3-D QSAR STUDY OF COMBRETASTATINS FUSED WITH HETROCYCLIC RING AS TUBULIN BINDING AGENTS

Project Report Submitted to the Central University of Punjab

For the award of

Master of Science

Chemical Sciences (Medicinal Chemistry)

In

Department
of

Pharmaceutical Sciences and Natural Products

BY

AJIT KUMAR DHANKA

Supervisor

Dr VINOD KUMAR

Department of Pharmaceutical Sciences and Natural Products

School of Basic and Applied Sciences

Central University of Punjab, Bathinda

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DECLARATION

I Ajit Kumar Dhanka, declare that the project report entitled **"3-D QSAR STUDY OF COMBRETASTATINS FUSED WITH HETROCYCLIC RING AS TUBULIN BINDING AGENTS"** has been prepared by me under the guidance of Dr. Vinod Kumar, Assistant Professor, in the Department of Pharmaceutical Sciences and Natural products, School of Applied Sciences, Central University of Punjab. No part of this project work has formed the basis for the award of any degree or fellowship previously.

(Ajit Kumar Dhanka)
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Central University of Punjab,
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Date:

CERTIFICATE

I certify that Ajit Kumar Dhanka has prepared his project report entitled **"3-D QSAR STUDY OF COMBRETASTATINS FUSED WITH HETROCYCLIC RING AS TUBULIN BINDING AGENTS"** for the award of M. Sc. (Medicinal Chemistry) degree from the Central University of Punjab, under my guidance. He has carried out his work at the Department of Pharmaceutical Sciences and Natural products, School of Basic and applied Sciences, Central University of Punjab, Bathinda.

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Date:

ABSTRACT

3-D QSAR STUDY OF COMBRETASTATINS FUSED WITH HETROCYCLIC RING AS TUBULIN BINDING AGENTS

Name of student	:	Ajit Kumar Dhanka
Registration Number	:	16mscchs12
Degree for which submitted	:	M.Sc. (Medicinal Chemistry)
Supervisor	:	Dr. Vinod Kumar
Centre	:	Department of Pharmaceutical Sciences and Natural Products
School of Studies	:	Basic and Applied Sciences

Combretastatin A4 (CA4) is a leading agent in vascular disrupting strategies and tubulin polymerization inhibitor for the tumour therapy. A large number of combretastatin derivatives have been synthesized as potent inhibitors of Tubulin which are responsible for the anticancer activity. Combretastatins bind with the colchicine binding site of the tubulin and disrupt the dynamic equilibrium of tubulin. IN the current research proposal we have performed 3D-Field based QSAR on Combretastatins analogue in order to recognize structural features which are responsible for the tubulin inhibitors activity. The designed compounds are expected to show good inhibitory activity against tubulin when electrostatic group is attached in case of compounds 16 and 18, Bulky group is attached in case of compound 26 and hydrophobic group is attached in case of compound 51 respectively.

Ajit Kumar Dhanka **Dr. Vinod Kumar**

Acknowledgments

Praise God, the most merciful and compassionate forgiving me the strength in the completing the project work

Next and the for most , I would like to express my deepest gratitude to my advisor, **Dr. Vinod Kumar**, Assistant Professor of pharmaceutical sciences and natural products, central University of Punjab, Bathinda for the thesis advice, supervision, guidance and patience throughout my research work. This thesis would not be possible without his unmatched support and enthusiasm. I consider myself lucky as I got chance to work under his guidance. “Thank you for everything”.

First of all, I would like to express my very sincere thanks to **Prof. Dr. P. Ramarao**, Dean, Academic Affair, Central University of Punjab, Bathinda, in this direction, valuable suggestions and listening my queries.

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I would like to thanks **Dr. Vikas Jaitak**, Assistant Professor, **Dr. Pradeep Kumar**, Assistant professor, **Dr. Vankata Rao Kaki**, assistant Professor, Department of Pharmaceutical sciences and Natural products, for their help and support throughout the dissertation work.

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Last but not least, I want to acknowledge my family who were on my side in the whole my life in the academic carrier in general and my master studies in Central University of Punjab in particular.

(AJIT KUMAR DHANKA)

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INTRODUCTION

1. Introduction

1.1 Cancer

Despite decades of tremendous clinical and fundamental research, cancer is one of the most challenging diseases. Traditional chemotherapy depends on the basis that the rapid growth of tumor cells is more likely to be destroyed by cytotoxic agents than normal cells. In reality, however, these cytotoxic agents do not have very little or no specificity, which leads to systematic toxicity due to undesirable side effects. Accordingly, dramatic improvements in the effectiveness of the development of tumor-specific drug distribution system for the separation of antisense agents, general and cancerous cells or tissues, and efficacy of cancer chemotherapy. In order to solve this problem, various drug delivery systems have been studied in the past few decades(Wiener, Kazak, Noll, Patenaude, & Kupst, 2015)**.**

There are many types of cancer and many types of treatment. Likes Bladder Cancer, Breast Cancer, Colorectal Cancer, Kidney Cancer, Lung Cancer - Non-Small Cell, Lymphoma - Non-Hodgkin, Melanoma.

The types of cancer treatments likes common treatments for Cancer Surgery, Chemotherapy, Radiation Therapy, Targeted Therapy, Immunotherapy and Other Procedures and Techniques Stem Cell Transplant, Hyperthermia, Photodynamic Therapy, Blood Transfusion and Donation, Lasers in Cancer Treatment.

1.2 Hallmarks of cancer

Hanahan and Weinberg reported that there are six biological capabilities acquired during the multistep development of human tumors which are involved in hallmarks of cancer. They include inducing angiogenesis, enabling replicative immortality, sustaining proliferative signaling, evading growth suppressors, resisting cell death, and activating invasion and metastasis. After that conceptual progress in the last decade, they added new hallmarks to this list, they are reprogramming of energy metabolism, evading immune destruction, genomic instability and mutation, and tumor promoted inflammation (fig.1.1)(Hanahan & Weinberg, 2011).

There are many effective methods available to treat diseases of cancer. The major methods used in the treatment of cancer today are surgery, radiation

therapy and chemotherapy. Primary tumors and large metastases are often treated by surgery and radiation therapy. The chemotherapy is mainly used to treat some disseminated tumors such as breast, prostate and colorectal cancers. (Ke & Shen, 2017).

The anticancer drugs have been classified in consonance with their mechanism of action as molecular targeting hybrids, antitubulin, anti-metabolites and DNA-interactive hybrids, hormones and monoclonal antibodies(Kerru, Singh, Koorbanally, Raj, & Kumar, 2017) Based on this mechanism of action, there are number of anticancer drugs are reported and many cancer chemotherapeutic drugs currently in clinical use. However, the major problems associated with the chemotherapeutic agents are poor patient compliance, drug resistance and drug induced toxicities. Therefore we need to give strong impetus for discovery and development of novel cancer chemotherapeutic agent(Avendano & Menendez, 2015)

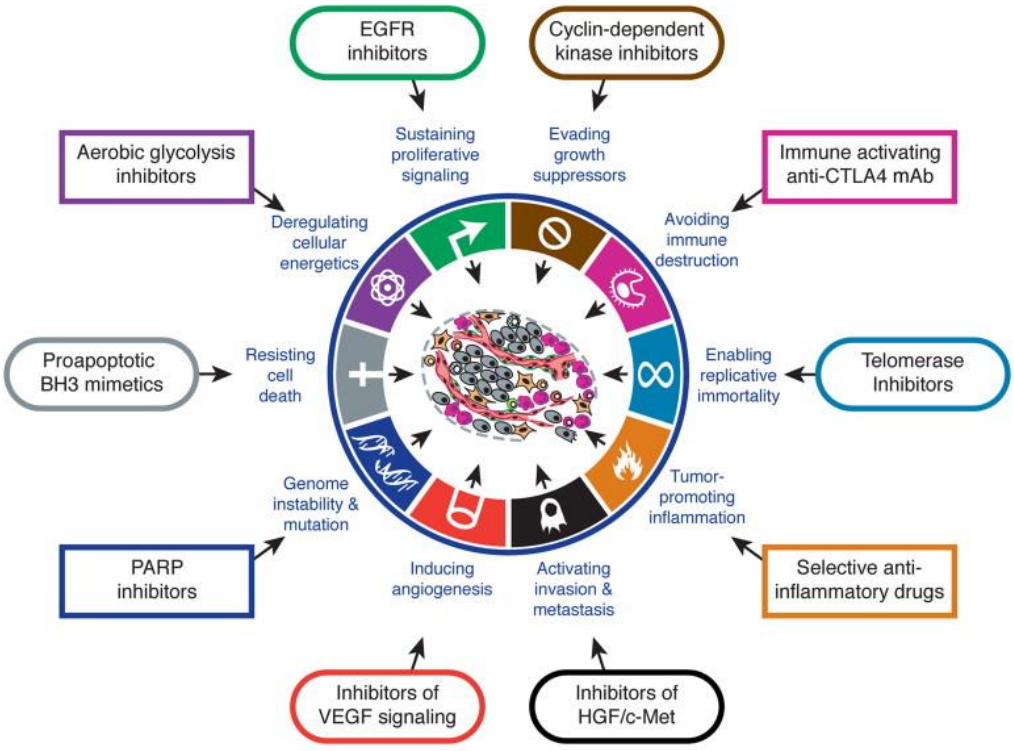


Fig.1.1 Illustration of the approaches employed in developing therapeutics targets to the promising and known hallmarks of cancer, adapted from (Hanahan & Weinberg, 2011).

Microtubules are essential constituents of the cytoskeleton in eukaryotic cells which are formed by α and β tubulin heterodimers. They are involved in a number of important structural and regulatory functions, such as cell division, cell shape maintenance, vesicles transportation and motility regulation. During the cellular mitosis, microtubules are responsible for forming the mitotic spindle and directing the movement of chromosomes(Kamal et al., 2015). Interference of microtubule dynamics causes cell cycle arrest in the G2/M phase and produce abnormal mitotic spindles (Kaur, Kaur, Gill, Soni, & Bariwal, 2014), leading to mitotic catastrophe and finally cellular apoptosis. The important role of microtubules in cell growth and cell division, tubulin dynamics has been a well-established target for developing anticancer drugs(Wu, Wang, & Li, 2016).Over the past few years, microtubule-active drugs are successfully used in the oncology clinic for a wide spectrum of malignancies. However, several problems associated with their clinical use, such as non-specific toxicity, drug resistance and water insolubility. Therefore, the discovery of novel molecule is required to overcome these problems (Mishra et al., 2015).

The combretastatin was separated from the bark of the South African tree Combretum *caffrum* and it is most potent anti-mitotic agents.

Molecules that fall into the Combretastatin family generally share 3 common structural features: a trimethoxy "A"-ring, a "B"-ring containing substituents often at C3' and C4', and often an ethene bridge between the two rings which provides necessary structural rigidity. Molecules with such an ethene bridge are also stilbenoids, molecules with a non-ethene bridge are dihydrostilbenoids (Marrelli et al., 2011).

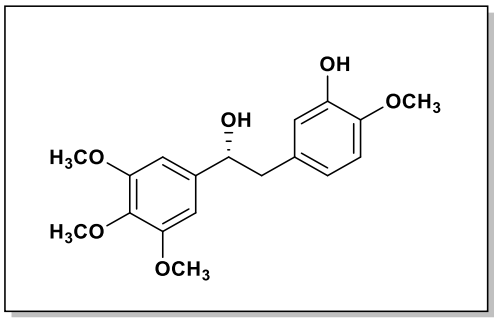


Figure 1.2 Structure of combretastatins

The most potent of these is combretastatin A-4 which has been found to be a potent cytotoxic agent which strongly inhibits the polymerization of tubulin by binding to the

colchicine site. Combretastatin A-4 is also able to elicit irreversible vascular shutdown within solid tumours, leaving normal vasculature intact. A prodrug of combretastatin A-4, the water soluble phosphate derivative4 2 is now in phase II of clinical trials(Gaukroger, Hadfield, Hepworth, Lawrence, & McGown, 2001).

Quantitative structure activity relationship (QSAR) is a theoretical model that can be used to predict biological properties of molecules. QSAR suggests that if a group of chemicals show the same mechanism of action towards a target, then alteration in the biological activity also alters chemical, structural and physical properties(Johnson & Maggiora, 1990).

QSAR is the process through which chemical structure is measurably connected with a well-outlined process like biological operation and chemical reactivity. QSAR are the methods that are not only used in drug designing but are widely used in other sciences like in biology, toxicology(Hansen, Telzer, & Zhang, 1995), environmental toxicology, agro chemistry, pharmaceutical chemistry, etc. The QSAR can also be used to determine the initial and final point of any synthesis(Bradbury, 1995).

CHAPTER-2

LITERATURE REVIEW

2. Literature review

2.1 Cancer scenario in India

The number of male, female and the total cancer patients in 2004 were 390808, 428546 and 819353 respectively (Ali, Wani, & Saleem, 2011)and the number of male and female cancer patients increased in 2009, with 454843, 507991 and 962831 cases for male, female and total cancer patients, respectively. In 2010 , 461408 male cancer patients and 517378 female cancer patients were reported , with a total number of 978786 patients .(Pal & Mittal, 2004). Also a prediction of cancer patients in 2015 and 2020 has also been made.

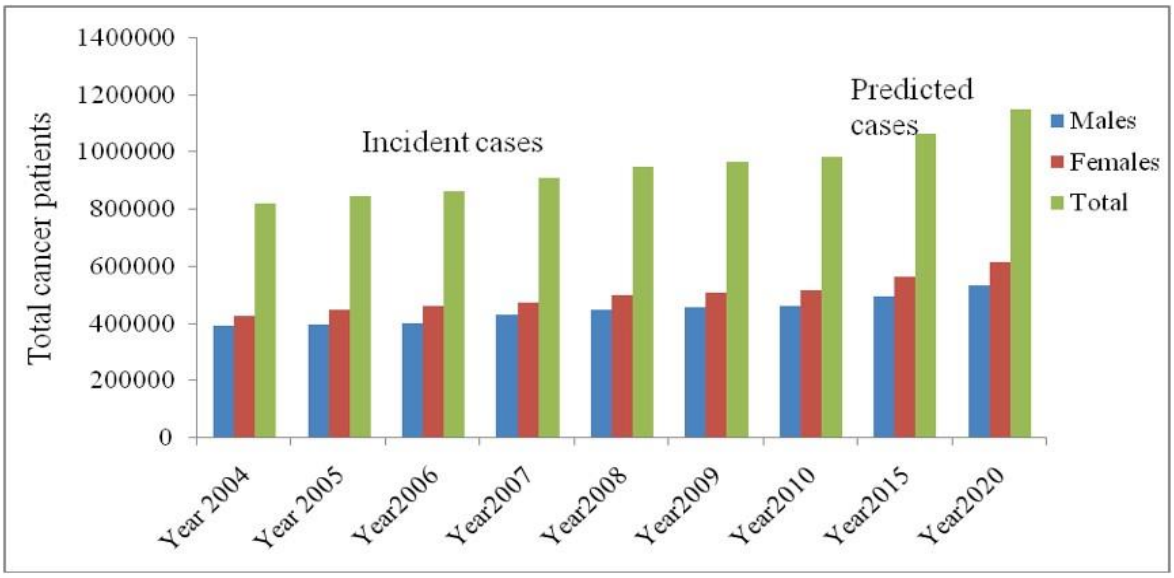


Figure 2.1 : Year wise total cancer prevalence in India (Mehrotra, Pandya, Chaudhary, Kumar, & Singh, 2008).

2.2 Microtubules

Microtubules, key components of the cytoskeleton are long, filamentous, tube-shaped protein polymers that are essential in all eukaryotic cells(Magnani, Maccari, Andreu, Diaz, & Botta, 2009).

2.2.1 Structure of microtubules

Microtubules are crucial in the development and maintenance of cell shape, in the transport of vesicles, mitochondria and other components throughout cells, in cell signalling, and in cell division and mitosis. Microtubules composed of α-tubulin and β-tubulin heterodimers (of dimensions 4 nm ×5 nm ×8 nm and 100,000 Daltons

in mass) arranged in the form of slender filamentous tubes that can be many micrometres long.

Generally 6 they are hollow structures formed by 13 parallel Protofilaments that grow and shorten by the reversible, noncovalent addition of tubulin dimers at their ends. Tubulin is a protein that contains two subunits called α and β in a head to tail arrangement. The microtubule polymerization proceeds via the nucleation elongation mechanism. The nucleation is slow in comparison with the elongation of the microtubule. The heterodimer polymerization and degradation is not only simple equilibrium process and the microtubules show dynamics that use for assembly energy obtained from GTP hydrolysis(Jordan & Wilson, 2004).

2.2.2 Functions of microtubules

Microtubules include nucleic and cell division, organization of intracellular structure, and intracellular transport, as well as silvery and Flagler mobility. Since the functions of microtubules are very important for the existence of eukaryotic cells(Kumar, Abbas, Fausto, & Aster, 2014).

2.2.3 Building blocks of microtubules – tubulins:

Blocking micro-organisms using tubulin targeting agent is a legitimate approach to anticancer therapy. Tubulin protein always been an important and realistic approach for anticancer drug discovery. Microtubules, major structural components in cells, are the target of various synthetic, semi artificial and naturally occurring anticancer drugs and thousands antitubulin compounds have been exposed (Dall'Acqua, 2014).

