CHAPTER-2

LITERATURE REVIEW
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2. General

2.1 Quinone

Quinones are described as a class of cyclic organic compounds comprising of a six-membered unsaturated ring to which two oxygen atoms are bonded as carbonyl groups. The name quinone is applied to the whole group, but it is often used specifically to refer to (1) p-benzoquinone (2) o-benzoquinone (Figure 2.1.) is also known but the meta-isomer does not exist.

![Figure 2.1. Structures of p-benzoquinone (1) and o-benzoquinone (2)](image)

This structure plays an important role in theories of chemical structure and color, since quinones occur as pigments in bacteria, fungi, and certain higher plants; animals containing quinones obtain them from plants they eat. Quinones are obtained in the form of colored crystals, which have a sharp odor. For example, para-benzoquinone (4), with a melting point of 116°C, and 1,4-naphthoquinone (5), with a melting point of 128.5°C, are yellow, while ortho-benzoquinone (3), (Figure 2.2.) with a melting point of 70°–80°C (with decomposition), and anthraquinone are red. Quinonoids compounds are widely distributed in nature, mainly as secondary metabolites, in all respiring animal and plant cells. Some of
these quinones can act as vital links in the electron transport chain playing important roles in the bio-chemistry of energy production in their natural hosts, while many others show pronounced cytotoxic and allergic actions that might enable the hosts to define themselves against invading pathogens. A number of natural quinonoids as well as their synthetic analogues have been found to possess significant antitumor activity by virtue of their facile redox cycling capacity. Incidentally, quinonoids comprise the second largest class of antitumor agents currently in use, e.g. the daunomycin group of drugs based on anthracyclin antibiotics (Powis 1987). Recent studies have been demonstrated that this drug, kill tumor cells through apoptosis and has been recognized as a target for cancer therapy. Various pigments in nature are derivatives of quinones; for example, muscapharain, the dye substance of the fly agaric, is a derivative of para-benzoquinone. Quinones are readily reduced to yield diatomic phenols [for example, in industry, hydroquinone is obtained from (I) in this way]. They actively enter in diene synthesis, as well as form molecular complexes with phenols. They are also used in analytical chemistry.

![Figure 2.2. Structures of o-benzoquione (3), p-benzoquione (4) and 1,4-naphthoquinone (5)](image)

Quinones are obtained by several methods, including the oxidation of the aromatic hydrocarbons or of their hydroxy or amino derivatives; for example, (I) is obtained by the oxidation of aniline, while (II) and anthraquinone are obtained by the oxidation of naphthalene and anthracene, respectively.
Quinones and their derivatives are intermediate products in the production of dyes. These are also used as fungicides, insecticides, and tanning agents. 2-Methyl-1,4-naphthoquinone is a vitamin of the vitamin K group. The K vitamins (see vitamin K) are naphthoquinones. The term quinone often specifically denotes para-benzoquinone \((\text{C}_6\text{H}_4\text{O}_2)\), a bright yellow solid with a sharp odor used in manufacturing dyes and fungicides and in photography.

2.2. Review of previous research on naphthoquinones and bi-naphthoquinones

2.2.1 Naphthoquinones

Naphtho-1, 4-quinones are widely available in nature, mainly in plants, fungi and bacteria. These classes of compounds have various properties and applications. These properties and applications have been extensively reviewed (Thomson 1971; Patai 1974), they isolated as yellow, orange, red, or purple solids, and are sparingly soluble in water but readily soluble in most organic solvents.

2.2.1.1 Naphthoquinones as Privileged Molecules

Naphthoquinones are considered privileged structures in medicinal chemistry due to their biological activities and structural properties. They are present in various families of plants and serve as vital links in the electron transport chains in the metabolic pathway, participating in multiple biological oxidative processes. The fundamental feature of quinone chemistry is its ease of reduction and, therefore, its ability to act as an oxidizing or dehydrogenating agent. This redox property is driven by the formation of a fully aromatic system. In folk medicine, plants containing naphthoquinones are often employed for the treatment of various diseases, and several quinonoids isolated from traditional medicinal plants are being investigated for their anticancer properties.
The redox cycling of quinones may be initiated by either a one- or two-electron reduction. The one electron reduction of quinones is catalyzed by NADPH-cytochrome P450 reductase, and yields unstable semiquinones. Quinones transfer electrons to molecular oxygen (O$_2$), and return to their original quinoidal formation, thus generating a superoxide anion radical (O$_2^-$). Superoxide can be converted to hydrogen peroxide (H$_2$O$_2$) via a superoxide dismutase (SOD)-catalysed reaction, followed by the formation of a hydroxyl radical (OH) by the iron-catalysed reduction of peroxide via the Fenton reaction. All of these highly reactive species may react directly with DNA or other cellular macromolecules, such as lipids and proteins, leading to cell damage (Figure 2.3.).

Figure 2.3. Representation of the redox cycle and metabolites by quinones
Owing to their molecular structure and their redox properties, they exhibit interesting physical properties, as well as a wide range of biological activities. Extracts from plants containing mixtures of naphtha-1, 4-quinone derivatives have been used for centuries not only as dyes or ingredients for cosmetics but also in traditional medicine for the treatment of a great number of diseases (Thomson 1971). Nowadays, a number of naphtha-1, 4-quinone, such as phylloquinone (regulation of blood coagulation, bone metabolism and vascular biology), lawsone (natural dye), naphthazarin (natural dye), atovaquone (antineumococcal) (Williams and Clark 1998) are used as drugs or ointments although the exact mode of action of these compounds has not been completely elucidated, the biological activity is probably due to their redox properties.

2.2.1.2. Anti fungal, antimicrobial and anti-bacterial quinones

2-arylamino-3-chloro-1, 4-naphthoquinone derivatives have been prepared and studied for their antifungal and antibacterial activities, chloro, methoxyphenyl and amino derivatives of the compounds were showing potent antifungal and antibacterial activities (Tandon et al. 2004). Chloro derivative showed better anti-fungal properties than clinically prevalent anti-fungal drug Fluconazole (MIC50-2.0 g/mL) against Sporothrix schenckii (MIC50-1.56 g/mL) potent profile against Candida albicans (MIC50-1.56g/mL), C. neoformans (MIC50-0.78g/mL) and same anti-fungal activity when compared to Amphotericin-B against C .neoformans (MIC50-0.78g/mL). Lapacol and its derivatives have two fold greater activities on Staphylococcus aureus Claudia (Oliveira et al. 2001). In 2-aryl amino naphthalene derivatives at position three showed more potent activity compared to position two. Different compounds have been synthesized for antifungal and antiviral activity. All the compounds having thiol group showed potent activities. Alpha-amino acid ester, hetero alkyl and aryl substituted 1,4-naphthoquinone derivatives having antifungal and antibacterial activities where as amino ester and hetero have potent effect among all for anti-
fungal activity (Tandon et al. 2005). Naphtho [2, 3] isoxazole-4,9-dione have evaluated against ATCC and PYCC strains of candida (Santos et al. 2010). This system contains electron withdrawing group at position three.

Sulphur and nitrogen containing napthoquinone also have potent activity against fungal and bacterial stain. C-2 substituted and C-2, C-3 disubstituted derivatives have synthesized with their reaction with amines, thiols and halogen acids and their use for the study of bacterial growth inhibition has also been demonstrated [60] (Figure 2.4.). 2-substituted-3mercapto-1,4-napthoquinones have been evaluated for anti-microbial activities (Stasevych M.V. et al. 2006). Morpholino and piperidino derivatives showed greater antibacterial activity than well known oxacillin.

