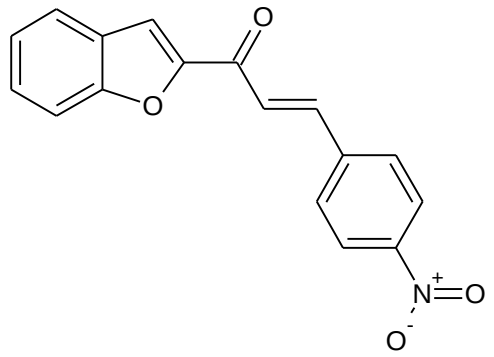
Polarizability = 33.27 ± 0.5 $10^{-24}cm^3$

Monoisotopic Mass = 278.094294 Da

Nominal Mass = 278 Da

Average Mass = 278.302 Da

COMPOUND 5d:

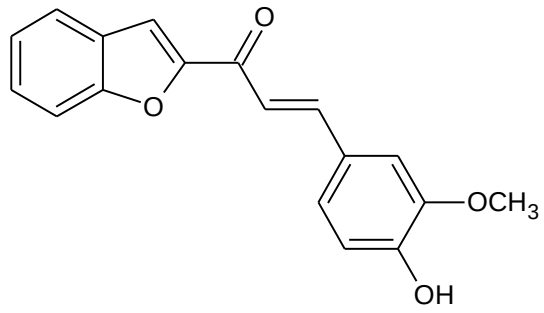


(2*E*)-1-(1-benzofuran-2-yl)-3-(4-nitrophenyl)prop-2-en-1-one

PROPERTIES:

Molecular Formula	= $C_{17}H_{11}NO_4$
Formula Weight	= 293.27354
Composition	= C(69.62%) H(3.78%) N(4.78%) O(21.82%)
Molar Refractivity	= 83.79 ± 0.3 cm^3
Molar Volume	= 218.5 ± 3.0 cm^3
Parachor	= 604.2 ± 4.0 cm^3
Index of Refraction	= 1.692 ± 0.02
Surface Tension	= 58.4 ± 3.0 dyne/cm
Density	= 1.341 ± 0.06 g/cm^3
Dielectric Constant	= Not available
Polarizability	= 33.21 ± 0.5 $10^{-24}cm^3$
Monoisotopic Mass	= 293.068808 Da
Nominal Mass	= 293 Da
Average Mass	= 293.2735 Da

COMPOUND 5e:



(2*E*)-1-(1-benzofuran-2-yl)-3-(4-hydroxy-3-methoxyphenyl)prop-2-en-1-one

PROPERTIES:

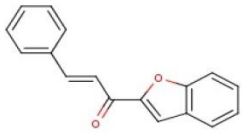
Molecular Formula	= $C_{18}H_{14}O_4$
Formula Weight	= 294.30136
Composition	= C(73.46%) H(4.79%) O(21.75%)
Molar Refractivity	= 85.81 ± 0.3 cm^3
Molar Volume	= 229.1 ± 3.0 cm^3
Parachor	= 620.4 ± 4.0 cm^3
Index of Refraction	= 1.672 ± 0.02
Surface Tension	= 53.7 ± 3.0 dyne/cm
Density	= 1.284 ± 0.06 g/cm^3
Dielectric Constant	= Not available
Polarizability	= 34.01 ± 0.5 $10^{-24}cm^3$
Monoisotopic Mass	= 294.089209 Da
Nominal Mass	= 294 Da
Average Mass	= 294.3014 Da

SWISS TARGET PREDICTION REPORT:5a

SwissTargetPrediction report:

Reference:
Gfeller D., Michielin O. & Zoete V. Shaping the interaction landscape of bioactive molecules, *Bioinformatics* (2013) 29:3073-3079.

Query Molecule



Frequency of Target Class

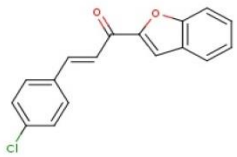
Target	Uniprot ID	Gene code	ChEMBL ID	Probability	# sim. cmpds (3D / 2D)	Target Class
Microtubule-associated protein tau	P10636	MAPT	CHEMBL1293224		306 / 27	Unclassified
Receptor-type tyrosine-protein kinase FLT3	P36888	FLT3	CHEMBL1974		3 / 5	Tyr Kinase
Arachidonate 5-lipoxygenase *(by homology)*	P09917	ALOX5	CHEMBL215		14 / 2	Enzyme
Muscleblind-like protein 1	Q9NR56	MBNL1	CHEMBL1293317		203 / 7	Unclassified
Muscleblind-like protein 2 *(by homology)*	Q5VZF2	MBNL2			203 / 7	Unclassified
Muscleblind-like protein 3 *(by homology)*	Q9NUK0	MBNL3			203 / 7	Unclassified
Tyrosyl-DNA phosphodiesterase 1	Q9NUW8	TDP1	CHEMBL1075138		55 / 6	Enzyme
Dual specificity tyrosine-phosphorylation-regulated kinase 1A *(by homology)*	Q13627	DYRK1A	CHEMBL2292		9 / 1	Ser_Thr_Tyr Kinase
Amine oxidase [flavin-containing] A *(by homology)*	P21397	MAOA	CHEMBL1951		43 / 4	Enzyme
Amine oxidase [flavin-containing] B	P27338	MAOB	CHEMBL2039		43 / 4	Enzyme
Histone deacetylase 3 *(by homology)*	O15379	HDAC3	CHEMBL1829		3 / 8	Enzyme
Histone deacetylase 1	Q13547	HDAC1	CHEMBL325		3 / 8	Enzyme
Histone deacetylase 2 *(by homology)*	Q92769	HDAC2	CHEMBL1937		3 / 8	Enzyme
Prostaglandin G/H synthase 1	P23219	PTGS1	CHEMBL221		15 / 1	Enzyme
Prostaglandin G/H synthase 2	P35354	PTGS2	CHEMBL230		15 / 1	Enzyme

SWISS TARGET PREDICTION REPORT:5b

SwissTargetPrediction report:

Reference:
Gfeller D., Michielin O. & Zoete V. Shaping the interaction landscape of bioactive molecules, *Bioinformatics* (2013) 29:3073-3079.

Query Molecule



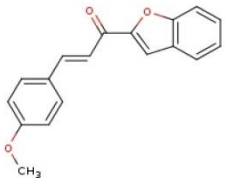
Frequency of Target Class

Target	Uniprot ID	Gene code	ChEMBL ID	Probability	# sim. cmpds (3D / 2D)	Target Class
Microtubule-associated protein tau	P10636	MAPT	CHEMBL1293224		260 / 16	Unclassified
Arachidonate 5-lipoxygenase	P09917	ALOX5	CHEMBL215		13 / 2	Enzyme
Receptor-type tyrosine-protein kinase FLT3	P36888	FLT3	CHEMBL1974		3 / 5	Tyr Kinase
Amine oxidase [flavin-containing] A	P21397	MAOA	CHEMBL1951		37 / 9	Enzyme
Amine oxidase [flavin-containing] B	P27338	MAOB	CHEMBL2039		37 / 9	Enzyme
Tyrosyl-DNA phosphodiesterase 1	Q9NUW8	TDP1	CHEMBL1075138		49 / 5	Enzyme
Arachidonate 15-lipoxygenase B *(by homology)*	O15296	ALOX15B	CHEMBL2457		3 / 1	Enzyme
Arachidonate 12-lipoxygenase, 12R-type *(by homology)*	O75342	ALOX12B			3 / 1	Enzyme
Arachidonate 15-lipoxygenase *(by homology)*	P16050	ALOX15	CHEMBL2903		3 / 1	Enzyme
Arachidonate 12-lipoxygenase, 12S-type *(by homology)*	P18054	ALOX12	CHEMBL3687		3 / 1	Enzyme
Epidermis-type lipoxygenase 3 *(by homology)*	Q9BYJ1	ALOXE3			3 / 1	Enzyme
Muscleblind-like protein 1	Q9NR56	MBNL1	CHEMBL1293317		207 / 6	Unclassified
Muscleblind-like protein 2 *(by homology)*	Q5VZF2	MBNL2			207 / 6	Unclassified
Muscleblind-like protein 3 *(by homology)*	Q9NUK0	MBNL3			207 / 6	Unclassified
Dual specificity tyrosine-phosphorylation-regulated kinase 1A *(by homology)*	Q13627	DYRK1A	CHEMBL2292		8 / 1	Ser_Thr_Tyr Kinase

SwissTargetPrediction report:

Reference:
Gfeller D., Michielin O. & Zoete V. Shaping the interaction landscape of bioactive molecules, *Bioinformatics* (2013) 29:3073-3079.

Query Molecule



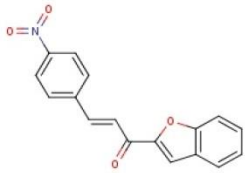
Frequency of Target Class

Target	Uniprot ID	Gene code	ChEMBL ID	Probability	# sim. cmpds (3D / 2D)	Target Class
Microtubule-associated protein tau	P10636	MAPT	CHEMBL1293224		1602 / 54	Unclassified
Dual specificity tyrosine-phosphorylation-regulated kinase 1A *(by homology)*	Q13627	DYRK1A	CHEMBL2292		54 / 3	Ser_Thr_Tyr Kinase
Tyrosyl-DNA phosphodiesterase 1	Q9NUW8	TDP1	CHEMBL1075138		169 / 7	Enzyme
ATP-binding cassette sub-family G member 2	Q9UNQ0	ABCG2	CHEMBL5393		25 / 33	Unclassified
Muscleblind-like protein 1	Q9NR56	MBNL1	CHEMBL1293317		751 / 9	Unclassified
Muscleblind-like protein 2 *(by homology)*	Q5VZF2	MBNL2			751 / 9	Unclassified
Muscleblind-like protein 3 *(by homology)*	Q9NUK0	MBNL3			751 / 9	Unclassified
Potassium voltage-gated channel subfamily A member 3	P22001	KCNA3	CHEMBL4633		50 / 11	Ion channel
Potassium voltage-gated channel subfamily A member 5	P22460	KCNA5	CHEMBL4306		50 / 11	Ion channel
Potassium voltage-gated channel subfamily A member 2 *(by homology)*	P16389	KCNA2	CHEMBL2086		50 / 11	Ion channel
Potassium voltage-gated channel subfamily A member 6 *(by homology)*	P17658	KCNA6	CHEMBL5279		50 / 11	Ion channel
Potassium voltage-gated channel subfamily A member 4 *(by homology)*	P22459	KCNA4	CHEMBL4205		50 / 11	Ion channel
Potassium voltage-gated channel subfamily A member 1 *(by homology)*	Q09470	KCNA1	CHEMBL2309		50 / 11	Ion channel
Potassium voltage-gated channel subfamily A member 10 *(by homology)*	Q16322	KCNA10			50 / 11	Ion channel
Potassium voltage-gated channel subfamily A member 7 *(by homology)*	Q96RP8	KCNA7	CHEMBL2773		50 / 11	Ion channel

SwissTargetPrediction report:

Reference:
Gfeller D., Michielin O. & Zoete V. Shaping the interaction landscape of bioactive molecules, *Bioinformatics* (2013) 29:3073-3079.

Query Molecule



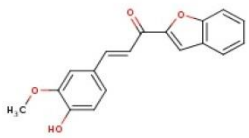
Frequency of Target Class

Target	Uniprot ID	Gene code	ChEMBL ID	Probability	# sim. cmpds (3D / 2D)	Target Class
Microtubule-associated protein tau	P10636	MAPT	CHEMBL1293224		351 / 28	Unclassified
Muscleblind-like protein 1	Q9NR56	MBNL1	CHEMBL1293317		244 / 8	Unclassified
Muscleblind-like protein 2 *(by homology)*	Q5VZF2	MBNL2			244 / 8	Unclassified
Muscleblind-like protein 3 *(by homology)*	Q9NUK0	MBNL3			244 / 8	Unclassified
Arachidonate 5-lipoxygenase *(by homology)*	P09917	ALOX5	CHEMBL215		10 / 2	Enzyme
Receptor-type tyrosine-protein kinase FLT3	P36888	FLT3	CHEMBL1974		7 / 4	Tyr Kinase
Prostaglandin G/H synthase 1 *(by homology)*	P23219	PTGS1	CHEMBL221		8 / 1	Enzyme
Prostaglandin G/H synthase 2 *(by homology)*	P35354	PTGS2	CHEMBL230		8 / 1	Enzyme
Dual specificity tyrosine-phosphorylation-regulated kinase 1A *(by homology)*	Q13627	DYRK1A	CHEMBL2292		11 / 1	Ser_Thr_Tyr Kinase
Tyrosyl-DNA phosphodiesterase 1	Q9NUW8	TDP1	CHEMBL1075138		53 / 5	Enzyme
Histone deacetylase 3 *(by homology)*	O15379	HDAC3	CHEMBL1829		4 / 6	Enzyme
Histone deacetylase 1	Q13547	HDAC1	CHEMBL325		4 / 6	Enzyme
Histone deacetylase 2 *(by homology)*	Q92769	HDAC2	CHEMBL1937		4 / 6	Enzyme
Epidermal growth factor receptor	P00533	EGFR	CHEMBL203		10 / 1	Tyr Kinase
Receptor tyrosine-protein kinase erbB-2	P04626	ERBB2	CHEMBL1824		10 / 1	Tyr Kinase

SwissTargetPrediction report:

Reference:
Gfeller D., Michielin O. & Zoete V. Shaping the interaction landscape of bioactive molecules, *Bioinformatics* (2013) 29:3073-3079.

Query Molecule



Frequency of Target Class

Target	Uniprot ID	Gene code	ChEMBL ID	Probability	# sim. cmpds (3D / 2D)	Target Class
Microtubule-associated protein tau	P10636	MAPT	CHEMBL1293224		658 / 49	Unclassified
Dual specificity tyrosine-phosphorylation-regulated kinase 1A *(by homology)*	Q13627	DYRK1A	CHEMBL2292		38 / 4	Ser_Thr_Tyr Kinase
Tyrosyl-DNA phosphodiesterase 1	Q9NUW8	TDP1	CHEMBL1075138		100 / 7	Enzyme
Potassium voltage-gated channel subfamily A member 3	P22001	KCNA3	CHEMBL4633		1 / 11	Ion channel
Potassium voltage-gated channel subfamily A member 2 *(by homology)*	P16389	KCNA2	CHEMBL2086		1 / 11	Ion channel
Potassium voltage-gated channel subfamily A member 6 *(by homology)*	P17658	KCNA6	CHEMBL5279		1 / 11	Ion channel
Potassium voltage-gated channel subfamily A member 4 *(by homology)*	P22459	KCNA4	CHEMBL4205		1 / 11	Ion channel
Potassium voltage-gated channel subfamily A member 5 *(by homology)*	P22460	KCNA5	CHEMBL4306		1 / 11	Ion channel
Potassium voltage-gated channel subfamily A member 1 *(by homology)*	Q09470	KCNA1	CHEMBL2309		1 / 11	Ion channel
Potassium voltage-gated channel subfamily A member 10 *(by homology)*	Q16322	KCNA10			1 / 11	Ion channel
Potassium voltage-gated channel subfamily A member 7 *(by homology)*	Q96RP8	KCNA7	CHEMBL2773		1 / 11	Ion channel
Histone deacetylase 3 *(by homology)*	O15379	HDAC3	CHEMBL1829		33 / 5	Enzyme
Histone deacetylase 1	Q13547	HDAC1	CHEMBL325		33 / 5	Enzyme
Histone deacetylase 2 *(by homology)*	Q92769	HDAC2	CHEMBL1937		33 / 5	Enzyme
ATP-binding cassette sub-family G member 2	Q9UNQ0	ABCG2	CHEMBL5393		18 / 33	Unclassified

LISTS OF CHEMICALS USED:

TABLE NO: 1

S.No	Name of Chemicals	Grade	Manufacture/Suppliers
1.	Salicylaldehyde	Laboratory Reagent	CD Lab
2.	Chloroacetone	Laboratory Reagent	CDH Fine Chemicals
3.	Potassium carbonate	Laboratory Reagent	CDH. Lab
4.	Ethanol 95%	Laboratory Reagent	Changshu yangyuan chemicals
5.	Benzaldehyde	Laboratory Reagent	Sisco Research. Lab
6.	4-Chloro benzaldehyde	Laboratory Reagent	Sisco Research. Lab
7.	4-Nitro benzaldehyde	Laboratory Reagent	S.d fine-chem
8.	4-Hydroxy 3-methoxy benzaldehde	Laboratory Reagent	CDH. Lab
9.	Anisaldehyde	Laboratory Reagent	Omega chemicals pvt.ltd
10.	Sodium hydroxide 50%	Laboratory Reagent	Spectrum reagent and chemicals
11.	Dil.Hcl	Laboratory Reagent	S.D.Fine chem
12.	Dry acetone	HPLC Grade	Sigma Aldrich chemicals

LIST OF INSTRUMENTS USED:

TABLE NO: 2

S.NO	NAME OF THE INSTRUMENT	MODEL	MANUFACTURER/SUPPLIER
1	Fourier transform IR Spectrometer	IR-Affinity-1	Shimadzu
2.	KBr press	M-15	Technosearch
3.	Mass Spectrometer	JEOL GC-MATE-II HR	Thermo fisher
4.	NMR Spectrometer	Avance-III HD	Bruker
5.	Thermostatically controlled water bath	PIC 101	M.C.Dalal
6.	Electronic Balance	M-D4404420019	Shimadzu
7.	Magnetic stirrer		
8.	UV-Chamber	CE102A	Deep vision
9.	Incubater	7441 sleudoc	Rays
10.	Melting point apparatus	Ce100	Labtronics

4.3 MOLECULAR SYNTHESIS:

COMPOUND-5a

Step 1:

Synthesis of 1-(1-benzofuran-2-yl)ethanone:

A mixture of salicylaldehyde (1g, 4.97mmol) and potassium carbonate (0.69 g, 4.97mmol) in dry acetone (10 mL) was stirred at 25°C for 1 hour. Reaction mixture was cooled at 0–5°C, and then chloroacetone (4 mL, 4.97mmol) was added dropwise. Reaction mixture was stirred at room temperature for ten minutes and then refluxed. Progress of the reaction was monitored by TLC. Upon completion, the reaction mixture was poured on crashed ice. The precipitated solid was filtered, washed with water, and dried. The product was crystallized from ethanol[23]