All eukaryotic cells produce the protein tubulin, in the usual way. The usual way is by transcription of genes coding for tubulin to produce messenger RNA, followed by the translation of mRNA by the ribosomes in order to produce protein. Cells maintain at least two types of tubulin called alpha **tubulin and beta tubulin**. These two types of tubulin can found in cells as individual proteins(Keene, 2007).

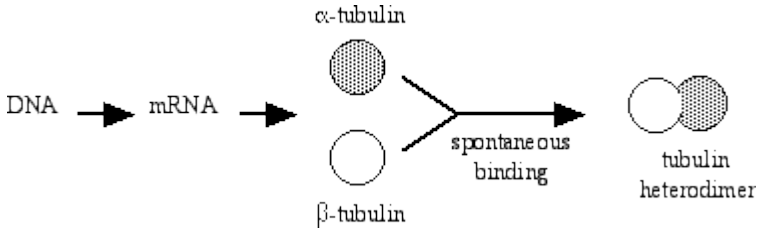


Figure 2.2: Tubulin of heterodimer

Alpha and beta tubulin spontaneously bind one another to form a functional subunit called a heterodimer. A heterodimer is a protein that consists of two different gene products. The term heterodimer consist of - the prefix *hetero-* means "different," the prefix *di-* means "two," and the suffix *-mer* refers to a unit, in this case a single polypeptide. Cells do not continue to make tubulin (or any other protein) until they run out of resources. A common regulatory mechanism is feedback inhibition.

The figure illustrates the inhibition of tubulin synthesis by the presence of heterodimers in the system.

2.2.4 Assembly of microtubules

When intracellular conditions favor assembly, tubulin heterodimers assemble into linear *Protofilaments*. Protofilaments in turn assemble into microtubules. All such assembly is subject to regulation by the cell.

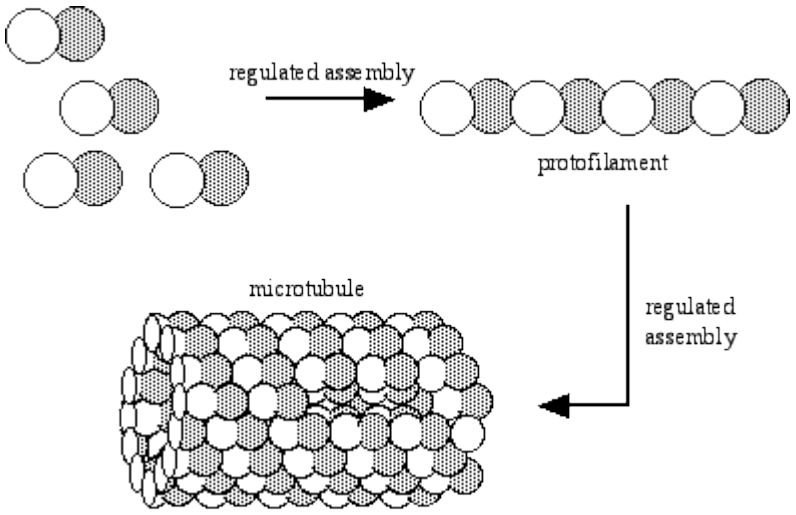


Figure 2.3: Assemble of microtubule

Microtubules form a framework for structures such as the spindle apparatus that appears during cell division, or the whip like organelles known as cilia and flagella.

Cilia and flagella are the well-studied models for microtubule structure and assembly.

2.2.5 Dynamic instability of microtubules:

Under steady state conditions a microtubule may appear to be completely stable, however there is action taking place constantly. Populations of microtubules usually consist of some that are shrinking and some that are growing. A single microtubule can oscillate between growth and shortening phases. During growth, heterodimers are added on to the end of a microtubule, and during shrinkage they come off as intact subunits. The same heterodimer can come off and go back on.

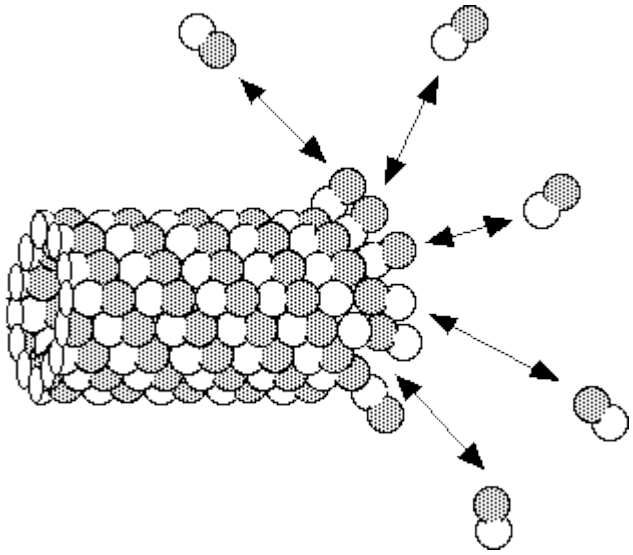


Figure 2.4: Dynamics instability of microtubules

Since obviously there is internal instability in static micro tubular structures, so they are considered in a dynamic equilibrium or stable condition.

In this kind of dynamics, microtubules switch between phases of growth and shortening. The changes in length at the plus end are greater than at the minus end. Microtubules can also interrupt growth and shortening and keep a constant length. This is known as a microtubule pause. Periods of slow lengthening are relatively long in contrast with brief periods of quick shortening. Dynamic instability is characterized by four variables: the rate of growth, the rate of shortening, catastrophe (frequency of transition from a growth or paused state to shortening)

and rescue (frequency of transition from shortening to growth or a paused state)(Bowne-Anderson, Zanic, Kauer, & Howard, 2013).

Tubulin is a 100 kD dimer of two 50 kD polypeptides designated alpha and beta. Each tubulin subunit composed of around 445 amino acid residues. These two chains are 36- 42% homologous to each other. The chains of stable alpha-beta tubulin heterodimers are called Protofilaments. Four separate small-molecule binding site are present on tubulin-system:

(i) Colchicine site

(ii) Vinca site

(iii) Toxoid site and

(iv) Laulimalide

Out of these colchicine and vinca site are located on monomeric unpolymerized α, β- tubulin and toxoid site on the polymerized microtubule. Ligands binding to colchicine site destabilizes the tubulin cytoskeleton by interfering with the dynamic instability of 7 microtubules, spindle poison arrest dividing cells in G2/M phase of the cell cycle, causing mitotic arrest and finally apoptotic cell death results(Lieu, North, & Rajak, 2013).

2.2.5.1 Colchicine site

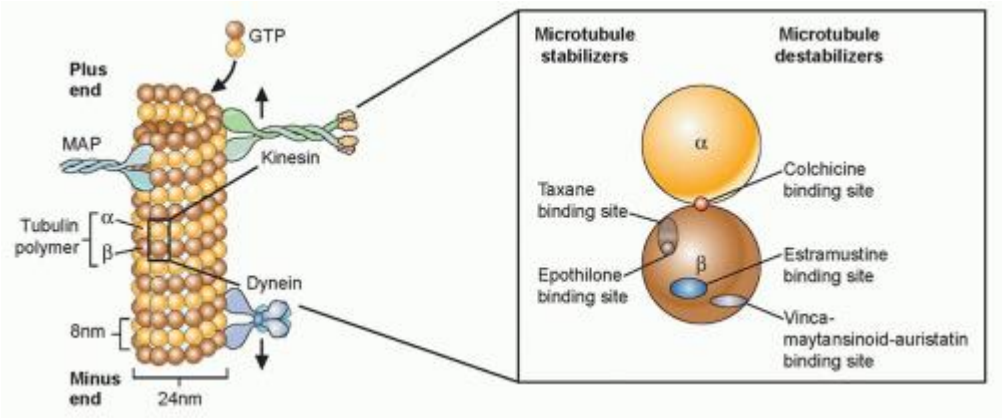


Figure 2.5 Antimicrotubule agents bind tubulin directly or inhibit its associated proteins. Texans and epothilones have distinct binding pockets within the same site on the interior surface of the tubule. Estramustine has a distinct site on β-tubulin, although it also directly binds microtubule-associated proteins (MAP)(Lieberman & Marks, 2009).

The structure elucidation of the α, β-tubulin complex with N-diacetyl-N-(2-mercaptoacetyl) colchicine (DAMA colchicine) and the α, β tubulin complex with podophyllotoxin confirmed the hypothesis that colchicine and podophyllotoxin bind to β- tubulin at its interface with α-tubulin. Several natural and synthetic small compounds with different structures have been shown to bind at the colchicine site of a tubulin protein, inhibit tubulin dynamics and polymerization and cause mitotic arrest. Large molecular diversities among colchicine site inhibitors are beneficial for drug design, but it is necessary to determine the structural features of drugs, which are responsible for ligand-protein interaction.

Due to structural variability of ligands binding to colchicine binding site, they are divided in to two groups:

(i) Prototypical and

(ii) Atypical colchicine binding site inhibitors.

Typical colchicine site inhibitors composed of similar structure elements as colchicine or podophyllotoxin. They are bi-aryl and rigid system having trimethoxyphenyl moiety. The molecules of the second group lack at least one of these features and usually contain other structural element. As the number of rotatable bond increases they show less interaction with colchicine binding site(Vilanova et al., 2014).

Among all the colchicine site agents, Combretastatin has received special attention in the last decade or example of typical colchicine binding site inhibitors in addition to its potent cytotoxicity and inhibitory activity on tubulin polymerization, Combretastatin are one of the few anti microtubule agents reported to have selective vascular targeting activity, and it is an agent that selectively destroyed tutor blood vessels and resulted in tutor cell death.

2.3 Role of tubulin in cancer

Members of the combretastatin family possess varying ability to cause vascular disruption in tumours. Combretastatin binds to the β-subunit of tubulin at what is called the colchicine site, referring to the previously discovered vascular disrupting agent colchicine. Inhibition of tubulin polymerization prevents cancer cells from producing microtubules(de Leeuw et al., 2015).

Microtubules are essential to cytoskeleton production, intercellular movement, cell movement, and formation of the mitotic spindle used in chromosome segregation and cellular division. The anti-cancer activity from this action results from a change in shape of vasculature endothelial cells. Endothelial cells treated with Combretastatin rapidly balloon in shape causing a variety of effects which result in necrosis of the tumour core(Dumontet & Jordan, 2010).

The tumour edge is supported by normal vasculature and remains, for the most part, unaffected. As a result it is likely that any therapeutic use will involve a combination of drugs or treatment options.

2.4 Mechanism of action of anti-mitotic agents

Microtubule inhibitor alters the dynamic of tubulin polymerization and depolymerisation. It involve in the inhibition of chromosome segregation in mitosis and inhibit the cell division. Microtubule- targeted antimitotic drug divided into two groups 1) microtubule destabilizing agent e.g. vinca alkaloids, colchicine and Combretastatins etc.

Vinca alkaloids used to treat leukaemia, non-Hodgkin's lymphoma and testicular germ cell tumours. Microtubule stabilizing agents (paclitaxel, epothilones and discodermolide) stimulate microtubule polymerization. Paclitaxel was clinically approved for used to treat breast cancer, ovarian cancer and Kaposi's sarcoma. Microtubule stabilizing and destabilizing drug supress the microtubule dynamics without alter the microtubule polymer mass. The suppression of microtubule dynamics leads to block mitosis and induced apoptosis(Stanton, Gernert, Nettles, & Aneja, 2011).

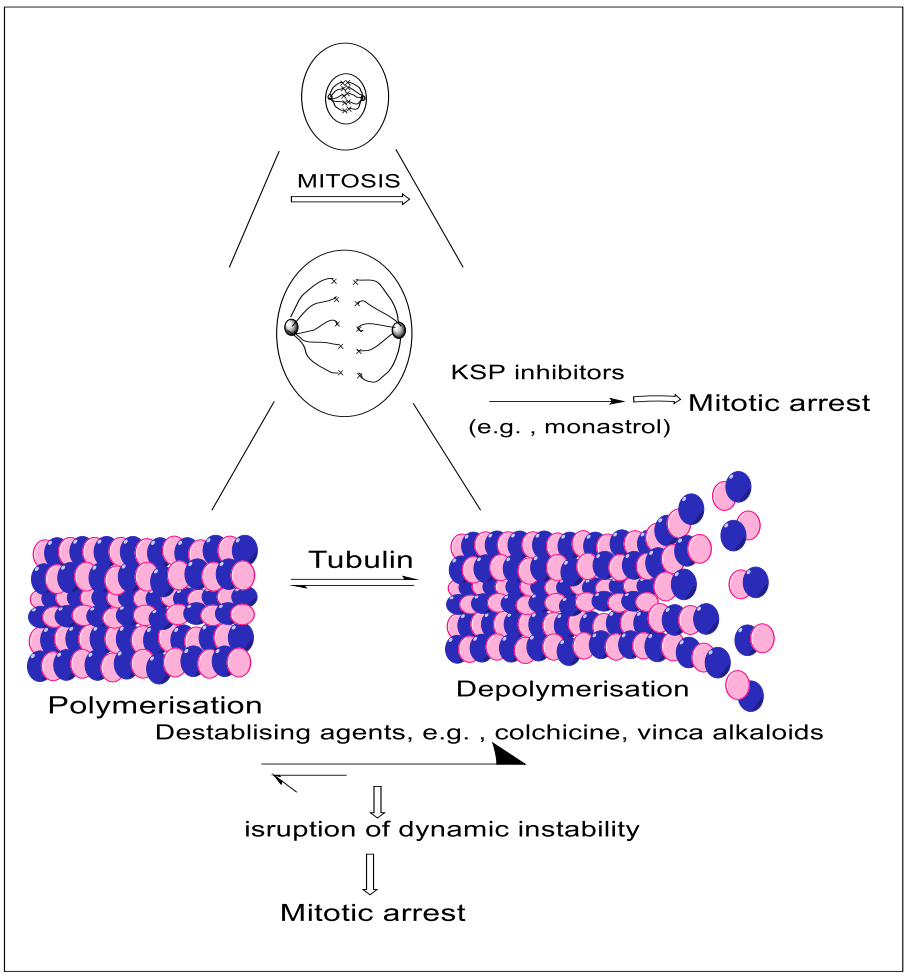


Figure 2.6 Mechanism of action of antimitotic drug

2.5 Introduction to combretastatins

Combretastatins are a class of natural phenols. Combretastatin is a small organic molecule found in the bark of the African bush willow tree *Combretum caffrum*, identified by Professor George R. Pettit, the Director of the Cancer Research Institute based at Arizona State University in the USA. Despite having a similar name, Combretastatins are unrelated to statins, a family of cholesterol lowering drugs(Cragg, Kingston, & Newman, 2011).

Scientists at the CRI have not only completed a total synthesis for Combretastatin, but have isolated it on scales large enough to allow clinical testing, and they have also produced water-soluble phosphate derivatives, which are helping the drug's delivery to patients. Professor Pettit, like many great scientists, is driven towards a goal, that of finding treatments for cancers.

2.5.1 Isolation

The combretastatin was separated from the bark of the South African tree Combretum caffrum and it is most potent anti-mitotic agents. The beautiful Bush Willow tree only grows on the banks of rivers in the Eastern Cape Province of South Africa. Local historians believe that for over 2000 years Arabians traded for the bark with the San people (Bushmen). The bark was probably used as a general tonic in those days, because, apart from its anti-cancer properties, the bark also produces a feeling of improved general wellbeing. Like many useful natural extracts it can be harmful in the wrong dose and it has been used as a poison for Zulu spears.

The most potent of these is combretastatin A-41 which has been found to be a potent cytotoxic agent which strongly inhibits the polymerization of tubulin by binding to the colchicine site.2 Combretastatin A-4 (1) is also able to elicit irreversible vascular shutdown within solid tumours, leaving normal vasculature intact.3 A prodrug of combretastatin A-4, the water soluble phosphate derivative4 2 is now in phase II of clinical trials(Gaukroger et al., 2001).

2.5.2 Natural combretastatins

Molecules that fall into the Combretastatin family generally share 3 common structural features: a trimethoxy "A"-ring, a "B"-ring containing substituents often at C3' and C4', and often an ethene bridge between the two rings which provides necessary structural rigidity. Molecules with such an ethene bridge are also stilbenoids, molecules with a non-ethene bridge are dihydrostilbenoids(Marrelli et al., 2011).

2.5.3 Structure of combretastatin

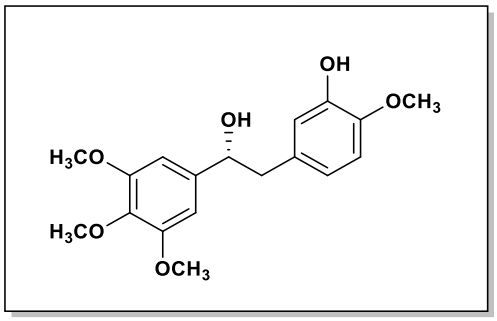


Figure 2.7 Combretastatin

Combretastatins are biaryls connected by an ethylene bridge. Restricted rotation about the olefin bridge seems crucial in these molecules. Professor Pettit and his colleagues have managed to add water-solubility to these natural products by replacing the phenolic H atom with phosphate groups.

