Aetiology of cancer

Cancer is known to arise within a single cell as a result of accumulation of abnormalities (mutations) within the DNA of that cell. When these mutations occur within the key genes, it can lead to uncontrolled cell growth and perhaps formation of a cell type that acquired the ability to metastasize (Spence and Johnston, 2001). Generally, many reasons were stated to cause genetic damage in a cell leading to neoplastic transformation, which can be summed up in three main categories:

1. Chemical carcinogens
2. Radiation
3. Viruses

Chemical carcinogens

About 80 to 90% of all human tumours are seen to develop due to chemical aetiology. Many experiments identified two main steps as essential for carcinogenesis: initiation and promotion.

Initiation occurs when a cell is exposed to an appropriate dose of a carcinogenic agent (the initiator) and is altered in such a way that it is likely to give rise to a tumour. The initiation step is usually rapid and irreversible and leads to DNA damage (mutations).

The promotion is the step in which there is proliferation and clonal expansion of the initiated cells. In contrast to initiators, promoters do not affect the DNA directly and their effects are reversible. The initiator should be applied before the promoters in

carcinogenesis process. There can be a long interval between the two steps. Therefore, promoters must be applied repeatedly and in regular intervals.

Initiation

Initiators can be categorized in direct-acting compounds being themselves carcinogenic like nitrogen mustard, dimethyl sulphate, beta-propiolactone and methyltyrosine and indirect-acting compounds which must be converted through metabolism first to become carcinogens, e.g. benzopyrene, vinyl chloride, aflatoxin B1 and benzidine.

All chemicals being able to transform a cell are reacting with the electron-rich sites within the cell; therefore, they are stated as highly reactive electrophiles. Despite the DNA being the main target of such electrophiles other electron-rich sites of proteins and RNA can be attacked leading to lethal damage. If dead does not occur, the DNA damage remains and is preserved transmitted to the daughter cells of the next generation. Certainly, the cell tries through its reparation mechanisms to prevent this by reconstituting the damage bevor the cells enters to division.

Inactive carcinogens are converted in vivo to ultimate carcinogens. It has also become apparent that nearly all chemicals undergo metabolism through several competing enzyme pathways, with differences in kinetics and saturation levels (Guengerich, 2000). A common group of enzymes involved with carcinogen metabolism are the cytochrome P450 isozymes; however, numerous other enzyme systems have been identified that participate in the metabolism of various carcinogens Although some of these metabolic processes lead to activation to reactive electrophiles, many actually lead to inactivation of the chemicals by increasing aqueous solubility and leading to their increased excretion either in the urine or in the feces (Mitchell and Smith, 2010).

Increasing knowledge of the multiple steps of carcinogenesis (Fig. 1) is leading to improved methods for screening chemicals for carcinogenic activity and for regulatory decision making. Improvements in assessment of modes of action involved in animal and in vitro models have led to more rational approaches to assessing relevance to humans. The advent of genomics and high-throughput technologies have contributed to investigations of mechanisms and is beginning to impact development of better methods for screening chemicals. Based on developments in basic research, epidemiology, and astute clinical observations, the major risk factors and etiologic agents have been identified for a majority of cancers, which is beginning to lead to methods to decrease cancer incidence overall and identify targets for early detection and treatment (Cohen and Arnold 2011). To increase cancer risk, a chemical either increases DNA reactivity leading to an increase of mutation with each DNA replication or increases the number of DNA replications in the target cell population. To reduce cancer, the reverse of this must occur. Either the amount of DNA reactivity must be decreased and/or the amount of DNA replication needs to be reduced (Cohen, 1991).

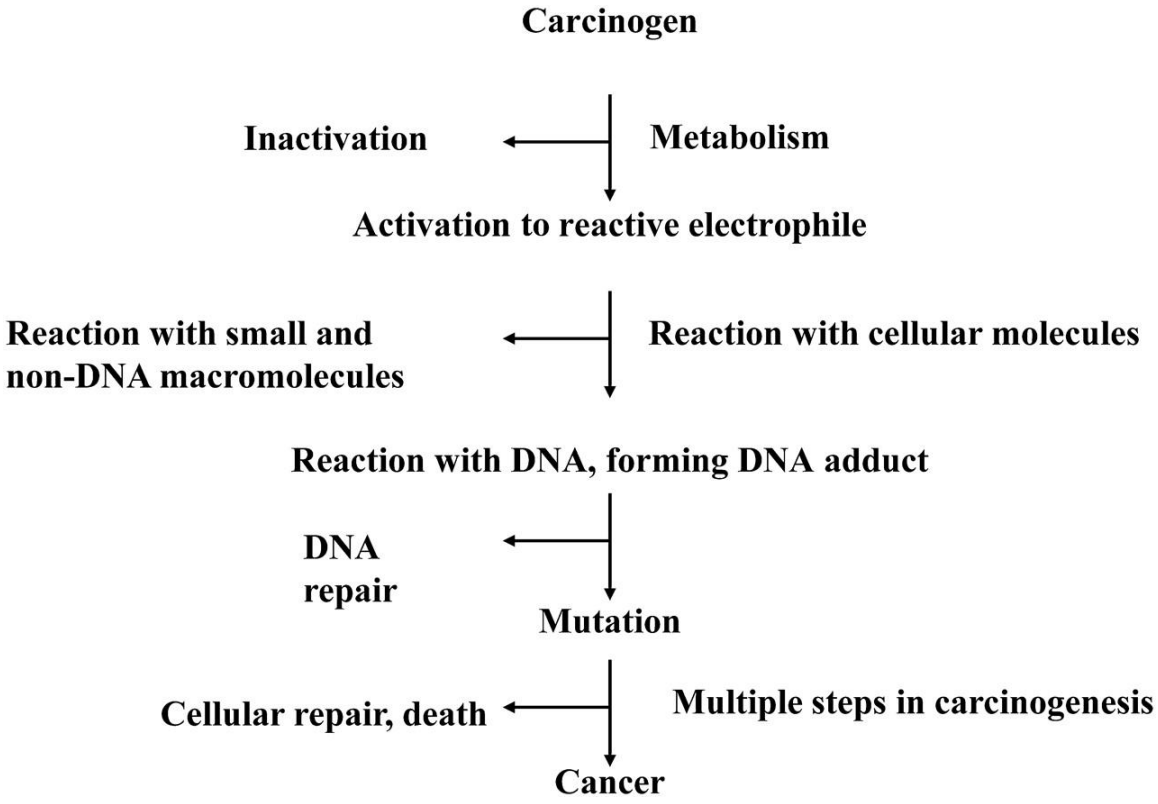


Figure 1: Competing activating and inactivating processes for DNA-reactive carcinogens. In addition, these must occur in a pluripotential (tissue stem) cell, and the cell must replicate to fix the DNA alteration produced by a DNA adduct as a permanent mutation (Cohen and Arnold 2011).

A major class of enzymes involved in this metabolic activation is the family of cytochrome P-450- dependent mono oxygenase isoenzymes (CYPs). Among general population these enzymes have different activities and ways of being induced. CYP1A1 is given as an example: About 10% of general population are stated to carry high inducible forms of this enzyme. It is responsible for the metabolism of polycyclic aromatic hydrocarbons (like benzopyrene) and associated with an increased lung cancer risk in smokers. So, the exposure to certain carcinogens is also influenced by gene polymorphisms (Roy A. J. Spence .et and Patrick G. Johnston 2001).

The second class is the enzyme glutathione-S-transferase which detoxifies polycystic aromatic hydrocarbons. 50% of population have a completely deleted locus of this locus. Hence, they have a higher risk to develop lung cancer when exposed to tobacco smoke.

Promotion

Promoters do not act as electrophiles and are not metabolized to active compounds. The action of promotion can be seen as a proliferation of paraneoplastic gaining additional abnormalities through mutations in every step resulting in developing a malignant tumour. Thus, promoters are not mutagenic but contribute to the tumorigenesis by stimulating cell proliferation through activating protein kinase C which leads to phosphorylation of various target proteins (Spence and Johnston, 2001).

Radiation

Radiation- induced carcinogenesis has similar mechanisms like chemical carcinogens: radiant energy acts mutagenic by DNA damage. Usually years have to pass between exposure to the radiation dose and the appearance of malignant signs. This condition may suggest that promotion also plays a role in proliferation of malignant cells.

Ultraviolet (UV) radiation is divided into three wavelength ranges: UV-A (320-400 nm), UV-B (280-320nm) and UV-C (200-280nm). Damages occur through generation of dimers in the DNA strand (between pyrimidine residues) which interfere in DNA transcription and replication. As a counteraction, the cell tries repairing the damage by excising the wrong nucleotides (Spence and Johnston, 2001).

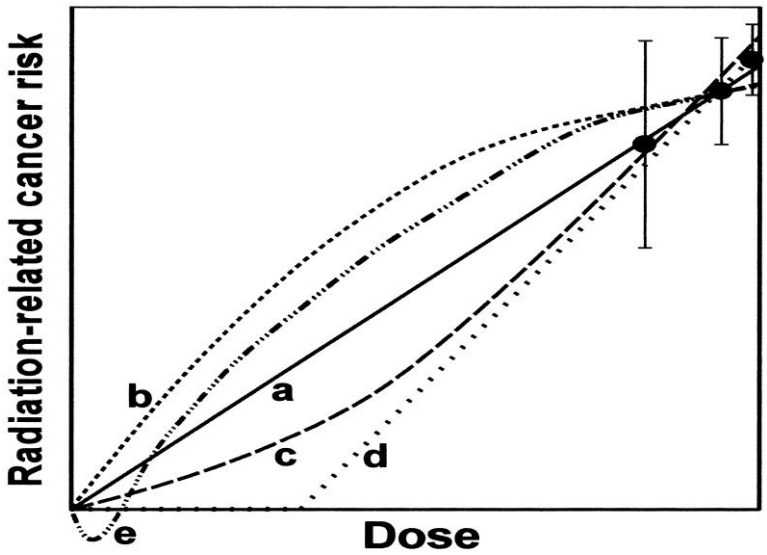


Figure 2: Schematic representation of different possible extrapolations of measured radiation risks down to very low doses, all of which could, in principle, be consistent with higher-dose epidemiological data. Curve a, linear extrapolation; curve b, downwardly curving (decreasing slope); curve c, up wardly curving (increasing slope); curve d, threshold; curve e, hermetic (Brenner, et al., 2003).

The linear no-threshold (LNT) model assumes a curvature at moderate doses, but linearity at low doses or low dose rates. However, for the low doses and dose rates relevant to diagnostic radiology, the curve can be assumed to be linear (Fig. 2, curve c). It is consistent with the data for solid tumours at doses 1.5 Gy in the Life Span Study. Cancer risk owing to one-track action is therefore proportional to dose, with any dose, no matter how small, able to induce cancer (although extremely unlikely to do so) the LNT model still remains the most robust model for making decisions about medical radiation exposure vs cancer risk, and one of the safest (Shah, et al., 2012).

It is postulated that high dose of UV radiation overwhelms the repairing mechanisms so that some DNA damage is remaining. The disease Xeroderma pigmentosum is characterized by a mutation of one of many genes responsible for excision repair. Patients

of this disease are extremely photosensitive and have a 2000x higher risk to develop skin cancer in sun exposed skin.

Ionizing radiations are part of our natural environment and come from the radiation background (cosmic rays, radon...). They are called as universal carcinogens because of their ability to induce cancer in nearly all tissues.

Furthermore, they are sufficient to induce tumour development though only one single exposure. This fact was demonstrated by the survivors of Hiroshima and Nagasaki: 6 years afterwards leukaemia appeared and after 20 years' solid tumours were recorded.

But also, therapeutic radiation is able to induce cancer: head and neck irradiation led in 10% to thyroid cancer (Spence and Johnston, 2001).