![Figure 2.4. Structures of different sulphur and nirrogen containing napthoquinones](image-url)
2.2.1.3. Naphthoquinone and inflammation

Lapachol, a natural organic compound isolated from the lapachol tree (*Tabebuia avellanedae*) identified as naphthoquinone group and is known for its anti-inflammatory, analgesic and antibiotic properties (Costa et al. 2011). It is also an anti-tumor agent. *Cipura paludosa* (Iridaceae) is a plant that forms lapachol and is distributed in the north region of Brazil. Its bulbs are used in folk medicine to treat inflammation and pain. It is having four naphthalene derivatives which have been isolated from the bulbs of the plant. Three of them were identified as naphthalene derivatives, eleutherine, Iso-eleutherine and hongkonin. The structure of the fourth was new and elucidated as 11-hydroxyeleutherine (Batista et al. 2011) by NMR. In-vivo effect of two major compounds eleutherine and iso-eleutherine, was evaluated in carrageenan-induced hypernociception and inflammation in mice. Eleutherine and iso-eleutherine (1.04-34.92 mol/kg), dosed i.p. or orally, decreased the carrageenan-induced paw edema (i.p. - inhibitions of 36 ± 7 % and 58 ± 14 %, resp.; p.o -inhibitions of 36 ± 7 % and 58 ± 14 %, resp.). Iso-eleutherine, but not eleutherine, significantly reduced (inhibitions of 39 ± 4 %) the plasma extravasation induced by intradermal (i.d.) injection of carrageenan. Likewise, eleutherine and iso-eleutherine (1.04- 34.92 mol/kg,i.p. or p.o.) were also effective in preventing the carrageenan-induced hypernociceptive response (i.p.- inhibition of 59 ± 4 % and 63 ± 1 %, resp.; p.o. - inhibitions of 36 ± 7 % and 58 ± 14 %, resp.).

It was also suggested that the anti-inflammatory and anti-hypernociceptive effects of eleutherine or iso-eleutherine partly depend on the interference with the synthesis or activity of mast cell products, kinins, cytokine, chemokines, prostanoids, or sympathetic amines. Two major compounds of *C. paludosa* contain pharmacologically active constituents that possess antinociceptive and anti-inflammatory activity, justifying, at least in part, its
Vitamin K3, which consists of a quinone component, inhibits the activity of human DNA polymerase (Kazuki et al. 2011). In this study, the inhibitory effects of 1, 4-quinone derivatives, (1,4-benzoquinone (BQ), 1,4-naphthoquinone (NQ), 9,10-anthraquinone and 5,12-naphthacenequinone) on the activity of mammalian polymerase has been shown. BQ and NQ potently inhibited the activity of all the polymerase species. NQ was a stronger polymerase inhibitor than BQ.

These quinone derivatives could inhibit inflammatory 12-o-tetradecanoylphorbol -13 acetate (tpa) induced acute responses. BQ and NQ caused a marked reduction in ion in mouse ear. These anti-inflammatory responses of NQ were more potent than those of BQ. In conclusion, this study has identified several quinone derivatives, such as NQ, that are promising anti-inflammatory candidates (Cherng et al. 1997).

A series of 2-substituted 3-chloro-1, 4-naphthoquinones (Figure 2.5.) were synthesized, and the antiplatelet, anti-inflammatory, and antiallergic activities of these compounds were evaluated (Tadashi et al. 1996). The structure-activity relationships in this series were also examined. The title compounds, I \([R1, R2 = \text{H, OH}; R3 = \text{acyl, etc.; } R4 = \text{H, etc.; or } NR3R4 = Q1, \text{etc.; } r, t = 1 - 3; Y = \text{CH, N, etc.; } R10 = \text{alkoxyphenyl, etc.; } X = \text{halo}]\) are prepared. The title compd. II in vitro showed IC\(_{50}\) -0.22 \(\mu\)M against neutrophil expression of CD11b. Naphthoquinone showed potent activities with similar trends in each of the activities evaluated.
Figure 2.5. Structures of 2-substituted -3-chloro naphthoquinones

Novel naphthoquinone and naphthothiazole compounds I (where in X and Y are independently halo, (un) substituted aryloxy, (un) substituted aryl, etc.), and methods of treating disorders related to methionine aminopeptidases (Figure 2.6.) (Liu et al. 2011). The disease or disorder associated with methionine aminopeptidase is selected from: tuberculosis, bacterial infection, tumor or cancer growth, skindisorders, neovascularization, inflammatory, arthritic diseases, retinoblastoma, cystoid macular edema, exudative age-related macular degeneration (AMD), diabetic retinopathy, diabetic macular edema, or ocular inflammatory disorders. Synthetic procedures for preparation of I and related compounds are exemplified. Compound-II was prepared by reacting 4-fluoro phenol and 2,3-dibromonaphthoquinone.
Figure 2.6. Structure of novel naphthoquinone derivatives

Three naphthoquinone derivatives, rhinacanthin-C (1), -D (2) and -N (3) were isolated from the leaves of *Rhinacanthus nasutus* extract and were tested for anti-inflammatory activity (Supinya et al. 2009). The result indicated that all three compounds possessed very potent anti-inflammatory activity against lipopolysaccharide (LPS)-induced nitric oxide release with IC\textsubscript{50} values of 1.8, 6.2 and 3.0 µM, resp. In addn., the effects of rhinacanthin-C, -D and -N on LPS induced release of prostaglandin E2 (PGE2) and tumor necrosis factor (TNF-) were also examined. It was found that rhinacanthin-C exhibited the most potent on PGE2 release with an IC\textsubscript{50} value of 10.4M, followed by rhinacanthin-D (IC\textsubscript{50} = 14.4M) and rhinacanthin-N (IC\textsubscript{50} = 52.1 M). Aethiopinone (I), an o-naphthoquinone diterpene from *Salvia aethiopis* L. roots and two hemisynthetic derivatives (II) and (III) were evaluated for toxicity, anti-inflammatory, analgesic, antipyretic, and hemostatic activities (Margarita et al.1995).

The compounds tested showed low toxicity and pharmacology profile similar to other NSAIDs on reducing the edema induced by carrageenan and contractions induced by phenyl-p-quinone. On the TPA-induced ear inflammation model, the three compounds showed a moderate reduction of edema. Compound I and II showed significant inhibition. Compound I produced
a significant increase in the reaction time against thermal painful stimuli in the tail immersion test. The results demonstrated strong anti-inflammatory, peripheral and central analgesic properties for I, as well as antiedema topical action and peripheral analgesic properties for II and III.

Figure 2.7. Structure of Aethiopinone (I), an o-naphthoquinone diterpene from *Salvia aethiopis* L. roots and two hemisynthetic derivatives (II) and (III)

Phylloquinone (K1), menaquinone- 4 (K2), menadione (K3), 2,3-dimethoxy-1,4-naphthoquinone (DMK) and a synthetic product of vitamin K catabolism, 2-Me-3-(2'-methyl)-hexanoic acid -1, 4- naphthoquinone (KCAT). All compounds are inhibiting IL-6 production responsible for inflammation with a rank order of potency: KCAT > K3 > DMK > K2 > K1. The most potent compound KCAT inhibited IL-6 production with an IC₅₀ of 3×10⁻⁷µM. The mechanism of action of these naphthoquinones on fibroblast IL-6 production
however remains unknown. It was concluded by research that this activity is not essential for the inhibition of IL-6 production and that activity may be related to the redox capacity of these naphthoquinones. The naphthoquinones I \([R = \text{alkylamino, AcNH, EtCONH;} R1 = \text{H, MeOCH}_2\text{CH}_2, \text{EtO, etc.; } R2 = \text{H, H}_2\text{NSO}_2]\) are prepared as anti-inflammatory agents [73] (Wolfgang et al. 1985). Thus, I \((R = \text{NHCH}_2\text{CH}:\text{CH}_2, R1 = \text{H, R2 = SO}_2\text{NH}_2)\) (II) (Figure 2.8) was prep. from 1, 4-dioxo-1,4- dihydronaphthalene-6-sulfonic acid by conversion into the sulfamide and reaction with \(\text{CH}_2:\text{CHCH}_2\text{NH}_2\) in the presence of air. Oral administration of II at 2.5 mg/kg inhibited by 39% the carrageenan-induced edema in rats.