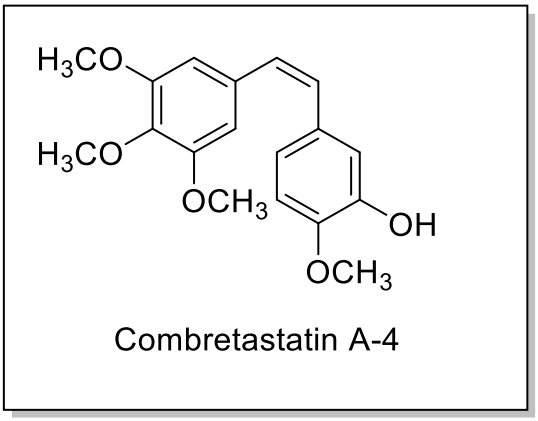


Figure 2.8 Combretastatin A-4

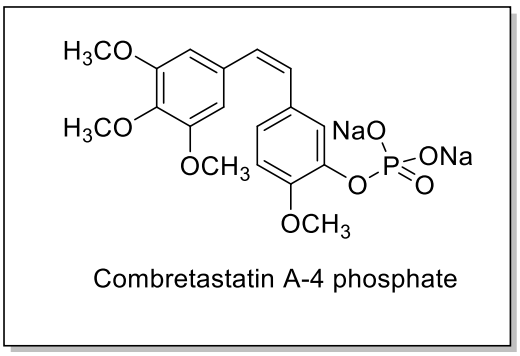


Figure 2.9 Combretastatin A-4 phosphate

2.5.4 Synthesis of combretastatins

2.5.4.1 Palladium-catalyzed Suzuki cross-coupling

Synthesis of the Z Isomer of Combretastatin A-4 Using the Palladium-Catalyzed Suzuki Cross-Coupling of (Z)-5-(2′-Bromoethenyl)-2-methoxyphenol and 3,4,5-Trimethoxybenzene Boronic Acid(Gaukroger et al., 2001).

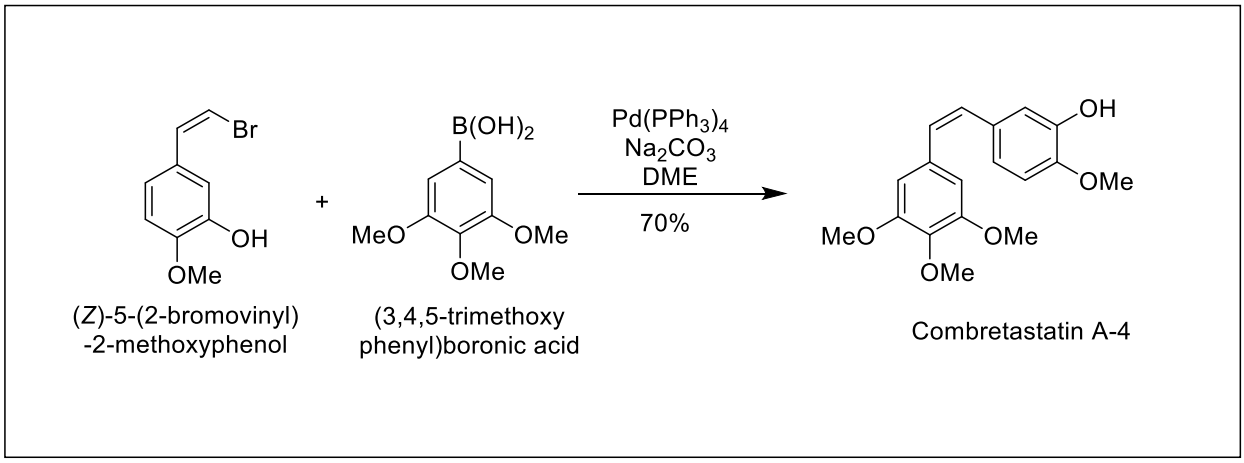


Figure 2.10: Palladium-Catalyzed Suzuki Cross-Coupling

2.5.4.2 Perkin condensation

Perkin Condensation of 3, 4, 5-Trimethoxyphenylacetic Acid and 3-Hydroxy-4-methoxybenzaldehyde

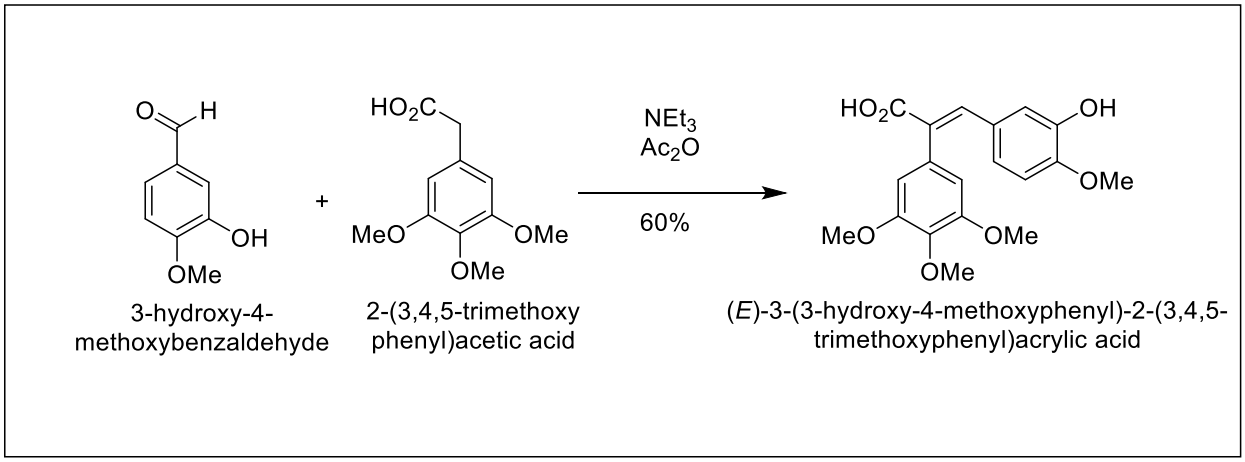


Figure 2.11: Perkin condensation

(E)-5-(2-Bromoethenyl)-2-methoxy-phenol was reacted with 3, 4, 5-trimethoxybenzene Boronic acid as described above. The reaction produced Trans

combretastatin A-4 in moderate yield (ca. 40%) and 3, 3′, 4, 4′, 5, 5′-hexamethoxybiphenyl in low yield (ca. 6%).

2.6 Computational chemistry

Use of computational methods in the field of chemistry has increased importantly in near in time years. Different applications of computational chemistry are possible. Computational chemistry gives details of molecular bonding and it is not possible to get these details by experimental methods. Modelling a molecular system before synthesizing that molecule is one of the computational chemistry's best applications(Leo & Hoekman, 1995).

With the putting into effect of computational chemistry, great change took place in almost every field of chemistry which has opened up different new things in the operation of making observations and development part of-the-day pharmaceutical chemistry. In our time computational methods are generally used in the qualitative and able to be measured put value of substances in all fields of knowledge of chemistry(Corwin Hansch, Leo, Hoekman, & Livingstone, 1995).

The drug discovery process began in 1905 by John Langley. The pharmaceutical operation of making observations and development process is time taking and faces many questions. In order to undergo growth a single new medical substance, almost < 15 years are needed to make it reachable to persons getting care, therefore the mean price of each with a good outcome medical substance sometimes used for amusement increases having different forms and uses.

This value also includes the price of coming short of one's hopes of thousands of those makes adjustment about payment of debt those which were included for operation of making observations and development system of pipes, but finally only one medical substance sometimes used for amusement gets approval. Thus the medical substance sometimes used for amusement discovery way gives hope and rest to millions of persons getting care(C Hansch & Leo, 1995).

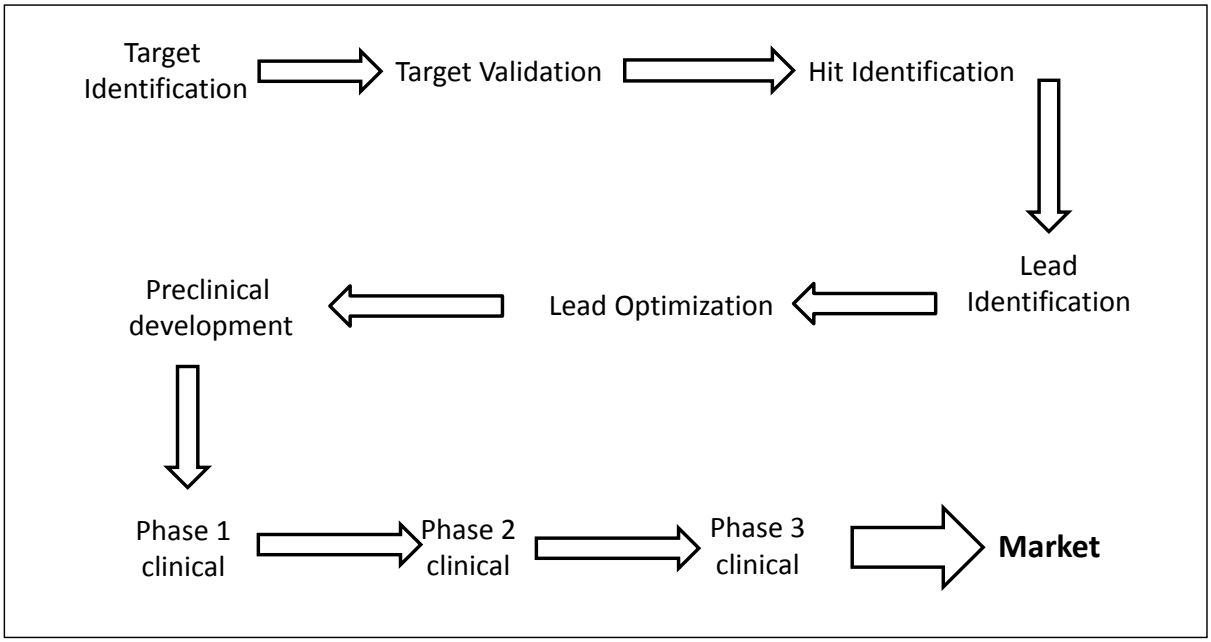


Figure 2.12: The drug development procedure: From discovery to commercialization

2.6.1 Discovery phase

2.6.1.1 Recognition processes (Puzyn, Leszczynski, & Cronin, 2010)

1) Target Identification
2) Target Validation
3) Hit Identification
4) Lead Identification
5) Lead Optimization

1) Target Identification:

Initially, the main reason of diseased are found, then pharmaceutical researchers choose a “target” for the creation of new medicines for it. Generally, “target’ is a single molecule, for example, gene or protein, which are the main outburst of particular diseases. That protein is taken as target which is “drug able” that is which allured and drug molecules is affected by it.

2) Target validation

The use of prior methods for validating targets is still a big question because the medicine is failed in clinical trials. Therefore, scientists select such a target which really gets involved in the disease and drug can work on this diseased. With the help of jumbled experiments, researchers demonstrates that a specific target is applied to the ailments.

3) Hit identification:

In this step, “hit” compounds are identify for choosing target like receptor, enzyme, from various libraries. To test new chemical entities (NCEs), high –throughput screening (HTS) and in silico evaluations are used with desirable pharmacodynamics (PD) activity. The biochemical and physiochemical effects of the molecular entity in the body are described by pharmacodynamics (PD) properties. Generally, the drug discovery and development process are separate denture-clinical and clinical phases.

4) Lead identification:

In the next step, analysis of a group of molecules is done for their activities in order to test suitability. The “hit” molecules with good physiochemical and ADME are recognized and taken further as lead compounds.

1. Molecular structure of the drug
2. Behaviour of the drug in the bio phase
3. Geometry of the receptor
4. Drug-receptor interaction
5. Changes in the structure on binding
6. Observed biological response

5) Lead optimization:

To nominate the drug candidates, different attributes of lead analogues are valuated. Consequently, by applying various structural modifications to lead molecular scaffold, lead analogues are generated. Selection of drug candidates is done on the basis of optimal potency, solubility and ADME properties. Detection and optimization of lead compound are performed using a computational method which includes quantitative structure activity relationship (QSAR) and molecular docking.

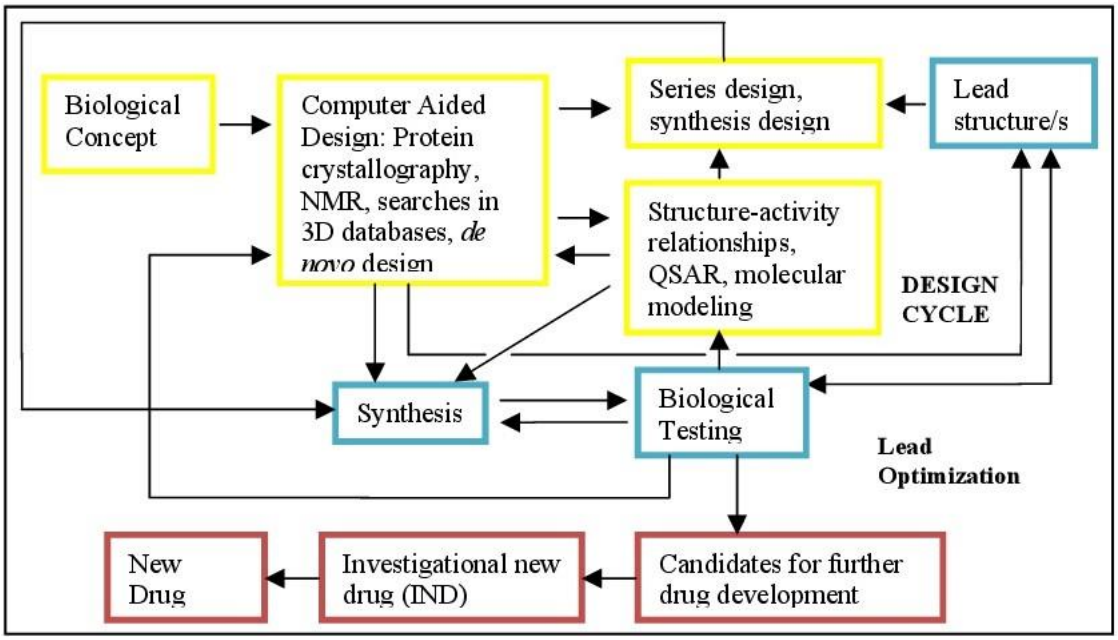


Figure 2.13:-Flow for the lead optimization

2.6.2 Technologies in CADD:

1. Ligand-based drug design (Pharmacophore-based approach),
2. Structure-based drug design (Molecular docking based approach) and quantitative structure-activity and quantitative structure-property relationships (QSAR/QSPR).

2.6.2.1 Ligand-based drug design

In the absence of an experimental 3D structure and pIC_{50} value of the target compound, Ligand-based drug design methods are used. Due to the lack of an experimental structure, the known ligand molecules that bind to the drug target are studied to understand the structural and physicochemical properties of the ligands that correlate with the desired pharmacological activity of those ligands. Besides knowing ligand molecules, ligand based methods may also include natural products or substrate analogues that interact with the target molecule yielding the desired pharmacological effect.

2.6.2.2 Structure-based drug design

Structure-based drug design is the design and optimization of a chemical structure with the goal of identifying a compound suitable for clinical testing—a drug candidate. It is based on knowledge of the drug's three-dimensional structure and

how its shape and charge cause it to interact with its biological target, ultimately eliciting a medical effect.(Ferreira, dos Santos, Oliva, & Andricopulo, 2015)

2.7 QSAR (Quantitative structure activity relationship)

Quantitative structure activity relationship (QSAR) help us to predict biological properties of molecules. QSAR suggests that if a group of chemicals show the same mechanism of action towards a target, then alteration in the biological activity also alters chemical, structural and physical properties(Johnson & Maggiora, 1990).