Viruses

Observations in animal experiments showed that viruses are able to integrate their genome into the host cell DNA and consequently cause a production of viral proteins who maintain in the cell.

Viruses with a DNA genome enter the cell and can directly interact with the genome of the host cell and integrate their DNA which is then transcripted to mRNA and translated to viral proteins (Spence and Johnston, 2001).

Viruses have been demonstrated to be the causative agents of approximately 10%–15% of all cancers worldwide. Carcinogenic viruses utilize a variety of mechanisms to transform human cells. Their genome encodes proteins that reprogram the normal functioning of cells so as to favor virus production, and the most common outcome for this virus-induced reprogramming is the induction of genomic instability, as seen by the accumulation of point mutations, aberrations and DNA damage. Genomic instability caused by viruses serves as a major step on the pathway leading to carcinogenesis and can be induced by viral infection and inflammation, by viral gene

expression and by exogenously derived factors (Chen, et al., 2014). Inflammation is referred to as a cancer “promoter”, because it induces cell proliferation, recruits inflammatory cells, increases cellular levels of ROS, thereby leading to oxidative DNA damage, and reduces DNA repair (Coussens and Werb, 2002).

In contrast RNA viruses are not able to integrate their genome into the host cell DNA; first they have to convert their RNA into DNA (so called provirus) through the enzyme reverse transcriptase. Then the provirus can be integrated into the genome of the host cell. Afterwards viral proteins are produced by transcription to mRNA and the finally translation. Because of this conversion from initially RNA to DNA to RNA again, those viruses are so called retroviruses (Spence and Johnston, 2002). Because viral integration requires the breakage of both the viral and the host DNA, the integration rate is believed to be linked to the levels of DNA damage. A better understanding of virus-mediated carcinogenesis, the networking of pathways involved in transformation and the relevant risk factors, particularly in those cases where tumorigenesis proceeds by way of virus integration, will help to suggest prophylactic and therapeutic strategies to reduce the risk of virus-mediated cancer. (Chen, et al., 2014)

Tumour markers:

Tumor markers are soluble glycoproteins that are found in the blood, urine, or tissues of patients with certain types of cancer (Sturgeon, et al., 2008, Lai, et al., 2003, Duffy, 2007).

Most of tumour markers are proteins. However, more recently, patterns of gene expression and changes to DNA have also begun to be used as tumour markers. They are typically produced by tumor cells, but in some cases, they may be produced by the body in response to malignancy or to certain benign conditions. Serum tumour markers may aid cancer diagnosis, assess prognosis, guide choice of treatment, monitor progress during and after treatment, and/or be used as screening tests (Duffy, (2013); Duffy, (2001).

The cancer biomarkers include genomic, epigenetic, proteomic and metabolomic markers among which genomic and proteomic have highly contributed to the biomarker discovery. The proteins involved in oncogenesis, angiogenesis, development, differentiation, proliferation, apoptosis, hematopoiesis, immune and hormonal responses, cell signaling, nucleotide function, hydrolysis, cellular homing, cell cycle and structure and hormonal control can serve as better candidates for biomarker discovery (Polanski and Anderson, 2006).

Cell lines, especially cancer cell lines are contributing important insights into the cancer mechanisms, drug discovery and biomarker identification (Ravi, et al 2014).

However, more recently, patterns of gene expression and changes to DNA have also begun to be used as tumour markers. Biological markers can be used as an adjunct to staging and histopathological classification of Tumours. Some of these markers are specific to one cancer, while others are seen in several types of cancer. Different types of Tumour markers such as CEA,β -HCG, ACTH, alpha –fetoproteins (AFP), prostate specific antigen (PSA) and CA-125 help further define the histopathological classification of Tumours. Some of these markers help differentiates between epithelial and lymphoid malignancies, in case of epithelial membrane antigen and common leucocyte antigen. In addition to some of them may also be useful in monitoring a patient's response to treatment. (Spence and Johnston, 2001)

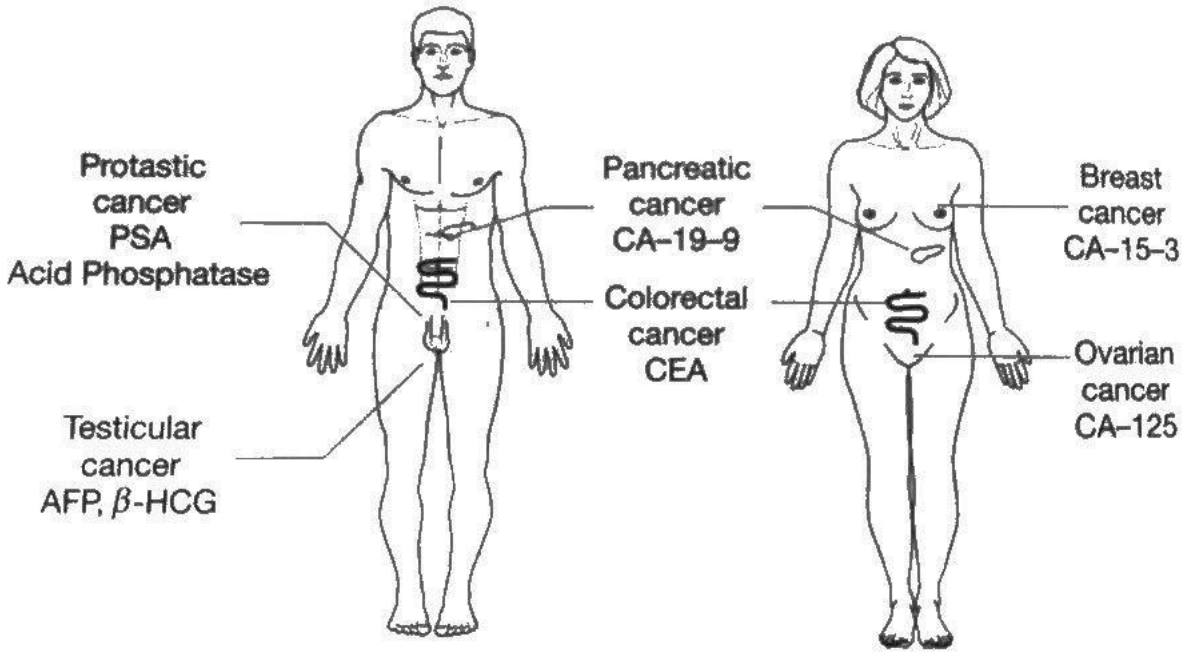


Figure 3: Tumour markers used in the diagnosis and evaluation of Cancer (Spence and Johnston 2001).

The Molecular biology of Cancer:

After the development of high throughput sequencing methods Genomics is now shifting towards the study of gene function and expression. High throughput genomic analyses can provide a basis for the discovery of clinically relevant biomarker signatures and give a new comprehensive view. Carcinogenesis is a multistep process which involves activation of oncogenes (K-ras, Raf, Cyclin D1 & E, β-Catenin) (Chial, 2008) and inactivation of tumor suppressor genes (p53, Bcl2, APC). Single gene mutations are the major causes which leads to the over expression of oncogenes and under expression of tumor supressors in cancer conditions. Molecular profiling of genome is now possible through the advent of new advanced technigues such as DNA microarray. Microarray offer analyses of expressions of 1000s of genes involved in carcinogenesis. This global

gene profiling can help diagnose subtypes of disease and predict patient survival (Konstantinopoulos, et al., 2008).

Several lines of evidence have combined to develop the concept of Cancer as a genetic disease at the cellular level. In addition to the recognition of heritable cancer syndromes such as familial retinoblastoma and familial polyposis coli, the observation of chromosomal abnormalities in neoplastic cells has recently become apparent that human tumours arise as a result of the accumulation of mutations in two classes of cellular genes- proto-oncogenes. Cancer cells nearly always contain abnormal numbers of chromosomes, a phenomenon called aneuploidy, which leads to altered dosage and expression of all of the genes carried on a given chromosome. As cancer cells rapidly grow and divide, they do not cope well with extra chromosome; which leads to abnormal chromosome segregation and additional changes in chromosome number as the cells continue to divide leading to what is termed genetic instability (Chial, 2008).

Proto-oncogenes are a group of genes that cause normal cells to become cancerous when they are mutated (Adamson, 1987; Weinstein and Joe, 2006). Mutations in proto-oncogenes are typically dominant in nature, and the mutated version of a proto-oncogene is called an oncogene. Often, proto-oncogenes encode proteins that function to stimulate cell division, inhibit cell differentiation, and halt cell death. All of these processes are important for normal human development and for the maintenance of tissues and organs. Oncogenes, however, typically exhibit increased production of these proteins, thus leading to increased cell division, decreased cell differentiation, and inhibition of cell death; taken together, these phenotypes define cancer cells. Thus, oncogenes are currently a major molecular target for anti-cancer drug design.

In common solid tumors, such as those derived from the colon, breast, brain, or pancreas, an average of 33 to 66 genes display subtle somatic mutations that would be expected to

alter their protein products. About 95% of these mutations are single-base substitutions such as (C>G), whereas the remainder are deletions or insertions of one or a few bases such as (CTT>CT) (Vogelstein, et al., 2013).

Most cancer-critical genes code for components of the pathways that regulate the social behavior of cells in the body in particular, the mechanisms by which signals from a cell's neighbors can impel it to divide, differentiate, or die. In fact, many of the components of cell-signaling pathways were first identified through searches for cancer-causing genes, and a full list of proto-oncogene products and tumor suppressors includes examples of practically every type of molecule involved in cell signaling—secreted proteins, transmembrane receptors, GTP-binding proteins, protein kinases, gene regulatory proteins, and so on. Many cancer mutations alter signal pathway components in a way that causes them to deliver proliferative signals even when more cells are not needed, switching on cell growth, DNA replication, and cell division inappropriately. Mutations that inappropriately activate a receptor tyrosine kinase, such as the EGF receptor, or proteins in the Ras family, which lie downstream from such growth factor receptors, act in this way.

(Alberts, et al., 2002) Other signaling pathways can function to inhibit cell division, the best-known example being the antigrowth effect of the TGFβ family of signaling proteins. Loss of growth inhibition through TGFβ-mediated pathways contributes to the genesis of several types of human cancers Alberts, et al., 2002).

The recognition that certain tumors contain activating mutations in driver genes encoding protein kinases has led to the development of small-molecule inhibitor drugs targeting those kinases.

Representative examples of this type of genome-based medicine include the use of EGFR kinase inhibitors to treat cancers with EGFR gene mutations, the aforementioned anaplastic

lymphoma kinase (ALK) inhibitors to treat cancers with ALK gene translocations, and specific inhibitors of mutant BRAF to treat cancers with BRAF mutations (Vogelstein, et al., 2013).

Biochemical functions of Proto-oncogenes:

More than 60 proto -oncogenes have been identified which have ability to act in biochemical pathways by which growth factors stimulate cellular proliferation. There are Three main chemical mechanisms of action have been identified as the following:

1. Protein phosphorylation:

Consequently, phosphorylation of serine, threonine and tyrosine residues which concerned as a one of the main mechanisms of activating intercellular signal transduction pathways.

Furthermore, several proto- oncogenes with kinase (phosphorylase) activity have been identified. for example, the growth factor receptor (met) and the cytoplasmic signal transductions factors (src, raf). Mutations of these genes have ability to regulate their kinase activity and hence the proliferative signal to the cell.

2. GTP- binding proteins:

Ras GTP-binding proteins are a large superfamily of signal transduction factors (Downward, 1990). The most important in tumorigenesis are undoubtedly H-ras, K-ras and N-ras genes mutated in approximately 30% of all tumours (Bos, 1989). Approximately 95% of pancreatic cancer ,60% of thyroid cancer, 50% of colon carcinomas ,40% of adenocarcinomas of the lung and 30% of acute myeloid leukaemia's possess ras gene mutations. These genes act as molecular switches in signal transduction pathways. They

bind and hydrolyse GTP, which are almost active in the GTP bound form and inactive in the GDP bound form. Their intrinsic GTPase activity is regulated by GTPase activating proteins (GAP). Ras genes approximately invariable activated Form by point mutations at codons 12, 13 and 61 , within the GTP binding domain. Such mutations lock ras proteins in the active GTP bound state by inhibiting GAP mediated GTP hydrolysis. The product of the NF1 tumour suppressor gene has activity as a ras GTPase activating protein and the bcr gene is a GTPase activating protein for a ras-related protein called rac.