![Figure 2.8. Structure of naphthoquinones I](image)

**Figure 2.8. Structure of naphthoquinones I** [where \(R = \text{alkylamino, AcNH, EtCONH;} R1 = \text{H, MeOCH}_2\text{CH}_2, \text{EtO, etc.; } R2 = \text{H, H}_2\text{NSO}_2\)]

### 2.2.1.4 Anti-leishmanial naphthoquinones

Naphthoquinones are also having anti-leishmanial activity. A series of naphthoquinones was tested for activity against both extracellular promastigotes and intracellular amastigotes Leishmania major GFP in vitro (Ahmad et al. 2011). In parallel, the compounds were evaluated for cytotoxic effects against bone marrow-derived macrophages as a mammalian host cell control. Most of the compounds inhibited the growth of extracellular parasites \((IC_{50} 0.5 \text{ to } 6 \mu\text{M})\) and the intracellular survival of L. major GFP amastigotes \((IC_{50} 1 \text{ to } 7\mu\text{M})\),
when compared with the antileishmanial drug amphotericin B (IC_{50} of 2.5 and 0.2 µM, resp.). Introduction of a methyl or methoxy group at C-2 of the parent 1, 4-naphthoquinone slightly increased the antileishmanial activity against clinical relevant amastigotes, while the presence of a hydroxyl function in this position dramatically reduced the effectiveness. In contrast, hydroxylation at C-5 and dihydroxy substitution at C-5 and C-8 significantly enhanced the antiprotozoal activity. Within the series of naphthoquinones tested, the dimeric mixture of varforhizin and isovarforhizin showed the highest activity in vitro against the clinically relevant intracellular amastigote with an IC_{50} of 1.1 µM. With IC_{50} values mostly in the range of 1-3 µM, the shikonin/alkannin derivatives proved to be considerably leishmanicidal. The mode of action apparently depended on the substitution pattern, associated with the electrophilicity of the naphthoquinone or the efficiency of redox cycling. Pterocarpanquinones and homologous series of derivatives compounds were evaluated on breast cancer cell line and parasites *Leishmania amazonesis* and *Plasmodium falciparum* (Silva et al. 2009). 2-phenoxy-1,4-naphthoquinone and 2-phenoxy-1,4-anthraquinone derivatives have inhibitory activity towards Trypanosoma or leishmania species. Where three of them were active against *Leishmania donovani*, *Trypanosome cruzi*, *Trypanosoma brucei rhodesisence* (IC_{50} = 50 nM, IC_{50} = 0.28 µM, and IC_{50} = 1.26 µM). The efficacy of different formulations of the naphthoquinone buparvaquone and two phosphate prodrugs against vivo models of both visceral and cutaneous leishmaniasis is described. Buparvaquone-3-phosphate was shown to be the most effective antileishmanial (P = 0.0003, 50 mg buparvaquone molar equivalent/kg/day five times), reducing the liver parasite burden by ~34% when compared with the untreated control. The introduction of a topical formulation, such as buparvaquone (or its prodrug), would be a significant advance for the treatment of simple cutaneous lesions. Lapachol exhibited an anti-amastigote effect. Monomeric and dimeric naphthoquinones were found active in vitro for treatment of Leishmania infections using a direct cytotoxicity assay against promastigotes of Leishmania.
donovani, L. infantum, L. enriettii and L. major. Some naphthoquinones were active in the microgram range (EC$_{50}$ 0.9-17.0 µg/mL) (Kayser et al. 2000).

The stem barks of *P. benensis* are employed by the Chimane Indians in the Bolivian Amazonia as treatment of cutaneous leishmaniasis caused by the protozoan *Leishmania braziliensis* (Alain et al. 1992). The chloroform extracts containing quinones were found to be active against the promastigote forms of leishmania donovani and the epimastigote forms of *Trypanosoma cruzi* at 10 µg mL$^{-1}$. The activity guided fractionation of the extract by chromatography afforded active compounds. Their structures were elucidated, by spectral and chemical studies, as known naphthoquinones, plumbagin, 3, 3’-bipplumbagin, 8, 8’-bipplumbagin, and triterpene, lupeol.

The activity in vitro of each compound was evaluated against 5 strains of Leishmania (promastigote), 6 strains of *T. cruzi* (epimastigote) and the intracellular form (amastigote) of *Leishmania amazonensis*. The baseline drugs used were Glucantime and pentamidine (Leishmania spp.), nifurtimox and benznidazole (*T. cruzi*). Plumbagin was the most active compound in vitro. This study has demonstrated that *Pera benensis*, a medicinal plant used in folk medicine is an efficient treatment of cutaneous leishmaniasis.

### 2.2.1.5. Anti-cancer and tumor quinones

The mannich reaction involving lawsone and certain amines with formaldehyde and acetaldehyde and the condensation product of lawsone with 4-bis (2-chloroethyl) aminobenzaldehyde has been described. Two isomers of naphthoquinones derivatives 6-(1-azidoalkyl)-DMNQ and 2-(1-azidoalkyl)-DMNQ exhibited higher cytotoxic activity against L1210 mouse leukemia cells and stronger inhibition of DNA topoisomerase-I (Chae et al. 1999). These molecules contain N- substituted- pyridino [2,3-f] indole-4,9-dione and 6-(α-diethoxy carbonyl methyl)7-substituted amino quinoline 5,8-dione, which
contain the active quinoline 5,8-dione moiety. This moiety have been tested against SRB (sulphorodamine B) assay against the cancer cell lines of A-549 (human lung cancer), SK-MEL-2 (human melanoma cancer), SK-OV3 (human ovarian cancer), XF-498 (human brain cancer) and HCT (human colon cancer). This moiety showed higher activity than cis-platin. Rhinacanthone and 1,2-pyranonaphthoquinones were synthesized and showed very potent cytotoxicity against three cancer cell lines (KB, HeLa and HepG2) with IC_{50} values of 0.92-9.63µM (Kongkathip et al. 2003).

CDC25 dual-specificity phosphatases are essential key regulators of eukaryotic cell cycle progression and the CDC25A and B isoforms are overexpressed in different tumors. Polyfluoro derivatives of 1,4-naphthoquinones are highly potent inhibitors of Cdc25A and Cdc25B phosphatases and growth of tumor cells and their cytotoxicity in human myeloma, human mammary adenocarcinoma, mouse fibroblasts and primary mouse fibroblast cells as well as their mutagenic and antioxidant properties in a Salmonella tester strain were studied (Brun et al. 2005). The β-lapachone based 1,2,3-triazoles showed the best cytotoxicity profile and emerge as promising anti-cancer prototypes.

The anti-tuberculcular activity and cytotoxicity of juglone derivatives were analyzed with the topological and molecular surface features from a web based server, MODEL(Molecular Descriptor Lab). Novel compounds derived from vitamin K_3 that inhibit CDC25B activity with IC_{50} values in the low micromolar range. Polymamine naphthoquinone conjugates by neuclophilic displacement of 2-methoxy lawsone, 2-methoxy lapachol, 2-methoxynorlapachol with the polymamine N1- Boc- N5- Bn- spermidine 4. 2-methyl-1,4-naphthoquinone derivatives especially vitamin K_3 Retardation of cytotoxicity and cell proliferation by 2-amino alkyl moiety with terminal bromo, chloro, hydroxyl, mercapto groups were examined on model murine hepatoma cell line-22A. Most active compound were the hydroxyl and bromo derivatives(Stasiauskaite et al. 2006).
A series of 2-chloro-3-arylsulfanyl-[1, 4] naphthoquinones 2, 3-bis-arylsulfanyl-[1,4] naphthoquinones and 12H-benzo [b] phenothiazine- 6,11-diones and their analogs were evaluated for their antiproliferative activity against human cervical cancer cells (Silva et al. 2011). 1,5-Diazaanthraquinone derivatives were synthesized employing single and double hetero Diels–Alder strategies. Their in-vitro antitumor activity was assayed using three cell lines. Some of these compounds, especially those bearing methyl or ethyl groups at the C-3,7 positions or chloro at C-4 and methyl at C-7, showed IC$_{50}$ values in the 10$^{-8}$ µM range for human lung carcinoma and human melanoma, which makes them attractive candidates for further development as anticancer agents.