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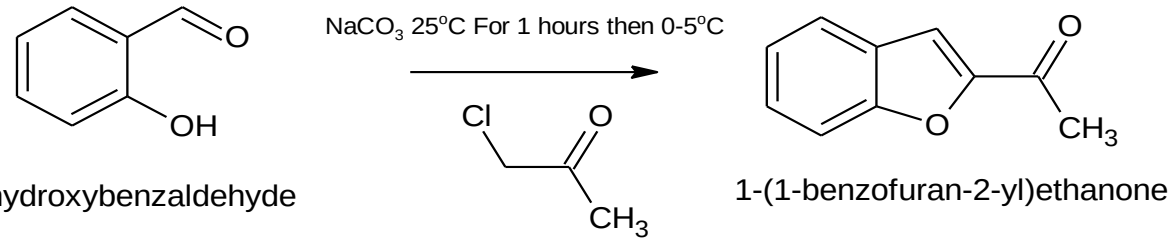
Step 2:

Procedure for Synthesis of Chalcone derivative Hybrid (5a) Claisen–Schmidt condensation:

A solution of 1-(1-benzofuran-2-yl)ethanone (1 g, 4.18mmol) and Benzaldehyde (4.18mmol) in MeOH (10 mL) was cooled at 0–5°C and then 6mL of aqueous NaOH (1 mol/L) was added to this solution and stirred at room temperature for 3 hours. The reaction mixture was poured on crushed ice. The precipitated solid was filtered after neutralization with diluted HCl and was washed several times with water and then dried. The product was recrystallized from ethanol.[23]

Step-1



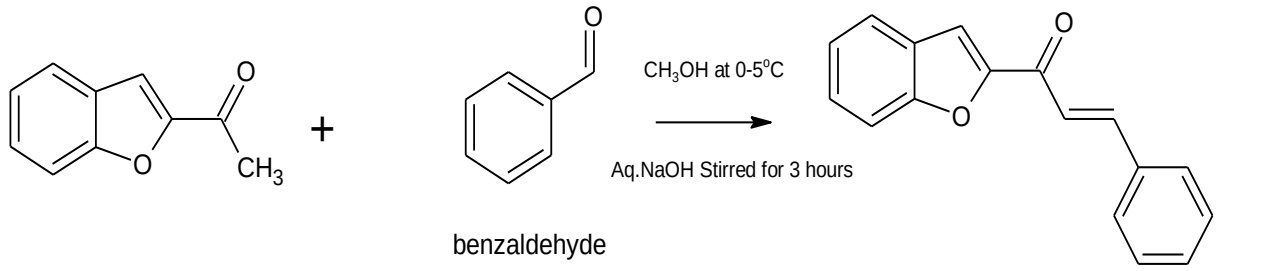
2-hydroxybenzaldehyde

1-chloropropan-2-one

1-(1-benzofuran-2-yl)ethanone

(1) (2) (3)

Step-2:



1-(1-benzofuran-2-yl)ethanone

benzaldehyde

(2*E*)-1-(1-benzofuran-2-yl)-3-phenylprop-2-en-1-one

(3) (4) (5a)

COMPOUND-5b

Step 1:

Synthesis of 1-(1-benzofuran-2-yl)ethanone:

A mixture of salicylaldehyde (1g, 4.97mmol) and potassium carbonate (0.69 g, 4.97mmol) in dry acetone (10 mL) was stirred at 25°C for 1 hour. Reaction mixture was cooled at 0–5°C, and then chloroacetone (4 mL, 4.97mmol) was added dropwise. Reaction mixture was stirred at room temperature for ten minutes and then refluxed. Progress of the reaction was monitored by TLC. Upon completion, the reaction mixture was poured on crashed ice. The precipitated solid was filtered, washed with water, and dried. The product was crystallized from ethanol[23]

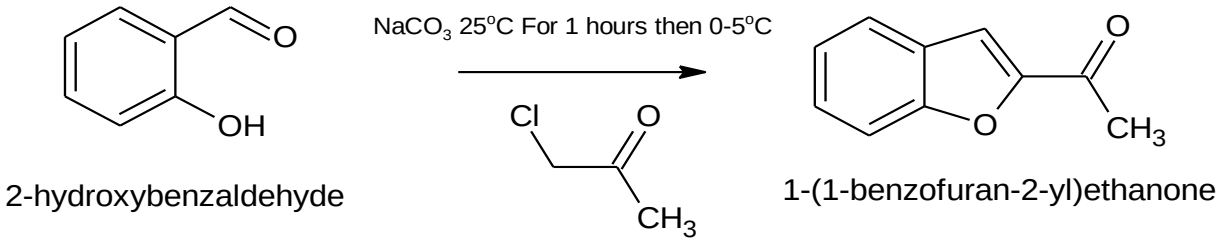
.

Step 2:

Procedure for Synthesis of Chalcone derivative Hybrid (5b) Claisen–Schmidt condensation:

A solution of 1-(1-benzofuran-2-yl)ethanone (1 g, 4.18mmol) and 4-Chloro Benzaldehyde(4.18mmol) in MeOH (10 mL) was cooled at 0–5°C and then 6mL of aqueous NaOH (1 mol/L) was added to this solution and stirred at room temperature for 3 hours. The reaction mixture was poured on crushed ice. The precipitated solid was filtered after neutralization with diluted HCl and was washed several times with water and then dried. The product was recrystallized from ethanol.[23]

Step-1



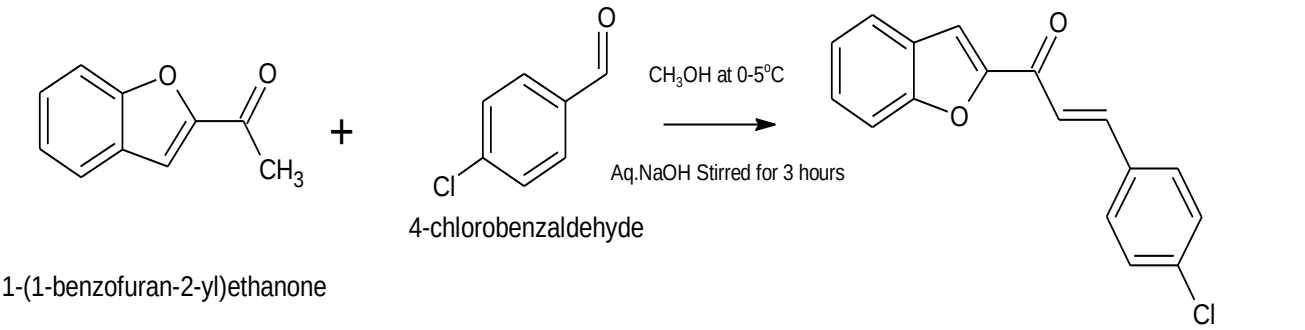
2-hydroxybenzaldehyde

1-chloropropan-2-one

1-(1-benzofuran-2-yl)ethanone

(1) (2) (3)

Step-2:



1-(1-benzofuran-2-yl)ethanone

4-chlorobenzaldehyde

(2*E*)-1-(1-benzofuran-2-yl)-3-(4-chlorophenyl)prop-2-en-1-one

(3) (4) (5b)

COMPOUND-5c

Step 1:

Synthesis of 1-(1-benzofuran-2-yl)ethanone:

A mixture of salicylaldehyde (1g, 4.97mmol) and potassium carbonate (0.69 g, 4.97mmol) in dry acetone (10 mL) was stirred at 25^{o}C for 1 hour. Reaction mixture was cooled at 0–5°C, and then chloroacetone (4 mL, 4.97mmol) was added dropwise. Reaction mixture was stirred at room temperature for ten minutes and then refluxed. Progress of the reaction was monitored by TLC. Upon completion, the reaction mixture was poured on crashed ice. The precipitated solid was filtered, washed with water, and dried. The product was crystallized from ethanol[23]

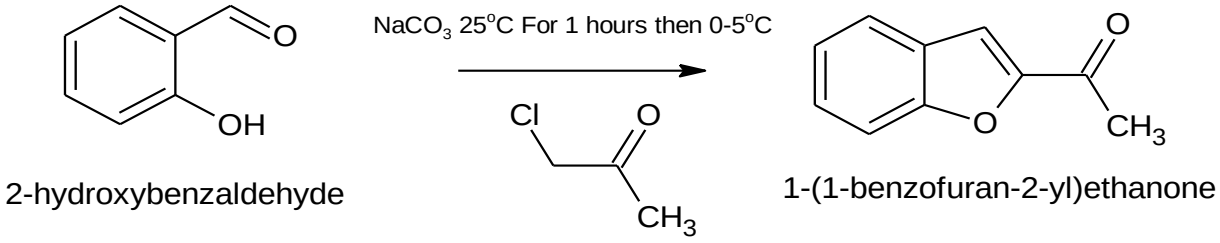
.

Step 2:

Procedure for Synthesis of Chalcone derivative Hybrid (5c) Claisen–Schmidt condensation:

A solution of 1-(1-benzofuran-2-yl)ethanone (1 g, 4.18mmol) and 4-methoxy Benzaldehyde(4.18mmol) in MeOH (10 mL) was cooled at 0–5^{o}C and then 6mL of aqueous NaOH (1 mol/L) was added to this solution and stirred at room temperature for 3 hours. The reaction mixture was poured on crushed ice. The precipitated solid was filtered after neutralization with diluted HCl and was washed several times with water and then dried. The product was recrystallized from ethanol.[23]

Step-1



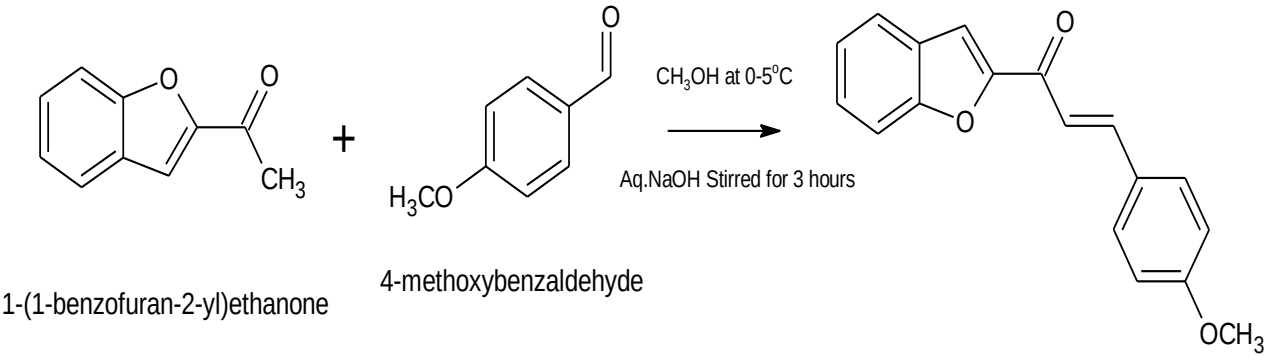
2-hydroxybenzaldehyde

1-chloropropan-2-one

1-(1-benzofuran-2-yl)ethanone

(1) (2) (3)

Step-2:



1-(1-benzofuran-2-yl)ethanone

4-methoxybenzaldehyde

(3) (4) (5c)

COMPOUND-5d

Step 1:

Synthesis of 1-(1-benzofuran-2-yl)ethanone:

A mixture of salicylaldehyde (1g, 4.97mmol) and potassium carbonate (0.69 g, 4.97mmol) in dry acetone (10 mL) was stirred at 25°C for 1 hour. Reaction mixture was cooled at 0–5°C, and then chloroacetone (4 mL, 4.97mmol) was added dropwise. Reaction mixture was stirred at room temperature for ten minutes and then refluxed. Progress of the reaction was monitored by TLC. Upon completion, the reaction mixture was poured on crashed ice. The precipitated solid was filtered, washed with water, and dried. The product was crystallized from ethanol[23]

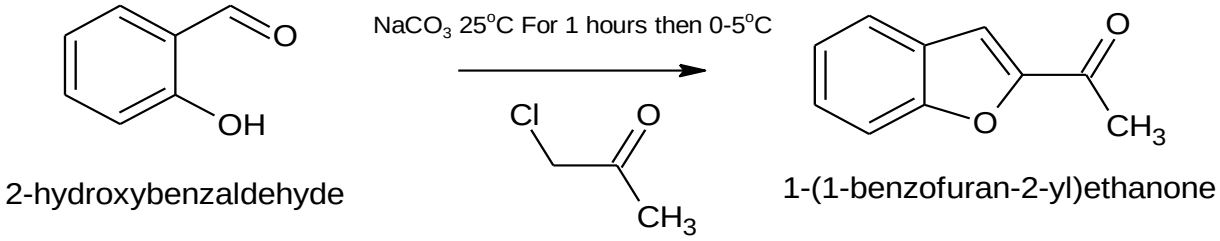
.

Step 2:

Procedure for Synthesis of Chalcone derivative Hybrid (5d) Claisen–Schmidt condensation:

A solution of 1-(1-benzofuran-2-yl)ethanone (1 g, 4.18mmol) and 4-nitro benzaldehyde (4.18mmol) in MeOH (10 mL) was cooled at 0–5°C and then 6mL of aqueous NaOH (1 mol/L) was added to this solution and stirred at room temperature for 3 hours. The reaction mixture was poured on crushed ice. The precipitated solid was filtered after neutralization with diluted HCl and was washed several times with water and then dried. The product was recrystallized from ethanol.[23]

Step-1



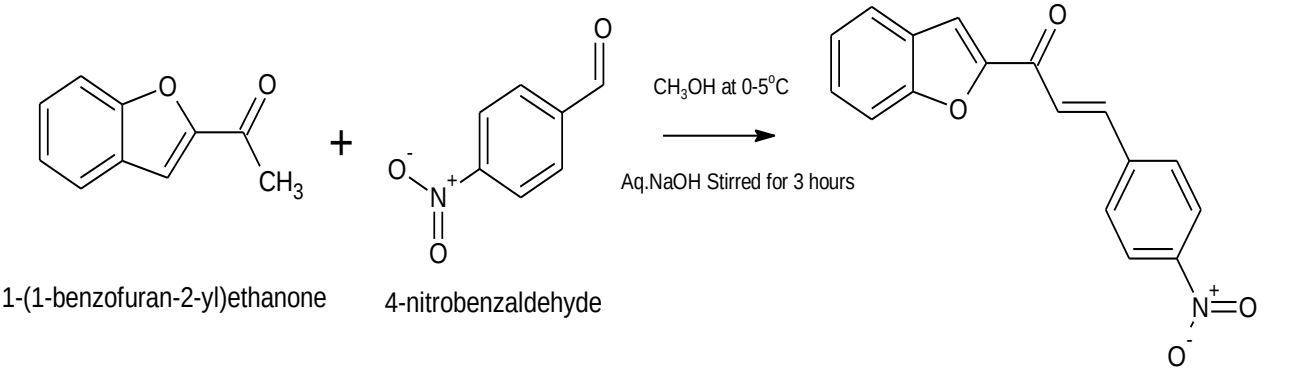
2-hydroxybenzaldehyde

1-chloropropan-2-one

1-(1-benzofuran-2-yl)ethanone

(1) (2) (3)

Step-2:



1-(1-benzofuran-2-yl)ethanone

4-nitrobenzaldehyde

(2*E*)-1-(1-benzofuran-2-yl)-3-(4-nitrophenyl)prop-2-en-1-one

(3) (4) (5d)

COMPOUND-5e

Step 1:

Synthesis of 1-(1-benzofuran-2-yl)ethanone:

A mixture of salicylaldehyde (1g, 4.97mmol) and potassium carbonate (0.69 g, 4.97mmol) in dry acetone (10 mL) was stirred at 25°C for 1 hour. Reaction mixture was cooled at 0–5°C, and then chloroacetone (4 mL, 4.97mmol) was added dropwise. Reaction mixture was stirred at room temperature for ten minutes and then refluxed. Progress of the reaction was monitored by TLC. Upon completion, the reaction mixture was poured on crashed ice. The precipitated solid was filtered, washed with water, and dried. The product was crystallized from ethanol[23]

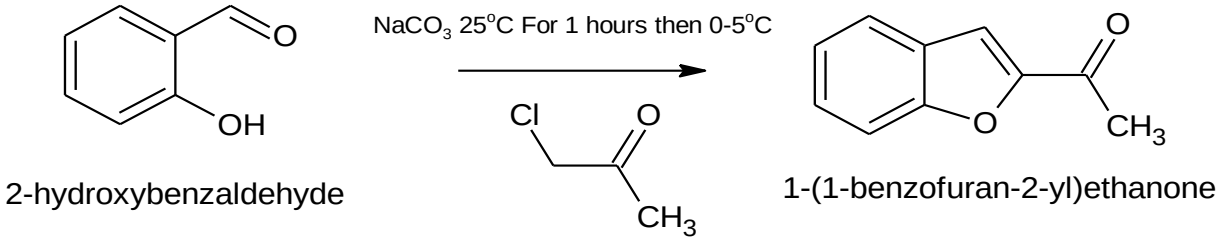
.

Step 2:

Procedure for Synthesis of Chalcone derivative Hybrid (5e) Claisen–Schmidt condensation:

A solution of 1-(1-benzofuran-2-yl)ethanone (1 g, 4.18mmol) and 4-hydroxy 3-methoxy benzaldehyde (4.18mmol) in MeOH (10 mL) was cooled at 0–5°C and then 6mL of aqueous NaOH (1 mol/L) was added to this solution and stirred at room temperature for 3 hours. The reaction mixture was poured on crushed ice. The precipitated solid was filtered after neutralization with diluted HCl and was washed several times with water and then dried. The product was recrystallized from ethanol.[23]

Step-1



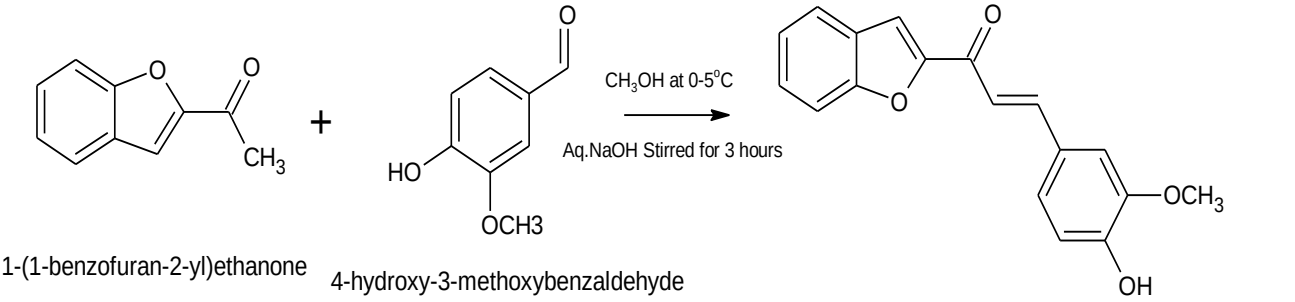
2-hydroxybenzaldehyde

1-chloropropan-2-one

1-(1-benzofuran-2-yl)ethanone

(1) (2) (3)

Step-2:



1-(1-benzofuran-2-yl)ethanone

4-hydroxy-3-methoxybenzaldehyde

(2*E*)-1-(1-benzofuran-2-yl)-3-(4-hydroxy-3-methoxyphenyl)prop-2-en-1-one

(3) (4) (5e)

4.4 ANALYTICAL TECHNIQUES [39,46]

Physical data

The physical data such as solubility and melting point was determined. The compound was soluble in DMSO, Chloroform and insoluble in water. The melting point of synthesized compounds were determined by the capillary tube method.