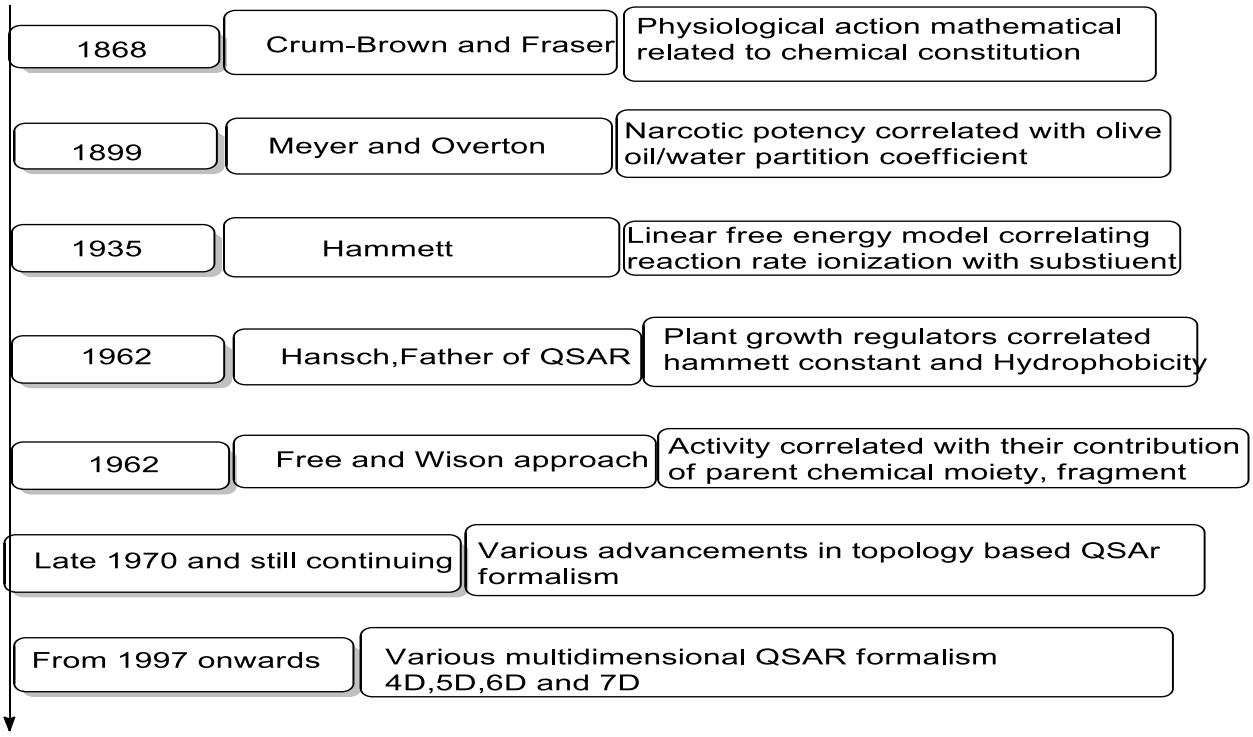
QSAR methods are not only used in drug designing but are widely used in other sciences too, i.e., biology, toxicology(Hansen et al., 1995), environmental toxicology, agro chemistry, pharmaceutical chemistry, etc. The QSAR is also used to determine the initial and final point of a synthesis(Bradbury, 1995).

This in turn reduces the number of compounds that could be practically /experimentally synthesized. This part of QSAR is not only beneficial for the pharmaceutical industry, but also to the environmental regulatory authority and human beings for reduction in toxic effects(Tong et al., 1999).

QSAR models are not only used for the prediction of properties but are also helpful in selection of alternative mechanism of action, determination of useful structural characteristics, projecting new design methodologies and help in proposing new hypotheses for future research work.

Thus QSAR decreases the cost, time and human resources to make that drug reachable to patient. QSAR models are also used in anticipation of pharmacokinetic and pharmacodynamics properties. QSAR also anticipates properties like permeability, and solubility.

2.7.1 Evolution of QSAR



2.7.2 Brief history of QSAR

More than a century ago QSAR originated in toxicological field. In year 1863, Cross proposed the relationship between the toxicity of primary aliphatic alcohols with their water solubility. In 1868-1869, Crum-Brown and Fraser postulated the idea that the chemical composition and constitution of any substance helps to determine the physiological action of that selected substance. After a few decades later Richet in 1893 showed the inverse relationship between the cytotoxicities of various organic molecules to their corresponding water solubilities(Hammett, 1935).

Hammett in year 1935 introduced an equation which tells the reaction mechanisms by considering substituent constant and reaction constant. Ferguson in 1939 correlates the depressant action with their relative saturation of volatile compounds in the vehicle using thermodynamic generalization. In bacteriostatic activity the importance of ionization of bases and weak acids was given by Albert, Bell and Roblin in between 1942-1985. In year 1935 and 1970 Hammett gave “sigma-rho” for the delineation of substituent effects on organic reactions.

Taft in 1952 introduced the first stearic parameter, Esfor separating polar, steric and resonance effects. Both Hammett and Taft contribute for mechanistic basis for QSAR paradigm development. Hansch and Muir published in year 1962 the structure activity relationships of plant growth regulators and its dependency on Hammett constants and hydrophobicity. In 1975 a new hydrophobic scale was introduced by giving the whole series of partition coefficients which was measured using octanol/water system (Smith, Hansch, & Ames, 1975).

In 1960's Hansch, Fujita and co-workers established the modern field of QSAR. They correlated the whole molecular properties with their biological activity using statistical methods. They introduced an equation named as Hansch equation which relates the biological activity to certain electronic characteristics and the hydrophobicity of a set of structures.

$$\text{Log}\left(\frac{1}{c}\right) = K_1 \text{logP} - K_2 (\text{logP})\ 2 + K_{3s} + K_4$$

Here, C is the minimum effective dose

P is the octanol-water partition coefficient

S is the Hammett substituent constant

Ks is the constants derived from regression analysis.

Besides Hansch approach, Free-Wilson approach were also introduced in year 1964 by Free and Wilson. Free-Wilson access for structure activity studies in an agnate sequences are shown by the given equation:

$$BA=\sum a_i x_i + u$$

Where BA stands for biological activity

U stands for the average contribution of the parent molecule

ai stands for the contribution of each structural feature

Xi indicates the presence of X_i =1 or X_i = 0 for a particular structural fragment.

Due to some limitations Fujita-Ban equation was introduced in 1971. Fujita-Ban equation used logarithm of activity to get the activity criterion in line with other free energy related terms.

$$\text{Log BA} = \sum G_i X_i + u$$

Where *u* is called the calculated biological activity value of unsubstituted scaffold compound of a particular series.

G_i is called for biological activity addition of substituents

X_i is give with a value of one if the substituent is present and zero when substituent is not present.

After 1960's so many changes has been done on Hansch original approach for solving various problems of structure activity/property relationship by using QSAR analysis. Topological methods to address the relationships between molecular structure and physical/biological activity, connectivity indices based on hydrogen–suppressed molecular structures and also electropological indices encoding significant structured information on topological state of atoms etc. are also applied to biological and toxicological data. In modern drug designing QSAR/QSPR analysis is one of the best fundamental tools for modern drug designing(Veith, Call, & Brooke, 1983).

2.7.3 Requirements of QSAR

All the molecules being considered for QSAR study should belong to a congeneric Series and act through the same mechanism of action. Normally molecules in a series of compounds have the similar basic moiety but they have different substituents, they can bind to the same target in the same manner and hence all analogues are supposed to interact in a comparable manner(Martinez et al., 2005). In order to perform QSAR, we require following conditions

1) Wide range in observed activities.
2) Identical mode of action.
3) Concentration in molar units.
4) Activity data as a function of concentration (IC_{50}).
5) Activity data in percentage.
6) Possible time dependency.

2.7.4 Development of QSAR model

For constructing a QSAR/QSPR model mainly four steps are involved as shown in below given(Nantasenamat, Isarankura-Na-Ayudhya, Naenna, & Prachayasittikul, 2009).

1. Data collection;
2. Pre-processing (like data curation, descriptors calculation, descriptors filtering, outliers removal);
3. Model generation;
4. Model validation

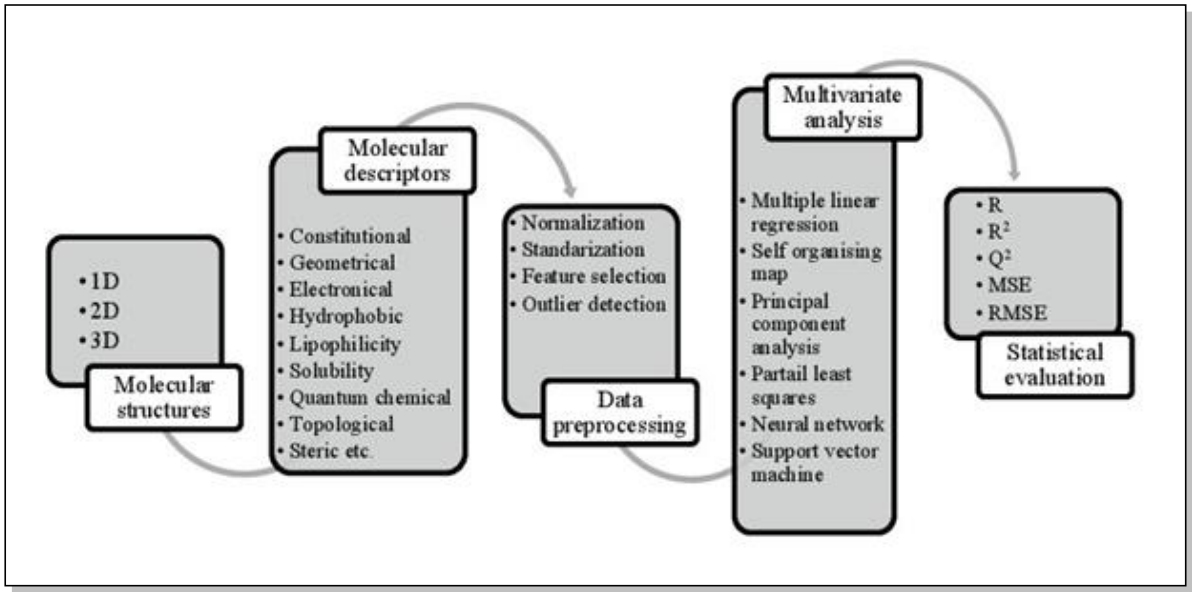


Figure 2.14:-Schematic Overview of QSAR Process

2.7.5 Classification of QSAR

Based on dimensions, QSAR are of following types

0. 0D-QSAR: These descriptors are calculated by using molecular formula e.g. atomic number, type of atom, molecular weight etc.

1. 1D-QSAR: Affinity correlates with pKa, logP, etc.

2. 2D-QSAR: Affinity correlates with a structural pattern (e.g., chemical connectivity). Connectivity indices, 2D-pharmacophones etc., without considering the 3D representation of these properties.

-Hansch analysis (linear free energy relationships)

-Martin-kubinyi analysis (non-linear free energy relationship)

-Free Wilson analysis

3. 3D-QSAR: 3D-QSAR correlates the activity with non-covalent interaction fields which surrounds the molecules

-Comparative molecular field analysis (Co MFA)

-Molecular shape analysis

-Hypothetical active site lattice (HASL) technique

4. 4D-QSAR: As with 3D, but with multiple representations of ligand conformation/orientation.

5. 5D-QSAR: As with 4D, but with multiple representations of induced-fit scenarios.

6. 6D-QSAR: As with 5D, but with multiple representations of solvation models. (Source: Based on information provided by Biographic Laboratory 3R)

2.7.6 Applications of QSAR (Jhanwar, Sharma, Singla, & Shrivastava, 2011)

- It is basically used to correlate chemical and physical properties of compounds with their biological activity.
- It helps in Differentiating drug molecules from nondrug molecules
- It helps in prediction of physicochemical properties (e.g. lipophilicity, water solubility)
- It helps in study of ADME (adsorption, distribution, metabolism, and excretion) prediction
- It helps in to study Drug resistance
- It helps in prediction of Toxicity

2.8 3D-Quantitative Structure-Activity Relationships (3D QSAR)(Matyus & Borosy, 1998)

3D-QSAR is used for the calculation of force field of three-dimensional structures. It depends on the protein crystallography and molecule superimposition. It provides the information of whole molecule instead of a single substituent and requires computed potentials (Lennard-Jones potential) instead of experimental constants.

Based on the applied energy function, 3D-QSAR calculates the electrostatic fields, hydrophobic, H-bond donor, H- bond accepter and the steric fields. The 3D-QSAR delivers the analysis of quantitative relationship between the three-dimensional properties of a compounds with their biological activity through statistical correlation methods. The 3D QSAR approach is categorized into two approaches i.e. The

ligand-based and structure-based approaches. The ligand-based 3D QSAR is applied when the fine 3D structure of the particular target protein is not obtained experimentally.

CHAPTER-3
RATIONALE

3. Rationale

Tubulin is important target in the anticancer drug development. Out of the four binding site on the tubulin there is no drug available in the market targeting colchicine binding site. QSAR is important strategies for the drug development. Current research purpose will help in the identification of some potential ligands targeting colchicine binding site.

CHAPTER-4
OBJECTIVES

4. Objectives

With this literature background we set the following objectives:

1. To perform 3D-Field based QSAR on Combretastatins analogue in order to recognize structural features which are responsible for Tubulin inhibitors activity.
2. Design of proposed compounds containing the following nucleus.

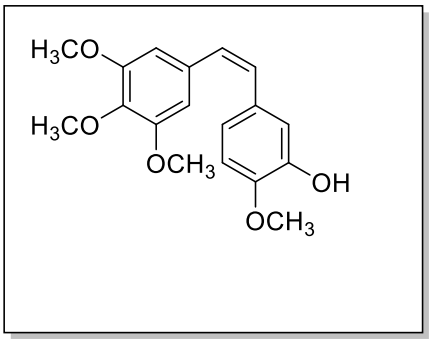


Figure 4.1 Combretastatins analogue

CHAPTER-5
METHODS AND MATERIALS

5. Methods and materials

5.1 Procedure of 3D QSAR Program:

There are many programs available for QSAR and molecular modelling. One of them is discussed below is Using Software for QSAR Study **Schrödinger Software Release 2017-2(MAESTRO11.1)** and the following steps are given as**:**

Collect the structure of the tubulin inhibitors which contains combretastatin nucleus through literature search.

QSAR software requires pIC_{50} value instead of IC_{50} value. This conversion can be done by using given formula

$$\mathbf{pIC_{50} = 6\text{-}log10\ (IC_{50})}$$

Draw structure of tubulin inhibitors (collected through literature search) using Chem Draw and save file of collected structure in SDF format in any folder.

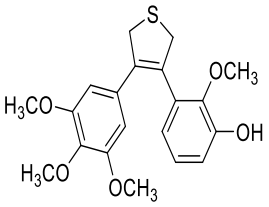
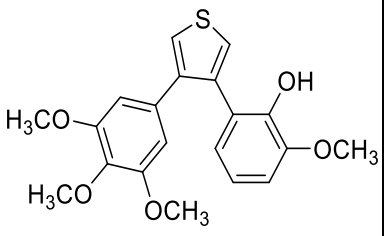
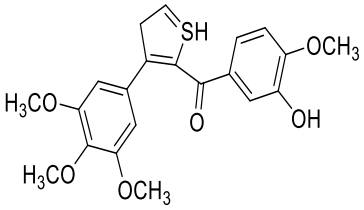
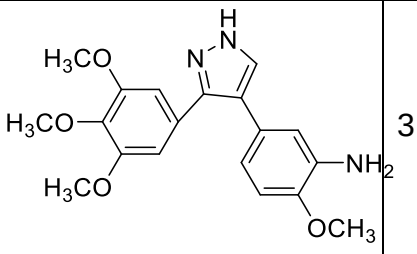
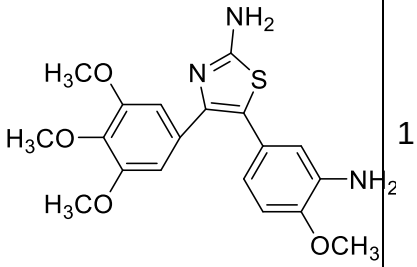
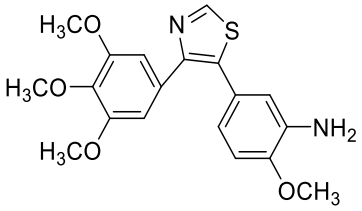
For the purpose of QSAR study of tubulin inhibitors, open **MAESTRO 11.1** software and click on the file and then select new project.

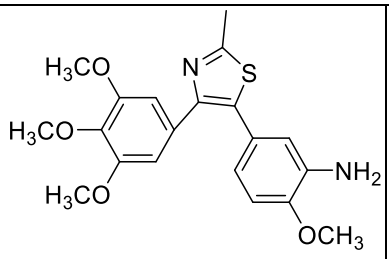
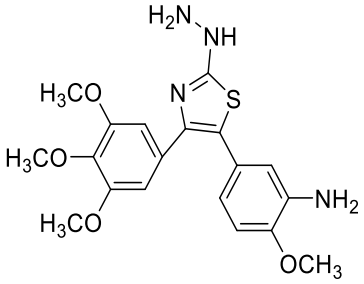
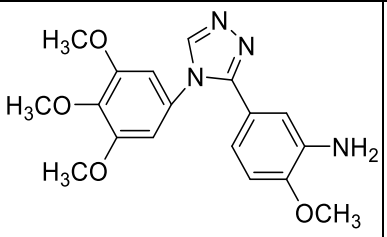
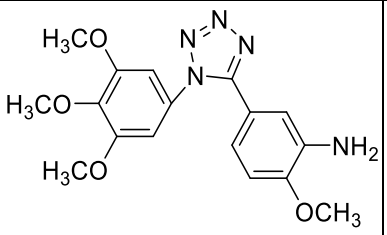
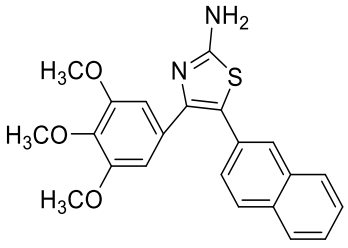
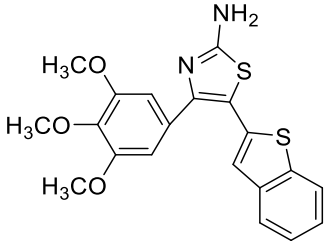
Next step is to import the structure from folder of collected structure. This can be done by on clicking file then click on import structure and select the folder of collected structure.