2.2.2. Bis-naphthoquinones

Bis-naphthoquinones and higher quinone oligomers are a unique group of natural products, which possess a diverse array of biological activities (Actinorodins et al. 1996). Their structures are based on two or more quinone units linked together at the quinine double bond. In almost all cases they possess an element of symmetry due to their biosynthetic mechanism of origin, which probably involves oxidative coupling of a common naphthol intermediate in the key step of the oligomerization process (Laatsh 1994) [85]. One intriguing member of this class is conocurvone isolated from the Western Australian smoke bush (Decosterd et al. 1993). Conocurvone (Figure 2.9.) was shown to inhibit the cytopathogenic effects of HIV-1 in human T-lymphoblastic cells over a broad concentration range (ID$_{50}$=0.02 µM; TD$_{50}$= 50 µM) (Decosterd et al. 1993). More recently, it was suggested that conocurvone 1 may be a dual inhibitor of both HIV integrase and HIV mediated cell fusion (Kearney et al. 2001).
Over the past decade, extensive efforts have been made resulting in the discovery of a large number of molecules that can inhibit replication of HIV (Yang et al. 2001). An essential step in the HIV life cycle is integration of the viral DNA into the host cell genome. The step is catalyzed by the viral enzyme, HIV integrase, which is absolutely required for productive infection and therefore, inhibition of integrase can half the viral life cycle. Integrase catalyses two separate steps known as 3’-prossessing and DNA strand transfer. In 3’-prossessing, integrase removes a dinuclotide next to a conserved cytosine-adenine sequence from each 3’-end of the viral DNA.

Integrase then attaches the processed 3’-end of the viral DNA to the host cell DNA in the strand transfer reaction. An important result of the structural and biochemical studies on integrase has been the development of practical assays used to identify novel HIV integrase inhibitors. These HIV inhibitors not only represent potential chemotherapeutic lead compounds (Mazumder et al. 1996), but as a collection, they are also useful in databases for pharmacophore searching. The most promising inhibitors are proposed to bind to the active site of the integrase enzyme and chelate important metal cofactors such as Mn$^{2+}$ or Mg$^{2+}$. Sidhu. and Pardhasaradhi. (1967a) (1970) established the structure of diospyrin, a bisnaphthoquinone with a benzene-quinone linkage. Bisnaphthoquinone may arise either by the oxidation of a bis-naphthol formed.
by the radical coupling of two naphthol units or by the condensation of a naphthoquinone unit with a naphthol (quinol) unit and subsequent oxidation. Diospyrin, a bis-naphthoquinonoid natural product, and three synthetic derivatives have been tested for their action in four human cancer cells lines: acute myeloblastic leukemia (HL-60), chronic myelogenic leukemia (K-562), breast adenocarcinoma (MCF-7) and cervical epithelial carcinoma (HeLa). Diospyrin was found to show significant tumor inhibitory effect against Ehrlich ascites carcinoma in vivo. Subsequently, synthesis of some derivatives of diospyrin led to the isolation of more potent inhibitors against murine tumors. Cells grown in appropriate media several derivatives elicited cytotoxicity as assessed by Typan Blue dye exclusion, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide reduction and DNA synthesis. Diethyl ether derivative was most effective in this regard while the parent diospyrin was least active.

![Structures of Diospyrin and its derivatives](image)

Diospyrin : \( R = H \)    Hydroquinonoid derivative

Diospyrin dimethyl ether : \( R = CH3 \)

Diospyrin diethyl ether : \( R = C2H5 \)

**Figure 2.10. Structures of Diospyrin and its derivatives**
Methyl and ethyl ethers (Figure 2.10.) of this compound were prepared by alkylation- the dark red residue with the alkyl iodide and silver oxide in chloroform and separating the ether from the corresponding diospyrin ether by TLC on deactivated silica gel plates with chloroform: methyl ether crystallized from methylene chloride/petroleum ether gives brown red hexagonal crystals mp 78°C. A comparison of the PMR spectra of this dihydro-diospyrin (Figure 2.11.) and its methyl and ethyl ethers with those of diospyrin and its dimethyl and diethyl ethers shows it to have structure II (R=H).

![Molecular structure](image)

Diospyrin. R=H

β’ – Dihydroxydiospyrin. R=H

Figure 2.11. Structures of Diospyrin and β’-Dihydroxydiospyrin

One of these derivatives, (-)-isodiospyrin (Figure 2.12.) bearing an unsymmetrical 1, 2’-binapthoquinonoid chromophore was isolated from the roots of diospyros and Euclea species. 2, 2’-binsaphthalene derivative and its monomer, 7-methyl-juglone were revealed to have potent anti-tumor activity against human KB epidermic carcinoma, A-549 lung carcinoma, HCT-8 and co-115 colon carcinoma cells in vitro. Isodiospyrin is also a dual DNA topoisomerase I & II α inhibitor. The inhibition of the catalytic activity of human topoisomerase I by isodiospyrin is 10-fold more potent as compared to
camptothecin, a potent anti neoplastic natural product and topoisomerase I inhibitor.

The isomer, diospyrin was also cytotoxic to several human tumor cell lines in culture. Ray et al. reported that diospyrin significantly inhibited the growth of *Leishmania donovani* promastigotes. This agent also inhibited the catalytic activity of DNA topoisomerase-I of the parasite and induced DNA Topoisomerase I-mediated cleavage in vitro, suggesting that the bi-naphthoquinonoids derivatives exert their inhibitory effect binding to the enzyme and stabilizing the Topoisomerase-I-DNA cleavable complex. However, diospyrin did not inhibit topoisomerase-II of *L. donovani* and required much concentrations to inhibit calf-thymus topoisomerase-I. Based on the biological properties of isodiospyrin and diospyrin, they can be exploited for rational drug design to develop new anticancer agents or drugs human leishmaniasis. Neo-diospyrin is a structural analogue of diospyrin and isodiospyrin having potent inhibition against mycobacterium tuberculosis as well.

![Figure 2.12. Structures of Isodiospyrin and Neodiospyrin](image)

Gossypol (Figure 2.13.) was isolated from Gossypium species (Dechary. and Pradel.1971) and has been studied as a male antifertility agent in china. The two representative naphthyl –isoquinoline alkaloids, ancistrocladine
were found in lianas of the genera Ancistrocladus and Triphyophyllum peltatum, respectively. The latter has been found to have fungicidal, insect growth retarding and anti-feedant activity, and in particular activity against malaria parasites.

Figure 2.13. Structures of Gossypol, Ancistrocladine and 8′-Hydroxy isodiospyrin
2.2.3. Protein Targets of Naphthoquinones

2.2.3.1. DNA Topoisomerase-I

Figure 2.14. Crystal structure of 2B9S and 1SC7 of DNA Topoisomerase-I
2.2.3.1.1. Function of the enzyme

Releases the supercoiling and torsional tension of DNA introduced during the DNA replication and transcription by transiently cleaving and rejoining one strand of the DNA duplex. Introduces a single-strand break via transesterification at a target site in duplex DNA. The scissile phosphodiester is attacked by the catalytic tyrosine of the enzyme, resulting in the formation of a DNA-(3'-phosphotyrosyl)-enzyme intermediate and the expulsion of a 5'-OH DNA strand. The free DNA strand then undergoes passage around the unbroken strand thus removing DNA supercoils. Finally, in the relegation step, the DNA 5'-OH attacks the covalent intermediate to expel the active-site tyrosine and restore the DNA phosphodiester backbone. This enzyme is also known to regulate the alternative splicing of tissue factor (F3) pre-mRNA in endothelial cells (D'Arpa et al. 1988; Interthal et al. 2004; Cushman et al. 2005).

![Diospyrin and Isodiospyrin](image)

**Figure 2.15. Structure Diospyrin and Isodiospyrin as DNA Topoisomerase-I inhibitor**
2.2.3.2 Topoisomerase-IIα

3L4J- Topoisomerase II-DNA cleavage complex, apo structure. (Narathip et al. 2012) (Figure 2.16.).