Thin Layer chromatography(TLC)

TLC analysis was carried out on commercially available silica gel plates of 0.5mm of thickness, as stationary phase. Petroleum ether:Ethyl acetate (1:1) mobile phase.

Instrumentation

The analytical instruments such as IR spectra, 1HNMR, MASS spectra were used for the characterization of synthesized compounds.

Infrared Spectra

The IR spectra of synthesized compounds K1-K10 were recorded by FTIR (Shimadzu IR affinityI) in the range of 4000-450cm-1.

Nuclear Magnetic Resonance

The bruker Avance II 400 NMR spectrometer is used to measure the chemical shift and reported in parts per million (δ ppm).

Mass spectroscopy

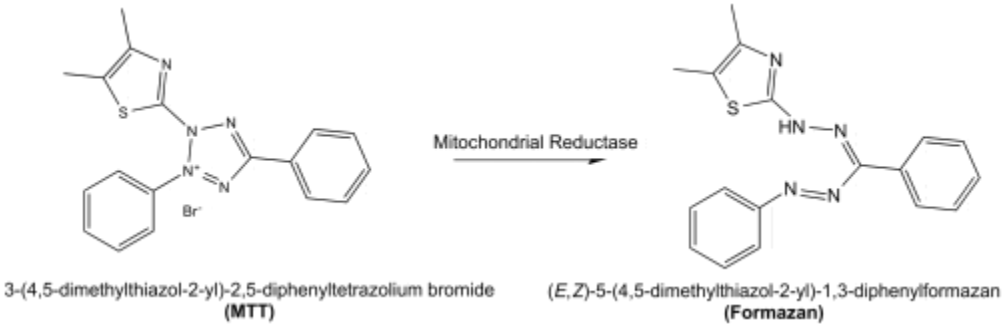
The molecular ion peaks are recorded by Mass spectroscopy and reported in m/z ratio.

4.5-*INVITRO* ANTICANCER ACTIVITY

MTT ASSAY:

PRINCIPLE:

MTT, a yellow tetrazole, is reduced to purple formazan in living cells. A solubilization solution (usually either dimethyl sulfoxide, an acidified ethanol solution, or a solution of the detergent sodium dodecyl sulfate in diluted hydrochloric acid) is added to dissolve the insoluble purple formazan product into a colored solution. The absorbance of this colored solution can be quantified by measuring at a certain wavelength (usually between 500 and 600 nm) by a spectrophotometer. The degree of light absorption depends on the solvent.



3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide **(MTT)**

(*E*,*Z*)-5-(4,5-dimethylthiazol-2-yl)-1,3-diphenylformazan **(Formazan)**

Tetrazolium dye reduction is dependent on NAD(P)H-dependent oxido reductase enzymes largely in the cytosolic compartment of the cell. Therefore, reduction of MTT and other tetrazolium dyes depends on the cellular metabolic activity due to NAD(P)H flux. Cells with a low metabolism such as thymocytes and splenocytes reduce very little MTT. In contrast, rapidly dividing cells exhibit high rates of MTT reduction.

PROCEDURE:

The MTT assay is a standard colorimetric non-radioactive assay for measuring viable cell and cytotoxicity through increased metabolism of the tetrazolium salt. Cancer cells (1 x 10^{-5} cells/ml) were seeded into 96 well plates and incubated for 24 hours incubation. Then the cells were treated with different concentration of drug formulation (10-200µg/ml). then, the cells were incubated in the presence of 5% CO2 at 37^0C for 24 hours. After incubation, MTT (0.5 mg/mL) was added to the incubated cells. Then cells were incubatedfor another 4 hours. Then 100µL of DMSO were added into each well and mixed well. Absorbance was measured in a multimode reader at 570 nm.(Jaya seema DM et al-2018)

4.6 SINSILICO DOCKING ANALYSIS

Geometry optimized molecular structures for all synthesized derivatives were obtained using AUTODOCK 4.0 (A Generic Evolutionary Method for molecular docking) automated docking program. AUTODOCK 4.0 is a software used for integrated structure based virtual screening, molecular docking, post screening analysis and visualization step. The 3-dimension (3D) coordinates of four cancer target proteins were selected and obtained from protein data bank (PDB). The PDB id of 1MOX (lung cancer) and 2DSQ (breast epithelian cancer) were selected for INSILICO study. The 3D structure coordinates of each therapeutic target protein and ligand molecules were implemented through the AUTODOCK 4.0 graphical environment interface. Before doing docking analysis, the output path was set. AUTODOCK 4.0 default parameters included the population size (n=200), generation (g=70) and number of solutions (s=10) to compute the probable ligand binding mechanism for each target protein. Then the docking run was started using AUTODOCK 4.0 scoring function. After docking, the individual binding pose of each ligand was observed and their binding affinity with the target proteins was analyzed. Visual examination of predicted binding geometries (docking poses) thereby contributes crucially to the further development of a lead compound. In the post docking screening the best binding pose and total energy of each ligand was analysed. The details of best binding pose and total energy values were saved in output folder. Protein-ligand binding site was analysed and visualized by using Discover Studio.

5.1 PHYCIAL CHARACTERISTICS OF SYNTHESISED COMPOUND:

TABLE :3

COMPOUND	MOLECULAR FORMULA	NATURE	SOLUBLE IN	% YIELD
5a	$C_{17}H_{12}O_2$	Brown solid	Chloroform, DMSO	74
5b	$C_{17}H_{11}ClO_2$	Brown solid	Chloroform, DMSO	76
5c	$C_{18}H_{14}O_3$	Brown solid	Chloroform, DMSO	72
5d	$C_{17}H_{11}NO_4$	Brown solid	Chloroform, DMSO	69
5e	$C_{18}H_{14}O_4$	Brown solid	Chloroform, DMSO	65

TABLE:4

COMPOUND	MELTING POINT (^{0}C)	Rf VALUE
5a	146	0.45
5b	149	0.48
5c	154	0.43
5d	138	0.52
5e	144	0.44

ELEMENTAL COMPOSITION OF COMPOUNDS

TABLE:5

COMPOUND	Elemental composition in percentage (%)				
	C	H	O	N	Cl
5a	82.24	4.87	12.89	-	-
5b	72.22	3.92	12.54	-	11.32
5c	77.68	5.07	17.25	-	-
5d	69.62	3.78	21.82	4.78	-
5e	73.46	4.79	21.75	-	-

5.2 RESULTS OF MOLECULAR DESIGN

Lipinski properties of synthesised compounds

TABLE:6

COMPOUND	MOLECULAR WEIGHT	LOG P	H-BOND DONAR	H-BOND ACCEPTOR	MOLAR REFRACTIVITY
5a	250.29	3.62	0	2	77.25
5b	282.72	3.62	0	2	82.14
5c	278.30	3.95	0	3	83.92
5d	293.27	3.58	0	4	83.79
5e	294.30	3.58	0	4	85.81

5.3 SPECTRAL ANALYSIS:

IR Data of Synthesised Compounds

TABLE: 7

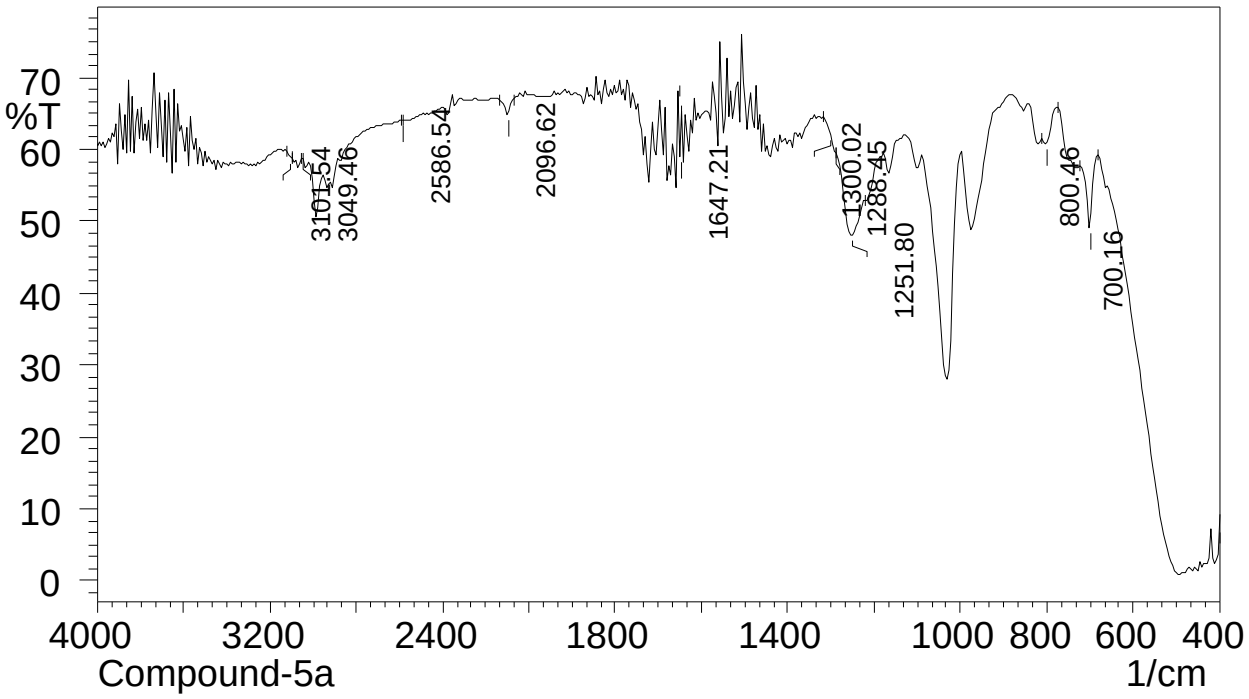
Compound 5a-5e

Compound	Vibration mode	Observed frequency
5a	Aromatic C-H C=O C=C	3049 1566 1647
5b	Aromatic C-H C=O C=C C-Cl	3057 1658 1652 835
5c	Aromatic C-H C=O C=C	3047 1577 1650
5d	Aromatic C-H C=O C=C C-NO	3062 1566 1658 617
5e	Aromatic C-H Aliphatic C-H C=O C-OH	2972 1658 1604 3400

KALASALINGAM ACADEMY OF RESEARCH AND EDUCATION (DEEMED TO BE UNIVERSITY)

Sir C.V. RAMAN KRISHNAN

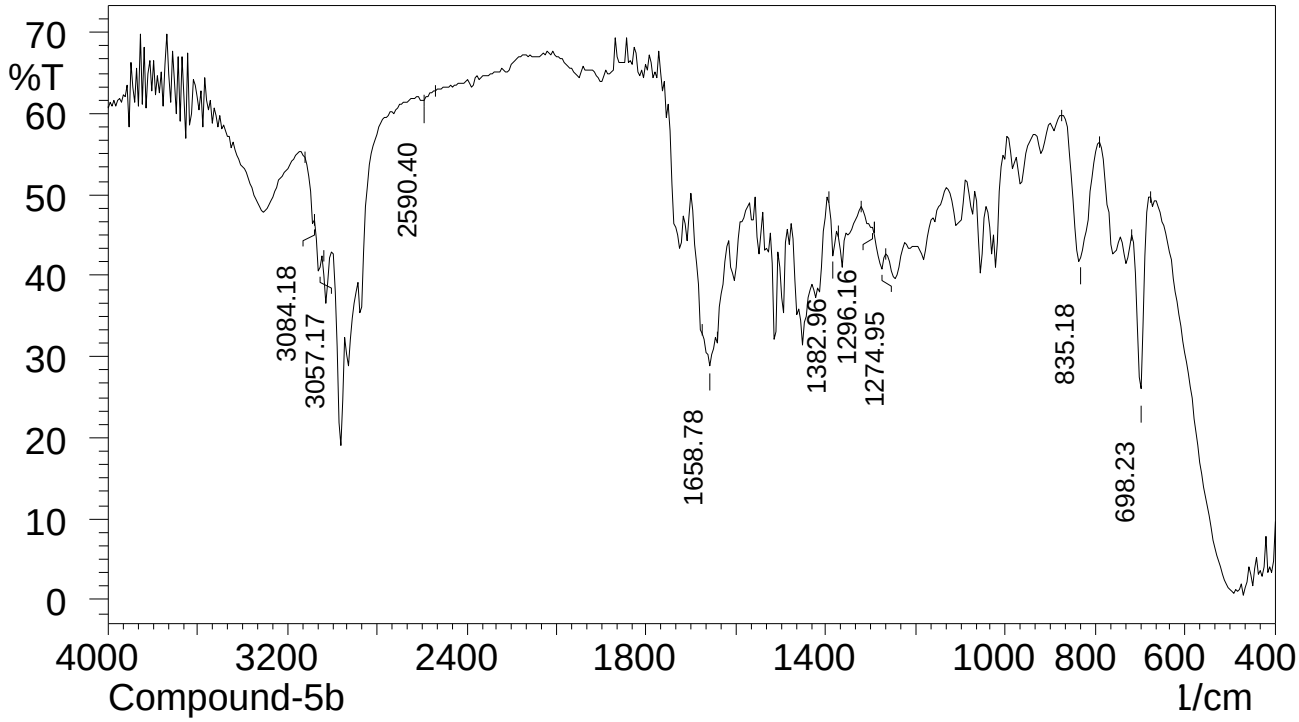
COMPOUND-5a



KALASALINGAM ACADEMY OF RESEARCH AND EDUCATION (DEEMED TO BE UNIVERSITY)

Sir C.V. RAMAN KRISHNAN

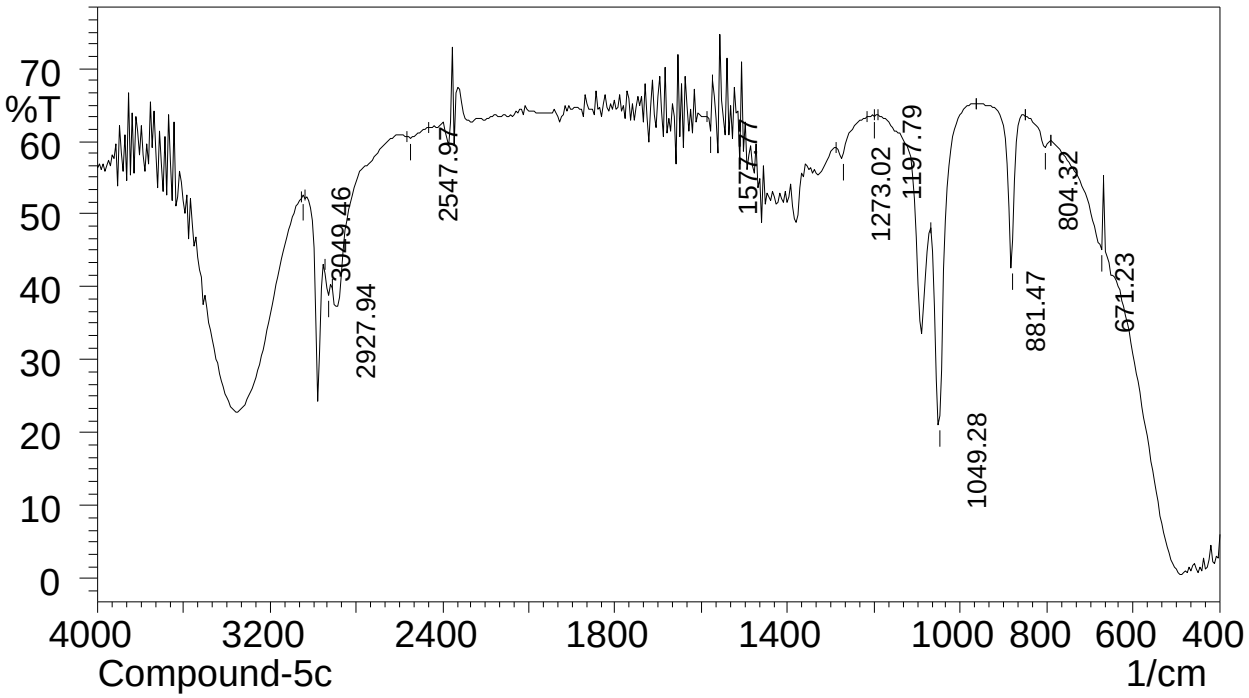
COMPOUND-5b



KALASALINGAM ACADEMY OF RESEARCH AND EDUCATION (DEEMED TO BE UNIVERSITY)