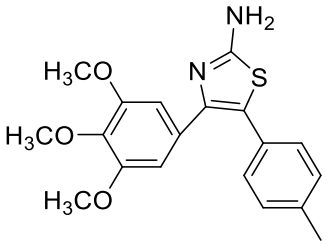
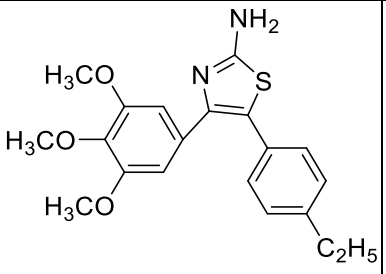
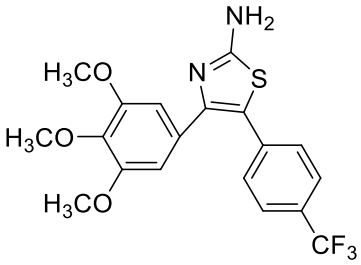
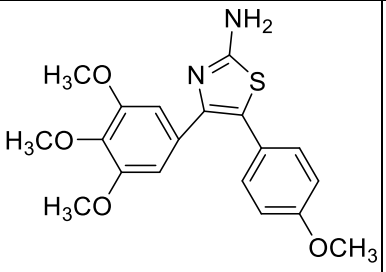
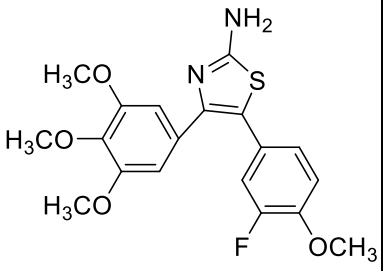
5.2 Structure used during the study of 3D-QSAR

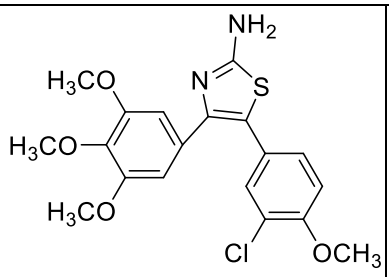
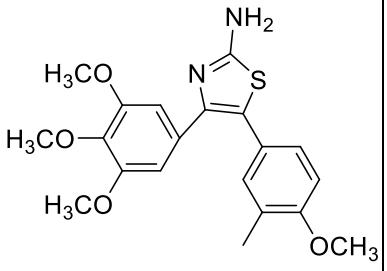
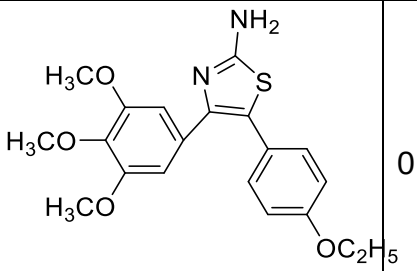
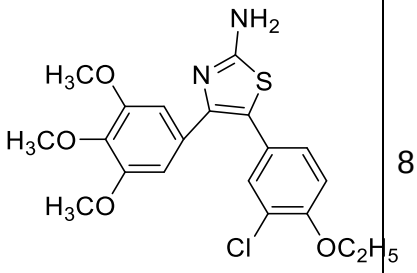
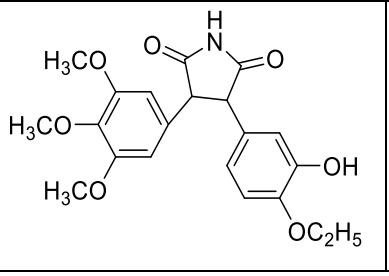
This table shows the conversion of IC_{50} into pIC_{50} of reported tubulin inhibitors which contains Combretastatin nucleus.

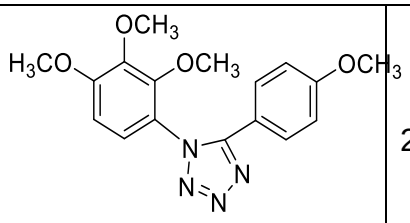
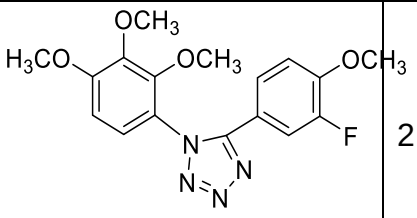
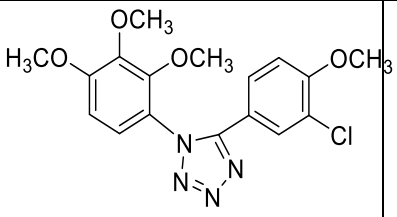
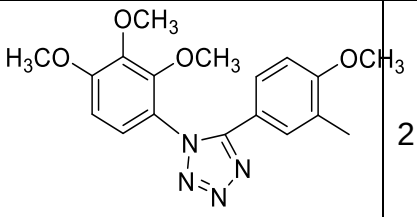
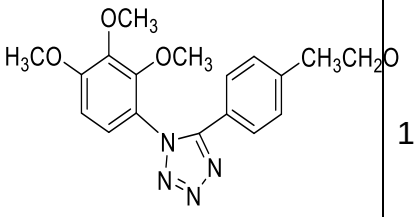
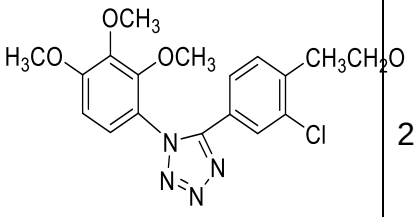
Table 5.1 Structure used

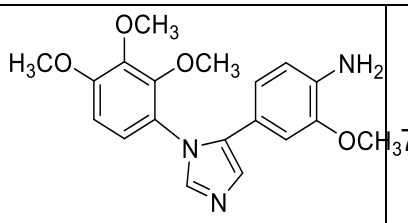
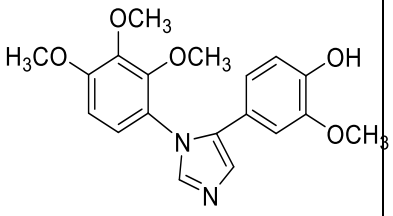
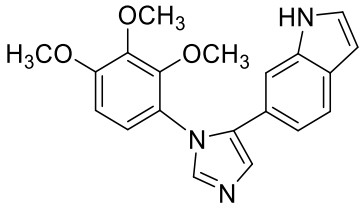
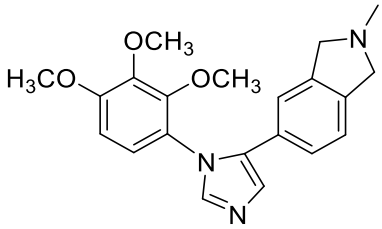
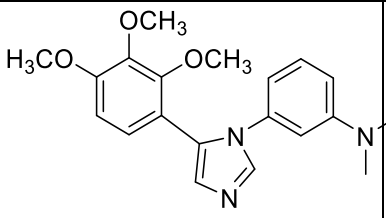
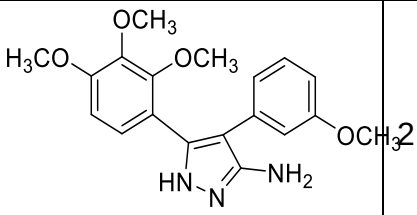
Sr.N.	Structure Name	Structures	IC_{50}	IC_{50}/ 1000000	pIC_{50}
1.	1		3.6	0.0000036	5.4436
2.	2		1	0.000001	6
3.	3		8.8	0.0000088	5.0555
4.	6		3	0.000003	5.5228
5.	7		1	0.000001	6
6.	8		3	0.000003	5.5228

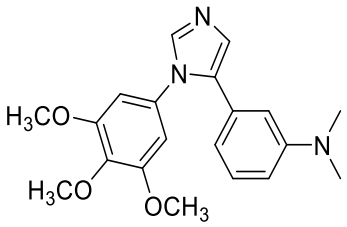
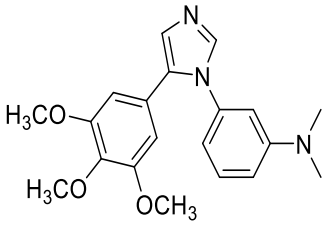
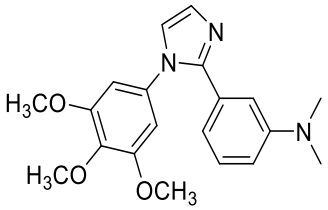
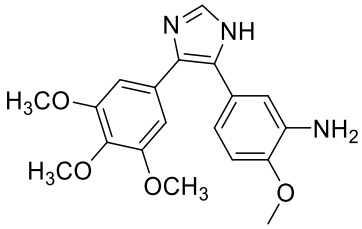
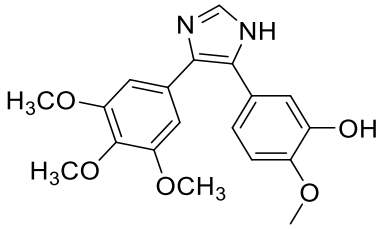
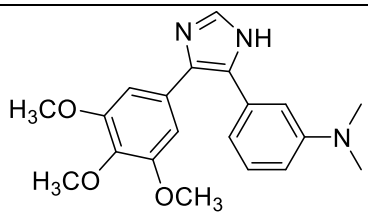
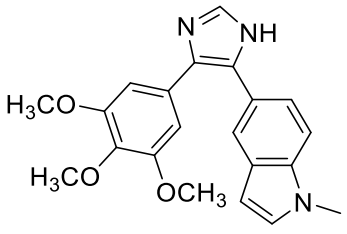
7.	9		2	0.000002	5.6989
8.	10		3	0.000003	5.5228
9.	11		12	0.000012	4.9208
10.	15		2	0.000002	5.6989
11.	16		0.74	0.0000007 4	6.1307
12.	17		2	0.000002	5.6989

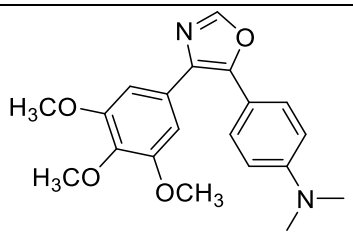
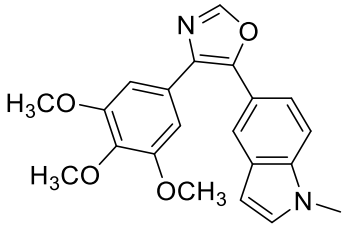
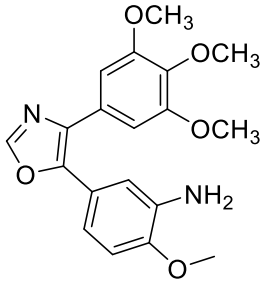
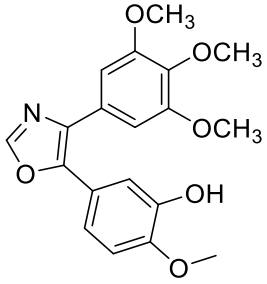
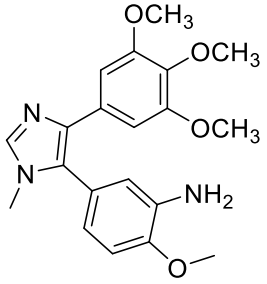
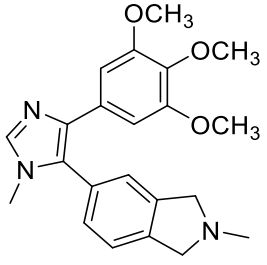
13.	18		0.44	0.00000044	6.3565
14.	19		1.1	0.0000011	5.9586
15.	20		4	0.000004	5.3979
16.	21		1.1	0.0000011	5.9586
17.	22		1.2	0.0000012	5.9208

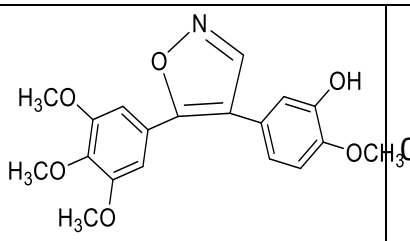
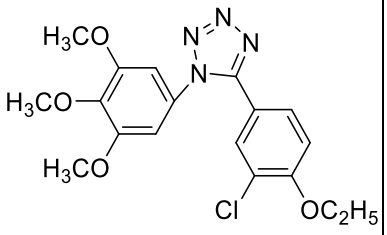
18.	23		0.88	0.00000088	6.0555
19.	24		1.1	0.0000011	5.9586
20.	26		0.61	0.00000061	6.2146
21.	27		8.9	0.0000089	5.0506
22.	28		7.6	0.0000076	5.1191

23.	30		2.5	0.0000025	5.6020
24.	31		2.1	0.0000021	5.6777
25.	32		3.5	0.0000035	5.4559
26.	33		2.5	0.0000025	5.6020
27.	34		1.1	0.0000011	5.9586
28.	35		2	0.000002	5.6989

29.	40		7.7	0.0000077	5.1135
30.	41		7.6	0.0000076	5.1191
31.	44		71	0.000071	4.1487
32.	45		1.8	0.0000018	5.7447
33.	46		48	0.000048	4.3187
34.	47		2.4	0.0000024	5.6197

35.	48		23	0.000023	4.6382
36.	49		130	0.00013	3.8860
37.	50		35	0.000035	4.4559
38.	51		0.68	0.0000006 8	6.1674
39.	52		0.73	0.0000007 3	6.1366
40.	53		3.7	0.0000037	5.4317
41.	55		1.1	0.0000011	5.9586

42.	56		0.87	0.00000087	6.0604
43.	57		0.79	0.00000079	6.1023
44.	59		0.92	0.00000092	6.0362
45.	60		0.98	0.00000098	6.0087
46.	64		31	0.000031	4.5086
47.	66		2	0.000002	5.6989

48.	82		0.75	0.00000075	6.1249
49.	84		2.8	0.0000028	5.5528

5.3 Ligand alignment

Ligand alignment can be done by selecting flexible shape based alignment in flexible ligand alignment and then click on selected entries in order to align the ligands so that we can see the binding site of all the ligands.

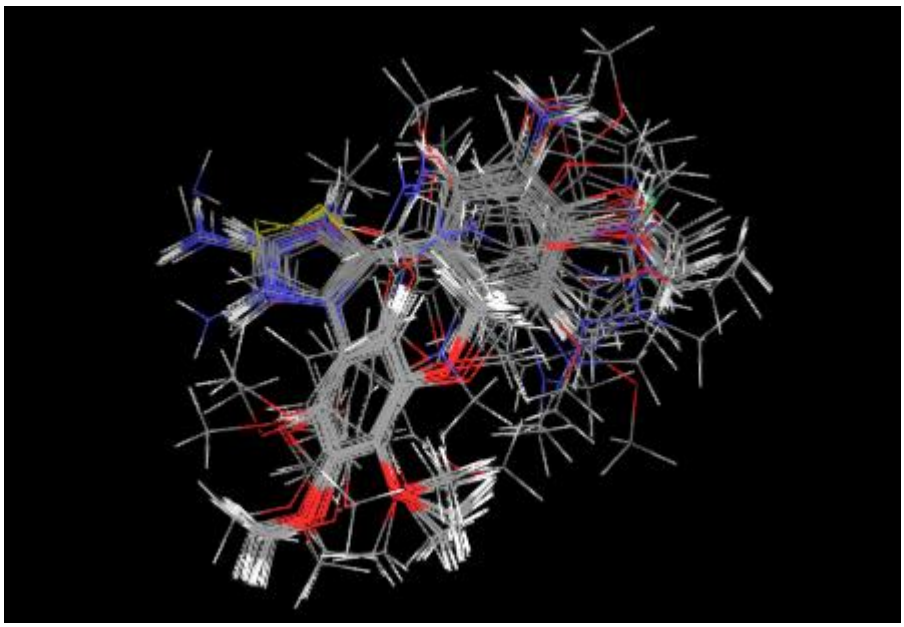


Figure 5.1 All 49 compounds were aligned with the best pharmacophore selected for 3D QSAR study for Tubulin Inhibitors

5.4 Add property

Click on add property in data and then click on property name (like IC50 value) in order to add any property. After adding property table will be shown which shows

the property like tautomer probability, ionization penalty, state penalty and shape sim in addition to pIC_{50} value.

5.5 Random training set

For best result, its percentage should be from 60-70 % in order to show good activity value but our result show best result at 70%.

5.6 Training Set and Test Set

The data set consists of the compounds with their biological activities, which is divided into two subset test set and training set. Approximately 30 % are selected by seeing wide span in activity and assigned as test set, with the remaining 70 % assigned to training set. The training set is used for QSAR model development and test set is used for model validation.

The QSAR in a generalised way consists of following steps:

(a) Calculation of various physiochemical and structural parameters.

(b) Correlation and regression analysis.

(c) Activity prediction of new designed compounds.

(d) Prediction error

5.7 Random seed

In this, we have to select a particular number which shows best activity value. Our best activity value shown by the ligand at random seed value of 999995909.

After clicking on apply our ligand dividing into training set and test set in 30 % and 70 % respective value.