3. Transcription factors:

Regulation of gene expression occurs as a result of growth factor stimulation and intracellular signal transduction which initially plays avital role in the control of cellular proliferation. Obviously, several oncogenes, for example c-myc, N-myc, fos and jun, have been presented to function as transcription factors. (Horwich ,1995))

Cyanobacteria metabolites Calothrixins, Biology, Ecology and Evolution

Marine Natural products

Natural products have played a major role in drug discovery, as the source of many earliest medicine such as a Morphine, penicillin, and paclitaxel (Taxol) , which have a highly potential activity against bacterial infection,Malaria ,Cancer……..etc. (Brown, et al., 2014)).

The marine environment is considered as a host to unparalleled biological and chemical diversity, which making it as an attractive resource of new many therapeutics for a plethora of diseases. Compounds which are extracted from cyanobacteria have represented a special interest due to their unique Structural modifications and Capacity to produce potent pharmaceutical and biotechnological trait (Xu, et al., 2016).

Cyanobacteria:

Cyanobacteria, also known as “blue-green algae “are a diverse group of gram-negative photosynthetic prokaryotes. They get their name from their blue-green pigment, c-PC (cphycocyanin) (Moreira, et al., 2014). This pigment is used for photosynthesis and is considered to have played a crucial role in releasing oxygen into the primitive atmosphere (Zanchett, et al., 2013 and Gabdulkhakov and Dontsova 2013). However, not all "blue-green" bacteria are blue; some common forms are red or pink from the pigment phycoerythrin.

These bacteria are often found growing on greenhouse glass, or around sinks and drains. The

Red Sea gets its name from occasional blooms of a reddish species of Oscillatoria, and African flamingos get their pink color from eating Spirulina.

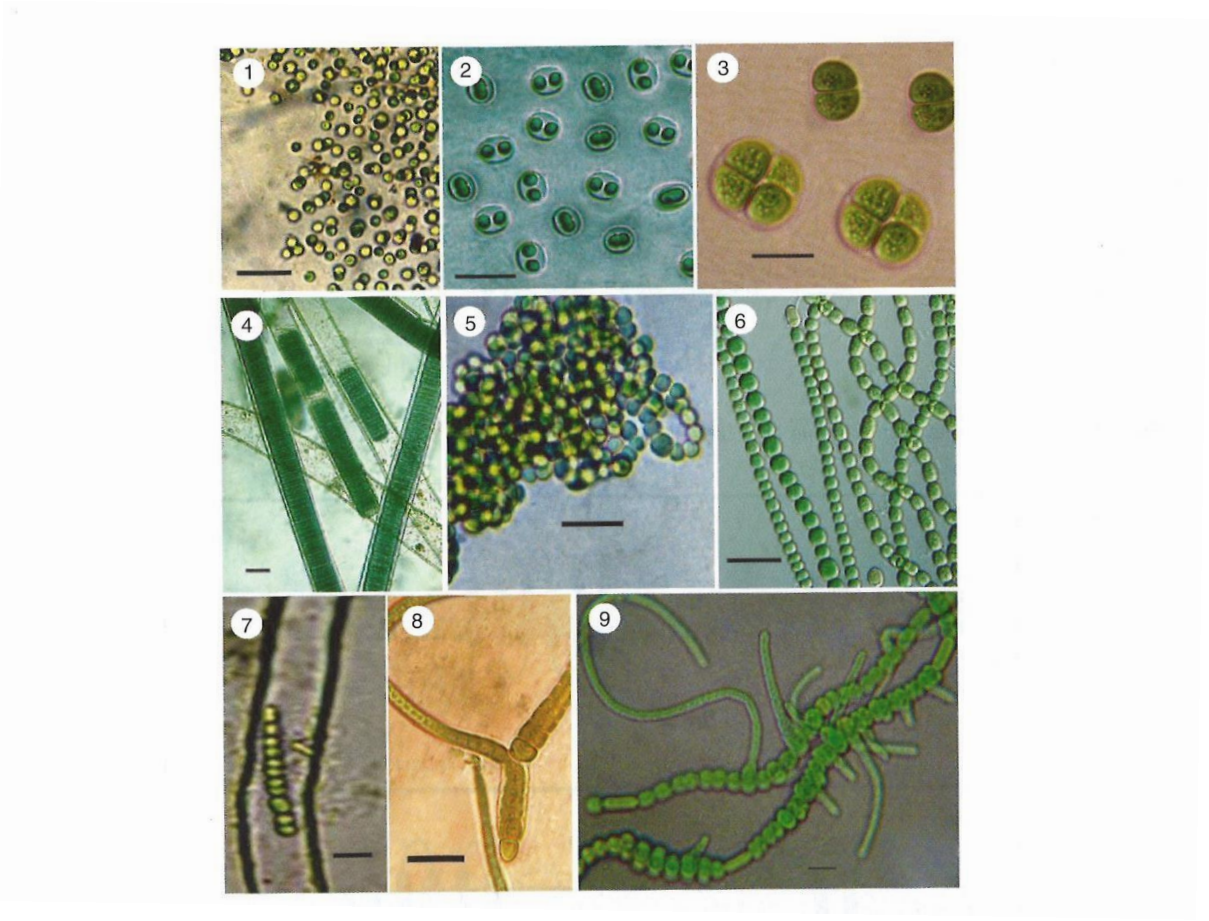


Figure 4: Microphotographs of Cyanobacteria isolated from rice and wheat field. (1) Aphanocapsa sp.; (2) Gloeocapsa fusco-lutea; (3) Chroococcus turgidus var. maximus; (4) Lyngbya magnifica; (5) Nostoc commune; (6) Anabaena sp.; (7) filament of Anabaena sp. inside the wheat roots; (8) Calothix parietina; (9) Westiellipsis prolifica (Bar indicates 20 µm in photographs 1-3, 6-9; and 50 µm in 4) (Naveen, et al., 2014).

Cyanobacteria possess a broad geographical distribution, but are most common in marine and freshwater habitats, in damp terrestrial habitats, and sometimes in association with fungi to form lichens (Gehringer and Wannicke 2014, Sobotka, 2014). This broad range of habitats mirrors the impressive ability of cyanobacteria to acclimate to changes of many different environmental factors (Tandeau de Marsac and Houmard, 1993). Based on their evolutionary history, Cyanobacteria have undergone several structural and

functional modifications, which are responsible for their wide ecological and physiological tolerance

(Whitton, 2012). Cyanobacteria represent an ancient monophyletic phylum of the domain Bacteria. Recent genome analyses seem to indicate that the primary endosymbiont was most closely related to present-day filamentous strains (Deusch et al., 2008). The clade consists of simple unicellular organisms with a frequent tendency for aggregation or colony formation and filamentous structures that either are plain unbranched or more elaborate and branched (Fay, 1992). They represent a unique bacterial phylum that shows many differences from classical Gram-negative bacteria such as Escherichia coli, including cell wall structure (Gupta and Griffiths, 2002). Like Gram-negative bacteria, cyanobacteria are surrounded by two membrane systems. The cell wall is enclosed by an outer membrane that surrounds the periplasmic space, whereas the cytoplasmic membrane surrounds the cytoplasm. Inside the cytoplasm, cyanobacteria contain a third membrane system. Thylakoid membranes, which originate from the cytoplasmic membrane, contain the photosynthetic complexes. In addition, the respiratory electron transport chain is also mostly situated at the thylakoid membranes (Hagemann, 2011).

Among organism's cyanobacteria play an undisputed, important role in earth´s history. Not only that they contribute to the carbon cycle as primary producers and to the nitrogen cycle by the ability of some species to fix nitrogen, but especially their ability to use water molecules as electron donors guarantees them an important place in the redox history (Flores and Herrero, 2009).

Cyanobacteria are widely accepted as the progenitor of oxygenic photosynthesis, and they have evolved into one of the largest and most diverse groups of bacteria on this planet (Whitton and Potts, 2000).

The origins of the essential machinery for conducting photosynthesis are not yet identified (Shi and Falkowski, 2008; Bekker, et al., 2004).

The evolution of eukaryotic photosynthetic organisms is thought to have occurred by the uptake of cyanobacteria into unicellular eukaryotes to form predecessors of plant cells by „primary endosymbiosis “. It is commonly accepted that the photosynthetic plastids, i.e. chloroplasts, have entered the cell by a process of phagocytotic uptake (Keeling, 2004).

Cyanobacteria are prokaryotes, but their photosynthetic apparatus is very similar to that found in higher plants.

Cyanobacteria have garnered a great deal of attention recently as biofuel-producing organisms. Their key advantage over other bacteria is their ability to use photosynthesis to capture energy from sunlight and convert CO_2 into products of interest. As compared with eukaryotic algae and plants, cyanobacteria are much easier to manipulate genetically and grow much faster. They have been engineered to produce a wide and ever-expanding range of products including fatty acids, long-chain alcohols, alkanes, ethylene, polyhydroxybutyrate, 2,3-Butanediol, ethanol, and hydrogen. These processes have been reviewed recently (Gronenberg et al., 2013).

Comparisons of the amino acid sequences of specific proteins from various species of cyanobacteria give clues about the evolution and taxonomy of the cyanobacteria (Krogmann, 1981).

Many blue-green algae produce toxins that can be dangerous to humans and to animals. Because they bloom in freshwater and marine habitats, they may cause mass mortalities of fish and other animals.

The photoautotrophic lifestyle made cyanobacteria dependent only on light, water, and a few inorganic nutrients. As a consequence, cyanobacteria became dominant organisms

with major impacts on geochemical processes of our planet. During their massive development, cyanobacteria generated the oxygen-containing atmosphere, which gave rise to the evolution of most oxygen-dependent organisms found today (Tandeau de Marsac and Houmard, 1993).

Biochemical and pharmaceutical roles of Cyanobacteria:

Cyanobacteria are also still very important players in biogeochemical cycles; it was estimated that they account for about 30% of global primary production, especially in the central oceans, which make them an important sink for CO_2. Additionally, because of their N2-fixing ability, many cyanobacterial strains are regarded as the major sources of combined nitrogen in the marine system (Scanlan et al., 2009).

Cyanobacteria have existed as oxygenic photosynthetic bacteria on earth for at least 2.7 billion years (Buick, 1992). During that time, they have endured a changing gaseous environment where CO_2 has declined and O_2 has risen. This has imposed evolutionary pressure on them to evolve strategies for efficiently acquiring inorganic carbon for photosynthesis. In response to this they have developed an effective photosynthetic CO_2 concentrating mechanism (CCM) for improving the carboxylation by their relatively inefficient Rubiscos (Badger and Price, 2003). This CCM is perhaps the most effective of any photosynthetic organism, concentrating CO_2 up to 1000-fold around the active site of Rubisco. In the past few years, there has been a rapid increase in the understanding of the mechanisms and genes involved in cyanobacterial CCMs (Badger and Price, (2003).

The most studied Species of marine cyanobacteria include Nostoc, Calothrix, Lyngbya and Symploca (Dixit, et al., 2013).

Several studies have investigated the pharmaceutical and biotechnological activity of the secondary metabolites isolated from Cyanobacteria .Many of these molecules possess antineoplastic chemotherapeutic properties, anti-inflammatory, anti-malarial, antiprotozoal, immunosuppressant, anti –cancer anti HIV, anti-coagulant, antibacterial, antifungal, anti-tuberculosis, antiviral, and antitumor activities (Dixit, et al., (2013), Jakubowska and Szelag-Wasielewska 2015, Wijffels, et al., (2013) Kock, et al., (2005)).