Sir C.V. RAMAN KRISHNAN

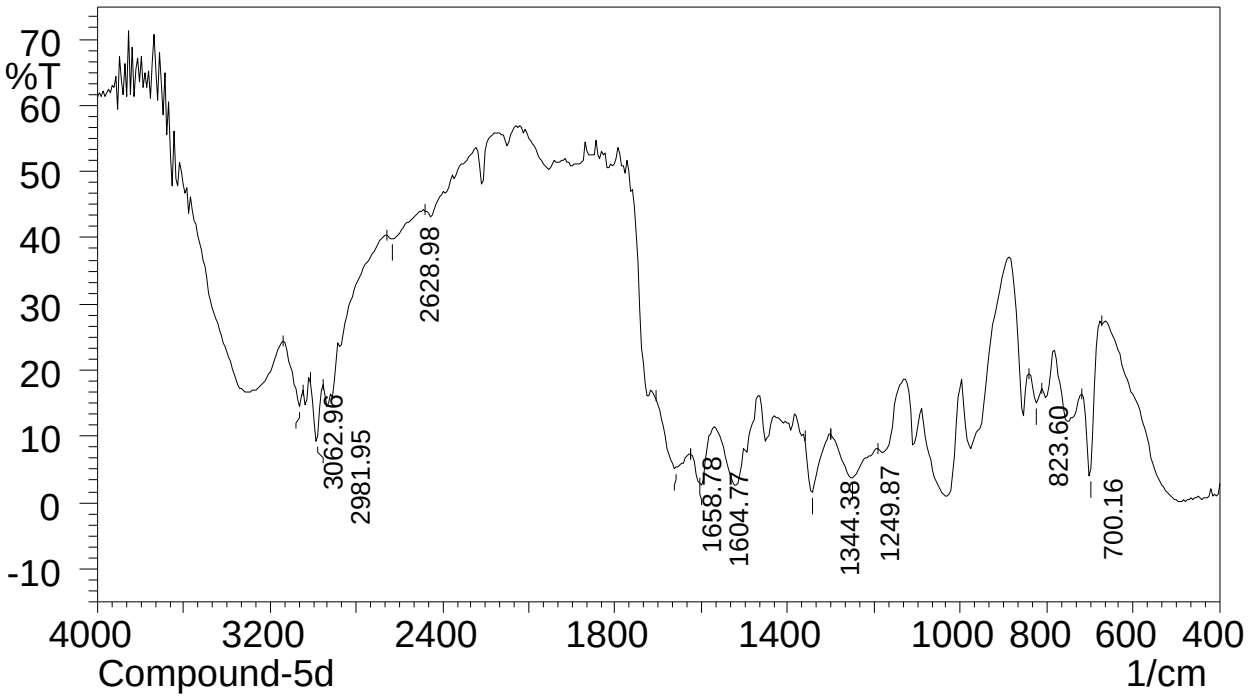
COMPOUND-5c



KALASALINGAM ACADEMY OF RESEARCH AND EDUCATION (DEEMED TO BE UNIVERSITY)

Sir C.V. RAMAN KRISHNAN

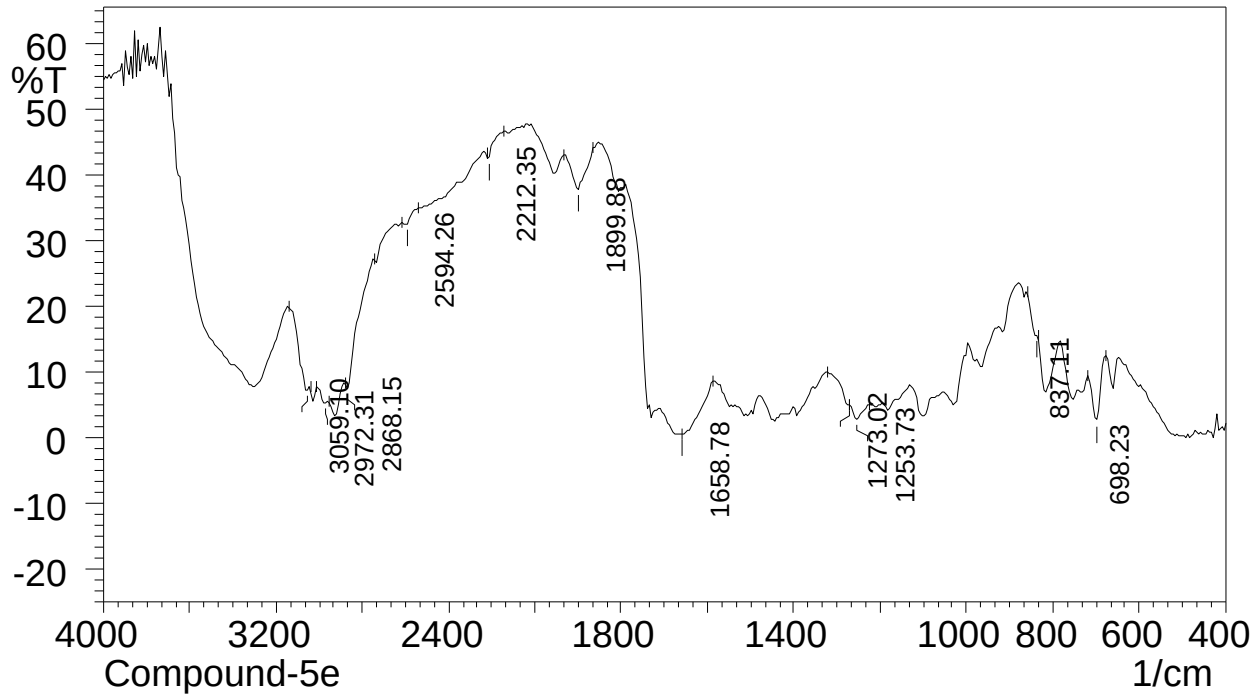
COMPOUND-5d



KALASALINGAM ACADEMY OF RESEARCH AND EDUCATION (DEEMED TO BE UNIVERSITY)

Sir C.V. RAMAN KRISHNAN

COMPOUND-5e

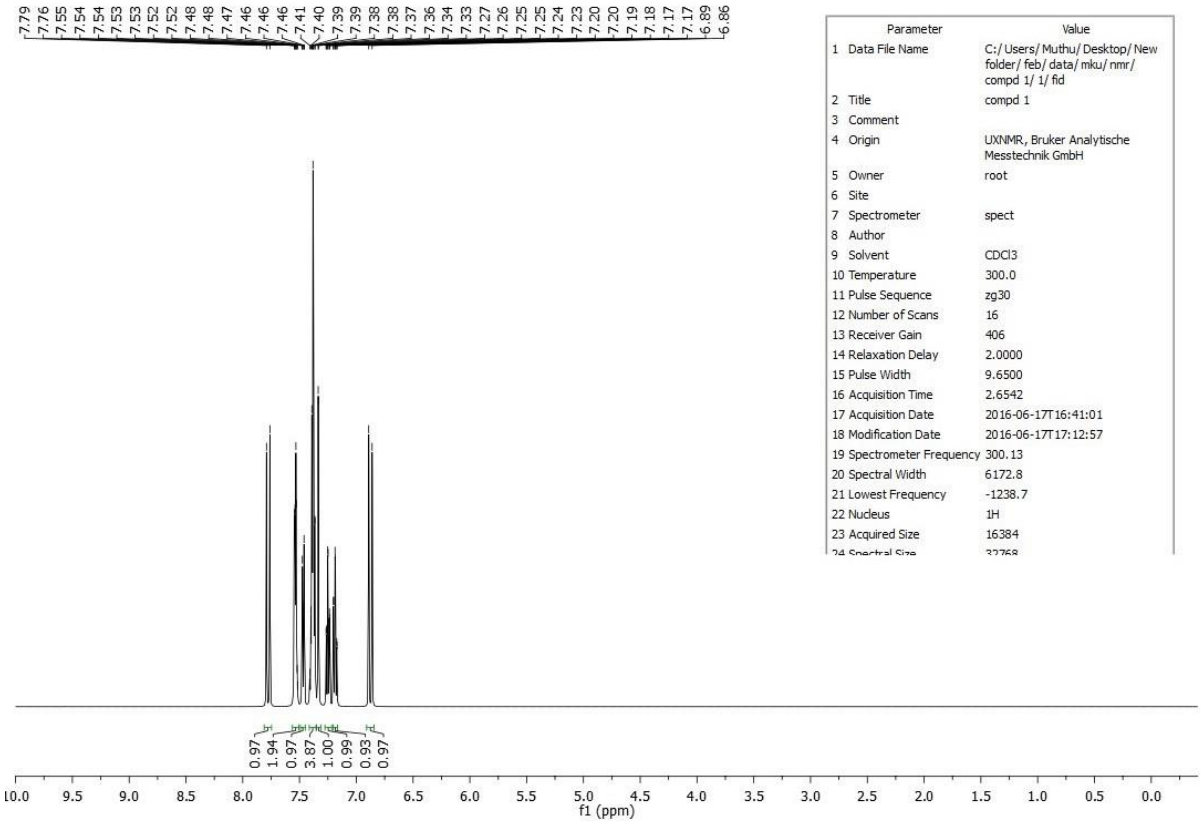


1H NMR of Synthesised Compounds

TABLE:8 (Compound 5a-5e)

Compound	Chemical shift value	Proton nature
5a	7.79-7.76 7.54 7.48 7.38 7.27 7.25 7.24-7.17 6-8	1H 2H 1H 4H 1H 1H 1H 1H
5b	8.19-8.16 7.67 7.52-7.5 7.48-7.41 7.24 7.16 6.84-6.81	1H 1H 3H 3H 1H 1H 1H
5c	7.75-7.72 7.53 7.51-7.48 7.46 7.38 7.27 7.18 7.02-7.0 6.81-6.78	1H 2H 1H 1H 1H 1H 1H 2H 1H
5d	8.31-8.28 7.84-7.82 7.76 7.50 7.48 7.25 7.18 7.11-7.07	3H 2H 1H 1H 1H 1H 1H 1H
5e	8.28-8.25 7.61 7.41 7.35 7.33 6.81 6.79 7.78 6.63	1H 1H 1H 1H 1H 1H 1H 2H 1H

COMPOUND-5a



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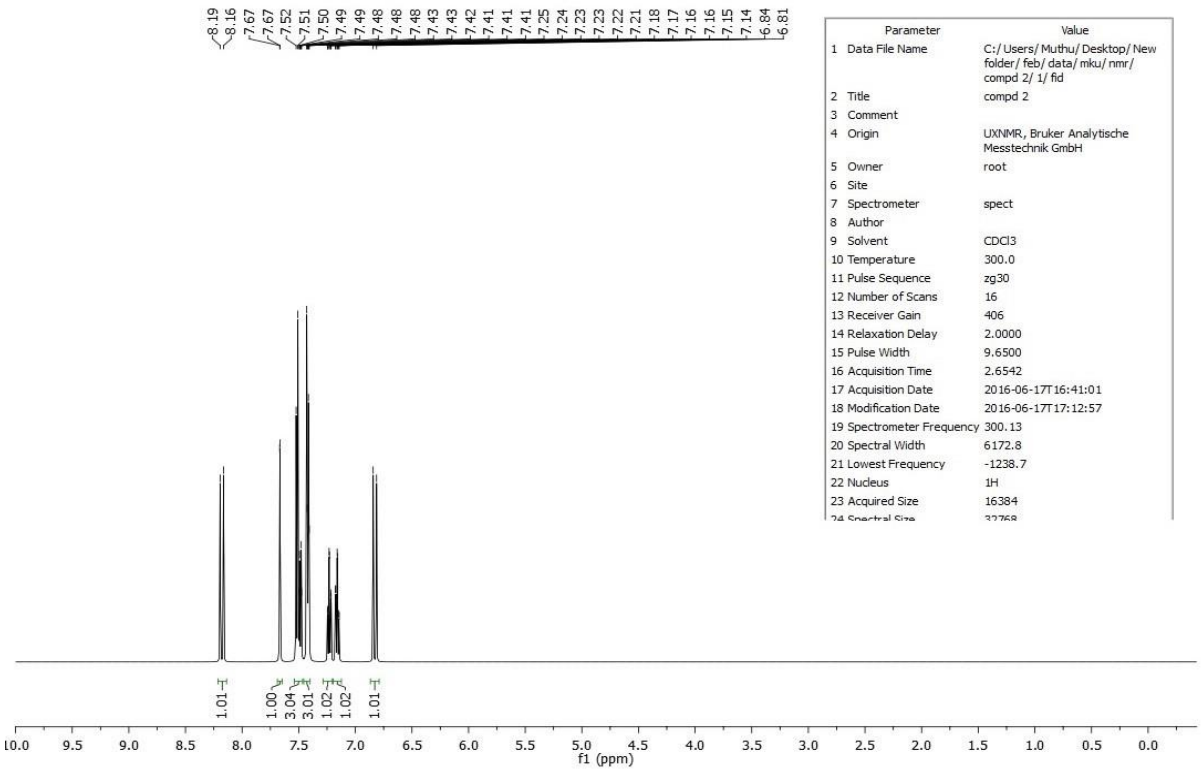
7 Spectrometer	spect
8 Author	
9 Solvent	CDCl3
10 Temperature	300.0
11 Pulse Sequence	zg30
12 Number of Scans	16
13 Receiver Gain	406
14 Relaxation Delay	2.0000
15 Pulse Width	9.6500
16 Acquisition Time	2.6542
17 Acquisition Date	2016-06-17T16:41:01
18 Modification Date	2016-06-17T17:12:57
19 Spectrometer Frequency	300.13
20 Spectral Width	6172.8
21 Lowest Frequency	-1238.7
22 Nucleus	1H
23 Acquired Size	16384
24 Spectral Size	32768

Compound-5b



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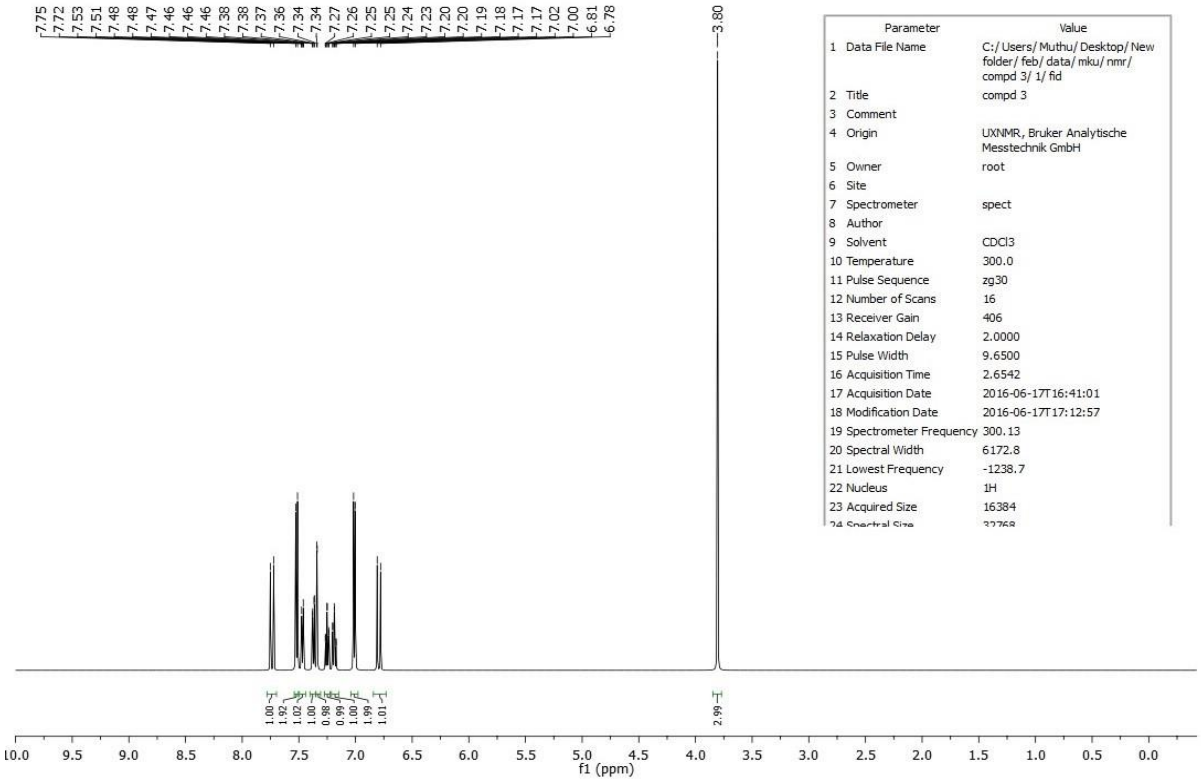
11 Pulse Sequence	zg30
12 Number of Scans	16
13 Receiver Gain	406
14 Relaxation Delay	2.0000
15 Pulse Width	9.6500
16 Acquisition Time	2.6542
17 Acquisition Date	2016-06-17T16:41:01
18 Modification Date	2016-06-17T17:12:57
19 Spectrometer Frequency	300.13
20 Spectral Width	6172.8
21 Lowest Frequency	-1238.7
22 Nucleus	1H
23 Acquired Size	16384
24 Spectral Size	32768

Compound-5c



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8 Author	
9 Solvent	CDCl3
10 Temperature	300.0
11 Pulse Sequence	zg30
12 Number of Scans	16
13 Receiver Gain	406
14 Relaxation Delay	2.0000
15 Pulse Width	9.6500
16 Acquisition Time	2.6542
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19 Spectrometer Frequency	300.13
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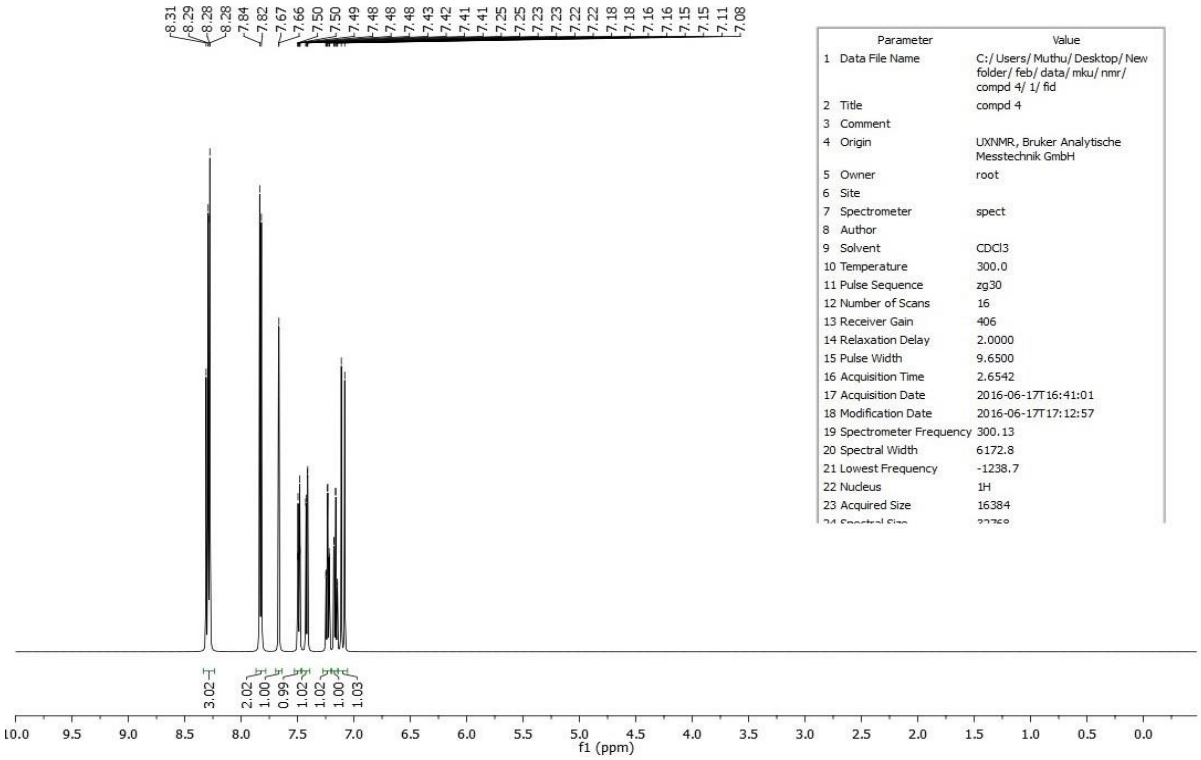
21 Lowest Frequency	-1238.7
22 Nucleus	1H
23 Acquired Size	16384
24 Spectral Size	32768