Figure 2.16. Crystal structure of 3L4J Topoisomerase II-DNA cleavage complex and its naphthoquinone inhibitors

2.2.3.2.1 Functions

Once cut, the ends of the DNA are separated, and a second DNA duplex is passed through the break. Following passage, the cut DNA is religated. This reaction allows type II topoisomerases to increase or decrease the linking number of a DNA loop by 2 units, and it promotes chromosome disentanglement. Reactions involving the increase in supercoiling require two molecules of ATP. Janet Lindsley has done much work to examine how the hydrolysis of ATP translates to topoisomerase function. For example, DNA gyrase, a type II topoisomerase observed in E. coli and most other prokaryotes, introduces negative supercoils and decreases the linking number by 2. Gyrase is also able to remove knots from the bacterial chromosome. Along with gyrase, most prokaryotes also contain a second type IIA topoisomerase, termed
Topoisomerase IV. Gyrase and topoisomerase IV differ by their C-terminal domains, which are believed to dictate substrate specificity and functionality for these two enzymes. Footprinting indicates that gyrase, which forms a 140-base-pair footprint and wraps DNA, allowing it to introduce negative supercoils, while topoisomerase IV, which forms a 28-base-pair footprint, does not wrap DNA. Eukaryotic type II topoisomerase cannot introduce supercoils; it can only relax them. The role of type IIB topoisomerase is less understood. Unlike type II topoisomerases, it cannot simplify DNA topology, but it shares several structural features with type IIA topoisomerases.

2.2.3.2.2. Topoisomerases as Drug Targets

Topoisomerases have been the focus for the treatment of certain diseases. Bacterial gyrase (topoisomerase II) and topoisomerase IV are the targets of two classes of antibiotic drugs: quinolones and coumarins. These antibiotics are used to treat an assortment of different diseases, such as pneumonia, tuberculosis and malaria, by inhibiting DNA replication in the bacteria responsible. Eukaryotic topoisomerases I and II are the targets of an increasing number of anti-cancer drugs that act to inhibit these enzymes by blocking the reaction that reseals the breaks in the DNA. Often the binding of the drug is reversible, but if a replication fork runs into the blocked topoisomerase, then a piece of the gapped DNA strand not bound by the topoisomerase could be released, creating a permanent breakage in the DNA that leads to cell death. Most of these inhibitors are selective against either topoisomerase I or II, but some can target both enzymes. Topoisomerase I inhibitors induce single-strand breaks into DNA, and can work by a variety of mechanisms. Some drugs, such as camptothecin, inhibit the dissociation of topoisomerase and DNA, leading to replication-mediated DNA damage, which can be repaired more efficiently in normal cells than in cancer cells (deficient for DNA repair). Topoisomerase I inhibitors can also cause gene inactivation.
through chromatid aberrations. Topoisomerase II inhibitors, such as anthracyclines, (Figure 2.15.) are amongst the most widely used anti-cancer agents. These drugs are potent inducers of double strand breaks in DNA, and can cause arrest in the cell cycle at the G2 stage, the latter occurring by disrupting the interaction between topoisomerase II and regulators of the cell cycle, such as Cdc2 (Eisenreich et al.2009). Topoisomerase II inhibitors can cause a wide range of chromosomal aberrations, and can act by either stabilizing topoisomerase II-DNA complexes that are easily cleaved, or by interfering with the catalytic activity of the enzyme, both resulting in double-strand breaks in the DNA. There are also dual inhibitors that target both topoisomerase I and II, which increases the potency of the anti-cancer effect. These drugs work by a variety of means: by recognizing structural motifs present on both enzymes, by linking separate topoisomerase inhibitors together into a hybrid drug, or by using inhibitors that bind to and intercalate DNA.

2.2.3.3. Mycobacterium tuberculosis DNA Gyrase

3IFZ ( Figure 2.17.).

![Crystal structure of Mycobacterium tuberculosis DNA Gyrase (3IFZ) and its inhibitor](image)

Figure 2.17. Crystal structure of Mycobacterium tuberculosis DNA Gyrase (3IFZ) and its inhibitor
2.2.3.4 COX-2

1PXX- crystal structure of diclofenac bound to the cyclooxygenase active site of COX-2 (Young Ahn et al. 2005).

![Crystal structure of Cyclooxygenase-2(IPXX) and its inhibitors](image)

Figure 2.18. Crystal structure of Cyclooxygenase-2(IPXX) and its inhibitors

2.2.3.4.1 Function

COX converts arachidonic acid (AA, an ω-6 PUFA) to prostaglandin H$_2$ (PGH$_2$), the precursor of the series-2 prostanoids. The enzyme contains two active sites: a heme with peroxidase activity, responsible for the reduction of PGG$_2$ to PGH$_2$, and a cyclooxygenase site, where arachidonic acid is converted into the hydroperoxy endoperoxide prostaglandin G$_2$ (PGG$_2$). The reaction proceeds through H atom abstraction from arachidonic acid by a tyrosine radical generated by the peroxidase active site. Two O$_2$ molecules then react with the arachidonic acid radical, yielding PGG$_2$. COX-2 is an inducible enzyme, becoming abundant in activated macrophages and other cells at sites of inflammation. More recently, it has been shown to be upregulated in various carcinomas and to have a central role in tumorigenesis.
2.2.3.5. Human HDAC6

3PHD- Crystal structure of human HDAC6 in complex with ubiquitin (Figure 2.19.).

![Crystal structure of Human HDAC6 and its inhibitor](image)

Figure 2.19. Crystal structure of Human HDAC6 and its inhibitor

2.2.3.5.1. Functions

Retracts the Cilium of the cell, which is necessary prior to mitosis of the cell. HDAC also encourages cell motility and catalyzes α-tubulin deacetylation. As a result the enzyme also encourages cancer cell metastasis. HDAC6 also affects transcription and translation by regulating the heat-shock protein 90 (Hsp90) and stress granules (SGs), respectively. HDAC6 is also known to bond with high affinity to ubiquitinated proteins. HDAC6 is also required in the formation of SG (Stress granule proteins, HDAC6 is instrumental in SG formation; pharmacological inhibition or genetic removal of HDAC6 abolished SG formation.
2.2.3.6 NAD (P)H: quinone oxidoreductase 1 (NQO1)

KBQ-Complex of Human NAD (P)H quinone Oxidoreductase with 5-methoxy-1,2-dimethyl-3-(4-nitrophenoxymethyl)indole-4,7-dione (ES936) (Figure 2.20.).

![Structure of NQO1](image)

**Figure 2.20.** Crystal Structure of Human NAD(P)H quinone Oxidoreductase with 5-methoxy-1,2-dimethyl-3-(4-nitrophenoxymethyl)indole-4,7-dione and benzo(a)pyrene

### 2.2.3.6.1. Functions

NQO1 catalyzes obligate two electron reduction of a wide variety of substrates. The most efficient substrates are quinones but the enzyme will also reduce quinone-imines, nitro and azo compounds. The enzyme functions via a hydride transfer mechanism and requires a pyridine nucleotide cofactor. Reduction proceeds with equal facility with both NADH and NADPH. NQO1 can generate antioxidant forms of both vitamin E and ubiquinone after free radical attack. The capability to protect cells from oxidative challenge and the ability to reduce quinones via an obligate two electron mechanism, which
precludes generation of reactive oxygen radicals, demonstrates that NQO1 is a chemoprotective enzyme. NQO1 knockout mice demonstrated increased susceptibility to benzo (a) pyrene and 7, 12-dimethylbenz (a) anthracene induced skin carcinogenesis. NQO1 has been proposed to stabilize the tumor suppressor gene p53 and has been shown to interact with p53 in a protein-protein interaction.

2.2.3.7a Plasmodium falciparum heat shock protein 70 (PfHsp70-1)

Figure 2.21. Crystal Structure of Plasmodium falciparum heat shock protein 70 (PfHsp70-1)
2.2.3.7b. Trypanosoma cruzi *Cruzain*

3LXS-Crystal structure analysis of cruzain bound to vinyl sulfone derived inhibitor (WRR483)

Cysteine protease of Trypanosoma cruzi (Figure 2.22.).

![Crystal structure of Cysteine protease of Trypanosoma cruzi and its inhibitor beta and alpha lapachone](image)

Figure 2.22. Crystal structure of Cysteine protease of Trypanosoma cruzi and its inhibitor beta and alpha lapachone

2.2.3.7.1. Functions

Trypanosoma cruzi proteases were object of intensive structural and functional characterization in the past decades. The celebration of the Chagas disease centenarian makes it opportune to review the foundations of molecular re-search on cruzipain, a major lysosomal cysteine protease. Acting as a virulence factor, cruzipain promotes intracellular parasitism. In addition, tissue culture trypomastigotes (TCTs) exploit the enzymatic versatility of cruzipain to liberate kinin peptides from kininogen molecules associated to heparan sulfate proteoglycans. Cruzain, also known as cruzipain and cruzaine, is a cysteine protease found primarily in the lysosomes of Trypanosoma cruzi. Cysteine proteases exhibit activity in much the same way as serine proteases. However, instead of the hydroxyl oxygen of the serine acting as the primary nucleophile in
the mechanism, the thiol sulfur of a cysteine residue is active. Cysteine proteases act by cleaving the peptide bond between bound amino acid residues. Cruzain’s specifically binds proteins with either basic or hydrophobic residues.

2.2.3.8. PPARγ

1FM9- Asymmetry in the PPARgamma/RXRalpha crystal structure reveals the molecular basis of heterodimerization among nuclear receptors (Figure.2.23.).

Figure 2.23. Crystal Structure of PPAR gamma and its naphthoquinone inhibitor

2.2.3.8.1. Functions

In the field of molecular biology, the peroxisome proliferator activated receptors (PPARs) are a group of nuclear receptor proteins that function as transcription factors regulating the expression of genes. PPARs play essential roles in the regulation of cellular differentiation, development, and metabolism (carbohydrate, lipid, protein), and tumorigenesis of higher organisms. The peroxisome proliferator activated receptors (PPARs) are ligands activated intra cellular transcription factors that have been implicated in important biological processes such as inflammation, tissue remodeling and atherosclerosis.
2.2.3.9 Protein-tyrosine-phosphatase-1B-(PTP1B)-

2NT7-Crystal structure of PTP1B-inhibitor complex (Figure 2.24.).

![Crystal structure of PTP1B-inhibitor complex](image)

**Figure 2.24. Structure of Crystal structure of PTP1B-inhibitor complex**

2.2.3.9.1. Functions

They play a very important role in cellular signaling within and between cells. PTPs work antagonistically with Protein Tyrosine Kinases (PTKs) to regulate signal transduction in a cell. PTKs phosphorylate tyrosine residues on a substrate protein and PTPs remove these phosphates from substrate tyrosines (dephosphorylation). Since the phosphorylation status of a protein can modulate its function, PTKs and PTPs work together to regulate protein function in response to a variety of signals, including hormones, mitogens, and oncogenes.
2.2.3.10. Filarial Glutathione S-transferase

**3T2U** - Structure of Wuchereria Bancrofti PI-class Glutathione S-Transferase. (Figure 2.25.).

![Crystal Structure of Filarial Glutathione S-transferase and its inhibitor](image)

**Figure 2.25. Crystal Structure of Filarial Glutathione S-transferase and its inhibitor**

### 2.2.3.10.1. Functions

Glutathione S-transferase (GST, EC 2.5.1.18) is a major phase-II detoxification enzyme comprised of multifunctional proteins. Helminths have limited detoxification enzymes and appear to lack the important cytochrome P-450 dependent detoxification reaction. GSH is proposed to constitute the antioxidant system responsible for the long term existence of filarial worms in mammalian hosts by protecting them from the reactive oxygen species (ROS) produced by normal metabolism and by the immune cells of the host.
2.2.3.11. CDC25 phosphatase

1QB0 HUMAN CDC25B CATALYTIC DOMAIN (Figure 2.26.).

![Figure 2.26. Crystal Structure of CDC25 phosphatase and 1, 4-naphthoquinone as CDC25 phosphatase inhibitor.]

2.2.3.11.1. Functions

Cdc25 activates cyclin dependent kinases by the removal of phosphates from residues in the Cdk active site. Also, the phosphorylation of M-Cdk (a complex of Cdk1 and cyclin B) activates Cdc25. Together with Wee1, M-Cdk activation is switch-like. The switch-like behavior forces entry into mitosis to be quick and irreversible. Cdk activity can be reactivated after phosphorylation by Cdc25. The Cdc25 enzymes Cdc25A-C are known to control the transitions from G1 to S phase and G2 to M phase.

2.3. Research Methodology

On the basis of published literature we choose different methods for the fulfillment of the proposed work. Our main methods are isolation, derivatisation of bis-naphthoquinone obtained from natural source, synthesis of
naphthoquinone derivatives from synthetic source and biological evaluation of synthesized/isolated compounds with in-vitro with different important protein targets and subsequent in-silico bio-molecular interaction studies with the help of molecular docking.

2.3.1. Ecofriendly method

We reviewed a numbers of methods for formulating a solvent free and single step reaction to synthesize naphthoquinone derivatives. Literature revealed the solvent free synthesis of aryl-5H-dibenzo [b,I] xanthenes-5,7,12,14(13H)-tetraone from a reaction of lawsone, aldehyde, and p-toulene sulphonic acid at 100°C. Xanthenes are also prepared in solvent free condition using silica sulphuric acid as a catalyst (Figure.2.27.). We formulated the Synthesis of 3, 3’-(aryl methylene) bis (2-hydroxynaphthalene1, 4-dione) derivatives from reacting lawsone and different substituted aromatic aldehydes in the presence of LiCl as catalyst and refluxing the contents over water for 12 h.

Indianium chloride has been used as a catalyst in the synthesis of fluorescent hydroxyl naphthalene-1, 4-dione derivatives by a single step, economical, three component reaction of lawsone, aromatic aldehydes and heterocyclic or aromatic amines with water as a solvent. In a separate attempt, the one-pot synthesis of 1,4-naphthoquinones by the Diels–Alder reaction of dienes with para-quinones generated in situ with laccase using water as solvent was developed. The para-quinones were generated in situ by the laccase oxidation of the corresponding 1, 4-hydroquinones which subsequently underwent the Diels–Alder reaction with dienes, which on further oxidation to generated 1, 4-naphthoquinones, in good yields. In both the reactions, the method adopted was ecofriendly using green chemistry techniques.
2.3.2. Suzuki coupling synthesis

Suzuki cross coupling is a versatile methodology for generation of carbon-carbon bonds. Dimerisation can occur through Suzuki coupling. This is a reaction involving an aryl-or vinyl-or an alkyl-halide which is catalyzed by palladium. For the synthesis of bis-naphthoquinone, Suzuki cross coupling between bromo naphtho quinone and boronic acids was carried out.

2.3.3. Dimerisation

Dimerisation of 2-methyl-1, 4-naphthoquinone by aqueous ethanolic NaOH was carried out to get dehydro dimmers. Synthesis of 1, 4-naphthoquinone dimer was accomplished through the irradiation and warming in acetic acid solvent containing pyridine. Symmetrical and non-symmetrical N, N-bis (quinonyl) amines have been prepared. Synthesis of dimer from diethylamine, dibutylamine, hexamethyleneimine was achieved. Primary aliphatic amines or low molecular weight amines yielded monomer instead of a dimer. The procedure to synthesize the dimer was achieved through addition of lawsone, 37% formalin and diethanolamine in absolute ethanol solution and stirring for 45 minutes in water bath at 30-35°C for 4 h during which time golden red precipitated. The mixture was filtered washed with ethanol and water. Precipitate was dissolved in 100 ml of 2% hydrochloric acid and treated with anhydrous sodium acetate. After overnight standing the precipitate was filtered
and dried at 50°C. Alternatively, ammonium metavanadate was also used as catalyst for the dimerisation of lawsone into lawsone dimer in dilute perchloric acid.

Dimerisation of 1,4-naphthoquinone reaction with lead tetraacetate in acetic acid gives 2-methyl- 3, 3” (1, 4-naphthoquinone), 3, 3” (2- methyl- 3, 3” (1,4-naphthoquinone) and 2-acetoxy-2’-methyl 3,3”(1,4-naphthoquinone). Hydrolysis with methanolic and ethanolic sulphuric acid of 2-acetoxy-2’-methyl-3, 3’ (1, 4-naphthoquinone) gives 2-methoxy-2’methyl-3, 3’ (1, 4-naphthoquinone). 2, 3-dichloro 1, 4-naphthoquinone react with potassium cyanate in DMSO or in DMF like a michael type addition reaction to form quinonyl isocyanate. In the second step the quinonyl isocyanate reacts with an amine or alcohol, to give a gives carbamate or uriedo product. Partial hydrolysis of this gives carbamic acid, which undergoes decarboxylation to yield the 2-chloro-3-amino-1, 4-naphthoquinone, under a slightly basic condition to give 3, N, N-Bis (2-chloro-3-amino-1, 4-naphthoquinone) (Figure 2.28.).
Figure 2.28. Synthesis of 3, N, N-Bis (2-chloro-3-amino-1, 4-naphthoquinone)

2.3.4. Extraction, isolation and derivatisation

Extraction and isolation of diospyrin a bis-naphthoquinonoid from the plant bark of *Diospyros montana* roxb. The bark was dried under shade and powdered in a mixer grinder. Bark powder was charged in a soxhlet apparatus and extracted with petroleum ether (60-80°C) for two days. The extracted stem bark with petroleum ether was again subjected to soxhlet using the chloroform for two days in usual way. The deep red chloroform extract was cooled and the solvent was removed. The residue was boiled with acetone for 30 min and filtered.

Derivatisation of diospyrin into several alkyl ether derivatives by stirring with appropriate alkyl iodide and silver oxide in chloroform at room temperature for 2 to 8 hours till aliquot of the reaction mixture no longer showed the characteristic purple color of diospyrin with a drop of aqueous sodium hydroxide solution. The mixture was filtered passed through a neutral alumina column using chloroform as eluent (Figure 2.29.).
A mixture of diospyrin dimethyl ether (2) in chloroform and p-chloroaniline in ethanol was refluxed at 80 °C for 10 h. The reaction mixture was diluted with dichloromethane and washed with water (3 × 10 mL). The pooled organic layer was dried over anhydrous sodium sulphate and the solvent was removed to get a crude product, which was chromatographed over silica gel (Figure 2.30.).
Figure 2.30. Synthesis of diospyrin derivatives from diospyrin’s dimethyl ether

Impatienol, a natural bis-naphthoquinone was isolated from the aerial part of Impatiens balsamina. L (balsaminaceae), which is used in Chinese herbal medicine to treat rheumatism, beri-beri, anti-platelet-activating factor. It is also used as anti-pruritic, anti-histaminic and anti-inflammatory drug (Figure 2.31.). Extraction and isolation of impatienol from fresh corollas of white flowers extracted twice with 35% EtOH at room temperature for a day, to give crude yellow crystals (Kaempferol). After the kaempferol had been obtained by filtration, the alcoholic solution was evaporated in vacuo to remove the EtOH and extracted with EtOAc. The EtOAc extracts were chromatographed repeatedly on silica gel with a CHCl₃-EtOAc and CHCl₃-MeOH gradient system and recrystallized to get impatienolate and balsaminolate.
2.3.5. Synthesis of naphthoquinones

Synthesis of different heterocyclic naphthoquinone can be done through cyclo-addition reaction with 1, 4-naphthoquinone, isatin or ninhydrin, alkene, (Chalcone or β-arylnitrostyrene) with different amino acids. It is a simple, fast and single pot reaction resulting in good yield. Reaction conditions are very simple. The purification of the product is also simple method. 2-Amino-5, 10, 15, 20-tetraphenylporphyrinato nickel (II) reacts with 1,4-benzoquinone, 1,4-naphthoquinone and 2-hydroxy-1,4-naphthoquinone, in the presence of a catalytic amount of sulfuric acid, to afford new porphyrin–quinone dyads and p-extended heterocyclic fused porphyrin derivatives.

Aryliodonium ylides of 2-hydroxy-1, 4-naphthoquinone react with amines in refluxing dichloromethane to afford good yields of indanedione 2-carboxamides, through a ring-contraction and R,R-dioxoketene formation reaction. These amides exist in solution in an unusual enol-amide form. In contrast, the same reactants in a copper-catalyzed reaction afford arylamines and 3-iodo-4-hydroxy-1,2-naphthoquinone.

Addition of tetrahydrofuran to Compound 2 (Figure 2.32.) and LiAlH₄ for 30 min and finally oxidation with CAN/CrO₃ [added with stirring] for an h at
room temperature led to synthesis of novel derivatives of naphthoquinones in good yields.

**Figure 2.32. Preparation of hetero-1, 4-naphthoquinone**

2.3.6. Methodology for biology and molecular docking

For biological evaluation standard procedures were adopted.

2.3.6.1 Plasmid relaxation assay

For enzyme assay plasmid relaxation method was selected. It is a well known method for the enzyme assay especially for DNA topoisomerase-I. Assay was run using agarose gel electrophoresis. Gel prepared from agarose, distilled water and TAE. Then agarose was boiled in hot water bath until the solution becomes clear and cooled to about 50-55°C. The melted agarose solution was poured into the casting tray and cooled until it is solid and the gel was loaded in the electrophoresis chamber with 1X TAE buffer at a constant voltage of 5-6 V/cm for 2-8 hrs. Different concentrations of compounds were added with
enzyme and DNA control. DNA was stained by soaking in 0.5 µg/ml of ethidium bromide solution, visualized in U.V. transilluminator and photographed.

Assays have differentiated into two methods: 1) Preincubation and 2) simultaneous. To investigate whether these compounds interact directly with the enzyme, it was preincubated with these compounds at different concentrations for 5 min at 37°C before the addition of substrate DNA. In Simultaneous assay addition of enzyme, compounds and DNA simultaneously. In simultaneous assay compounds formed DNA-enzyme-compound stable complex.

2.3.6.2. MTT assay

The MTT assay is a colorimetric assay for measuring the activity of cellular enzymes that reduce the tetrazolium dye, MTT, to its insoluble formazan, giving a purple color. Other closely related tetrazolium dyes including XTT, MTS and the WSTs, are used in conjunction with the intermediate electron acceptor, 1-methoxy PMS. With WST-1, which is cell-impermeable, reduction occurs outside the cell via plasma membrane electron transport. These assays measure cellular metabolic activity via NAD (P) H-dependent cellular oxidoreductase enzymes and may, under defined conditions, reflect the number of viable cells (cell proliferation). Tetrazolium dye assays can also be used to measure cytotoxicity (loss of viable cells) or cytostatic activity (shift from proliferative to resting status) of potential medicinal agents and toxic materials. MTT Assay usually done under dark area since MTT reagent is sensitive to light.

2.3.7. Molecular docking

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

Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to predict the affinity and activity of the small molecule (Figure 2.33). Hence docking plays an important role in the rational design of drugs. Given the biological and pharmaceutical significance of molecular docking, considerable efforts have been directed towards improving the methods used to predict docking.

![Figure 2.33. Schematic diagram illustrating the docking of a small molecule (brown) to a protein receptor (green) to produce a complex.](image)

2.3.7.1. Docking approaches

Two approaches are particularly popular within the molecular docking community. One approach uses a matching technique that describes the protein and the ligand as complementary surfaces. The second approach simulates the actual docking process in which the ligand-protein pair wise interaction energies are calculated. Both approaches have significant advantages as well as some limitations. These are outlined below.
2.3.7.2 Shape complementarity

Geometric matching shape complementarity methods describe the protein and ligand as a set of features that make them dockable. These features may include molecular surface complementary surface descriptors. In this case, the receptor’s molecular surface is described in terms of its solvent-accessible surface area and the ligand’s molecular surface is described in terms of its matching surface description. The complementarity between the two surfaces amounts to the shape matching description that may help finding the complementary pose of docking the target and the ligand molecules. Another approach is to describe the hydrophobic features of the protein using turns in the main-chain atoms. Yet another approach is to use a Fourier shape descriptor technique. Whereas the shape complementarity based approaches are typically fast and robust, they cannot usually model the movements or dynamic changes in the ligand/protein conformations accurately, although recent developments allow these methods to investigate ligand flexibility. Shape complementarity methods can quickly scan through several thousand ligands in a matter of seconds and actually figure out whether they can bind at the protein’s active site, and are usually scalable to even protein-protein interactions. They are also much more amenable to pharmacophore based approaches, since they use geometric descriptions of the ligands to find optimal binding.