Calothrixins A and B are two cyanobacterial metabolites which consist assembly of Quinone, Quinolone and indole pharmacophores that were first isolated from Calothrix in 1999 (Rickards, et al., (1999); Xu, et al., (2016)). Calothrixins, which are cyanobacterial metabolites, have demonstrated a diverse range of bioactivities that include antimalarial, anticancer, and antibacterial properties. They have been observed to target various aspects of RNA synthesis in bacteria (Xu, et al., 2016).

Topoisomerases:

Topoisomerases are ubiquitous proteins found in all three domains of life “bacteria, archaea and eukarya”, Baker, et al (2009). DNA topoisomerases are marvelous molecular machines that manage the topological state of the DNA in the cell. These enzymes accomplish this feat by either passing one strand of the DNA through a break in the opposing strand (type I subfamily) or by passing a region of duplex from the same or a different molecule through a double-stranded gap generated in a DNA (type II subfamily). Topoisomerases are known that relax only negative supercoils, that relax supercoils of both signs, or that introduce either negative (bacterial DNA gyrase) or positive supercoils into the DNA (reverse gyrase). Besides altering the supercoiling of a closed DNA domain, the strand passing activities of topoisomerases can promote the

catenation and decatenation of circular DNAs or the disentanglement of intertwined linear chromosomes.

The fundamental need for topoisomerases derives from the double helical structure of DNA. For most processes that must access the information stored in the DNA, the two strands of the helix must separate either temporarily, as in transcription or recombination, or permanently, as in replication. The circular nature of bacterial chromosomes and the large size of eukaryotic DNA molecules preclude a simple winding solution to many of the topological problems that accompany the DNA transactions that occur in the cell (Wang, 1998). Thus, during DNA replication, the two strands of the DNA must become completely unlinked by topoisomerases, and during transcription, the translocating RNA polymerase generates supercoiling tension in the DNA that must be relaxed (Wang, (1998); Wu, et al
(1988)). The association of DNA with histones and other proteins introduces supercoiling that requires relaxation by topoisomerases. In addition, transcription from some promoters in bacteria requires a minimal level of negative supercoiling, but too much supercoiling of either sign is disastrous (Kaguni and Kornberg (1984), Masse and Drolet (1999), Wallis, et al
(1989), Wang, (1990)). In all cells, completely replicated chromosomes must be untangled by DNA topoisomerases before partitioning and cell division can occur.

The immense interest in topoisomerases in recent years derives not only from the recognition of their crucial role in managing DNA topology, but also from two major advances in the field. First, a wide variety of topoisomerase-targeted drugs have been identified, many of which generate cytotoxic lesions by trapping the enzymes in covalent complexes on the DNA, these topoisomerase poisons include both antimicrobials and anticancer chemotherapeutics, some of which are currently in

widespread clinical use. Second, the crystal structures of numerous topoisomerase fragments have been published in the last few years that complement an extensive biochemical literature and provide key insights into how these machines work (Champoux, 2001).

Topoisomerases constitute a class of nuclear enzymes responsible for maintaining the topology of DNA and are involved in DNA repair, transcription, replication and segregation of chromosomes (Gobert, et al., 1996; Pommier ,et al., 1994). Inhibition and/or interference with topoisomerase functions lead to cell death. Several clinically active anticancer drugs (e.g., Etoposide, Adriamycin, and Camptothecin) target topoisomerases (Froelich-Ammon and Osheroff, 1995). Camptothecin (CPT), a topoisomerase I (topo I) poison, is effective against a wide variety of solid tumors. CPT stabilizes transient complexes formed between topo I and DNA (cleavable complexes) resulting in the formation of highly toxic double

strand breaks (Pommier, et al., 1994; Beidler and Cheng 1995; Nieves-Neira and Pommier, 1999).

Etoposide and doxorubicin are highly active anti-cancer agents in many different settings, that target topoisomerase II(Top2), an identification of a critical target of these drugs was a major landmark in the pharmacology of anti-cancer drugs. Although the biological functions of Top2 are important for insuring genomic integrity, the ability to interfere with Top2 and generate enzyme mediated DNA damage is an effective strategy for cancer chemotherapy. Drugs targeting Top2 are divided into two broad classes. The first class, which includes most of the clinically active agents including etoposide, doxorubicin, and mitoxantrone, lead to increases in the levels of Top2 DNA covalent complexes. Because these agents generate a “lesion” that includes DNA strand breaks and protein covalently

bound to DNA, these agents have been termed Top2 poisons. A second class of compounds inhibits Top2 catalytic activity, but do not generate increases in the levels of Top2 covalent complexes. This second class of agents is thought to kill cells through elimination of the essential enzymatic activity of Top2 and is therefore termed catalytic inhibitors (Nitiss, 2009).

Human DNA topoisomerase type 1:

Human topoisomerase I is a type IB enzyme composed of four major domains (N-terminal, core, linker, and C-terminal domains) and is involved in the relaxation of both positive and negative supercoiled DNA, reversibly nicking one DNA strand. The crystal structures of a reconstituted topoisomerase comprising the core and the C-terminal domain, in both covalent and non-covalent complexes with a 22-basepair duplex DNA, that have been reported (Redinbo et al., 1998; Stewart et al., 1998). Human topoisomerase I (Top1) is the molecular target of a diverse set of anticancer compounds, including the camptothecins, indolocarbazoles, and indenoisoquinolines.

Human topoisomerase I has been shown to be inhibited by camptothecin (CPT), a plant alkaloid with antitumour activity (Tamura, et al., (1991)). The crystal structures of human topoisomerase I comprising the core and carboxyl-terminal domains in covalent and noncovalent complexes with 22-base pair DNA duplexes reveal an enzyme that "clamps" around essentially B-form DNA. The core domain and the first eight residues of the carboxylterminal domain of the enzyme, including the active-site nucleophile tyrosine-723, share significant structural similarity with the bacteriophage family of DNA integrases. A binding mode for the anticancer drug camptothecin has been proposed on the basis of chemical and biochemical information combined with the three-dimensional structures of topoisomerase I-DNA complex (Redinbo, et al., (1998)).

Human DNA topoisomerase 1 brings about the transient breakage and relegation of the DNA phosphodiester backbone by catalyzing the relaxation of supercoiled DNA (Wang, (1996); Champoux, (2001)). The enzyme participates in DNA reactions, including replication and transcription, and has also been thought to have recombinative properties (Wang, (2002); Pommier, (2006)). DNA strand scission is co-occurring with the formation of a covalent topoisomerase I-DNA binary complex, which is recognized as having success in antitumor therapy (Hsiang, et al., (1985); Potmesil, (1994)).

The camptothecins are the prototype topoisomerase 1 poisons and are in use clinically. Other structural classes of topoisomerase I poisons have been reported, including aporphine alkaloids (Zhou, et al., 2000), indolocarbazoles (Bailly, et al., (1997)), indenoisoquinolines (Marchand, et al., (2006)), terbenzimidazoles (Khan and Pilch (2007)) and topopyrones (Kanai et al., (2000))

Khan, et al., (2009), studied the possible topoisomerase 1-mediated DNA cleavage induced by calothrixins and their methylated analogues. Khan, et al 2009, converted calothrixin B and calothrixin A to their N- and O-methylated derivatives in the yield of 65% and 70% respectively, by treating the calothrixin B with methyl iodide and calothrixin A with trimethyloxonium tetrafluoroborate. The calothrixin A, calothrixin B, N-metylcalothrixin B and Omethylcalothrixin A, all four calothrixins have shown to act as poisons of DNA topoisomerase 1 and calothrixin A, calothrixin B and N-methylcalothrixin B exhibited cytotoxicity toward cultured CEM leukemia cells with IC_{50} value ranging from 0.2 to 5,13 µM. The cell cycle effect of Calothrixin B produced G_1 arrest at 0.1 µM concentration.

Khan, et al., (2009) proved that the cell cycle effects produced by calothrixins were more readily reversible upon removal of the compounds than those produced by camptothecin.

Calothrixin B, Calothrixin A Murrayaquinone A, Ellipticine Quinones and Olivacine

Nature, Structure and their Role in Medicine:

Calothrixin B is a pentacyclic quinone that has an indolo phenanthridine or quinolino carbazole skeleton $C_{19}H_{10}N_2O_2$ (Fig. 1, a). It is a unique naturally occurring alkaloid. Calothrixin B and its N-oxide derivative Calothrixin A $C_{19}H_{10}N_2O_3$ (Fig. 1, b) were first isolated in 1999, from a bluegreen algae Calothrix cyanobacteria (Rickards, et al., (1999)).

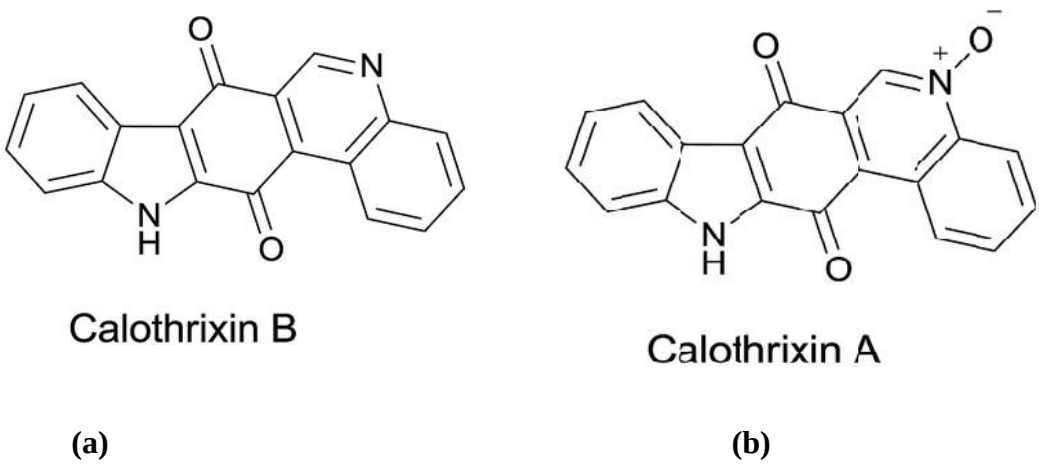


Figure 5: Structure of Calothrixin B and Calothrixin A.

Natural products have played a major role in drug discovery as the source of many of earliest medicines (Brown et al., (2014)). Although many natural products exhibit potent biological activities, their systematic biological evaluation is precluded because they are often isolated in very minute quantities, therefore development of new synthetic methods for natural products remains very important research area (Xu et al., (2014)). Butler, (2005) mentioned the shortage of drug leads progressing into clinical trials especially in the areas of oncology, immunosuppression and metabolic diseases.

Several natural and synthetic heterocyclic quinones have important biological activities; many of these molecules possess antineoplastic chemotherapeutic properties (Kock et al., (2005)).