Compound-5d



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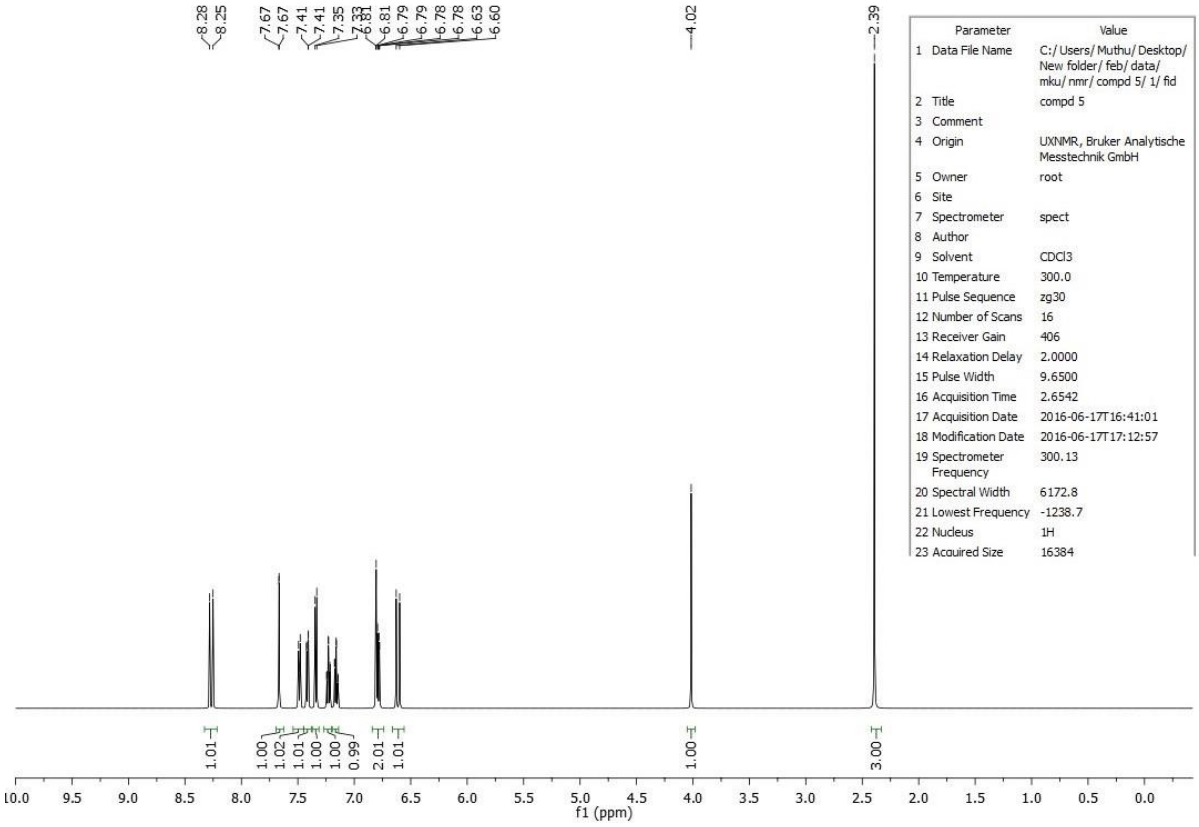
8 Author	
9 Solvent	CDCl3
10 Temperature	300.0
11 Pulse Sequence	zg30
12 Number of Scans	16
13 Receiver Gain	406
14 Relaxation Delay	2.0000
15 Pulse Width	9.6500
16 Acquisition Time	2.6542
17 Acquisition Date	2016-06-17T16:41:01
18 Modification Date	2016-06-17T17:12:57
19 Spectrometer Frequency	300.13
20 Spectral Width	6172.8
21 Lowest Frequency	-1238.7
22 Nucleus	1H
23 Acquired Size	16384
24 Spectral Size	32768

Compound-5e

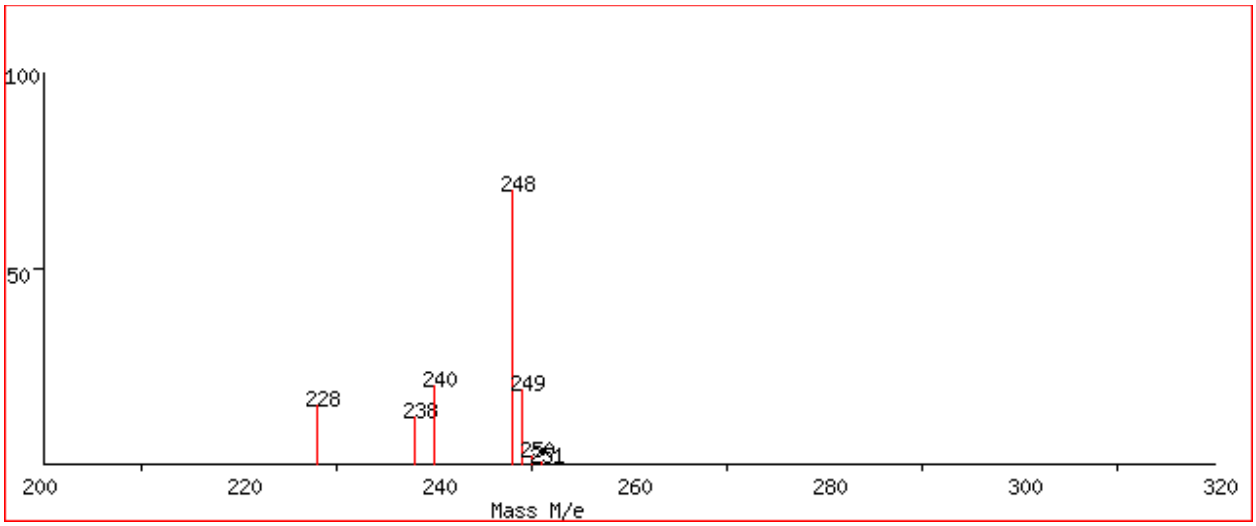

8.28
8.25
7.67
7.67
7.41
7.41
7.35
7.33
6.81
6.81
6.79
6.79
6.78
6.78
6.63
6.60
4.02
2.39
1.01
1.00
1.02
1.01
1.00
1.00
0.99
2.01
1.01
1.00
3.00
f1 (ppm)
Parameter Value
1 Data File Name C:/ Users/ Muthu/ Desktop/ New folder/ feb/ data/ mku/ nmr/ compd 5/ 1/ fid
2 Title compd 5
3 Comment
4 Origin UXNMR, Bruker Analytische Messtechnik GmbH
5 Owner root
6 Site
7 Spectrometer spect
8 Author
9 Solvent CDCl3
10 Temperature 300.0
11 Pulse Sequence zg30
12 Number of Scans 16
13 Receiver Gain 406
14 Relaxation Delay 2.0000
15 Pulse Width 9.6500
16 Acquisition Time 2.6542
17 Acquisition Date 2016-06-17T16:41:01
18 Modification Date 2016-06-17T17:12:57
19 Spectrometer Frequency 300.13
20 Spectral Width 6172.8
21 Lowest Frequency -1238.7
22 Nucleus 1H
23 Acquired Size 16384

Mass spectra of synthesised compounds:

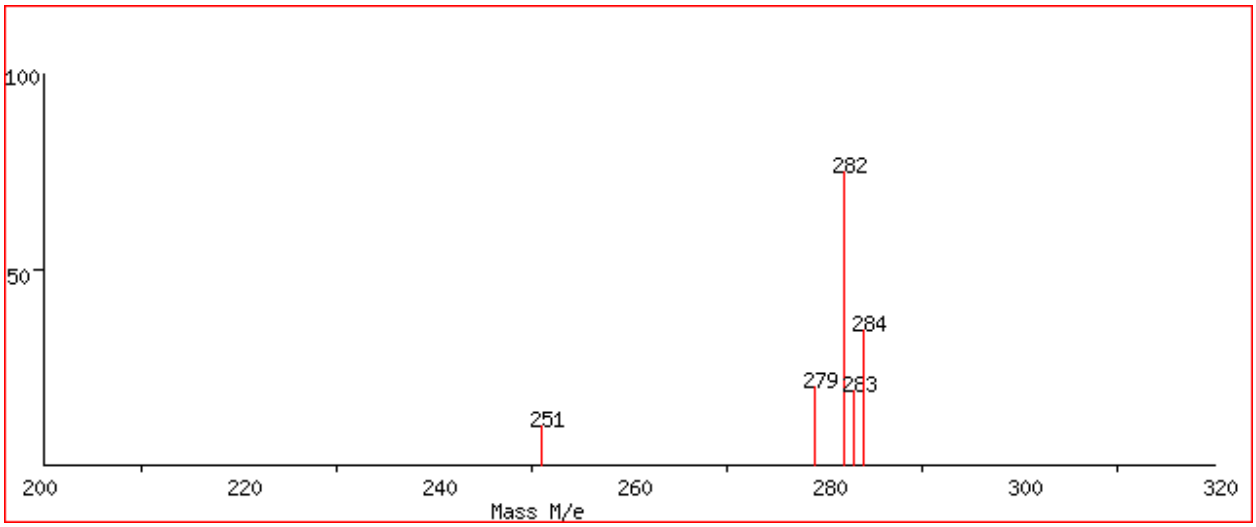
Table:9

COMPOUND	MOLECULAR ION PEAK
5a	248
5b	282
5c	278
5d	293
5e	294

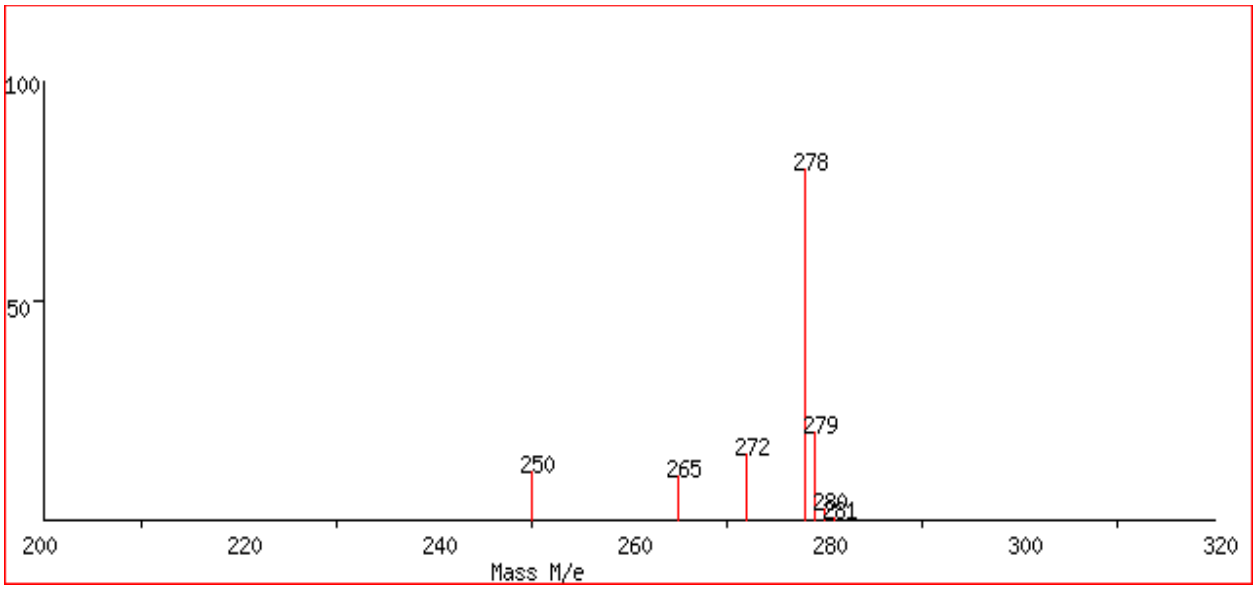
COMPOUND 5a



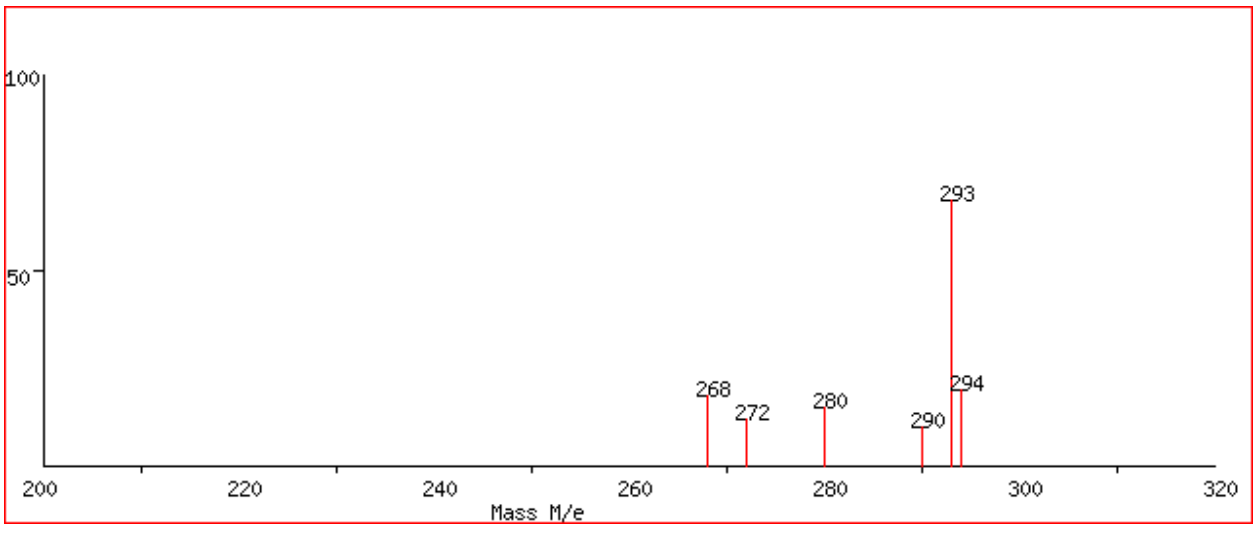
COMPOUND 5b



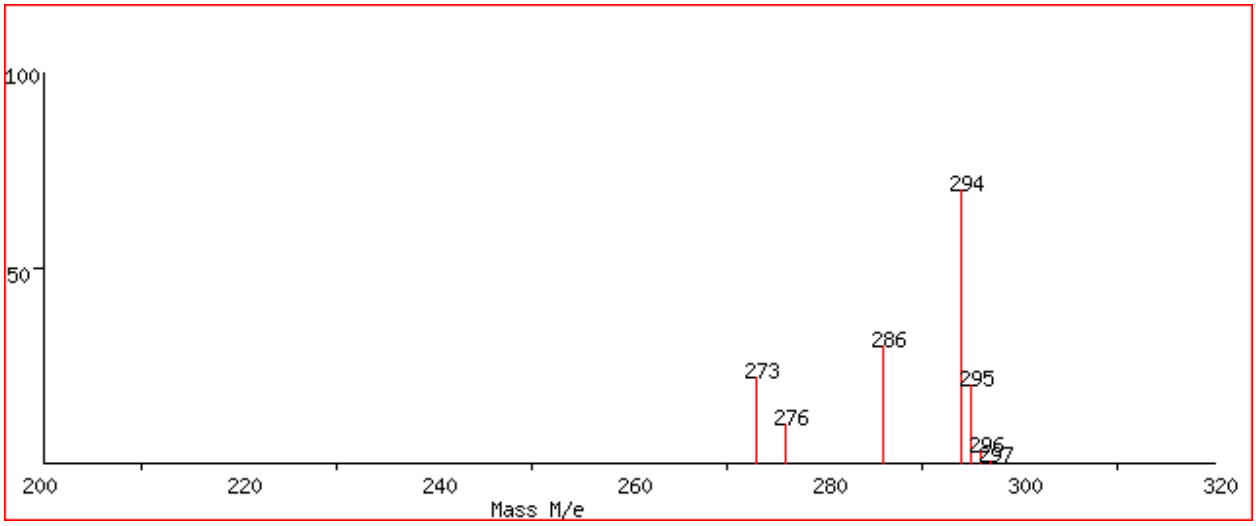
COMPOUND 5c



COMPOUND 5d



COMPOUND 5e

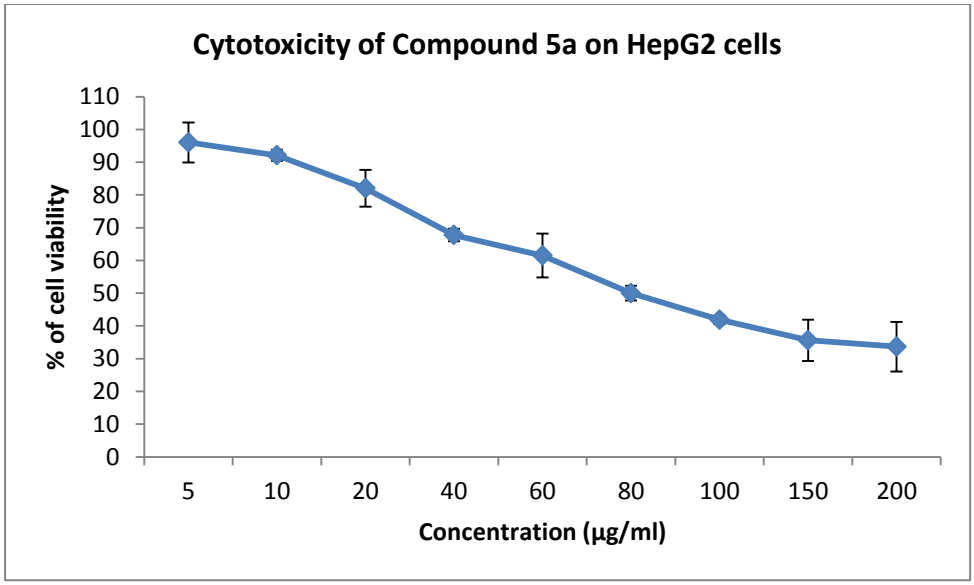

100
50
294
286
273
295
276
296
297
200
220
240
260
280
300
320
Mass M/e

5.4 RESULTS OF INVITRO-ANTICANCER ACTIVITY

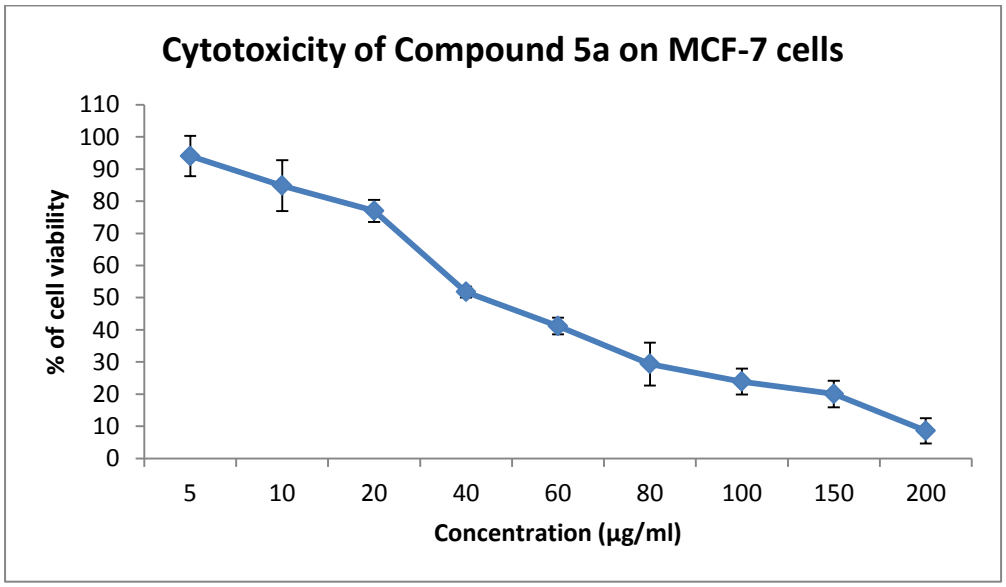
Invitro anti-cancer activity of Liver and Breast cancer cell lines by using MTT Assay method:

COMPOUND- 5a

HepG2 cell

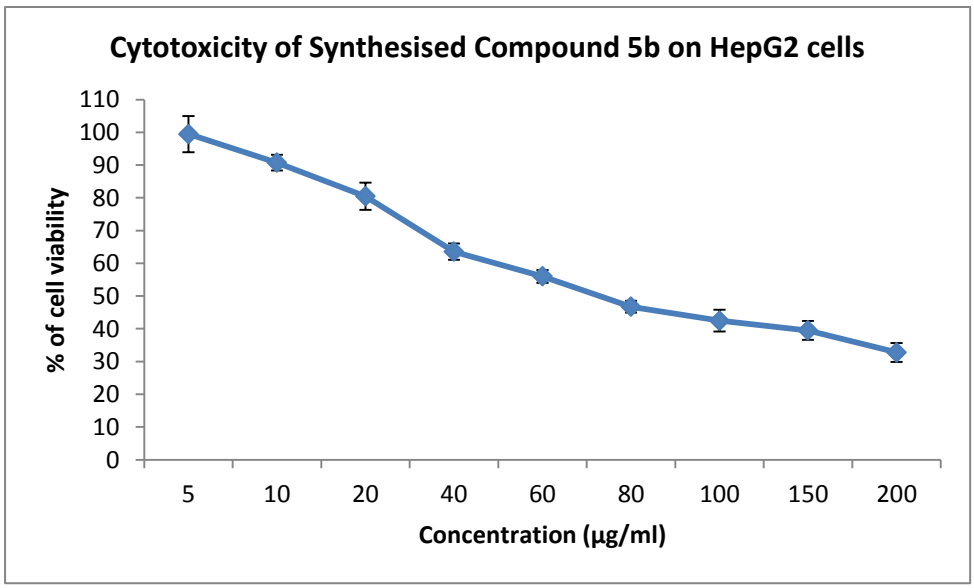


MCF-7

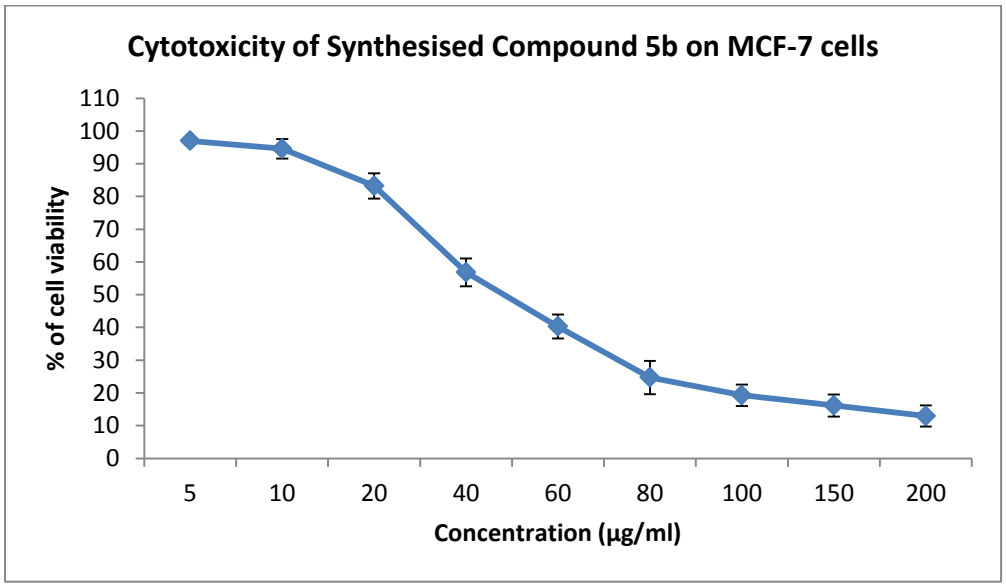


COMPOUND- 5b

HepG2 cell

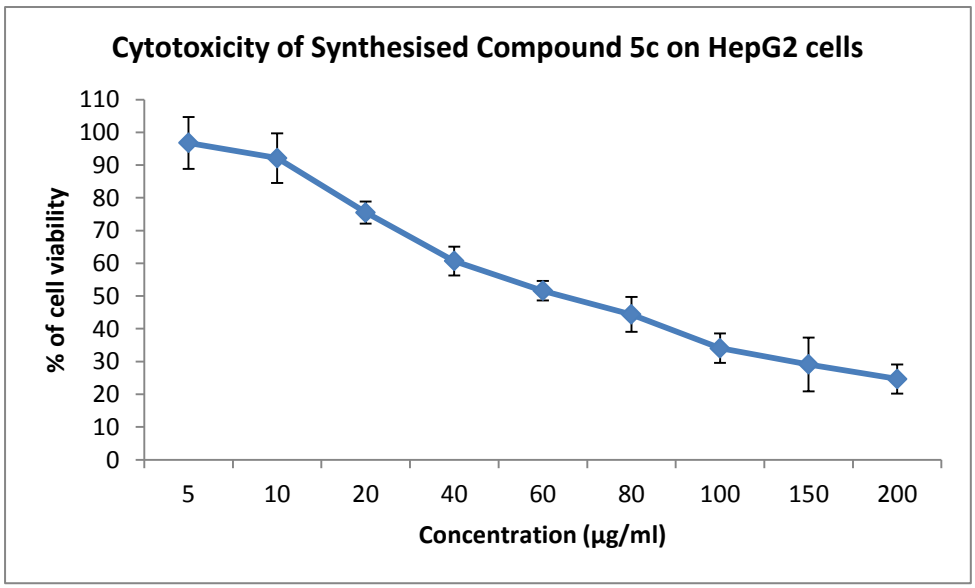


MCF-7

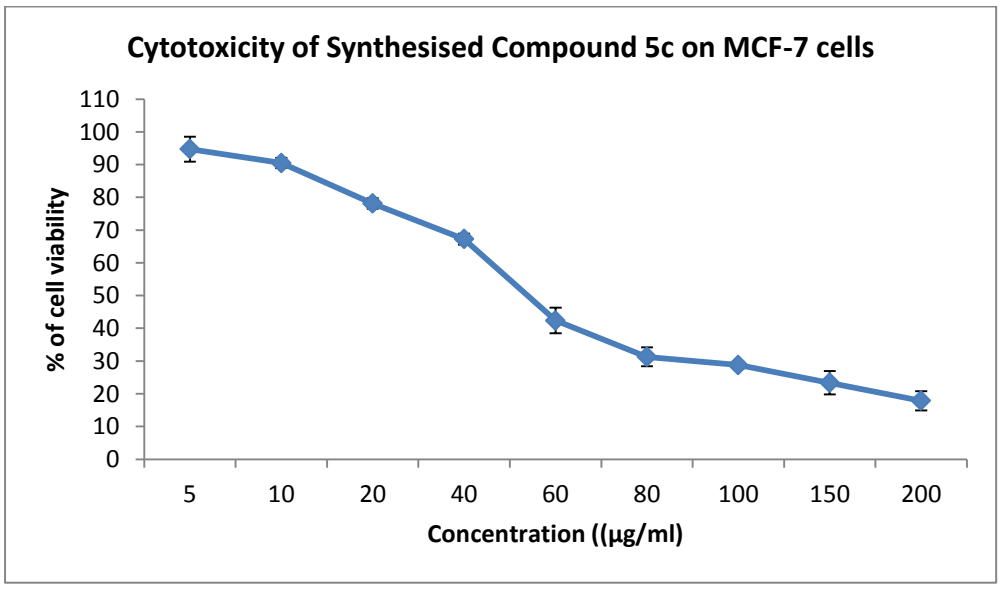


COMPOUND- 5c

Hep cell

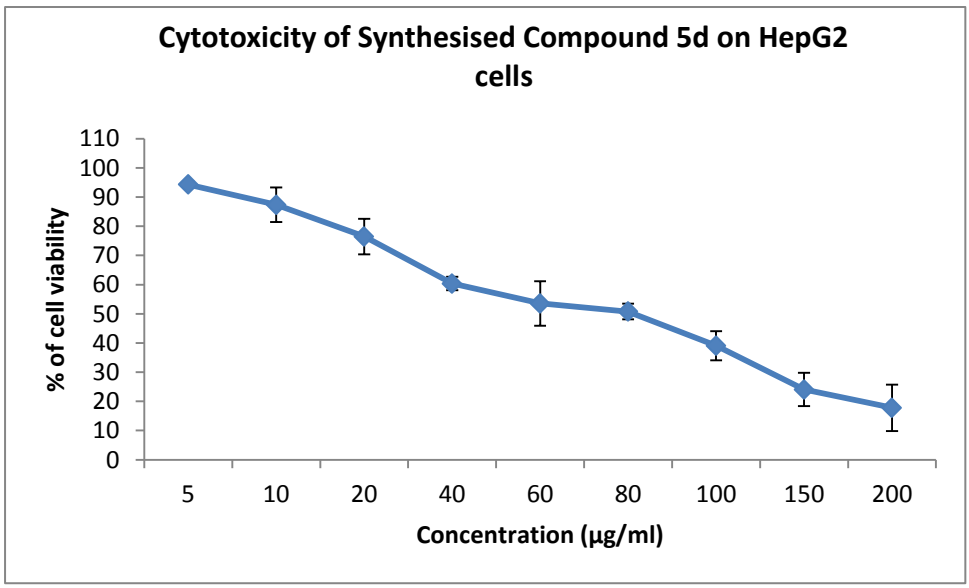


MCF-7

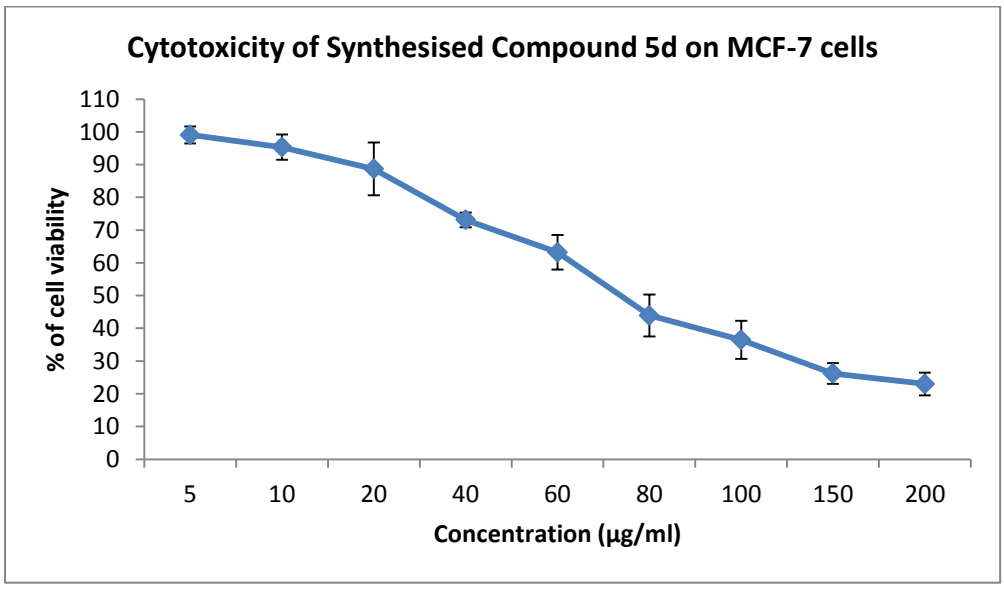


COMPOUND- 5d

Hep cell

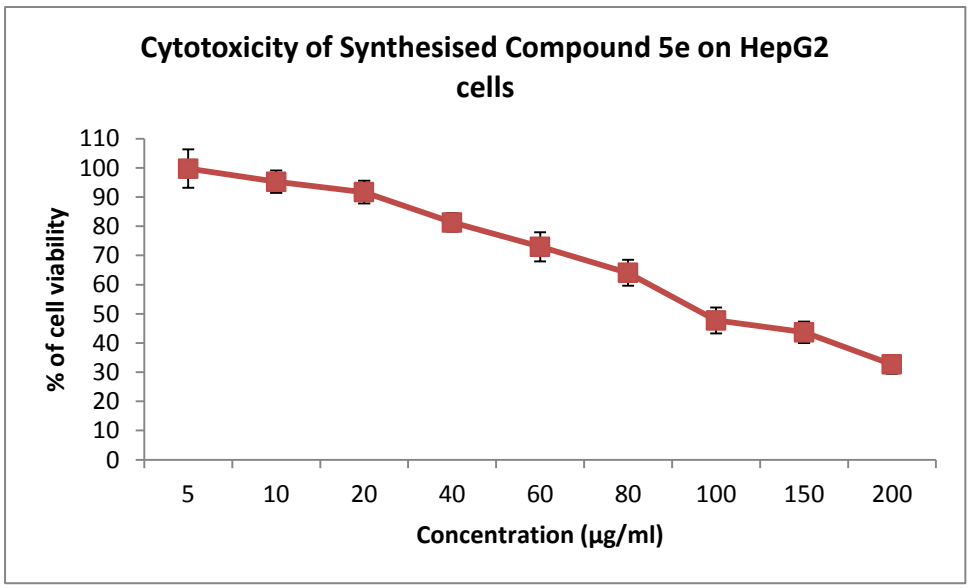


MCF-7



COMPOUND- 5e

Hep cell



MCF-7

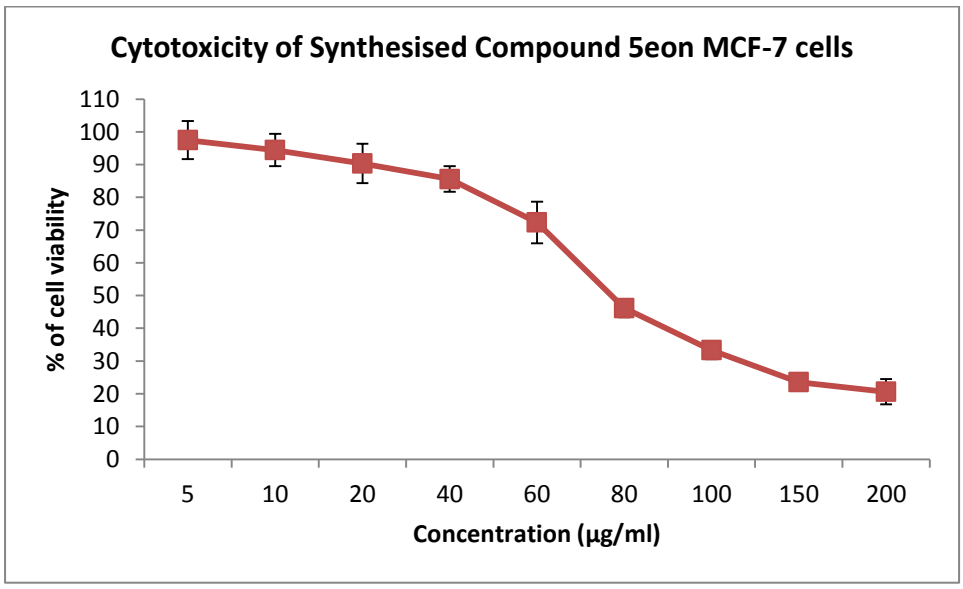


Table:10- PERCENTAGE CELL INHIBITION OF DIFFERENT CONCENTRATION OF COMPOUND 5a

COMPOUND	CELL LINE	CONC (µg/ml)	% Cell Inhibition	IC_{50} (µg/ml)
5a	HepG2	5	96.05±6.0	88.23
		10	92.11±1.17	
		20	82.01±5.6	
		40	67.74±1.91	
		60	61.48±6.7	
		80	50.00±2.2	
		100	41.87±1.3	
		150	35.61±6.3	
		200	33.64±7.5	
	MCF-7	5	94.02±6.2	53.39
		10	84.8±7.91	
		20	76.99±3.44	
		40	51.74±1.7	
		60	41.16±2.62	
		80	29.35±6.69	
		100	23.88±4.03	
		150	20.02±4.16	
		200	8.52±3.94	

Table:11- PERCENTAGE CELL INHIBITION OF DIFFERENT CONCENTRATION OF COMPOUND 5b

COMPOUND	CELL LINE	CONC (µg/ml)	% Cell Inhibition	IC_{50} (µg/ml)
5b	HepG2	5	99.45±5.50	90.76
		10	90.71±2.40	
		20	80.46±4.12	
		40	63.52±2.49	
		60	56.01±1.93	
		80	46.72±1.78	
		100	42.48±3.28	
		150	39.48±2.87	
		200	32.78±2.86	
	MCF-7	5	96.98±1.08	56.41
		10	94.57±2.96	
		20	83.23±3.87	
		40	56.82±4.27	
		60	40.26±3.68	
		80	24.69±5.12	
		100	19.27±3.25	
		150	16.16±3.35	
		200	12.95±3.18	