For best result R^2 and Q^2 value have a difference of 0.2 and value of R^2 and Q^2 should be nearer to 1.

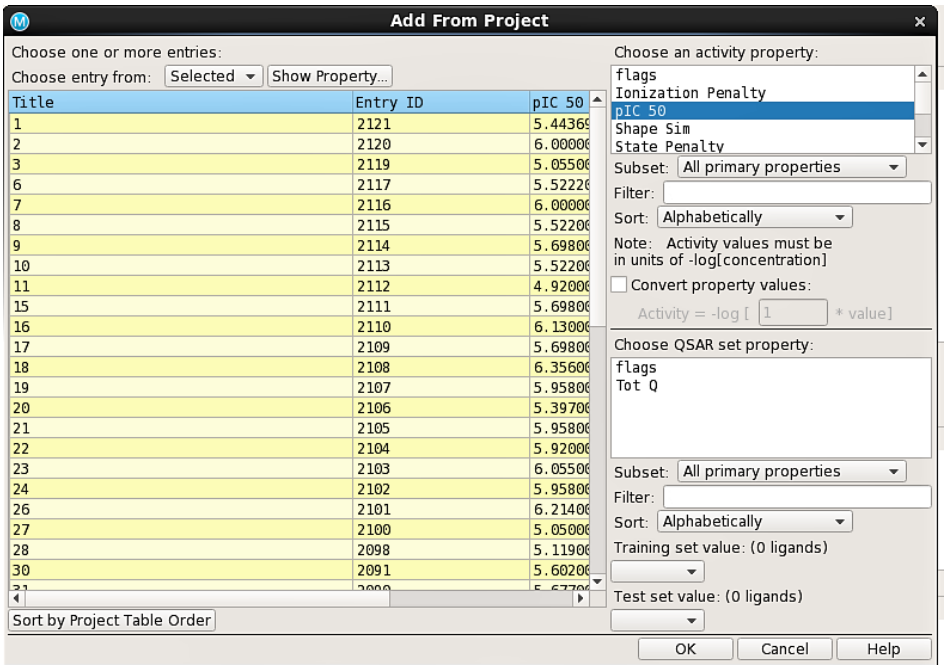


Figure 5.2 Adding the Ligands for the Model

5.8 Build field based QSAR model

This can be done by on clicking Build field based QSAR model in model icon. After that select the field style (Gaussian) and choose maximum PLS (partial least sequence) of 6 and then click ok. It will show QSAR statistics in which data is shown in the form of table which contains factors SD, RMSE, Pearson-r, R^2 and Q^2 etc.

Another table also shows that contains the value of factor like Gaussian steric, Gaussian electro static, Gaussian hydrophobic, Gaussian H-bond accepter and Gaussian H-bond donor.

If the difference in the value of R^2 and Q^2 is more than 2, then we have to plot called scatter plot which differentiate between the less active and more active ligands. Scatter plot is the graph between predicted value and experimental value.

5.9 3D QSAR and PLS analysis (Silverman & Platt, 1996)

3D QSAR models were then developed for the pharmacophore hypothesis using the training set structures that match the pharmacophore on three sites. However, we utilized the three models or the 3D QSAR studies by generating pharmacophore based 3D QSAR models and PLS analysis.

Hypotheses showed the highest survival score, all selected 49 molecules for 3D QSAR study were aligned with that selected pharmacophore as shown in Figure. To develop superlative 3D QSAR models which were meant to exhibit reliable

predictions it necessitated internal and external statistical validation. Models which were capable of fulfilling statistical validation parameter boundaries were expected to display more reliable predictions. Random training set is used 70% then apply to the random seed which is used in the Field-based QSAR as random seed number (999995909). When 70% random training set is applied to random seed, all the value of training set and test set changes. Some PLS values R^2 and Q^2 are shown after clicking the icon build, then next step is to select that value shows the difference of which around 2 between the values R^2 and Q^2.

A model with PLS factor six (6) was considered as the best statistical model. This model was validated by predicting activities of test set, training set and overall molecules. Randomly we chose compounds in the training set and in the test to develop 3D QSAR model. Important parameters obtained based on favoured the internal statistical validation by PLS analysis.

5.10 QSAR statistics term

Table 5.2 QSAR statistics term

Sr. no.	QSAR statistics Term	Meaning
1.	# Factors	It represents the number of factors in the partial least squares regression model.
2.	SD	It means Standard deviation of the regression.
3.	R^2	It represents the regression and value of R^2 is always between 0 and 1.
4.	R^2 CV	It represents Cross-validated R^2 value
5.	R^2 Scramble	It represents Average value of R^2 from a series of models built using scrambled activities.
6.	Stability	It represents Stability of the model predictions to changes in the training set composition and its Maximum value is 1.
7.	F	It represents the ratio of the model variance to the observed activity variance.
8.	P	It represents the significance level of F when applied on ratio of Chi-squared distributions. P value of 0.05 indicates that F is significant at the 95% level.
9.	RMSE	Root-mean-square error
10.	Q^2	It represents the Value of Q^2 for the predicted activities. And values if the variance in the errors is greater than the variance in the observed activity values thenQ^2 can take on negative values.
11.	Pearson-r	It represents correlation between the predicted and observed activity for the test set.

Among the models, the best hypothesis should show good external predictive ability for each combination as compared to others. Randomly software study only 15 compounds in the test set while 34 compounds in the training set. Important parameters obtained based favoured the internal statistical validation by PLS analysis.

A good R^2 value for the training set of 0.8953, good predictive power with Q^2 of 0.7111 for the test sets, with SD of 0.2019, and F value of 39.9.

5.11 Statistical parameters

Table 5.3 Statistical parameters

Statistical parameters	**Values**
PLS Value	999995909
# F	6
SD	0.2019
R^2	0.8953
R^2 CV	-1.5751
R^2 Scramble	0.8225
Stability	-1.54
F	39.9
P	1.85e-12
RSME (Root-mean-square error)	0.33
Q^2	0.7111
Pearson-r	0.8578

5.12 Field-Based QSAR

The field-based QSAR models are based on CoMFA (Comparative molecular field analysis) and CoMSIA (comparative molecular similarity index analysis). CoMFA field based models are constructed by calculating the field value like the electrostatic field It is require to add the ligand from the above the table which is created in add property step. There are two types of property

Show property – this property expresses about the shape and structure of the ligand. Show property can be done by choosing pIC_{50} from show property menu and then click ok.

Active property –this property expresses where we can add which group so that activity of ligand can be alter. Active property can be done by choosing pIC50 from active property menu and then click ok.

This experiment exercise for developing a field-based QSAR model from Maestro. In field based QSAR, we studied about three dimensional structure of ligands and also we can study the shape of each and every part of ligand. Field based QSAR also predicts which binding site of ligand is better for specific group in order to increase the activity.

5.13 Activity of ligands after Field-QSAR

The table are shown the ligands the build and after predicted value and prediction error. We have selected max PLS value of 6, and our results of activity and predicted activity also comes neared to 6.so we can conclude that our PLS value of 6 is best in order to study interaction of groups.

Table 5.4 Activity of ligands after Field-QSAR

Sr. No.	Ligands Name	QSAR Set	Activity	PLS	Predicted Activity	Prediction Error	%Extrapolated
1.	1	Training	5.444	1	5.43118	-0.01251	0.00
				2	5.09149	-0.35220	
				3	5.25535	-0.18835	
				4	5.52036	0.076663	
				5	5.51228	0.075121	
				6	5.45863	0.014930	
2.	2	Training	6.000	1	5.45224	-0.54776	0.00
				2	5.48697	-0.51302	
				3	5.79649	-0.20350	
				4	6.09866	0.098652	
				5	6.02691	0.026912	
				6	5.90809	-0.09190	

3.	3	Training	5.055	1 2 3 4 5 6	5.51971 4.96007 4.85547 4.84059 4.96541 5.14002	0.464712 -0.094928 -0.199526 -0.214405 -0.089585 0.085016	0.00
4.	6	Training	5.522	1 2 3 4 5 6	5.41394 5.61794 5.66677 5.68871 5.4092 5.40524	-0.10826 0.095739 0.144567 0.166506 0.113003 0.11696	0.00
5.	7	Test	6.000	1 2 3 4 5 6	6.00496 6.03192 6.10142 6.04553 6.01172 5.90634	0.004957 0.031917 0.101423 01045531 0.011715 -0.093664	0.51
6.	8	Test	5.522	1 2 3 4 5 6	5.80613 6.00217 6.11223 6.01132 5.98283 5.88717	0.279627 0.486174 0.590229 0.489318 0.460835 0.365174	0.25
7.	9	Training	5.698	1 2 3 4 5 6	5.83598 5.96672 6.04873 5.91821 5.86508 5.71598	0.137985 0.268723 0.35073 0.220208 0.167082 0.017982	0.00
8.	10	Test	5.522	1 2 3	5.78911 5.65123 5.76034	0.267112 0.129228 0.238341	3.59

				4	5.76781	0.245813	
				5	5.88991	0.367912	
				6	5.76685	0.244851	
9.			4.920	1	5.50784	0.587836	0.00
				2	5.48282	0.562824	
				3	5.47664	0.556644	
	11	Training		4	5.2233	0.3033	
				5	5.1265	0.206497	
				6	5.01949	0.099492	
10.			5.698	1	5.61023	-0.087772	0.27
				2	5.84638	0.148383	
				3	5.86716	0.169155	
	15	Test		4	5.67156	-0.026440	
				5	5.59489	-0.103111	
				6	5.53257	-0.165429	
11.			6.130	1	5.91496	-0.21503	1.18
				2	5.71281	-0.417194	
				3	5.67176	-0.458241	
	16	Test		4	5.66946	-0.460539	
				5	5.66281	-0.46719	
				6	5.60361	-0.526386	
12.			5.698	1	6.00276	0304758	0.00
				2	5.82371	0.125713	
				3	5.81441	0.116407	
	17	Training		4	5.86729	0.169287	
				5	5.89285	0.194845	
				6	5.82399	0.135993	
13.			6.356	1	5.94258	-0.41342	0.00
				2	5.78602	-0.56998	
				3	5.80168	-0.55431	
	18	Training		4	5.96567	-0.39033	
				5	6.05193	-0.30407	
				6	6.08951	-0.26648	

14.	19	Test	5.958	1 2 3 4 5 6	5.97089 5.80205 5.75373 5.77515 5.80468 5.78896	0.01288 -0.15594 -0.20426 -0.18285 -0.15331 -0.16903	0.00
15.	20	Training	5.397	1 2 3 4 5 6	5.96079 5.63248 5.5663 5.6784 5.6909 5.62714	0.563792 0.235485 0.169297 0.281405 0.293904 0.23014	0.00
16.	21	Test	5.958	1 2 3 4 5 6	5.94314 5.86513 5.85454 5.89008 5.92551 5.95304	-0.014861 -0.092871 -0.103459 -0.06792 -0.032487 -0.004960	0.13
17.	22	Test	5.920	1 2 3 4 5 6	5.96369 5.8775 5.85341 5.82607 5.82127 5.81733	0.043687 -0.04249 -0.06658 -0.093933 -0.098734 -0.102667	0.08
18.	23	Training	6.055	1 2 3 4 5 6	5.96663 5.85313 5.79716 5.74121 5.73257 5.7502	-0.08836 -0.20187 -0.25783 -0.31379 -0.32243 -0.30479	0.00
19.	24	Training	5.958	1 2 3	5.95768 5.81426 5.78217	-0.00032 -0.14374 -0.17582	0.00

				4 5 6	5.77464 5.79765 5.85504	-0.183363 -0.160353 -0.10295	
20.	26	Training	6.214	1 2 3 4 5 6	6.00518 6.05559 6.04191 5.99443 6.00495 6.0314	-0.20882 -0.15840 -0.172092 -0.219568 -0.209053 -0.182603	0.00
21.	27	Training	5.050	1 2 3 4 5 6	6.0107 5.96351 5.87711 5.72927 5.69818 5.71765	0.9607 0.91350 0.827105 0.679265 0.648175 0.667654	0.00
22.	28	Training	5.119	1 2 3 4 5 6	5.05573 5.34258 5.16252 5.16318 5.15097 5.07606	-0.063271 0.223577 0.043517 0.044179 0.103197 -0.042941	0.00
23.	30	Training	5.602	1 2 3 4 5 6	5.3578 5.77804 5.52752 5.7774 5.78779 5.83119	-0.2442 0.176044 -0.074481 0.175402 0.185791 0.229191	0.00
24.	31	Test	5.677	1 2 3 4 5	5.4007 5.7435 5.4948 5.6518 5.6368	-0.27630 0.066582 -0.06658 -0.025159 -0.040169	1.72

				6	5.6696	-0.007353	
25.	32	Training	5.455	1 2 3 4 5 6	5.35816 5.73179 5.89657 5.61804 5.41494 5.48255	-0.09684 0.276787 0.441566 0.163037 -0.040063 0.027552	0.00
26.	33	Training	5.602	1 2 3 4 5 6	5.40431 5.67967 5.41449 5.57966 5.59063 5.6848	-0.197688 0.077665 -0.187511 -0.023363 -0.011373 0.082797	0.00
27.	34	Training	5.958	1 2 3 4 5 6	5.4255 5.84171 5.50113 5.61719 5.6107 5.70516	-0.532502 -0.116287 -0.456874 -0.34081 -0.347303 -0.252839	0.00
28.	35	Training	5.698	1 2 3 4 5 6	5.4028 5.89738 5.61688 5.77007 5.76127 5.80742	-0.295205 0.199381 -0.081118 0.072067 0.063265 0.109424	0.00
29.	40	Test	5.113	1 2 3 4 5 6	5.23263 5.40329 5.26173 5.53583 5.50332 5.43769	0.119635 0.290288 0.148733 0.422831 0.390316 0.324695	3.92
30.	41	Training	5.119	1 2	5.09683 5.06258	-0.022172 -0.056422	0.00

				3	5.1426	0.023647	
				4	5.03691	-0.082085	
				5	5.15374	0.034736	
				6	5.11457	-0.004427	
31.			4.148	1	4.9365	0.788496	0.00
				2	4.53945	0.391453	
				3	4.15934	0.011338	
	44	Training		4	4.33792	0.189918	
				5	4.38077	0.23277	
				6	4.16629	0.018286	
32.			5.744	1	4.82191	-0.922091	0.00
				2	5.15344	-0.590556	
				3	5.62917	-0.114826	
	45	Training		4	5.54388	-0.200119	
				5	5.72801	-0.015994	
				6	5.7788	0.034797	
33.			4.318	1	5.08653	0.768535	0.00
				2	4.34249	0.024490	
				3	4.34496	0.026965	
	46	Training		4	4.2763	-0.041702	
				5	4.09366	-0.224342	
				6	4.3114	-0.006596	
34.			5.619	1	5.40437	-0.214635	0.00
				2	5.13837	-0.480628	
				3	5.50998	-0.109016	
	47	Training		4	5.84574	0.226742	
				5	5.69318	0.074183	
				6	5.7805	0.161496	
35.			4.638	1	5.43724	0.799242	4.85
				2	5.16038	0.522384	
	48	Test		3	5.07105	0.433048	
				4	4.91557	0.277567	

				5	4.89956	0.261562	
				6	4.78582	0.147825	
36.	49	Test	3.886	1	4.8352	0.949202	15.59
				2	4.70976	0.823759	
				3	4.51202	0.626023	
				4	4.66993	0.78393	
				5	4.67263	0.786629	
				6	4.60073	0.71473	
37.	50	Training	4.455	1	5.27906	0.824056	0.00
				2	4.76403	0.309034	
				3	4.6747	0.2197	
				4	4.54894	0.093942	
				5	4.43608	-0.01892	
				6	4.28278	-0.17222	
38.	51	Training	6.167	1	5.51063	-0.656368	0.00
				2	5.5609	-0.606099	
				3	5.72727	-0.439729	
				4	5.83382	-0.333182	
				5	6.0821	-0.084901	
				6	6.08697	-0.080034	
39.	52	Test	6.136	1	5.76311	-0.372894	0.83
				2	6.02594	-0.110055	
				3	6.22156	0.085562	
				4	6.37957	0.243574	
				5	6.49866	0.362662	
				6	6.59704	0.461042	
40.	53	Test	5.431	1	5.25764	-0.173358	1.14
				2	4.8476	-0.583098	
				3	4.88747	-0.543532	
				4	4.93295	-0.498052	
				5	5.11111	-0.319888	
				6	5.06534	-0.365659	
41.	55	Training	5.958	1	5.71475	-0.243252	0.00

				2	5.67923	-0.278768	
				3	5.73773	-0.220267	
				4	5.77273	-0.18527	
				5	5.86436	-0.093639	
				6	5.93226	-0.257382	
42.	56	Training	6.060	1	5.81829	-0.241715	0.00
				2	6.23606	0.17606	
				3	0.36385	0.30385	
				4	6.31942	0.259423	
				5	6.30551	0.245511	
				6	6.23172	0.171723	
43.	57	Training	6.102	1	5.65902	-0.442978	0.00
				2	5.84067	-0.261329	
				3	5.90964	-0.192364	
				4	5.90736	-0.194637	
				5	5.97775	-0.124246	
				6	5.96628	-0.135722	
44.	59	Test	6.036	1	5.75991	-0.276092	2.95
				2	6.07602	0.040024	
				3	6.20402	0.168018	
				4	6.1715	0.135498	
				5	6.19856	0.162563	
				6	6.17729	0.141387	
45.	60	Training	6.124	1	5.73466	-0.389335	0.00
				2	5.94027	-0.183732	
				3	6.06496	-0.059038	
				4	6.11349	-0.010507	
				5	6.15512	0.03112	
				6	6.18116	0.05763	
46.	64	Training	6.008	1	5.73878	-0.269216	0.00
				2	5.91584	-0.92155	
				3	6.02793	0.019932	
				4	5.91063	-0.097369	

				5	5.91099	-0.097012	
				6	5.85306	-0.154944	
47.	66	Training	4.508	1	5.21172	0.703722	0.00
				2	4.93727	0.459267	
				3	4.90003	0.392029	
				4	4.43207	-0.075933	
				5	4.62742	0.119422	
				6	4.61655	0.108551	
48.	82	Training	5.698	1	5.73018	0.032176	0.00
				2	5.8394	0.141403	
				3	5.87739	0.179394	
				4	5.79793	0.099934	
				5	5.77669	0.078388	
				6	5.78365	0.085652	
49.	84	Training	5.552	1	5.16328	0.061284	0.00
				2	5.75094	0.198935	
				3	5.6163	0.064302	
				4	5.32032	-0.231677	
				5	5.23162	-0.300382	
				6	5.32437	-0.22763	

5.14 Scatter plot

Those ligands which are closer to the regression line are more active ligands and those which are far away from the regression line are called less active ligands.