Quinones are compounds found in many drugs that are used in clinical therapy of solid tumors (Morton, (1965) and Chabner et al., (1996)), their biological modes of action differ according to their particular structure and they can act as DNA intercalators, bioreductive alkalators of biomolecules and/or generators of reactive oxygen species through redox cycling (Bolton et al., (2000); O'Brien, (1991)). Among them, carbazolequinone alkaloids such as Ellipticine Quinones, Olivacine and Calothrixin B,display notable biological properties such as cardiotonic, antituberclosis and neutronal cell-protecting activities (Kazumi et al., (1989); Choi et al., (2008); Knolker and Reddy, (2003) and Shin-Ya et al., (1997)). Ellipticine (5,11-dimethyl6H-pyrido[4,3-b]carbazole) 3 and 9-methoxyellipticine 4 were isolated from the leaves of Ochrosia elliptica Labill by Goodwin et al. (1959). Olivacine was isolated from different members of the Apocynaceae family (Schmutz and Hunziker, (1958); Ondetti and Deulofeu, (1959); Gorman et al., (1960)). Quinones are recognized as being among the most effective antitumor agents that have attracted the attention of researchers looking to synthesize anticancer compounds (Monks et al., (1992) Halliwell and Gutteridge, (1999), the anti-cancer properties if quinines are well established with compounds such as mitomycin C, streptonigrin and adriamycin in clinical use. Many cofactors of enzymes such as menadione and ubiquinone are known to have a crucial function in cellular respiration and/or photosynthesis in prokaryotic and eukaryotic organisms. Large number of biologically active secondary metabolite quinones, have been isolated from various sources such as plants, fungi and marine organisms. These compounds often display selective toxicity towards foreign organisms. Some naturally

occurring quinines possess anti-oxidant, anti-inflammatory and anti-tumor activities (Thomson, 1971).

Quinones and Menadione showed micromolar EC_{50} values against HeLa cells, (Rickards et al., (1999); Bernardo et al., (2007); Khan et al., (2009); Bernardo et al., (2004) and Chen et al., (2003)).

Bernardo et al., (2007) in their study to elucidate the biological mode of action of calothrixin A and B, showed that similar analogus of calothrixin B display varying degrees of potency against different cell lines. Indolophenanthrenedione and benzocarbazoledione are the most selective in their activity against Hela cells and the differences in EC_{50} values obtained for calothrixin B against the different cell lines also highlight its specific utility against Hela cells.

Calothrixin possess a wide array of biological activities, they display nanomolar antiproliferative activity against certain human cancer cell lines such as human cervical cancer cell, CEM leukemia cells and human Jurkat cancer cells, Calothrixins act as a new class of human DNA topoisomerase I poisons, they stabilize the topoisomerase I-DNA binary complex to prevent DNA relegation and cause DNA damage that result in apoptosis (Doan et al., (2000)). Calothrixin A binds to DNA quadruplex to inhibit DNA replication and DNA directed RNA synthesis resulting in impaired protein synthesis and cell death (Owen and Keniry, (2009)). Calothrixins exhibit in vitro antiparasitic activity against chloroquine resistant strains of Plasmodium falciparum which is the causative organism of malaria, in a dose-dependent manner (Rickards et al., (1999), Chen et al., (2003), Doan et al., (2000)). These biological activities make Calothrixins the potential lead compounds for anticancer and antiparasitic drug discovery. Preliminary mechanistic studies suggest that the biologicalmode of action of the calothrixinsmay be linked to their ability to undergo redox cycling.

For a number of quinine systems, a relationship between biological activity and redox potential has been suggested, such as between the redox potential of azaanthraquinones and their inhibitory effects on Epstein-Barr virus early antigen activation (Koyama et al., (2001)), also the cytotoxicity of naturally occurring quinines against human breast adenocarcinoma (Inbaraj et al., 1999) and the higher cytotoxic potency of mitomycin A versus mytomycin C, which has been tentatively correlated with the large difference in the quinine redox potential of both drugs (Paz et al., 2001). Different investigations have showed that calothrixin A is a redox-active and induces the intracellular formation of the reactive oxygen species (Chen et al., (2003)). Bernardo et al., (2004), compared the bioactivities of calothrixins with those of structurally related quinines to identify the structural feature of the calothrixins essential for biological activity. The reduction potentials of the calothrixins and some related quinines were measured electrochemically, Calothrixin A and B, as well as N-MOM-calothrixin B showed comparable redox potentials, with calothrixin A proving the easiest to reduce. N-MOMellipticine quinone was found to be significantly easier to reduce than its benzo analogue NMOM-benzocarbazoledione. In their biological activity, quinines showed micromolar EC_{50} values against Hela cells. The most active of these quinines were, calothrixins and ellipticine quinine followed by benzocarbazoledione. The comparison of the E_{12} values with EC_{50} of the different quinines tested showed no direct correlation between reduction potentials and biological activities of these quinines.

Total synthesis of Calothrixins B and A:

Different synthesis methods have been reported for the production and isolation of Calothrixins such as electrocyclization, metallation strategy Kelly et al., (2000); Bernardo et al., (2002), Watanabe and Snieckus (1980); Ketcha and Gribble (1985), Pd-catalyzed coupling hetero Diels-Alder (bonds C6-C6a and C-C13b bonds), Friedel-Crafts acylation/alkylation, reaction, radical reaction, (Ramkumar and Nagarajan, (2013), Xu et al., (2014), Sissouma et al., (2004)). Total synthesis of calothrixin employing ortholithiation methods was first reported by Kelly, et al., (2000), their method based on ortho-lithiation reactions to create the C_{6a}-C_7 and C_{12a}-C_{13} bonds. One of the widely used method of synthesis of Calothrixin B is the sonogashira coupling reaction, in which 1,n-enyne system is synthesize which is an important unit in biologically active natural compounds.

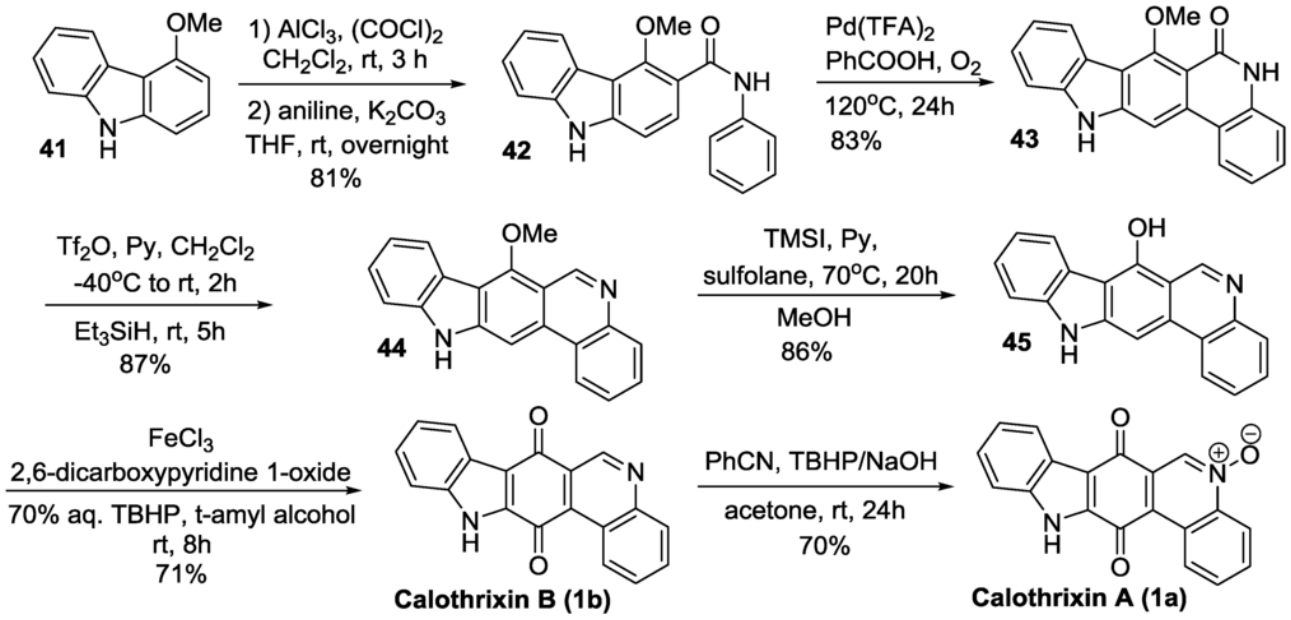
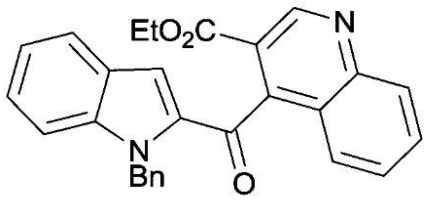


Figure 6: Synthesis of Calothrixin A and B by exploiting Pd-catalyzed intramolecular cross-coupling reaction via C-H activation using an appropriately substituted carbazole derivative (Ramkumar and Nagarajan (2013).

Ramkumar and Nagarajan, (2015) reported the synthesis of Calothrixin B through sequential sonogashira coupling and copper- catalyzed oxidative cyclization reactions with an overall yield of 40.6 to 41.8% over 5 steps, first ketoester 11 (Fig. 2.) was produced in two ways of intramolecular and intermolecular copper-catalyzed oxidative cyclization reactions, then Calothrixin B is produced through subsequent ester hydrolysis, cyclodehydration and debenzylation of ketoester 11.



Ketoester 11

Figure 7: Ketoester 11.

Ramkumar and Nagarajan (2014) used the methods of Friedel-Crafts hydroxyalkylation followed by oxidation and directed ortho-lithiation reactions of readily available indole-2carboxylate esters, with appropriately substituted pyridine and quinoline carboxaldehydes to synthesize ellipticine quinine and calothrixin B in three –step sequences of 67 % and 38 % overall yield respectively and also synthesized olivacine in 16 % overall yield in six steps.

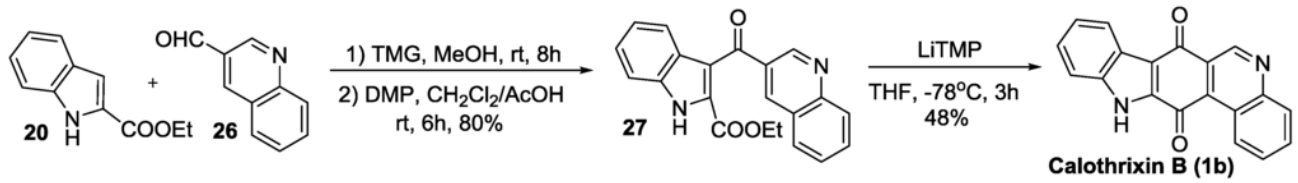


Figure 8: Synthesis of Calothrixin B using Friedel-Crafts hydroxyalkylation method followed by oxidation and directed ortho-lithiation reactions of indole-2carboxylate esters (Ramkumar, 2014).

Ramalingam et al., (2013) synthesized calothrixin B and its analogs by involving a $FeCl_3$mediated domino reaction of enamines in dry DMF at reflux. The interaction of the enamines with $CuBr_2$ in DMF at reflux led to the formation of 1-phenylsulfony-2-(2-nitroaryl)-4hydroxycarbazole-3-carbaldehydes in excellent yield.