Table:12- PERCENTAGE CELL INHIBITION OF DIFFERENT CONCENTRATION OF COMPOUND 5c

COMPOUND	CELL LINE	CONC (µg/ml)	% Cell Inhibition	IC_{50} (µg/ml)
5c	HepG2	5	96.76±7.87	73.63
		10	92.14±7.60	
		20	75.50±3.34	
		40	60.70±4.39	
		60	51.61±3.00	
		80	44.37±5.33	
		100	34.04±4.48	
		150	29.12±8.21	
		200	24.65±4.44	
	MCF-7	5	94.71±3.83	69.65
		10	90.48±1.56	
		20	78.11±1.59	
		40	67.23±1.67	
		60	42.38±3.87	
		80	31.28±2.87	
		100	28.75±1.01	
		150	23.30±3.53	
		200	17.86±2.92	

Table:13- PERCENTAGE CELL INHIBITION OF DIFFERENT CONCENTRATION OF COMPOUND 5d

COMPOUND	CELL LINE	CONC (µg/ml)	% Cell Inhibition	IC_{50} (µg/ml)
5d	HepG2	5	94.29±1.10	72.95
		10	87.34±5.90	
		20	76.49±6.07	
		40	60.36±2.29	
		60	53.54±7.60	
		80	50.76±2.68	
		100	39.08±5.0	
		150	24.06±5.69	
		200	17.80±7.93	
	MCF-7	5	99.06±2.63	80.22
		10	95.32±3.85	
		20	88.66±8.08	
		40	73.13±2.25	
		60	63.20±5.30	
		80	43.92±6.38	
		100	36.44±5.83	
		150	26.16±3.18	
		200	23.01±3.45	

Table:14- PERCENTAGE CELL INHIBITION OF DIFFERENT CONCENTRATION OF COMPOUND 5e

COMPOUND	CELL LINE	CONC (µg/ml)	% Cell Inhibition	IC_{50} (µg/ml)
5e	HepG2	5	99.74±6.61	123.45
		10	95.26±3.84	
		20	91.67±3.92	
		40	81.30±3.19	
		60	72.98±4.99	
		80	64.02±4.45	
		100	47.75±4.45	
		150	43.66±3.65	
		200	32.65±3.14	
	MCF-7	5	97.48±5.78	84.36
		10	94.44±4.95	
		20	90.34±6.00	
		40	85.58±3.91	
		60	72.35±6.35	
		80	46.16±2.78	
		100	33.33±2.77	
		150	23.54±1.39	
		200	20.63±3.82	

Insilico docking report:

TABLE 15

With Breast epithelian cancer-2DSQ

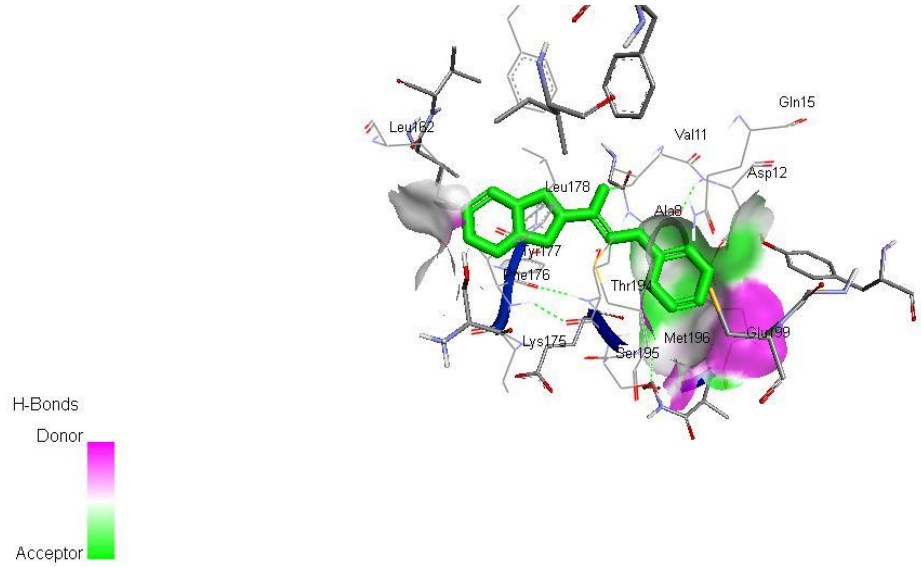
compound	Target protein	Binding energy
5a	2DSQ	-10.63
5b	2DSQ	-7.14
5c	2DSQ	-6.83

TABLE 16

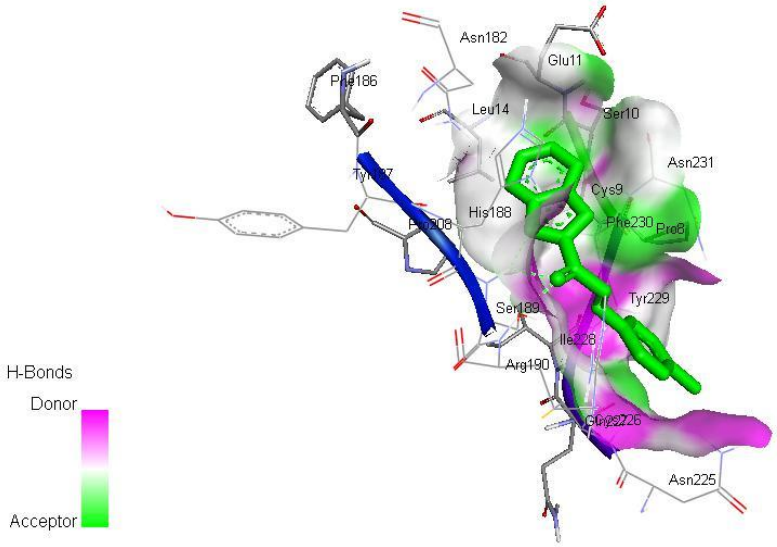
With Lung cancer-1MOX

compound	Target protein	Binding energy
5a	1MOX	-6.13
5b	1MOX	-10.74
5c	1MOX	-4.11

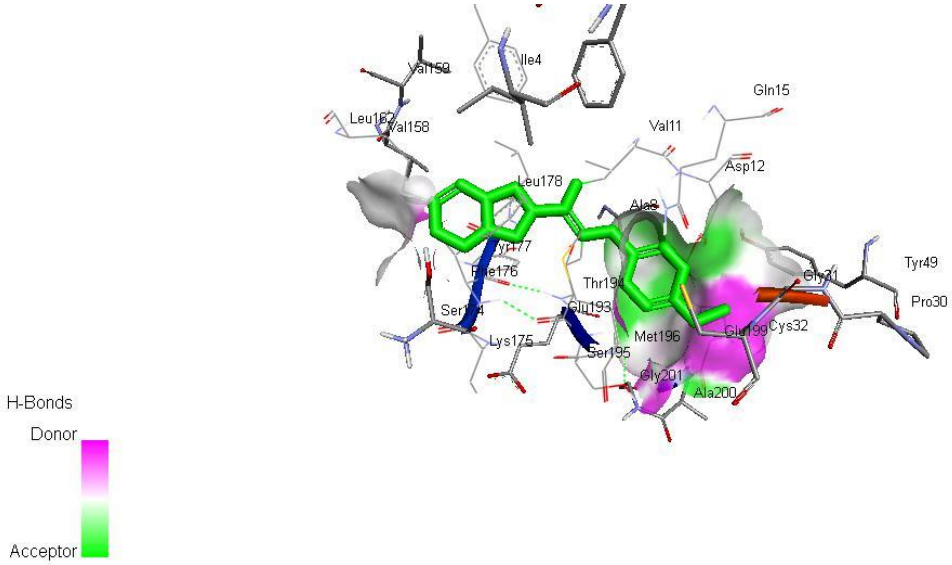
Binding of Compound 5a with 2DSQ



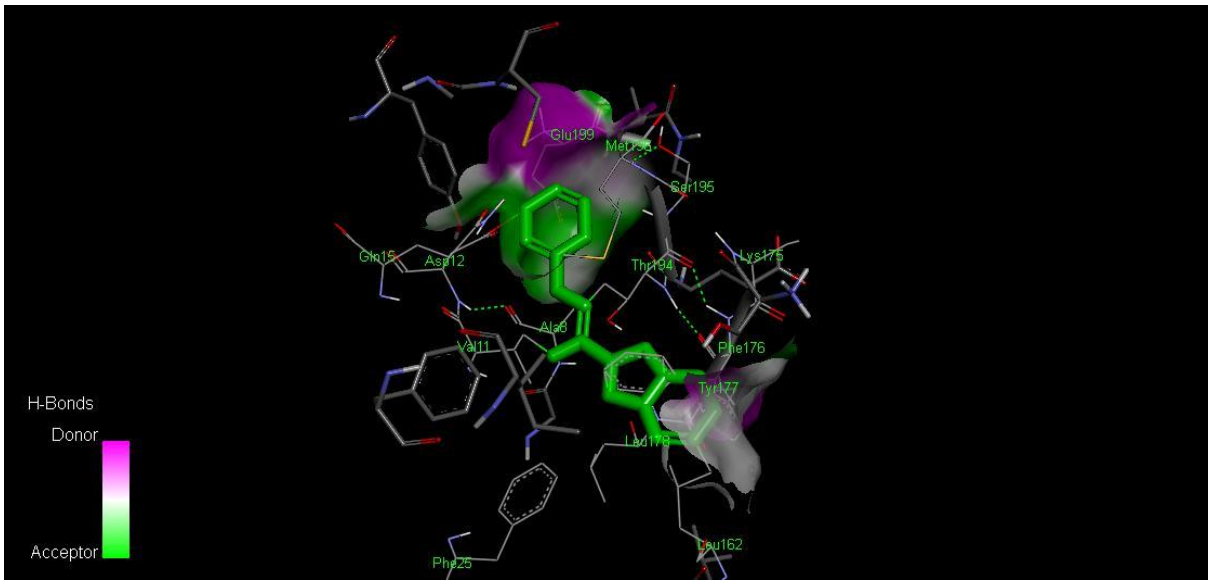
Binding of Compound 5b with 2DSQ



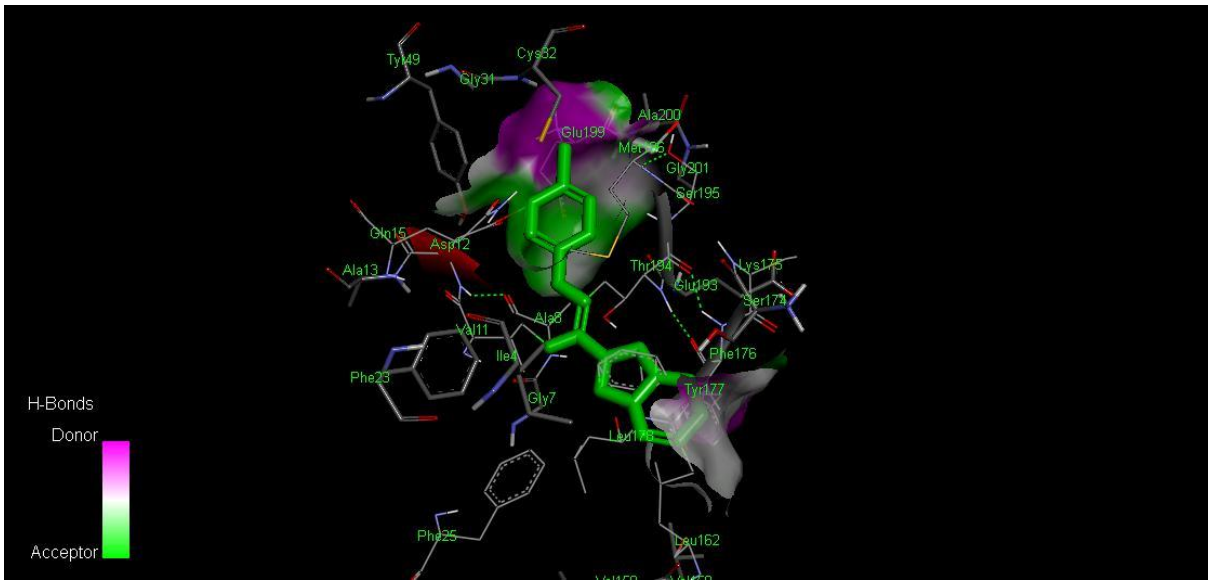
Binding of Compound 5c with 2DSQ



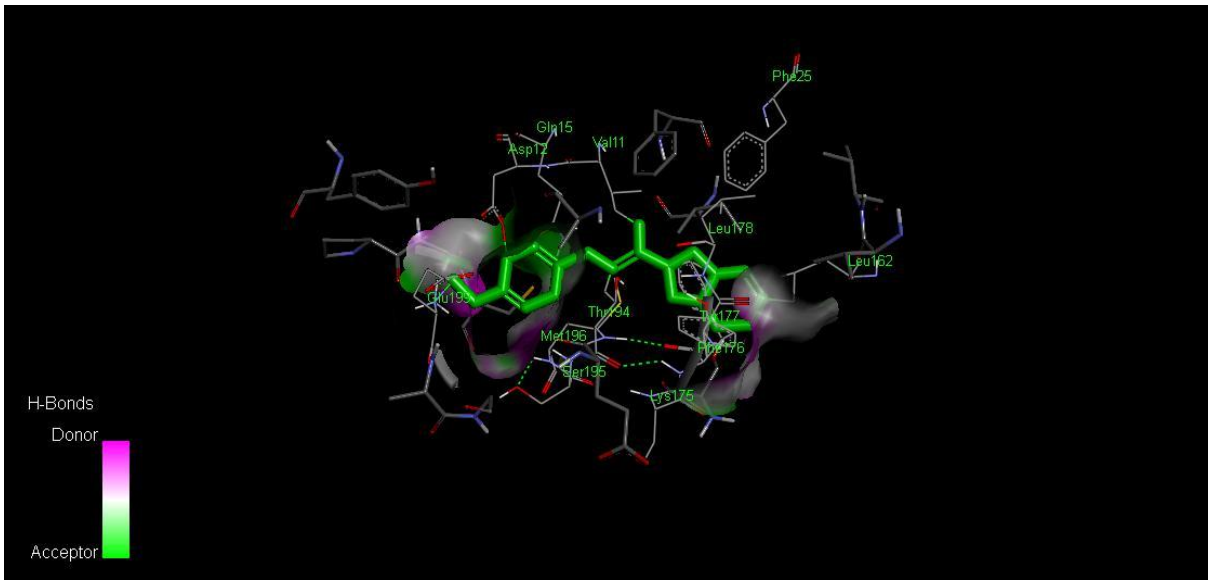
Binding of Compound 5a with 1MOX



Binding of Compound 5b with 1MOX



Binding of Compound 5c with 1MOX



5.5 DISCUSSION:

The molecular design of all synthesized compounds were done by using different software such as Chemdoole, Chemsketch and Molinspiration.

- The lipinski rule was predicted for all synthesized compound using CHEMDOODLE.
- It shows no violation in basic properties .The results were shown in **Table.No: 6**
- The pecentage yield, Mocular Formula, solubility and appearance of the compounds were pridicted and shown in **Table.No;3**
- The purity of the compounds were found out by TLC and Rf value was calculated. The results are shown in **Table.No:4**
- Melting points of compounds were predicted and shown in **Table.No:4**
- Elemental compostion were found and calculated in percentage and results obtained were shown in **Table. No:5**
- The Characterisation of synthesized compounds were confirmed by IR spectra,NMR spectra and Mass spectra.
- IRspectra interpret value shown in **Table**.**No:7**
- NMR specctra interpret value shown in **Table**.**No:8**
- Mass spectra results are shown in **Table.No.9**
- All synthesized compounds were evaluated for anti-cancer activity.
- Anti-cancer activity of all synthesized compounds were evaluated and results were shown in **Table.No:10,11,12,13 & 14**
- From the biological activity report further addition predict with insilico docking. It results compound-5a,5b and 5c were fair binding energies with two targets 2DSQ (breast epithelian cancer) and 1MOX (lung cancer). **Table.No:15 & 16.**

6. CONCLUSION

- The molecules were designed by the software tools and the lead molecules of Benzofuran-chalcone were synthesized by " CLAISEN-SCHMIDT REACTION"

- The formation of molecules was confirmed by TLC.

- The structure of synthesized compounds (5a-e) were confirmed by FT-IR, 1HNMR, MASS Spectroscopy.

- The IR data's showed relavant peaks for C=C, C-H, C=N, C=O groups. The 1HNMR also showed relavant proton peaks for all synthesized compounds. The MASS spectrum confirm the molecular ion peak of all synthesized compounds (5a-e).

- The synthesized compounds (5a-e) were evaluated invitro anti-cancer activity of Liver cancer (HepG2) and Breast cancer (MCF-7) two cell lines by using MTT Assay

- Invitro anticancer activity reported the synthesised compounds **5a** and **5b** were potent activity against breast cancer cells **(MCF-7)**

- And the synthesised compounds **5c** and **5d** were significant activity against Liver cells **(HepG2)**

- Based on results of invitro anticancer activity additionally predict insilico molecular docking study for having the major potent compounds 5a, 5b and 5c.

- The compounds 5a, 5b and 5c were dock with the two major taget proteins. The PDB id of 1MOX (lung cancer) and 2DSQ (breast epithelian cancer) were selected and obtained from protein data bank (PDB). were selected for INSILICO study

- In the post docking screening the best binding pose and total energy of each ligand was analysed. The details of best binding pose and total energy values were saved in output folder.

- Protein-ligand binding site was analysed and visualized. Its results majorly compounds 5a and 5b are the best binding pose with targets.

REFERENCES:

1. R.S.Myers,MEDCH,401, Spring,2006,1-16

2. Phrmacological Significance of Synthetic Heterocycles Scaffold: A Review Rajiv Duatal., Advances in Biological Research 5(3) 120-144, 2011

3. Y.-L. Chen, S.-Z. Lin, J.-Y. Chang et al., "In vitro and in vivo studies of a novel potential anticancer agent of isochaihulactone on human lung cancer A549 cells," *Biochemical Pharmacology*, vol. 72, no. 3, pp. 308–319, 2006.