5.14.1 Scatter Plot of training set and test set molecules

Scatter plot are showed the training set and test set data. And the scatter plot looks between predicted activity verses experimental activity. In scatter plot showed the training set and test set nearly the point of line the showing the best activity molecule and the far of line they points are showing the molecules least activity. The scatter plot appeared both training set and test set activity.

(The green round of show the least active molecules and the red round of showing the most active molecules)

In the scatter plot, those ligands which shows less activity, their activity can be increased by exchanging the training set with test set ligands.

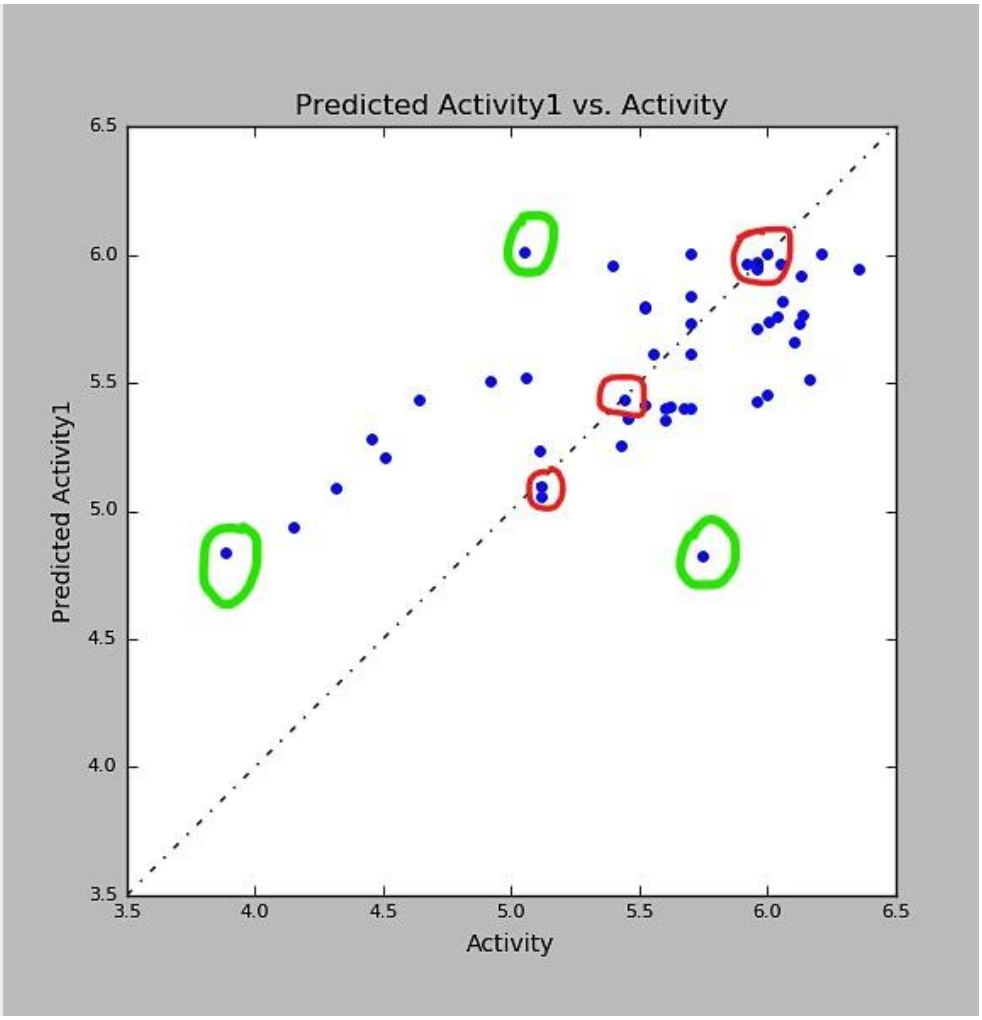


Figure 5.3 Scatter plot of the experimental activity vs predicted activity of tubulin inhibitors of training set and test set.

5.14.2 Scatter plot of test set of molecules

These are the molecule which are most active and shown in the given scatter plot.

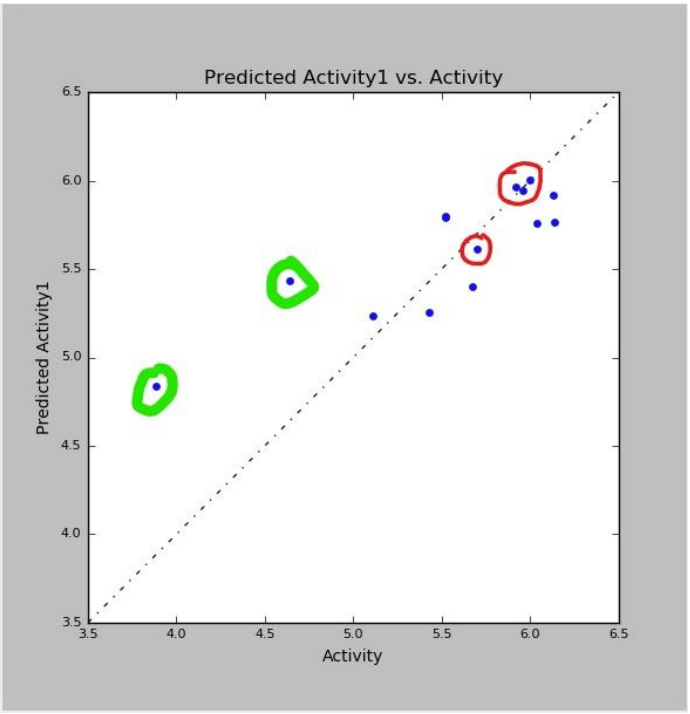


Figure 5.4 Scatter plot of the experimental activity vs predicted activity of tubulin inhibitors of test set.

5.14.3 Scatter plot of training set of molecules

These are molecules which are most active and shown in the given scatter plot.

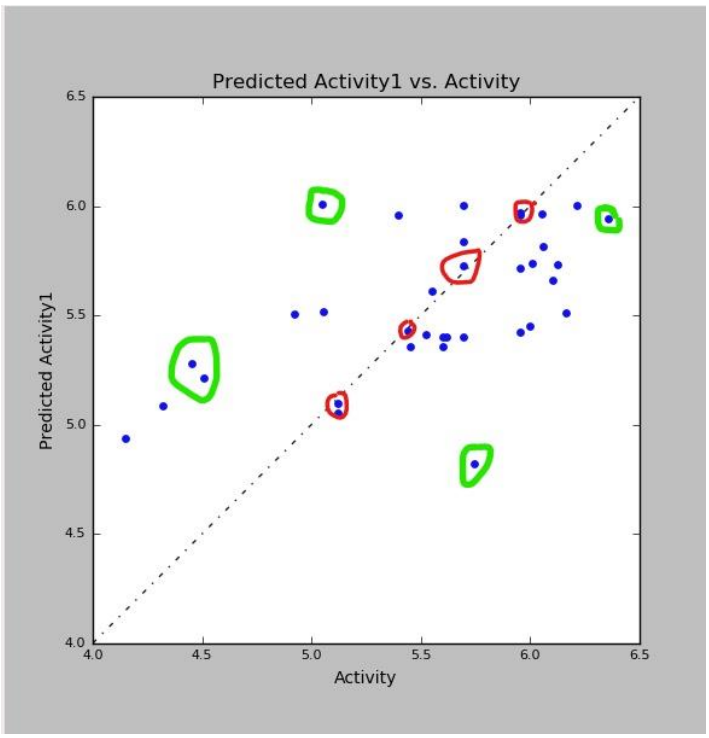


Figure 5.5 Scatter plot of the experimental activity vs predicted activity of tubulin inhibitors of test set.

CHAPTER-6

RESULTS AND DISCUSSION

6. Results and discussion

6.1 QSAR visualization

It shows where we add which group on which position so that activity of the ligands can be increased.

6.2 Interaction

By the help of the interaction we identify the activity of the ligands**.** The 5 best active ligands (16, 18, 26, 51, 52, and 60) out of 49, showed good interaction like steric (Bulky group), electrostatic, hydrophobic, H-bond donor and accepter group interactions. These interaction show the position and the effect of activity. (Activity increase and activity decrease)

6.2.1 Colours for field contours

Table 6.1 Colours for field contours

Field-Based QSAR from Maestro 11.1 **Colours for field contours**		
Field Type	**Positive**	**Negative**
Gaussian Steric	Green	Yellow
Gaussian Electrostatic	Blue	Red
Gaussian Hydrophobic	Magenta	White
Gaussian H bond Acceptor	Red	Pink
Gaussian H bond Donor	Purple	Cyan

6.2.2 Site responsible for activity

The given figure represents how any group (may be electrostatic, hydrophobic etc.)Alters the activity of combretastatin containing nucleus of tubulin inhibitor.

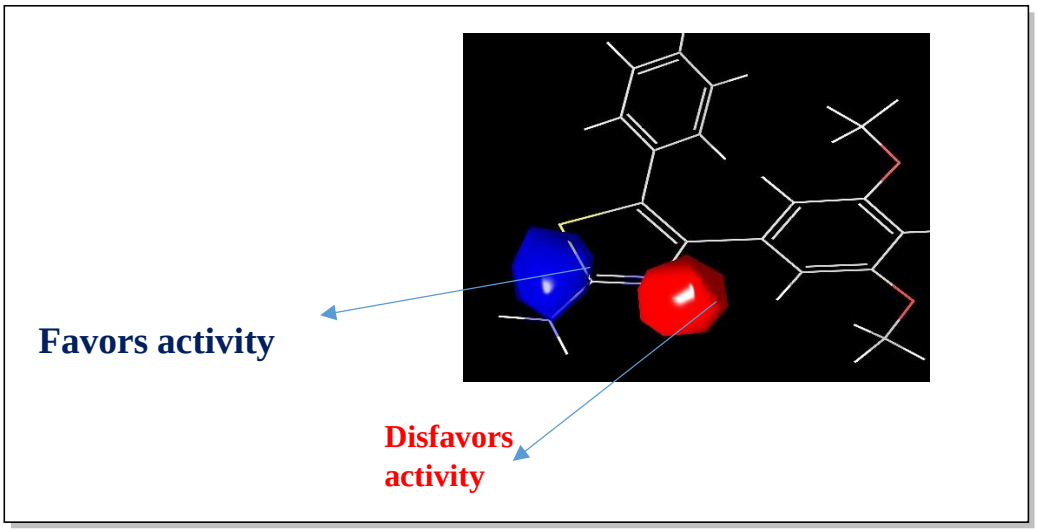


Figure 6.1 Site responsible for activity

6.3 Contour map

Activity will depends upon substitution of any group in the specific region selected by the 3D-QSAR study.

6.3.1 Contour map of Gaussian Electrostatics

In electrostatic interactions both attractive and repulsive forces are taken for association with the making of charge in a molecule. Electrostatic interactions are not strong forces which are useful for the traditional bonds and also halt the formation of a traditional bond, if consider the Electrostatics effect.

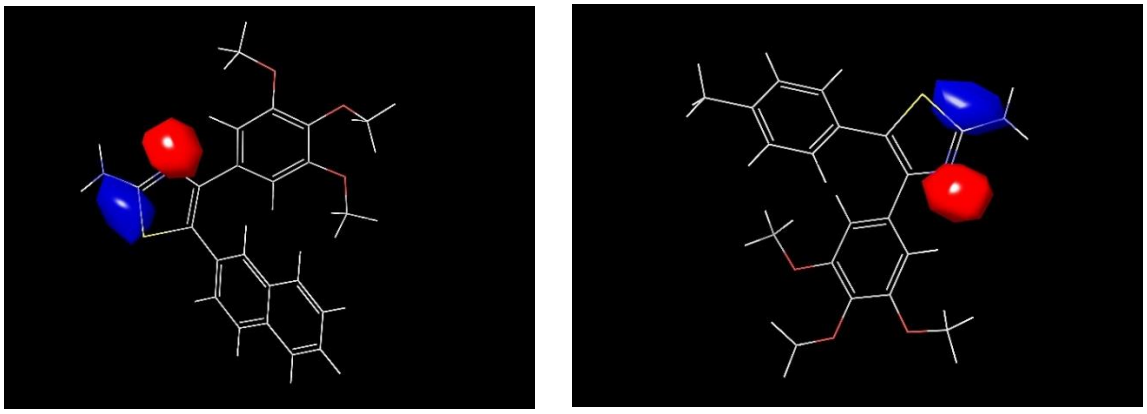


Figure 6.2 Contour map of Gaussian Electrostatics of best active ligands16 and 18

The figure shows how Gaussian Electrostatics group alters the activity of the best active two ligands (16, 18) of combretastatin containing nucleus. If we add Electrostatics group on the red coloured binding site of combretastatin containing nucleus, activity will be increased and if we add Electrostatics group on the blue coloured binding site of combretastatin containing nucleus, activity will be decreased.

6.3.2 Contour map of Gaussian steric

Steric effects are the effects seen in molecules that come from the fact that atoms occupy space. When atoms are put close to each other, this costs energy. The electrons near the atoms want to stay away from each other. This can change the way molecules want to react. It can also change the shape (or conformation) of the molecule. The amount of space that a group of atoms takes is called the steric bulk. An example of steric effects is steric hindrance. This is when a large group in a molecule makes reactions not work.

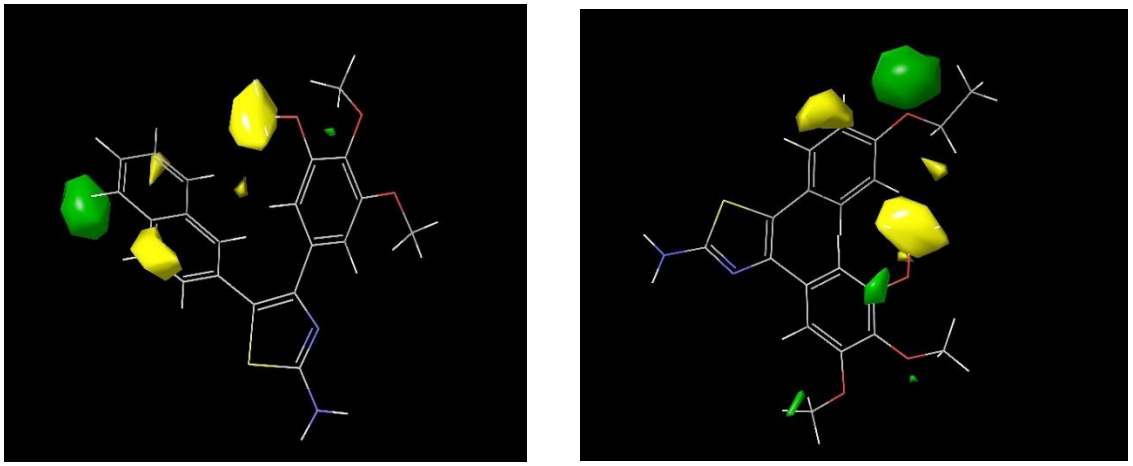


Figure 6.3 Contour map of Gaussian Steric of best active ligands 16 and 26

The above figure shows how Gaussian steric (bulk) group alters the activity of the best active two ligands (16, 26) of combretastatin containing nucleus. If we add steric (bulk) group on the green coloured binding site of combretastatin containing nucleus, activity will be increased and if we add steric (bulk) group on the yellow coloured binding site of combretastatin containing nucleus, activity will be decreased.

6.3.3 Contour map of Gaussian Hydrophobic

Hydrophobic compounds and solvents are nonpolar. Consequently they not form hydrogen bonds with water and with other polar solvents, but these compound are not highly soluble or partially soluble in polar solvents like alcohols. Hydrophobic compounds not contain the polar functional groups that which can formed weak hydrogen bonds with water or alcohols.

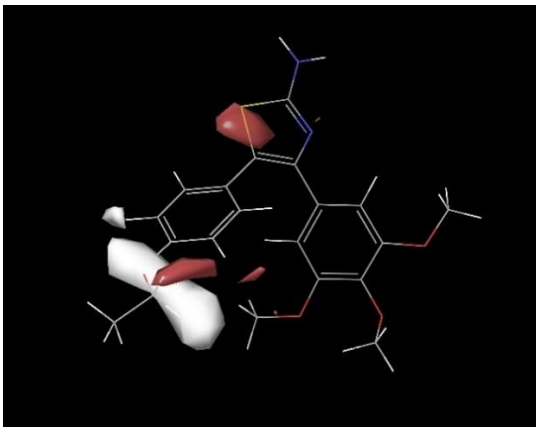
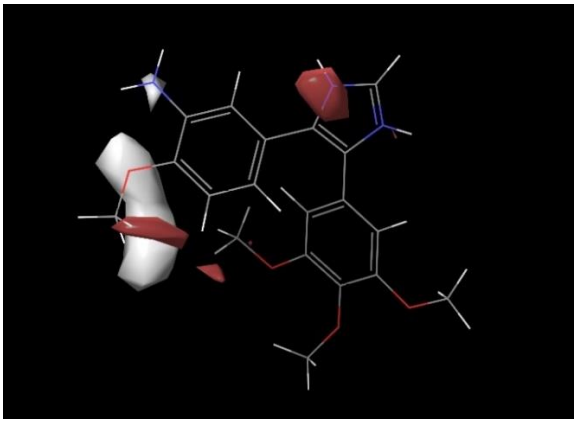



Figure 6.4 Contour map of Gaussian hydrophobic of best active ligands 26 and 51

The figure shows how Gaussian hydrophobic group alters the activity of the best active two ligands (26, 51) of combretastatin containing nucleus. If we add hydrophobic group on the white coloured binding site of combretastatin containing nucleus, activity will be increased and if we add hydrophobic group on the magenta coloured binding site of combretastatin containing nucleus, activity will be decreased.

6.3.4 Contour map of Gaussian H-bond donor

The hydrogen bonding can be described as noncovalent intermolecular interaction. For hydrogen bonding the size of anion must be small and it has high electronegative and another small, highly electronegative atom with an unshared electron pair. The elements that usually participate in hydrogen bonds are small size and high electronegative like nitrogen, oxygen, and fluorine.

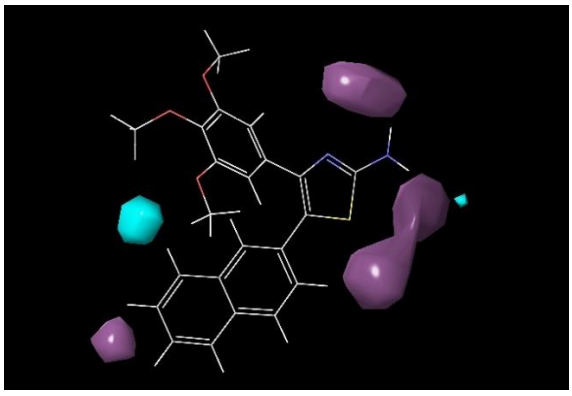
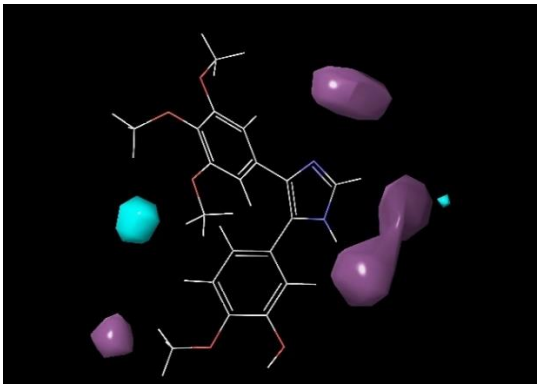



Figure 6.5 Contour map of Gaussian H-Bond donor of best active ligands 16 and 52

The figure shows how Gaussian H-Bond donor alters the activity of the best active two ligands (16, 52) of combretastatin containing nucleus. If we add Gaussian H-Bond donor group on the purple coloured binding site of combretastatin containing nucleus, activity will be increased and if we add Gaussian H-Bond donor group on the cyan coloured binding site of combretastatin containing nucleus, activity will be decreased.

6.3.5 Contour map of Gaussian H-bond acceptor

In the diagram at left below, the oxygen atom of the hydroxyl group is called the hydrogen bond donor, because it is donating its hydrogen to the nitrogen and formed the hydrogen bond. The nitrogen atom is called the hydrogen bond acceptor, because it is accepting the hydrogen from the oxygen. In the picture of two water molecules at lower right, the oxygen of the water molecule B is the hydrogen bond donor. The oxygen of water molecule A is the hydrogen bond acceptor.

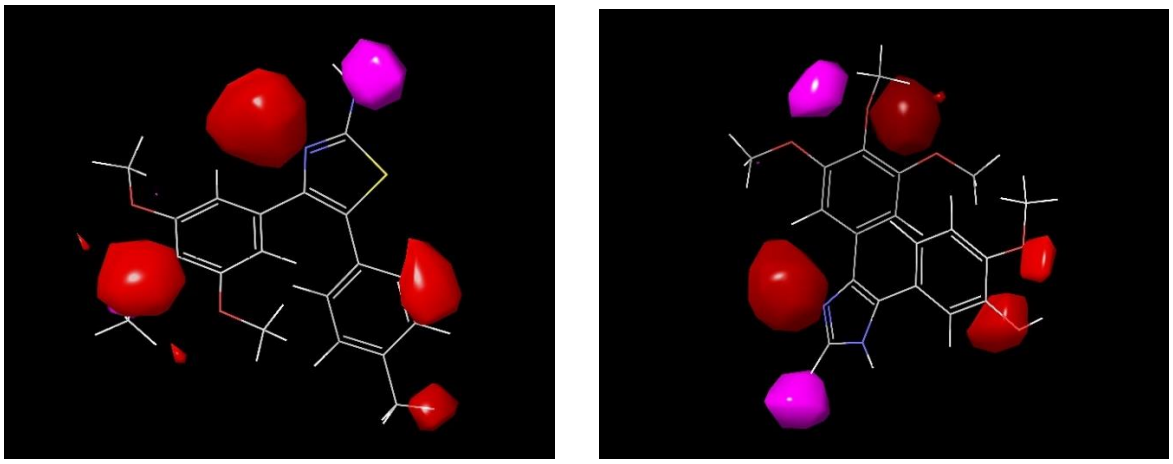


Figure 6.6 Contour map of Gaussian H-Bond Acceptor of best active ligands 18 and 52

The figure shows how Gaussian H-Bond Acceptor alters the activity of the best active two ligands (18, 52) of combretastatin containing nucleus. If we add Gaussian H-Bond Acceptor group on the red coloured binding site of combretastatin containing nucleus, activity will be increased and if we add Gaussian H-Bond Acceptor group on the pink coloured binding site of combretastatin containing nucleus, activity will be decreased.

6.3.6 Contour map of combined best ligands interaction of Gaussian Electrostatics

These are best activity showing the ligands in aligned form.

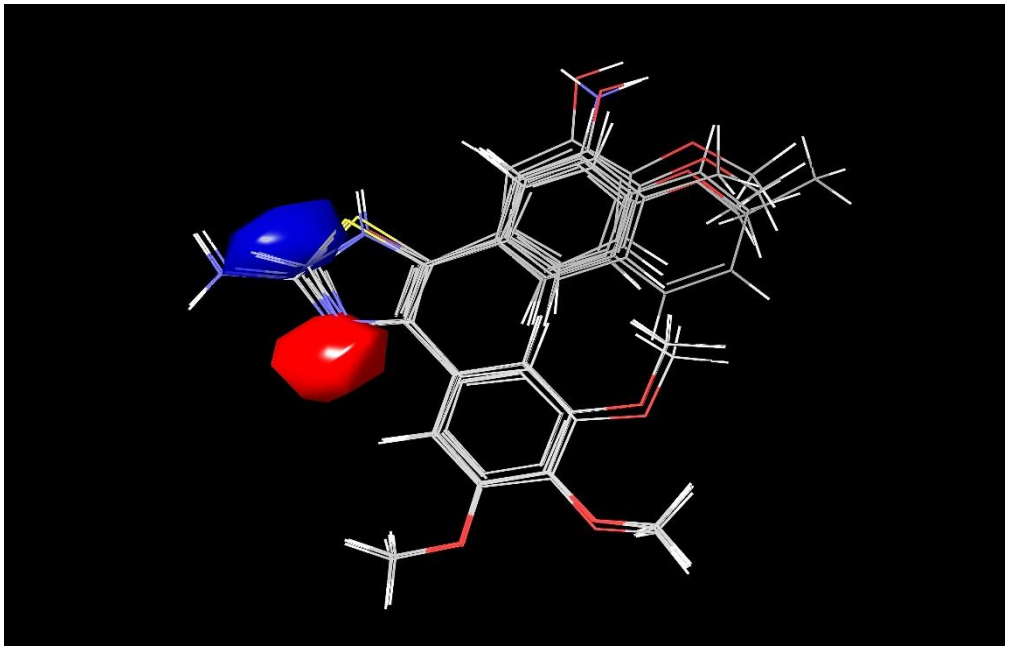


Figure 6.7 Contour map of combined best active ligands interaction of Gaussian Electrostatics

The above figure shows how electrostatic group alters the activity of the best active five combined ligands (16, 18, 26, 52, 60) of combretastatin containing nucleus .if we add electrostatic group on the red coloured binding site of combretastatin containing nucleus, activity will be increased and if we add electrostatic group on the blue coloured binding site of combretastatin containing nucleus, activity will be decreased.

6.3.7 Contour map of combined best ligands interaction of Gaussian H-Bond acceptor

These are best activity showing the ligands in aligned form.

The above figure shows how H-Bond acceptor group alters the activity of the best active five combined ligands (16, 18, 26, 52, 60) of combretastatin containing nucleus .if we add H-Bond acceptor group on the blue coloured binding site of combretastatin containing nucleus, activity will be increased and if we add H-Bond acceptor group on the cyan coloured binding site of combretastatin containing nucleus, activity will be decreased.

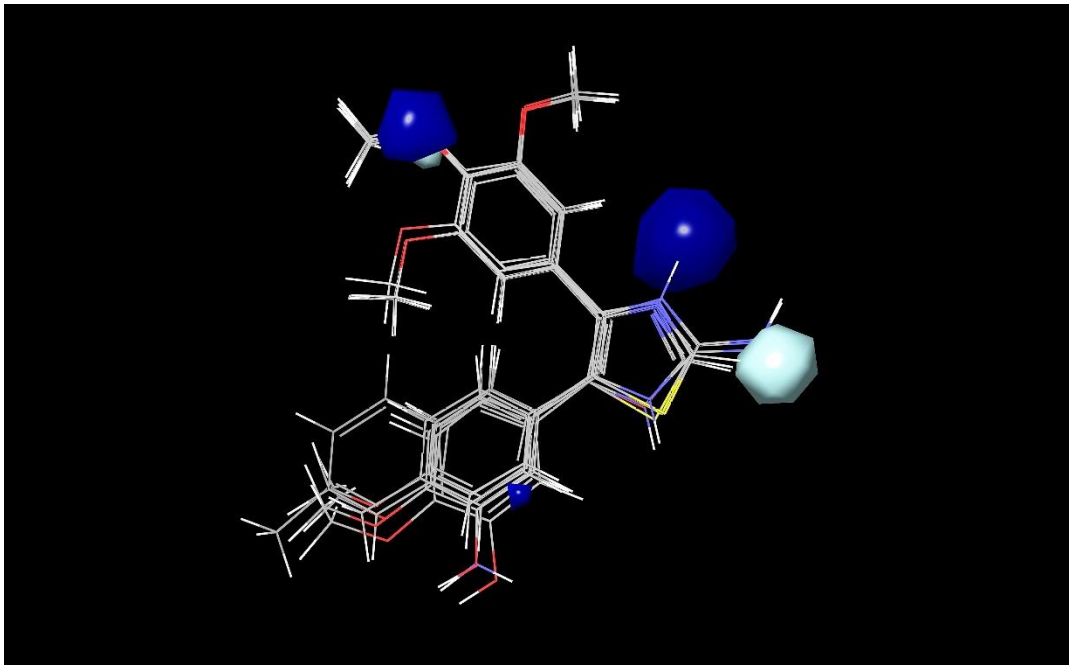


Figure 6.8 Contour map of combined best ligands interaction of Gaussian H-Bond acceptor

6.3.8 Contour map of combined less activity ligands interaction of Electrostatics

These are low activity showing the ligands in aligned form.

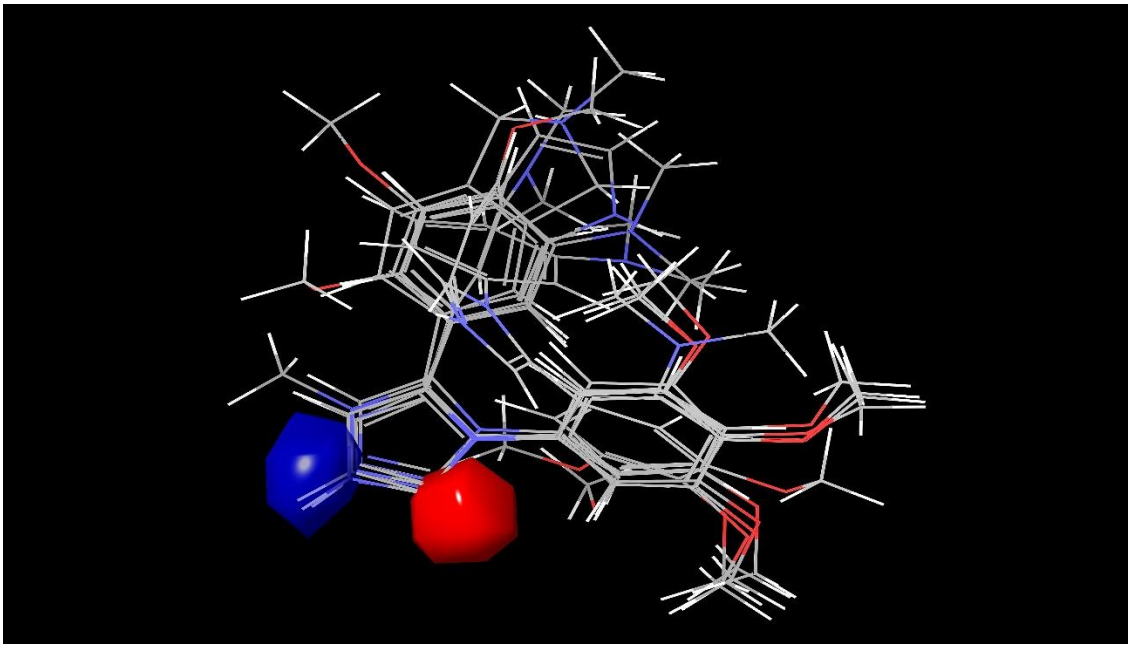


Figure 6.9 Contour map of combined less active ligands interaction of Gaussian Electrostatics

The above figure shows how electrostatic group alters the activity of the less active five combined ligands (44, 46, 48, 49, 50) of combretastatin containing nucleus .if we add electrostatic group on the red coloured binding site of combretastatin containing nucleus, activity will be increased and if we add electrostatic group on the blue coloured binding site of combretastatin containing nucleus, activity will be decreased.

CHAPTER-7

CONCLUSION

7. Conclusion

From the above discussion on the tubulin inhibitors, it's concluded that the activity of already available tubulin inhibitors can be increased by adding or removing: Electrostatic group, Steric group, H-bond Acceptor, H-bond donor and Hydrophobic group in the best selected 5 out of 49 ligands on basis of IC50 value deduced from Field Based QSAR Study.

On the basis of FQSAR study, we can design more potent tubulin inhibitor. Based on the QSAR studies it can be concluded that steric factors plays important role than the Electrostatic force of interaction out of Gaussian factors Gaussian hydrophobic group plays important role. So in future the molecules can be designed by introducing the steric and hydrophobic group in order to increase the activity. Similarly the H-bond acceptor plays a major role than the other Gaussian factors. So combretastatin based derivatives can be further modified in the future we have made structural modifications on 5-membered ring system and changes on the 6-membered and biological activity will be done in future.

We can conclude that electrostatic group alters the activity of the best active five combined ligands (16, 18, 26, 52, 60) of combretastatin containing nucleus .if we add electrostatic group on the red coloured binding site of combretastatin containing nucleus, then activity will be increased.

We can concluded that H-Bond acceptor group alters the activity of the best active five combined ligands (16, 18, 26, 52, 60) of combretastatin containing nucleus .if we add H-Bond acceptor group on the blue coloured binding site of combretastatin containing nucleus, activity will be increased and if we add H-Bond acceptor group on the cyan coloured binding site of combretastatin containing nucleus, activity will be decreased.

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