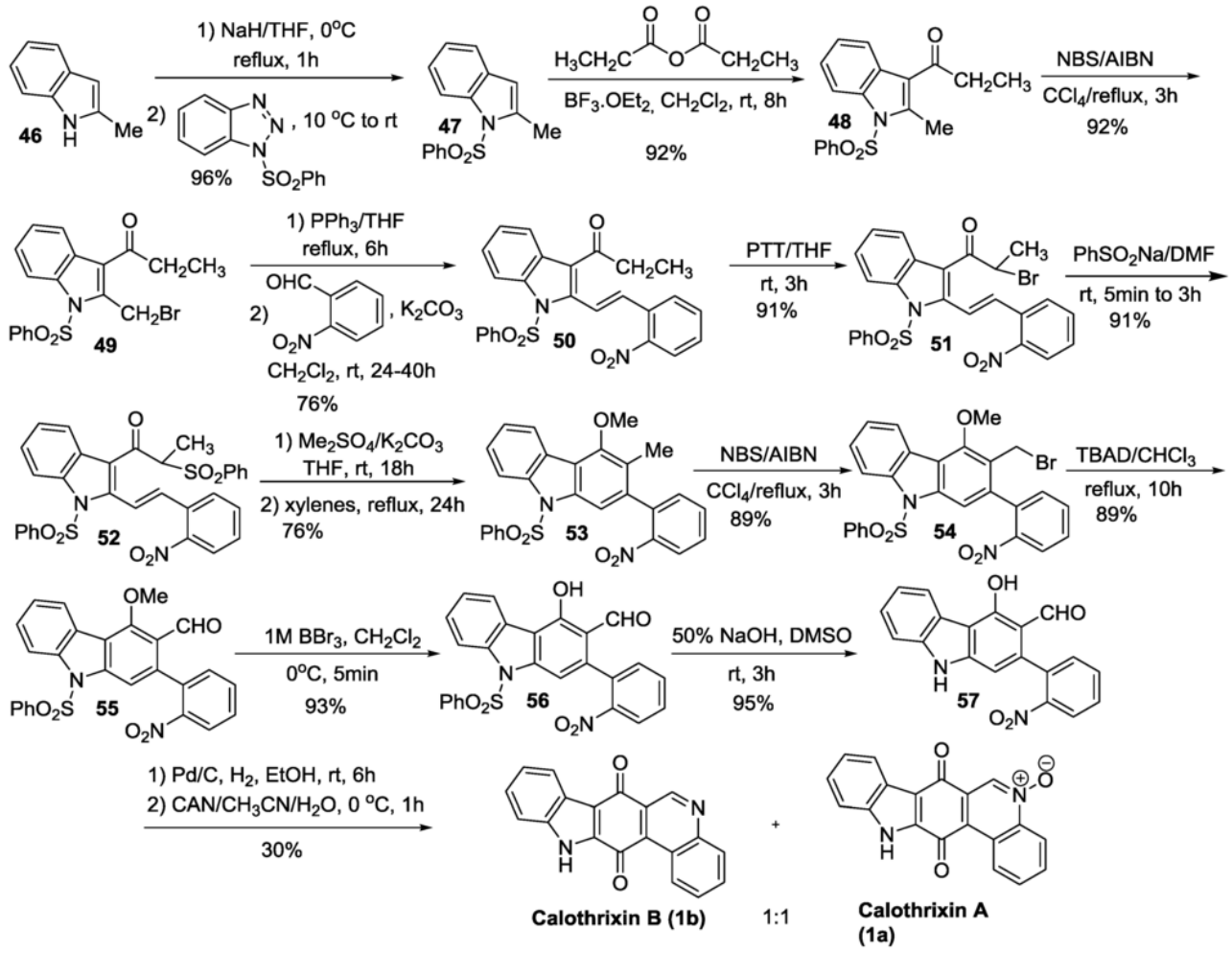


Figure 9: Synthesis of calothrixin B and its analogs using an FeCl3-mediated domino reaction of enamines in dry DMF at reflux. Alternatively, the enamines upon interaction with CuBr2 in DMF at reflux led to the formation of 1-phenylsulfony-2-(2-nitroaryl)-4-hydroxycarbazole-3-carbaldehydes Ramalingam et al., (2013).

Xu et al., (2014), developed synthetic approach that relies on the construction of the indole ring on to a phenanthridine dione via a novel oxidative free radical reaction mediated by $Mn(OAc)_3$, they synthesized Calothrixin B from commercially available 2,4,5trimethoxybenyaldehyde in only seven steps.

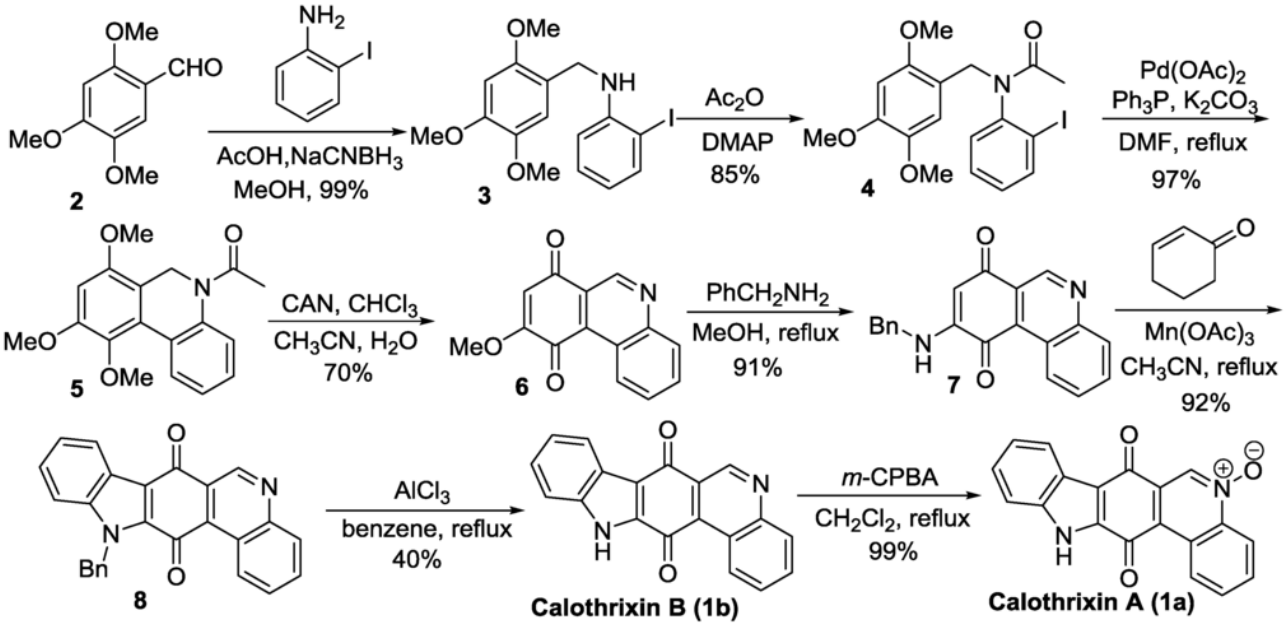


Figure 10 : Synthesis of Calothrixins B from commercially available 2,4,5trimethoxybenyaldehyde via a novel oxidative free radical reaction mediated by $Mn(OAc)_3$ (Xu et al., 2014).

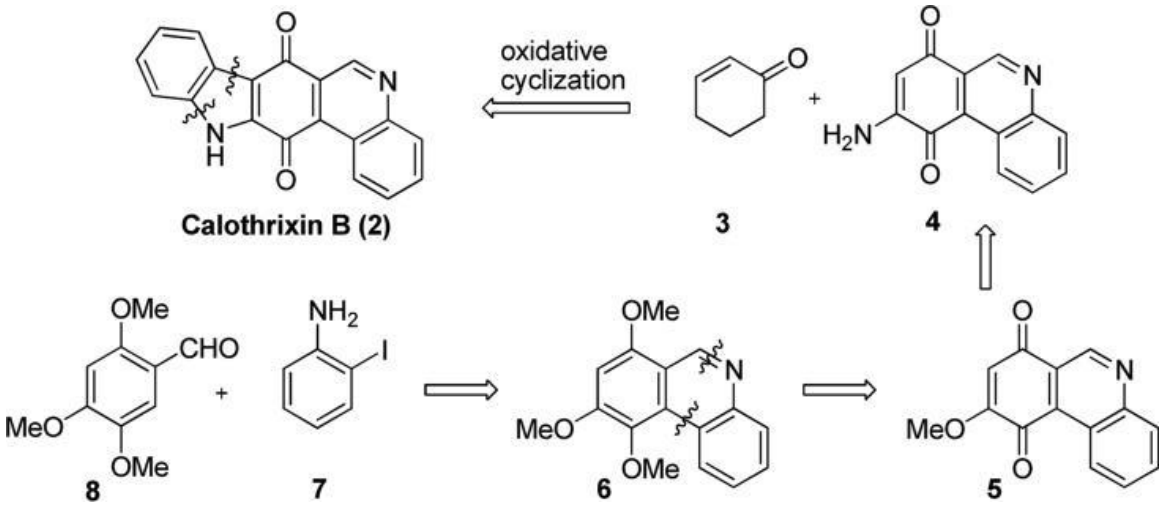


Figure 11: Retrosynthetic analysis of calothrixin B (Xu et al., 2014).

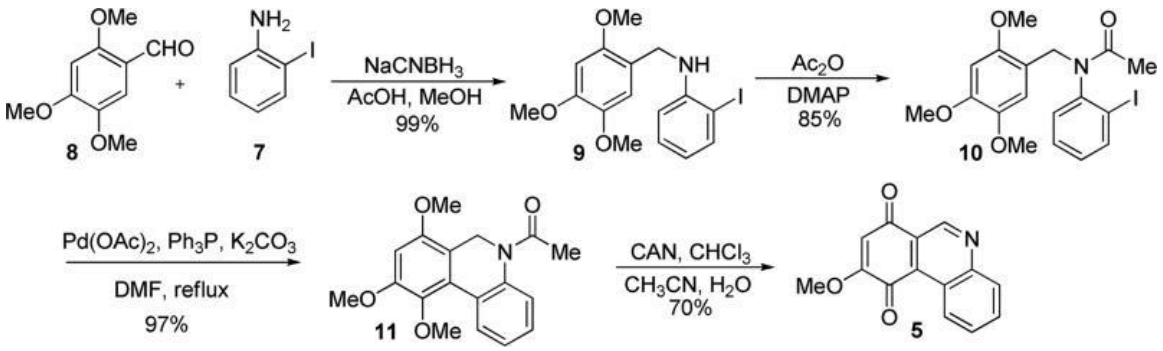


Figure 12: Synthesis of intermediate quinine 5 (Xu et al., 2014).

Kaliyaperumal et al., (2014) reported the total synthesis of Calothrixin B via multiple as well as step-wise palladium mediated intramolecular multiple C-X/C-H cross coupling reactions and murrayaquinone A synthesis by C-H activation and also by C-X/C-H cross coupling reaction from its basic starting material. Murrayaquinone A ($C_{13}H_9NO_2$) is an indole alkaloid isolated from *Murraya euchrestifolia* and has been used in folk medicine for analgesia, treatment of eczema, rheumatism and dropsy.

Kaliyaperumal et al., (2014) afforded Calothrixin B in excellent yield in two steps using regioselective palladium mediated intramolecular multiple C-X/C-H cross coupling reaction on N-(4-((2-bromophenyl)amino)-2,5-dimethoxybenzyl)-N-(2-iodophenyl)acetamide followed by CAN oxidation Utilizing C-H activation as well as intramolecular C-X/C-H reaction protocols, murrayaquinone A has also been synthesized (Kaliyaperumal et al) ,.2014)).

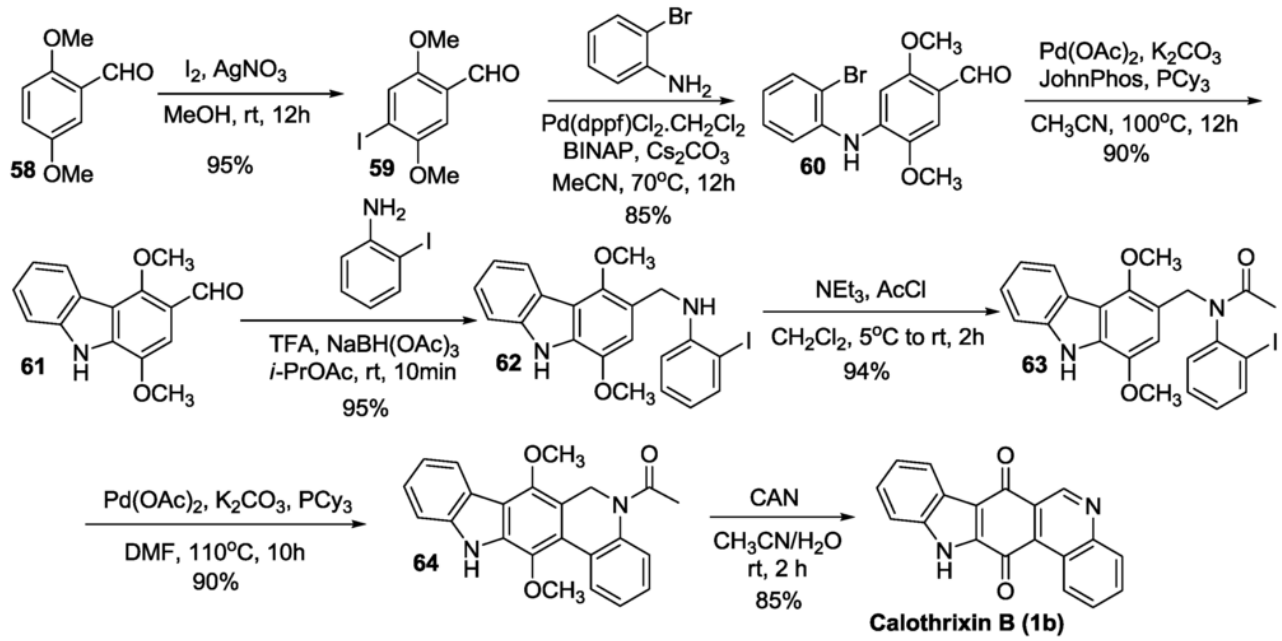


Figure 13: Synthesis of Calothrixin B by cross coupling reaction on N-(4-((2-bromophenyl) amino)-2,5-dimethoxybenzyl)-N-(2-iodophenyl)acetamide followed by CAN oxidation (Kaliyaperumal et al., 2014).