4. R. Abonia, D. Insuasty, J. Castillo et al., "Synthesis of novel quinoline-2-one based chalcones of potential anti-tumor activity," *European Journal ofMedicinal Chemistry*, vol. 57, pp. 29–40, 2012.

5. DeSantis, C.; Ma, J.; Bryan, L.; Jemal, A. Breast cancer statistics, 2013. CA Cancer J. Clin. 2014, 64, 52–62.[CrossRef] [PubMed]

6. Kennedy, M.J.; Donehower, R.C.; Rowinsky, E.K. Treatment of metastatic breast cancer with combination paclitaxel/cyclophosphamide. Semin. Oncol. 1995, 22, 23–27. [PubMed]

7. Singh, P.; Anand, A.; Kumar, P. Recent developments in biological activities of chalcones: A mini review. Eur. J. Med. Chem. 2014, 85, 758–777. [CrossRef] [PubMed]

8. Zhuang, C.; Zhang,W.; Sheng, C.; Zhang,W.; Xing, C.; Miao, Z. Chalcone: A privileged structure in medicinal chemistry. Chem. Rev. 2017, 117, 7762–7810. [CrossRef] [PubMed]

9. Aryapour, H.; Riazi, G.H.; Ahmadian, S.; Foroumadi, A.; Mahdavi, M.; Emami, S. Induction of apoptosis through tubulin inhibition in human cancer cells by new chromene-based chalcones. Pharm. Biol. 2012, 50,1551–1560. [CrossRef] [PubMed]

10. Mirzaei, H.; Emami, S. Recent advances of cytotoxic chalconoids targeting tubulin polymerization: Synthesis and biological activity. Eur. J. Med. Chem. 2016, 121, 610–639. [CrossRef] [PubMed]

11. Kerru, N.; Singh, P.; Koorbanally, N.; Raj, R.; Kumar, V. Recent advances (2015–2016) in anticancer hybrids. Eur. J. Med. Chem. 2017, 142, 179–212

12. Z. Nowakowska, "A review of anti-infective and anti-inflammatory chalcones," *European Journal of Medicinal Chemistry*, vol. 42, no. 2, pp. 125–137, 2007.

13. A. Agarwal, K. Srivastava, S. K. Puri, and P. M. S. Chauhan, "Antimalarial activity and synthesis of new trisubstituted pyrimidines," *Bioorganic and Medicinal Chemistry Letters*, vol.15, no. 12, pp. 3130–3132, 2005.

14. M. L. Go, X. Wu, and X. L. Liu, "Chalcones: an update on cytotoxic and chemoprotective properties," *Current Medicinal Chemistry*, vol. 12, no. 4, pp. 483–499, 2005.

15. P. M. Sivakumar, S. Ganesan, P. Veluchamy, and M. Doble, "Novel chalcones and 1,3,5-triphenyl-2-pyrazoline derivatives as antibacterial agents," *Chemical Biology & Drug Design*, vol.76, no. 5, pp. 407–411, 2010.

16. J. C. Aponte, M. Ver´astegui, E. M´alaga et al., "Synthesis, cytotoxicity and anti-*Trypanosoma cruzi* activity of new chalcones," *Journal of Medicinal Chemistry*, vol. 51, no. 19, pp. 6230–6234,2008.

17. M. Maggiolini, G. Statti, A. Vivacqua et al., "Estrogenic and antiproliferative activities of isoliquiritigenin in MCF7 breast cancer cells,"*The Journal of Steroid Biochemistry and Molecular Biology*, vol. 82, no. 4-5, pp. 315–322, 2002.

18. S. K. Kumar, E. Hager, C. Pettit, H. Gurulingappa, N. E.Davidson, and S. R. Khan, "Design, synthesis, and evaluation of novel boronic-chalcone derivatives as antitumor agents,"*Journal of Medicinal Chemistry*, vol. 46, no. 14, pp. 2813–2815, 2003.

19. V. Ugale, H. Patel, B. Patel, and S. Bari, "Benzofurano-isatins:search for antimicrobial agents," *Arabian Journal of Chemistry*, 2012.

20. C.-L. Kao and J.-W. Chern, "A convenient synthesis of naturally occurring benzofuran ailanthoidol," *Tetrahedron Letters*, vol. 42, no. 6, pp. 1111–1113, 2001.

21. R. Basawaraj, B. Yadav, and S. S. Sangapure, "Synthesis of some 1H-pyrazolines bearing benzofuran as biologically active agents," *Indian Journal of Heterocyclic Chemistry*, vol. 11, no. 1, pp. 31–34, 2001.

22. Aggeliki Politi A, Serdar Durdagib, George Kokotosa, Thomas Mavromoustakosa "Development of accurate binding affinity predictions of novel renin inhibitors through molecular docking studies. Journal of Molecular Graphics and Modelling 2010.

23. Demet coskun and Mechmet faith Coskun " Synthesis, Characterisation and Anticancer activity of New Benzofuran substituted Chalcones,"Journal of Chemistry, Hindawi publishing corporation, vol.2016, article ID 7678486, 8 Pages

24. H.K¨uc¸¨ukbay,A.Mumcu, S. Tekin, and S. Sandal, "Synthesis and evaluation of novel N,N'-disubstituted benzimidazolium bromides salts as antitumor agents," *Turkish Journal of Chemistry*, vol. 40, pp. 393–401, 2016.

25. N. Kolocouris, G. B. Foscolos, A. Kolocouris et al., "Synthesis and antiviral activity evaluation of some aminoadamantane derivatives," *Journal of Medicinal Chemistry*, vol. 37, no. 18, pp. 2896–2902, 1994.

26. A. O. Gorgulu, K. Koran, F. ¨Ozen, S. Tekin, and S. Sandal, "Synthesis, structural characterization and anti-carcinogenic activity of new cyclotriphosphazenes containing dioxybiphenyl and chalcone groups," *Journal of Molecular Structure*, vol. 1087, pp. 1–10, 2015

27. S. M. Rida, S. A. M. El-Hawash, H. T. Y. Fahmy, A. A. Hazzaa, and M. M. M. El-Meligy, "Synthesis of novel benzofuran and related benzimidazole derivatives for evaluation of *in vitro* anti-HIV-1, anticancer and antimicrobial activities," *Archives of Pharmacal Research*, vol. 29, no. 10, pp. 826–833, 2006.

28. R. K. Ujjinamatada, R. S. Appala, and Y. S. Agasimundin, "Synthesis and antimicrobial activity of new benzofuranyl-1,3- benzoxazines and 1,3-benzoxazin-2-ones," *Journal of Heterocyclic Chemistry*, vol. 43, no. 2, pp. 437–441, 2006.

29. Vagdevi H M.Latha K.P, Vaidya V.P, Vijayakumar M L & Pai K S R Indian J Pharm Csi, 2001 63,86

30. Antimicrobial Assay methods- Indian Pharmacopoeia published by Govt of India (2010) Vol-1.1 49-54

31. Mphahlele, M.J.; Paumo, H.K.; Choong, Y.S. Synthesis and in vitro cytotoxicity of the 4 (halogenoanilino)-6-bromoquinazolines and their 6-(4-fluorophenyl) substituted derivatives as potential inhibitors of epidermal growth factor receptor tyrosine kinase. Pharmaceuticals 2017, 10, 87. [CrossRef] [PubMed]

32. Organic Spectroscopy by Y.R.Sharma

33. A text book of Spectral analysis by Robert Silvestein

34. Sharma, B.Chakravarti,M. P.Gupt, J. A. Siddiqui, R.Konwar, and R. P. Tripathi, "Synthesis and anti breast cancer activity of biphenyl based chalcones," *Bioorganic and Medicinal Chemistry*, vol. 18, no. 13, pp. 4711–4720, 2010.

35. K. Juvale,V. F. S. Pape, andM.Wiese, "Investigationof chalcones and benzochalcones as inhibitors of breast cancer resistance protein," *Bioorganic and Medicinal Chemistry*, vol. 20, no. 1, pp. 346–355, 2012.

36. C.-L. Kao and J.-W. Chern, "A convenient synthesis of naturally occurring benzofuran ailanthoidol," *Tetrahedron Letters*, vol. 42, no. 6, pp. 1111–1113, 2001.

37. S. M. Rida, S. A. M. El-Hawash, H. T. Y. Fahmy, A. A. Hazzaa, and M. M. M. El-Meligy, "Synthesis of novel benzofuran and related benzimidazole derivatives for

evaluation of *in vitro* anti-HIV-1, anticancer and antimicrobial activities," *Archives of Pharmacal Research*, vol. 29, no. 10, pp. 826–833, 2006.

38. R. Romagnoli, P. G. Baraldi, T. Sarkar et al., "Synthesis and biological evaluation of 2-aroyl-4-phenyl-5-hydroxybenzofurans as a new class of antitubulin agents," *Medicinal Chemistry*, vol. 4, no. 6, pp. 558–564, 2008.

39. V. R. Solomon, C. Hu, and H. Lee, "Hybrid pharmacophore design and synthesis of isatin-benzothiazole analogs for their anti-breast cancer activity," *Bioorganic and Medicinal Chemistry*, vol. 17, no. 21, pp. 7585–7592, 2009.

40. R. A. Kabli, A. A. Khalaf, M. T. Zimaity, A. M. Khalil, A. M. Kaddah, and H. A. Al-Rifaie, "Synthesıs of a new serıes of furyl and thıenyl substıtuted pyrazolınes startıng wıth furyl and thıenyl chalcones," *Journal of the Indian Chemical Society*, vol. 68, pp. 47–51, 1991.

41. S. Holla, P. M. Akberali, and M. K. Shivananda, "Studies on arylfuran derivatives: part X. Synthesis and antibacterial properties of arylfuryl-Δ 2-pyrazolines," *Farmaco*, vol. 55, no. 4, pp. 256–263, 2000.

42. M. Cabrera, M. Simoens, G. Falchi et al., "Synthetic chalcones, flavanones, and flavones as antitumoral agents: biological evaluation and structure-activity relationships," *Bioorganic and Medicinal Chemistry*, vol. 15, no. 10, pp. 3356–3367, 2007.

43. N. S. Hari Narayana Moorthy, Sergio F. Sousa, Maria J. Ramos, and Pedro A. Fernandes 'Structural feature study of benzofuran derivatives as farnesyltransferase inhibitors' Journal of Enzyme Inhibition and Medicinal Chemistry, 26:6, 777-791 2011

44. Studies on some heterocyclic entities, Part-IV,studies on chalcones ., 85-109.

45. P.M.Gurubasavaraja Swamy* and y.s. agasimundin synthesis and antimicrobial screening of certain substituted chalcones and isoxazolines bearing hydroxy benzofuran rasayan j. chem vol.1,no.2(2008), 421-428

46. Kamelia M. Amin a, Yasmin M. Syam b,⇑, Manal M. Anwar b,⇑, Hamed I. Ali c, Tamer M. Abdel-Ghani d, Aya M. Serry Synthesis and molecular docking study of new benzofuran andfuro[3,2-g]chromone-based cytotoxic agents against breast cancer and p38a MAP kinase inhibitors, Bioorganic chemistry 76-2018(487-500)

47. Mahmoud reza heidaria,b*, alireza foroumadib,c , hojat noroozia,ali samzadeh-kermanid and behzad sarvar azimzadeh study of the anti-inflammatory and analgesic effects of novel rigid benzofuran-3, 4-dihydroxy chalcone by formalin, hot-plate and carrageenan tests in mice www.researchgate.net/publication/26840346

48. Mohammed Rayees Ahmad a,*, V. Girija Sastry a, Nasreen Bano b, Syed Anwar c Synthesis of novel chalcone derivatives by conventional and microwave irradiation methods and their pharmacological activities Arabian Journal of Chemistry (2016) 9, S931–S935

49. Lucas C.C. Vieira, Márcio Weber Paixão, Arlene G. Corrêa Green synthesis of novel chalcone and coumarin derivatives via Suzuki coupling reaction Tetrahedron Letters 53 (2012) 2715–2718

50. P..K.Gurubasavaraja swamy and y.s.agasimundin "synthesis and antimicrobial activity of some novel chalcones containg 3-Hydroxy benzofuran. Acta pharmaceutica science 50:197-202.

51. Ajit.k.nangare1 and amit.n.chavan application of the benzofuran ring system in chemical structure of different pharmaceuticals drug. world journal of pharmaceutical research volume 4, issue 7, 1734-1746.

52. Zahoor A. Wani1, Anup S. Pathania1, Girish Mahajan1, Akanksha Behl1, Mubashir J. Mintoo1, Santosh K. Guru1 anticancer activity of a novel quinazolinone-chalcone Derivative through cell cycle arrest in pancreatic Cancer cell line Journal of Solid Tumors, 2015, Vol. 5, No. 2

53. Mayank j. Mamtora, javed g. Mahetar, juvansinh j. Jadeja, rohit b. Manawar and Manish k. Shah synthesis and evaluation of antimicrobial activity of benzofuran derivative and its metal complexes **Journal of Chemical and Pharmaceutical Research, 2015, 7(4):1456-1460**

54. Raga basawaraj_ and s. s. sangapurea, 'synthesis of some 3-aryl-2-(5'-chloro-3'-methylbenzofuran-2'-yl)-1, 4-quinoxalyl methanes of biological interest' Int. J. Chem. Sci.: 6(1), 2008, 351-357

55. Krishnakumar and lohidashan , manju rajan ,andhale ganesh , Mathew paul and jithu jenin .Bangladesh J pharmacol .2018;13:23÷29

56. S.N.Mokale ,P.N. Dube ,S.A.Bhavale et al., Synthesis invitro screening and docking analysis of noval pyrolidine and piperidine –substituted ethoxy chalcone as anticancer agents "Medicinal chemistry Research ,vol 24 no.5 ,pp:1842 -1856 ,2015.

57. N.Ashok kumar ,G.Umarani .et al ,Green synthesis ,charecterisation ,Invitro molecular docking and invitro anticancer activity of 1,2,3-triazyl dihydro pyrimidine -2-thione hybrids ,Journal of pharmaceutical Advanced Research.2018;1(4):229-235

58. Koneni V.sathidhara ,Ram K .Modulcuri ,Pooja Jadiya Benzofuran –chalcone Hybrids as potential multifunctional Agent against Alzhemers disease :Synthesis and invivo studies with Transgenic caenorhabditis elegants chem med chem 1-15.

59. Shaik KA :International journal of pharmaceutical research scholors (IJPRS) VOL -3 ,Issue -3,2014

60. Jacek kyujawskiet ,Marelek ,Bernord ,Anna Janeq and Weranika kuzma ;J.chem .Edue;2012 ,89(1) pp 64-67

61. Clarisse G.Ricci and Paulo. A.Netz *J.Doucong students on DNA-Ligand intraction ,Building mode .chem.Inf.model.2009,49,1925-1935.

62. Wenying Ren ,Zhenhua Dino,Hongwei wang.Leizhu , Li zhang Flavanoids : Promoting Anticancer agents ,Medical research .Reviews ,vol.23 ,No 4 ,519 – 534 ,2003.

63. Paulo A Netz et al. "Docking studies on DNA ligand interactions: Building and application of a protocol to identify the binding mode." Chem.Inf.model 2009,49,1925-1935

64. Li zhang Flavonoids : Promising anti-cancer agents medicinal reviews, vol.23, no.4,519-534-**(2003)**

65. Hena Khanam, Shamsuzzaman Bioactive Benzofuran derivatives: A review European Journal of Medicinal Chemistry 97 (2015) 483-504

66. Jaya Seema D.M, Saifullah B, Selvanayagam M, Subbaih Sk, Mohd Esa N, Arulselvan P, Designing Of The Anticancer nanocomposite with sustained release properties by using graphene ocide nano carrier. Pharmaceutics, 2018 aug 1:10(3) e109,

67. Ayaz mahmood Dar and Shafia Mir; journal of analytical and Bioanalytical techniques 2017,8:2

68. Sheng tian, Junmei Wang, Young LI, Dan Li, Lei Xu, 'Advanced Drug Delivery Reviews-12735

69. .T. Sakai, R. N. Eskander, Y. Guo et al., "Flavokawain B, a kava chalcone, induces apoptosis in synovial sarcoma cell lines," *Journal of Orthopaedic Research*, vol. 30, no. 7, pp. 1045–1050, 2012.

70. S. K. Kumar, E. Hager, C. Pettit, H. Gurulingappa, N. E. Davidson, and S. R. Khan, "Design, synthesis, and evaluation of novel boronic-chalcone derivatives as antitumor agents," *Journal of Medicinal Chemistry*, vol. 46, no. 14, pp. 2813–2815, 2003

71. .B. Srinivasan, T. E. Johnson, R. Lad, and C. Xing, "Structure— activity relationship studies of chalcone leading to 3-hydroxy- 4,3!,4!,5!-tetramethoxy chalcone and its analogues as potent nuclear factor B inhibitors and their anticancer activities," *Journal of Medicinal Chemistry*, vol. 52, no. 22, pp. 7228–7235, 2009.

72. D. Kumar, N. M. Kumar, K. Akamatsu, E. Kusaka, H. Harada, and T. Ito, "Synthesis and biological evaluation of indolyl chalcones as antitumor agents," *Bioorganic & Medicinal Chemistry Letters*, vol. 20, no. 13, pp. 3916–3919, 2010.

73. Kim SH, Lee E, Beak KH, Chalcones, inhibitors for topoisomerase I and capthesin B and L, as potential anticancer agents, Bio org med cam Lett 2013 1;23(11):3320

74. N. S. Hari Narayana Moorthy, Sergio F. Sousa, Maria J. Ramos, and Pedro A. Fernandes 'Structural feature study of benzofuran derivatives as farnesyltransferase inhibitors' Journal of Enzyme Inhibition and Medicinal Chemistry, 26:6, 777-791 2011

75. Demet Coskun, Merve Erkisa, Engin Ulukaya, Mehmet Fatih Coskun, Ferda Ari "Novel 1-(7-ethoxy-1-benzofuran-2-yl) substituted chalcone derivatives: Synthesis, characterization and anticancer activity *European Journal of Medicinal Chemistry* (2017), doi: 10.1016/j.ejmech.2017.05.017

76. G.Krishnamuthy, S.Shakila Banu, r,senthamai, Ismail,"European journal of Pharmaceutical and med research 2017,4 (07) 704-709.

77. S.D.Tala, P.B.Vekariya, R.M.Ghetiya and joshi."synthesis and biological study of some new chalcone and pyrazole derivatives. Indian journal of chemistry Vol52b. 807-809. 2013.

78. Sachin Kumar[1], Sakshi Bajaj[2], Ramesh Babu Bodla[3] Preclinical screening methods in cancer" Indian journal of pharmacology (2016).

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