2.3.7.3 Simulation

The simulation of the docking process as such is a much more complicated process. In this approach, the protein and the ligand are separated by some physical distance, and the ligand finds its position into the protein’s active site after a certain number of “moves” in its conformational space. The moves incorporate rigid body transformations such as translations and rotations, as well as internal changes to the ligand’s structure including torsion angle rotations. Each of these moves in the conformation space of the ligand induces a total energetic cost of
the system, and hence after every move the total energy of the system is calculated. The obvious advantage of the method is that it is more amenable to incorporate ligand flexibility into its modeling whereas shape complementarity techniques have to use some ingenious methods to incorporate flexibility in ligands.

Another advantage is that the process is physically closer to what happens in reality, when the protein and ligand approach each other after molecular recognition. A clear disadvantage of this technique is that it takes longer time to evaluate the optimal pose of binding since they have to explore a rather large energy landscape. However grid-based techniques as well as fast optimization methods have significantly ameliorated these problems.

To perform a docking screen, the first requirement is a structure of the protein of interest. Usually the structure used has been determined using a biophysical technique such as x-ray crystallography, or less often, NMR spectroscopy. This protein structure and a database of potential ligands serve as inputs to a docking program. The success of a docking program depends on two components: the search algorithm and the scoring function.

![Figure 2.34. Small molecule docked to a protein](image)

Figure 2.34. Small molecule docked to a protein
2.3.7.4. Search algorithm

The search space in theory consists of all possible orientations and conformations of the protein paired with the ligand. However in practice with current computational resources, it is impossible to exhaustively explore the search space—this would involve enumerating all possible distortions of each molecule (molecules are dynamic and exist in an ensemble of conformational states) and all possible rotational and translational orientations of the ligand relative to the protein at a given level of granularity. Most docking programs in use account for a flexible ligand, and several attempt to model a flexible protein receptor. Each "snapshot" of the pair is referred to as a pose.

A variety of conformational search strategies have been applied to the ligand and to the receptor. These include:

- systematic or stochastic torsional searches about rotatable bonds
- molecular dynamics simulations
- genetic algorithms to "evolve" new low energy conformations

2.3.7.5. Ligand flexibility

Conformations of the ligand may be generated in the absence of the receptor and subsequently docked or conformations may be generated on-the-fly in the presence of the receptor binding cavity, or with full rotational flexibility of every dihedral angle using fragment based docking. Force field energy evaluation are most often used to select energetically reasonable conformations, but knowledge-based methods have also been used.

2.3.7.6. Receptor flexibility

Computational capacity has increased dramatically over the last decade making possible the use of more sophisticated and computationally intensive
methods in computer-assisted drug design. However, dealing with receptor flexibility in docking methodologies is still a thorny issue. The main reason behind this difficulty is the large number of degrees of freedom that have to be considered in this kind of calculations. Neglecting it, however, leads to poor docking results in terms of binding pose prediction. Multiple static structures experimentally determined for the same protein in different conformations are often used to emulate receptor flexibility. Alternatively rotamer libraries of amino acid side chains that surround the binding cavity may be searched to generate alternate but energetically reasonable protein conformations.

2.3.7.7. Scoring function

The scoring function takes a pose as input and returns a number indicating the likelihood that the pose represents a favorable binding interaction. Most scoring functions are physics-based molecular mechanics force fields that estimate the energy of the pose; a low (negative) energy indicates a stable system and thus a likely binding interaction. An alternative approach is to derive a statistical potential for interactions from a large database of protein-ligand complexes, such as the Protein Data Bank, and evaluate the fit of the pose according to this inferred potential.

There are a large number of structures from X-ray crystallography for complexes between proteins and high affinity ligands, but comparatively fewer for low affinity ligands as the later complexes tend to be less stable and therefore more difficult to crystallize. Scoring functions trained with this data can dock high affinity ligands correctly, but they will also give plausible docked conformations for ligands that do not bind. This gives a large number of false positive hits, i.e., ligands predicted to bind to the proteins that actually don’t when placed together in a test tube.
One way to reduce the number of false positives is to recalculate the energy of the top scoring poses using (potentially) more accurate but computationally more intensive techniques such as Generalized Born or Poisson-Boltzmann methods.

2.4 Conclusion from the literature

From the literature review we have concluded that green method or solvent free reaction is good approach for the dimerisation of naphthoquinones. Now-a-days green methods are the first choice of the chemist due to its benefit for environment safety, easy to handle, simple, one step method, less use of hazardous chemicals, less use of equipments, energy and time. Catalyst plays an important role in the reaction. Some catalyst increase yield, reaction rate and gives fair product. From the previous literature we came to know about the different catalyst which have used for the synthesis of bis-naphthoquinone. LiCl, p-TSA, Indinium Chloride, Triethylamine, silica sulphuric acid, ammonium meta vandate gives dimer of 1,4-naphthoquinone. In some reactions alkali (NaOH, KOH) and amines (dibutylamine, piperidine, morpholino, diethylamine) are also used as a catalyst. Extraction, isolation and derivatisation of bis-naphthoquinone, diospyrin was carried out from the the bark of Diospyros montana, a indigenous plant from family Ebenaeceae. This plant is found mainly in the forests of Bihar, West Bengal, Tamilnadu and other states of India. Extraction and isolation of diospyrin was carried out through slight modifications on the reported procedure. The first total synthesis of diospyrin has been reported through Suzuki coupling method by Kenji Mori research group. Consecutively, more latest synthesis of novel diospyrin analogues via a Suzuki cross coupling between bromonaphthoquinones and aryl or naphthyl boronic acids in presence of tetrakis (triphenylphosphine) palladium (0) as catalyst were also reported by Ivan R.Green and co-workers. Derivatisation of diospyrin with alkyl iodide and silver oxide and further derivatisation of alkyl
ether derivative by different methods also have been reported. Glucoside derivatives of diospyrin were also prepared. We have attempted further derivatisation of diospyrin alkyl ether into novel amino acid ester derivatives through modification on the published procedure. Further details are provided in following chapters on experimental section.

Mamegakinone dimethyl ether was prepared by Stille-type coupling reaction with the bromo-naphthoquinone in presence of bis( triphenylphosphine) palladium (II) chloride. Dimerisation of Bi-vitamin K3, 3, 3’-bijuglone,in presence of AgO-40% HNO₃. Bi-ramentaceone, a bis-naphthoquinone was prepared by oxidative coupling of 4-methoxy-1-naphthol compounds using lead oxide or silver oxide with 65% of HNO₃.

In literature review we studied different chemistries and synthetic procedures on different naphthoquinones and the biological evaluations, mostly anti-leishmanial, anti-cancer, and anti-bacterial, anti-fungal and anti-tumor activities of the same. Our two-fold aim was to synthesize novel naphthoquinone derivatives and subsequently conduct biological screening. For the fulfillment of this aim we had a thorough study of previous literature for both chemistry and selection of disease and its protein target against which these novel compounds were to be tested.

2.5. Conclusion from Biological methods

From the literature we studied different reported activities against protein targets for naphthoquinone and bis-naphthoquinones. Some targets were already established for naphthoquinone and bis-naphthoquinones. From review we found that some naphthoquinones were suitable for leishmanial Topoisomerase I-DNA cleavage complex, target was for anti-leishmanial studies and some other for human CDC25, which is a molecular target for anticancer studies. For docking studies we selected GOLD, GLIDE and Autodock Vina
docking software. All the softwares are well known and widely accepted by industrial as well as academic community. For biological evaluation we concluded to carry on MTT assay for cytotoxicity.

2.5.1 Significance of MTT assay

Tetrazolium dye reduction is dependent on NAD (P)H-dependent oxidoreductase enzymes largely in the cytosolic compartment of the cell.\textsuperscript{6,7} Therefore, reduction of MTT and other tetrazolium dyes increases with cellular metabolic activity due to elevated NAD(P)H flux. Resting cells such as thymocytes and splenocytes that are viable but metabolically quiet reduce very little MTT. In contrast, rapidly dividing cells exhibit high rates of MTT reduction. It is important to keep in mind that assay conditions can alter metabolic activity and thus tetrazolium dye reduction without affecting cell viability and that different tetrazolium dyes will give different results depending on whether they are reduced intracellularly (MTT, MTS) or extracellularly (WST-1).