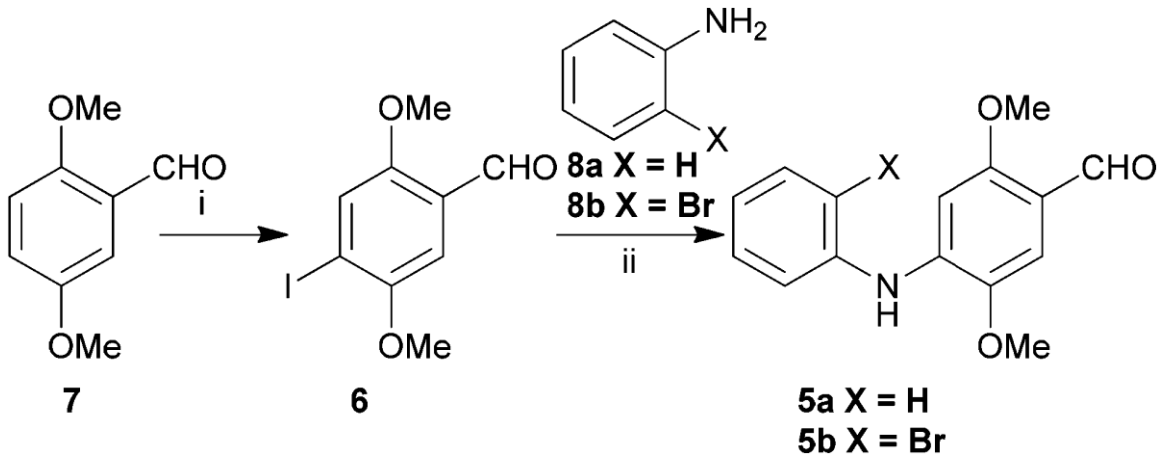


Figure 14:Buchwald Hartig amination. Reagents and conditions:(i) I2, AgNO3, MeOH,r.t.,12 h, 95%; Pd(dppf)l2.CH2Cl2.(+_)-BINAP.Cs2CO3, 8a or 8b, MeCN,70 °C, 12 h (X= Br,85%; X= H, 90%) (Kaliyaperumal et al., 2014).

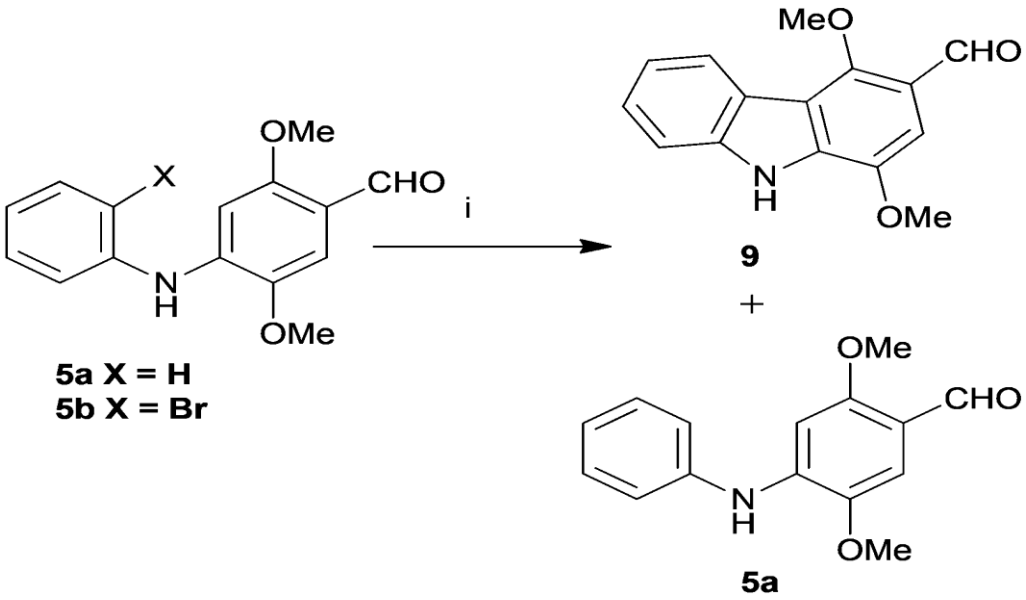


Figure 15: Synthesis of carbazole derivatives. Reagents and conditions: (i) X= H, $Pd(OAc)_2$ (2 equiv.), AcOH, 130 °C, 6 h, 91 %; X= Br, $Pd(OAc)_2$, K_2CO_3, 10 mol% JohnPhos, 20 mol% PCY3, MeCN, !00 °C, 12 h, 90%.

Mal, et al., (2014) reported remarkably selective annulation culminating in the total synthesis of both Calothrixin B and its geometric isomer isocalothrixin in only five steps from commercially available starting materials and in two steps from the known compounds 4bromoquinoline and 3-bromoquinoline in 41% and 35% overall yields respectively. The anionic annulations of MOM-protected furoindolone with 4-bromoquinoline followed by deprotection of the N-MOM group provides Calothrixin B. whereas that with 3bromoquinoline yields isocalothrixin B. The outcomes are explained by the divergence of the reaction mechanism from commonly perceived quinolyne intermediate. Mal, et al., (2014) proposed a sequence of addition-cyclization –elimination to account for the formation of Calothrixin from 4-bromoquinoline.

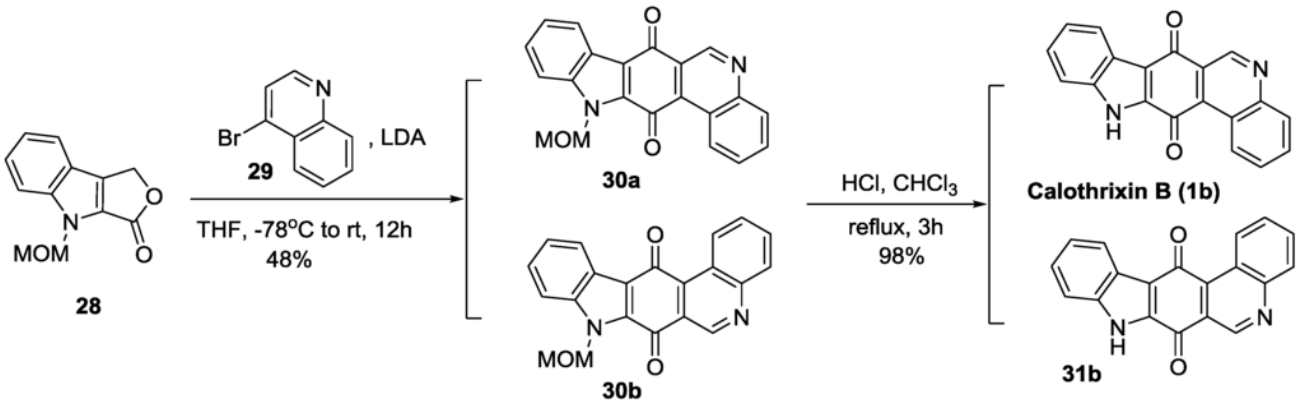


Figure 16: Synthesis of Calothrixin by the treatment of N-protected indololactone with 4-bromoquinoline in the presence of lithium diisopropylamide (LDA) in tetrahydrofuran (THF) at −78 °C (Mal, et al).

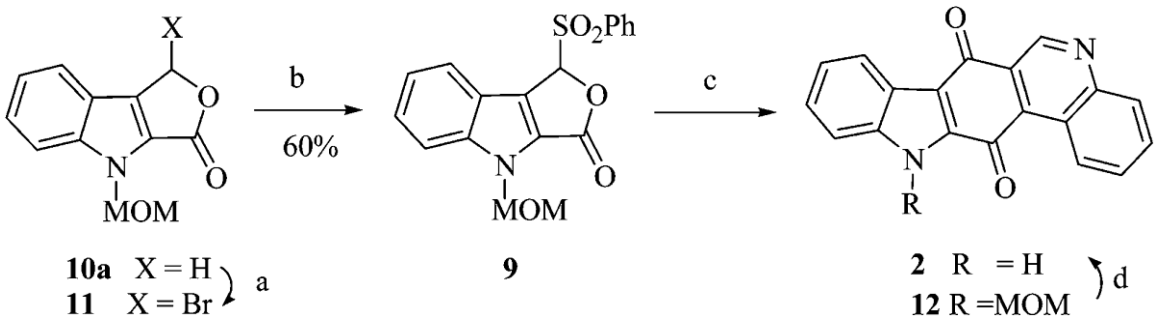


Figure 17: Synthesis of calothrixin B from 3-phenylsulfonylfuroindolone. Reagents and conditions: (a) NBS, CCl4, 1.5 h, reflux; (b) PhSO2Na, DMF, overnight, 60% over two steps; (c) 4-bromoquinoline (5), LDA, THF, -78 °C to rt, overnight, 7%; (d) conc. HCl, CHCl3, reflux, 3h, 97% (Mal, et al).

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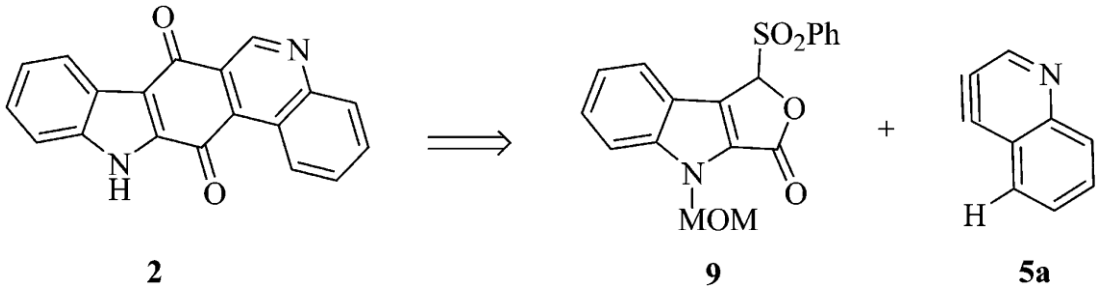

Figure 18: Retrosynthesis of calothrixin (2) by annulation.

Miyaura et al., (1985), Negishi et al., (1987) , Sharma et al., (1988), Ishikura and Terashima (1994) and Ishikur Takumi (2006) in their research studies have reported that trialkyl(indol-2yl) borates, which showed high reactivity in palladium-catalyzed cross-coupling reactions such as carbonylative cross-coupling and tandem cyclization cross-coupling reactions, have proven to be less practical for the palladium-catalyzed cross-coupling reactions.

Abe, et al., (2011) described a new approach to synthesis calothrixin A and B through the palladium-catalyzed tandem cyclization/cross-coupling reaction of triethyl(indol-2-yl)borate, while building the indolophenanthridine core via one-pot construction of a hexatriene as a key intermediate; Hexatriene then Transformed through 6-electrocyclization into indolophenanthridine, that later has been converted into calothrixin B using quinone.2iodoaniline with 2-(prop-2-2yn-l-yloxy) tetrahydro-2H-pyran. In their study Abe, et al., (2011) produced calothrixin B from pentacycle in 85% yield in three steps. Further the oxidation of calothrixin B produced calothrixin A in 70 % yield.

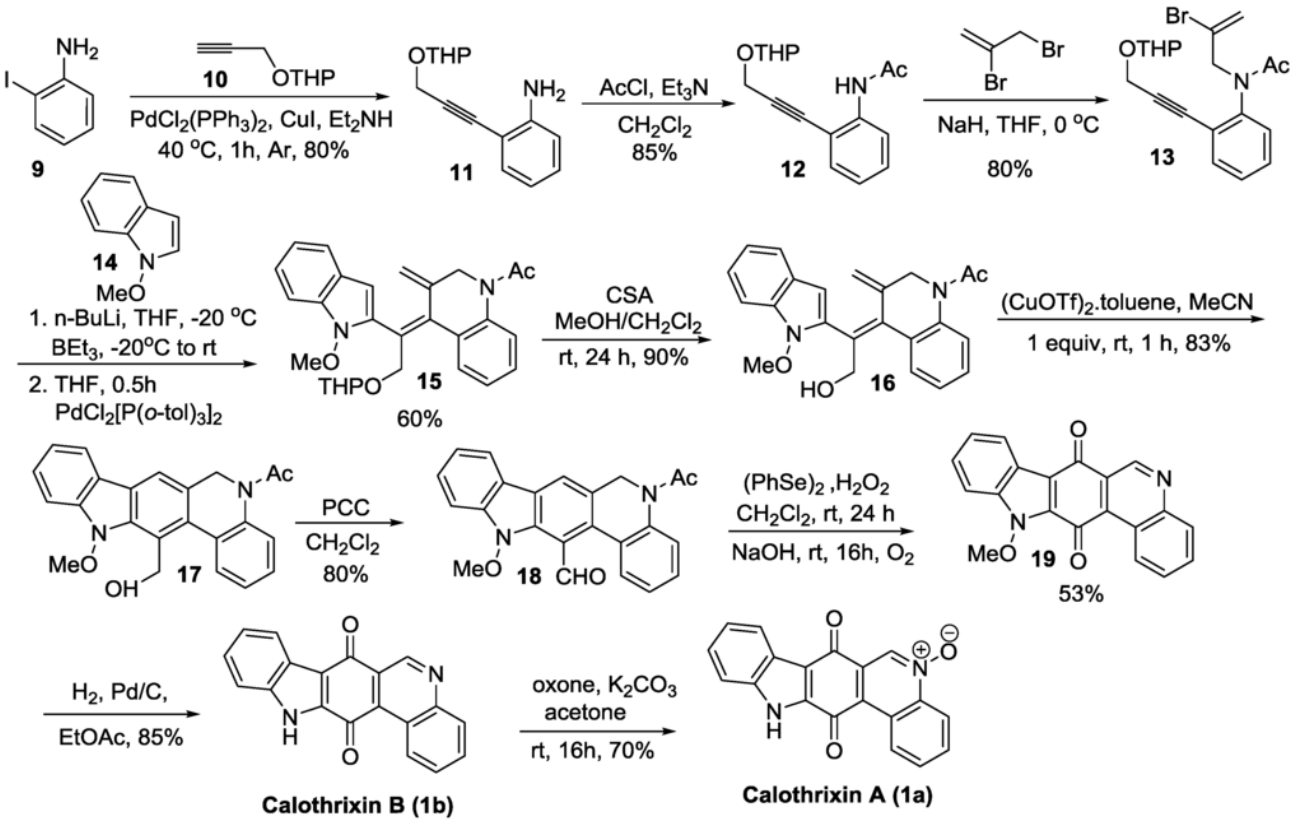

Figure 19: Preperation of Vinyl Bromide (13), Palladium-Catalyzed Tandem (Cyclization/ Cross-Coupling Reaction and Total Synthesis of Calothrixin A and B (Abe, et al 2011)

Bernardo et al., (2002) employed both a Friedel-Crafts reaction and a lithiation-cyclization reaction Hibino et al., (2005) accomplished the synthesis via an allene-mediated electrocyclic reaction with the creation of the C6a-C13a bond, while Bennasar et al., (2006) accomplished the synthesis of calothrixin B via regioselective cyclization of of an acyl radical while forming the C13- C13a bond simultaneously.

Sissouma et al., (2006) have prepared calothrixin B through the exploitation of a regioselective hetero-Diels-Alder cycloaddition to assemble the core structure of the molecule. The electronic nature of the indole moiety N-protecting group benzloxycarbonyl vs benzyl influenced the reactivity significantly. Using the N- benzloxycarbonyl protective group was more productive as it accelerated the cycloaddition process and could easily be

removed during the reaction under the action of the liberated dimethylamine hydrobromide. An overall calothrixin B yield of 17% from commercially available 1,2,3,4,9-tetrahydro-4H-carbazole-4one was produced. Sissouma et al., (2006) also reported that recourse to reprotection the indole nitrogen atom was unnecessary to achieve the transformation of the Diels-Alder adduct toward calothrixin B in nine steps.

Some researcher such as Sissouma, et al., (2004) and Tohyama, et al.,(2005) synthesized calothrixin B through the strategy of hetero Diels-Alder (bonds C_6-C_{6a} and C_{13a}-C_{13b}) and a final formation of the quinoline ring from an appropriately substituted carbazole bond (C_5-C_6) respectively.

Bennasar et al., (2001) observed that 2-indolylacyl radicals can bring about cyclization upon aromatic rings, but only under non-reductive conditions, resulting in moderate yields of benzocarbazolediones and ellipticine quinones.

Bennasar et al., (2005) developed a method to produce calothrixin B from a selenoester, a model radical precursor bearing the required (3-quinolyl)methyl moiety connected to the indole 3-position. Bennasar et al., (2005) have shown that synthetic entry to calothrixinrelated pentacycles (obtained in different oxidation states depending on the substitution at the indole nitrogen) can be enabled via the cyclization of 3-(3-quinolyl) methyl-2-indolylacyl radicals under tris(trimethylsilyl)silane (TTMSS) and azobisisobutyronitrile AIBN conditions. Cyclizations of 2-indolylacyl radicals display significant promise in constructing polycyclic indole compounds in aromatic systems. Using synthetic intermediates such as N(methoxymethyl)-selenoester, the researchers arrived at a yield of 42%. However, the Nsubstituted 2-indolylacyl radicals generated from selenoester brought about even greater efficiency: a fully aromatic tautomeric form was isolated in a yield of, at best, 90%. Bennasar et al., (2005) reported that a high-yielding totally regioselective intramolecular homolytic acylation of a quinoline ring constitutes the

key step in a new synthesis of the pentacyclic indolo[3,2-j]phenanthridine alkaloid calothrixin B.

Bernardo and Chai (2003) used Friedel-Craft acylation and metalation strategies to install the pentacyclic ring system to produce calothrixin. their method showed much better regioselectivity (5: 1 of 4-methyl ester vs 3-methyl ester) when dry methanol was used to effect the anhydride ring opening and a yield of 70 % of the desired quinoline was obtained, which was later quantitatively converted to acid chloride (3-Chlorocarbonyl-4methoxycarbonylquinoline). The indole 3 was then treated with $MeMgCl_3$ in the presence of $ZnCl_2$ and subsequent addition of the acid chloride to give the desired ketone (3-(3Indol)carbonyl-4- methoxycarbonylquinoline) in yield of 90%, from which the calothrixin B was produced. Calothrixin A was achieved by the oxidation of calothrixin B, using mchloroperbenzoic acid (m-CPBA) as an oxidant.

Quinolines are important class of antimalarial agents as mentioned above and chloroquine is widely used for the prevention and initial treatment of malaria, with the spread of malaria strains resistant to chloroquine and other drugs, lead the researchers to develop a new chemotherapeutic agents that are more effective.

Matsumoto, et al., (2012) synthesized calothrixin A and B using their developed biomimetic method, they evaluated in vitro the antimalarial potencies of the calothrixin A and B and their N-alkylated compounds against the Plasmodium falciparum FCR-3 strain. All test compounds exhibited antimalarial activity over a concentration range of 6.4×10^{-6}–1.2×10^{-7} M, where calothrixin B had the highest activity value. Antimalarial activity was reduced in both compounds by introducing an alkyl group in their indole nitrogen atom, whereas 2hydroxyethyl substitution proved more effective in the N-alklyated compounds than other substitutions. The IC_{50} value of N-alkyl-calothrixin A derivatives exceeded that

of derivatives for the calothrixin B compound. Grater Clog P compounds proved more potent in N-alkylated calothrixin A derivatives. This potency, however, had an inversely proportional relationship to hydrophobicity, decreasing as the latter increased in the final 4 runs. Matsumoto, et al., (2012) suggested that the pentacyclic indol[3,2-j]phenanthridine structure of calothrixin is advantageous for antimalarial medicinal chemistry.

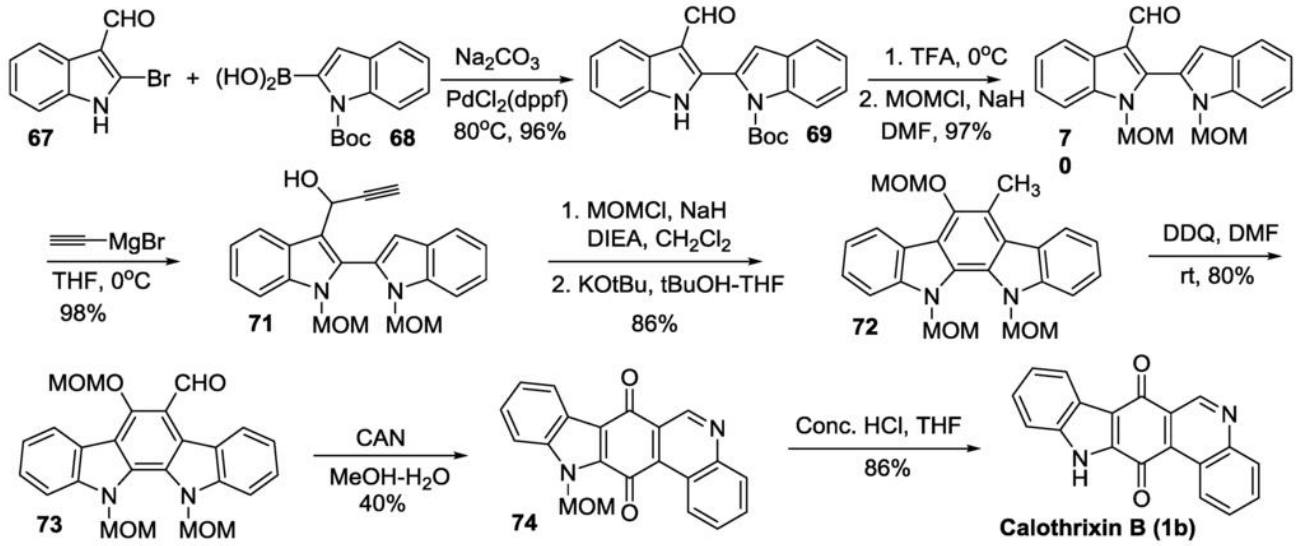

Figure 20: Synthesis of Calothrixin B from 2-bromoindole-3-carboxaldehyde using a developed biomimetic method (Matsumoto, et al., 2012).

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List of Figures: