CHAPTER 3
SYNTHESIS OF MITOXANTRONE ANALOGUES AND THEIR CYTOTOXIC ACTIVITIES.

3.1.1 Introduction;

For well over a century substituted anthraquinone or anthracene-9,10-diones such as those 1,4-bis(amino)anthracene-9,10-dione and 1,4-bis(amino) 5,8- dihydroxy anthracene9,10-dione, have been used as dyes or pigments because of their high chemical, photochemical and thermal stability. In the 1970s, new application emerged for these derivatives and Murdock and Child were the first to show that the N-alkyl derivatives of anthraquinone, were chemotherapeutically active as anti-cancer agents. Subsequently it was shown that functionalized N-alkyl derivatives such as Mitoxantrone (1) and Ametantrone (2) (figure 3.1) were highly effective anti-cancer drugs with an efficacy and therapeutic index exceeding doxorubicin (3), methotrexate, (4) or 5-fluorouracil (5). Mitoxantrone is anti-neoplastic agent, is active against breast cancer, acute leukemia, lymphoma, cervix carcinoma, and liver cell cancer. Unlike the anthracyclines that have a red color, the amino anthracene diones are deep blue color.

![Figure 3.1](image-url)
DOXORUBICIN (R = OH) (3)
DAUNORUBICIN (R = H) (6)

Epirubicin (7)

Idarubicin (8)

methotrexate (4)
However, while the additional hydroxyl groups present at 5-and 8-positions of mitoxantrone lead to tenfold increase in its antineoplastic activity over ametantrone, this beneficial enhancement is unfortunately countered by a tenfold increase in its cardiotoxicity. Anthracyclins are derivatives of 5,12-napthacene and these are antibiotics, which are used to treat to wide range of cancers, including (but not limited to) leukemias, lymphomas, breast, ovarian, and lung cancers. Anthracyclines inhibit DNA and RNA synthesis by intercalating between base pairs of the DNA/RNA strand, thus prevent the replication of rapidly growing cancer cells. Important anthracyclin drugs are doxorubicin, daunorubicin, epirubicin and idarubicin. The mechanism of the cytotoxicity of anthracyclines is pleiotropic and its significance in cell growth inhibition seems to be highly specific and dependent on cell type. The anthracycline antibiotics daunorubicin, and doxorubicin have been used widely as anticancer drugs for more than 30 years, but their cardiotoxicity limits their clinical use. The clinical use of anthracyclines like doxorubicin and daunorubicin can be viewed as a sort of double-edged sword. Doxorubicin and its analogues function as topoisomerase inhibitor, poisons through stabilization of the cleavable complex that forms as part of the catalytic cycle of enzyme. The detailed mechanism of how these compounds, all of which contain an anthraquinone core that forms between the DNA base pairs, the drug, and the enzyme in the minor groove has remained elusive. Anthracyclines and their anthraquinone derivatives remain evergreen drugs with broad clinical indications but have an improvable pharmacological index.

Mitoxantrone (1) demonstrate potent antitumor activity and has been used widely in clinic since 1980s. In addition, mitoxantrone has been the only drug approved by the FDA for the treatment of worsening relapsing-remitting multiple sclerosis (MS), secondary progressive MS, and progressive-relapsing MS since 2000. However,
these clinical applications are limited due to the accumulative and irreversible cardiotoxicity.\textsuperscript{18-20} Recently, Mitoxantrone was found to induce a progressive increase in mitochondrial mass in the cancer cells but not in the cardiac cells.\textsuperscript{21} This suggests the opportunities to develop novel anthraquinones with reduced cardiotoxicity. The planar tricyclic structure of antraquinone is essentional for intercalating into DNA base pairs.

The two sidechains of 1 and 2 could be used to connect with a variety of substituent’s, which may form additional interactions with the double-strand DNA (ds-DNA) or DNA-topoisomerase II (TOP2) cleavable complex to increase their binding affinities and selectivities. It is well known that precise recognition of defined DNA sequences in biological systems is mediated by enzymes and proteins having appropriate structure motifs.\textsuperscript{22} In the literature, various anthraquinone-peptide conjugates\textsuperscript{23-27}, were reported to demonstrate remarkable DNA binding and exhibit cytostatic or cytotoxic activities. In addition, mono-amino acid-substituted anthraquinone conjugates could inhibit the catalytic activity of TOP1 and TOP2.\textsuperscript{28,29} Theoretical design of anthraquinone-oligopeptide conjugates to selectively target the double-stranded oligonucleotides has also been published.\textsuperscript{30-31}

With the aim to discover more specific chemotherapeutic agents, a focused library of 1,4-bis(2-amino-ethylamino)anthraquinone-amino acid conjugates (BACs) was designed and synthesized\textsuperscript{32}. The alpha amino acids possessing a variety of side chains, which could be used to create specific interactions by one of the following mechanisms, were chosen for construction of BACs.

(a) Metal ions, such as magnesium and zinc, are involved in DNA synthesis and repair processes. Attaching the amino acids containing potential metal ion-chelating side chain (i.e., the methylsulfanyl group of methionine) to the core structure may be useful to interrupt DNA synthesis by stabilization of the DNA-TOP2 cleavable complex.

(b) The hydroxyl group of tyrosine residue in TOP2 plays an essential role in TOP2-mediated DNA strand cleavage.\textsuperscript{33,34} Furthermore, the side chains of 2 and 1 also contain the hydroxyl groups. Therefore, introducing hydroxyl group-containing
amino acids (i.e., serine and tyrosine) to anthraquinone may provide the opportunity to enhance the cytotoxicity of this series of BACs.

(c) The $\epsilon$-amino group of lysine is protonated under physiological conditions, which would be expected to produce strong electrostatic interactions with the negatively charged phosphate groups on DNA skeleton.\textsuperscript{35,36}

3.1.2 Synthetic mitoxantrone analogues and their application;

Pixantrone (9) (6,9-bis[(2-aminoethyl)amino]benzo[g]isoquinoline-5,10-dione; is an experimental antineoplastic drug, an analogue of mitoxantrone with fewer toxic effects on cardiac tissue. It acts as a topoisomerase II poison and intercalating agent, the code name BBR 2778 refers to pixantrone dimaleate, the actual substance commonly used in clinical trials\textsuperscript{36a}.

\begin{center}
\begin{tikzpicture}
\node[draw, circle] (a) at (0,0) {\text{O}};
\node[draw, circle] (b) at (0,1) {\text{O}};
\node[draw, circle] (c) at (1,0) {\text{NH(CH}_2\text{)}_2\text{NH}_2};
\node[draw, circle] (d) at (1,1) {\text{NH(CH}_2\text{)}_2\text{NH}_2};
\end{tikzpicture}
\end{center}

Pixantrone (9)

In an attempt to identify new molecular or pharmacophore targets for anticancer drugs, Hsu-Shan Huang et al\textsuperscript{37} attention has been focused on cytotoxicity and telomerase. Telomerase activity is thought to be required for the development of cellular immortality and oncogenesis. Thus, considerable interest is focused on telomerase because of its potential uses in assays for cancer diagnosis, research into cell biology and for anti-telomerase drugs as a strategy for cancer chemotherapy. At present, among different strategies pursued telomerase inhibitors in chemotherapy, the development of G-quadruplex stabilizers has emerged as a highly promising approach. Herein, Hsu-Shan Huang reported the design and synthesis of a series of non-nucleoside telomerase inhibitors, in an attempt to identify potent enzyme A series of 1,2-heteroannelated anthraquinones and anthra[1,2-d]imidazole-6,11-dione tetracyclic analogues (10, 11, 12,
13, 14) with different side chain were prepared using various synthetic route via acylation, cyclization, condensation, and intramolecular heterocyclization. Tetracyclic system containing alkyl and aryl, aromatic and heterocyclic, linear and cyclic polar and apolar, and basic and acids residues were incorporated. They were evaluated for their effects on telomerase activity, human telomerase reverse transcriptase (hTERT) expression, cell proliferations, and in vitro cytotoxicity against NCI’s 60 cell line human tumorscreen. It appeared that addition of a fourth planar aromatic system to a tricyclic chromophore might enhances potent cytotoxic agents, at a level equivalent to a second side chain in one of the tricyclic series.

Gouda, M. A. et al synthesized anthraquinone compounds (Figure 3.3) and were screened in vitro for their antimicrobial activity. The diameter of inhibition zone was measured as an indicator for the activity of the compounds. The results for antibacterial activities depicted below revealed that few compounds have exhibited good activities38.
Telomerase inhibitors have been touted as a novel cancer specific therapy, as most tumor cells have high expression of telomerase, whereas most normal somatic cells express low or undetectable levels of telomerase. Telomerase is a reverse transcriptase that prevents the mortality checkpoints evoked by programmed telomere shortening during each round of cell division. Expression hTERT, the catalytic subunit of telomerase, appears to be a key determinant for telomerase activity. Insufficient hTERT expression in most mortal somatic cells cannot produce enough telomerase to maintain telomere length during cycles of chromosome replication. The hTERT is highly expressed approximately 90% in stem cells, germ cell lines, and most human tumors, and its inhibition represents a strategy for the development of selective anti-cancer drugs. It is also well accepted that human cancer cells achieve immortalization in large part through the illegitimate activation of telomerase expression. The reactivation of telomerase activity in most...
cancer calls supports the concept that telomerase is a relevant target in oncology, and telomerase inhibitors have been proposed as new potential anti-cancer agents. The important roles of telomerase in tumor initiation and cellular immortalization have led to the identification of telomerase as a potentially important molecular target in cancer therapeutics. The development of telomerase inhibitors and G-quadruplex stabilizers has emerged as a highly promising approach. Telomerase is important in tumor initiation and cellular immortalization. Given the striking correlations between telomerase activity and proliferation capacity in tumor cells, telomerase had been considered as a potentially important molecular target in cancer therapeutics. A series of 2,7-diamidoanthraquinone were designed and synthesized. They were evaluated for their effects on telomerase activity, hTERT expression, cell proliferations and cytotoxicity. Few compounds shown bellow (19, 20, 21), potent telomerase inhibitory activity, while compounds activated hTERT expression in normal human fibroblasts. The results indicated that 2,7-diamidoanthraquinones represent an important class of compounds for telomerase-related drug developments.

![Figure 3.4](image_url)
Hsu-Shan Huang et al\textsuperscript{39} has designed and synthesized a series of 2,7-disubstituted amidoanthraquinone derivatives (figure 3.4) and evaluated their effects to telomerase activity as inferred from the TRAP assay and collated some selected compounds growth-percent against tumor cell lines in vitro (NC160 assays). The antiproliferative effect and hTERT repressing activity of these synthesized compounds were also determined results indicated that the 2,7-disubstituted amido-anthraquinones are potent telomerase inhibitors. Results indicated that only few compounds showed significant cytotoxic activity. The growth percent values at $10^{-5}$ molar for 60 cancer cell lines, it is especially notable that compound with side chain NHCO-\text{CH}_2\text{N(CH}_3)_2$ (19) displayed relatively potent and differential cytotoxic activity.

Telomerase is a ribonucleoprotein for telomere maintenance which utilizes its RNA component as the template to extend telomeric DNA length. The activity of telomerase could be detected in about 85-90\% of tumor cells, (where as it is low or not present in most somantic cells). Thus, the maintenance of telomere length is considered as a biological marker for determining the proliferation of cancer cells. Chia-Chung Lee et al\textsuperscript{40} group have reported a series of amidoanthraquinones at 1,4-, 1,5-, 2,6- and 2,7-position that showed diversely in vitro antitumor activity and telomerase inhibitory activities. They also reported the design and synthesis of a series of novel asymmetrical mono- or disubstituted 1,2-diamidoanthraquinone derivatives. These compounds were evaluated for cytotoxicity and telomerase inhibitory activity using Cell Cultures and Sulforhodamine B(SRB) assay, and repressing hTERT-expression, respectively. Among these derivatives, few compounds showed the highest potency against PC-3 (prostate cancer) with IC50 from 0.95 mM to 2.64 mM (figure 3.5)

![Figure 3.5](image-url)
In the course of their continuous search for new antitumor agents from anthraquinone moiety, Chia-Chung Lee et al described a method of synthesizing diversely symmetrical or asymmetrical substituted 1,2-diamidoanthraquinone derivatives and comparing to their cytotoxicity and telomerase activity. Focused attention on the role of systematic synthesized tricyclic pharmacophore bearing the symmetrical or asymmetrical side chain linked to the planar anthraquinone moiety and to understand the basis of tricyclic system selectivity. Forty new compounds have been prepared among which twenty-seven displayed a broad spectrum of antitumor activity below 10 mM range.

A single molecule containing features of both Intercalating (example; Mitoxantrone) and alkylating agent (example; Cyclophosphamide, 23) 1,4-Bis-(2,3-epoxypropylamino)-9,10-anthracenedione (25) was synthesized and was found to be a potent antitumor agent. Derivatives of this compound containing planar skeleton and diamino side chain substitution pattern of mitoxantrone and the alkylating epoxide moiety of teroxirone (24) and evaluated for in vitro cytotoxic activity in several cell lines. ED50 of less than 40ng/ml against human epidermoid carcinoma (KB cells) as shown in figure 3.6.
A red pigment that accumulates in cultures of a *Drechslera avenae* pathotype with specificity for *Avena sterilis* was isolated and identified as the anthraquinone cynodontin (3-methyl-1,4,5,8-tetrahydroxyanthraquinone)\(^{(26)}\). As shown in figure 3.7, satisfactory yield of compound was obtained with 20-60 day incubations at temperatures between 20 and 27°C. Cynodontin (26) was tested in vitro for fungitoxicity and was found to be a potent inhibitor of the growth of sclerotinia minor, *Sclerotinia sclerotiorum* and to a lesser extent, of *Verticillium dahliae*. The ED50 values obtained with these fungi were of the same order of magnitude as those of the commercial fungicides dicloran and carbendazim, which were used as reference chemicals. In contrast, the growth of a number of other fungi was not significantly inhibited by cynodontin. Anthraquinone and two other anthraquinone derivatives, emodin and chrysophanol, which were also included in the tests, did not affect the growth of the cynodontin-sensitive fungi. It thus appears that the type and position of the substitution at the C-ring play a role in the expression of antifungal activity.

![Figure 3.7](image)

The growth of the majority of the fungi used in the fungitoxicity tests was not affected by cynodontin at concentrations of up to 100 µg/ml. Antifungal activity would probably have not been detected if the three members of the family Sclerotiniaceae of the cup fungi (Discomycetes) had not been included in these tests. Cynodontin was recognized as a potent inhibitor of the mycelial growth of *B. cinerea*, *S. minor*, and *S. sclerotiorum*. The ED50 values of 5.25, 4.31, and 5.52, respectively, were comparable to those obtained with dicloran, which is used commercially to control diseases caused by these pathogens\(^{43}\).
The rapidly growing global energy demand and the increase of CO₂ emissions associated with the burning of fossil fuels are the key driving forces for the development of inexpensive renewable energy sources. The sun is the primary source of most forms of energy found on the earth and can give out a large amount of solar energy. Solar energy is one of most important renewable sources of energy in this century, which can be used over and over again. Besides, it causes little pollution and does not contribute to the greenhouse effect. In this context, polymeric solar cells (PSCs) have attracted considerable attention for their great advantages over the existing inorganic solar cells, such as ease of processing, light weight, flexibility, low cost, and so forth. The bulk heterojunction concept has improved the power conversion efficiency (PCE) of the PSCs significantly by forming a donor acceptor bicontinuous interpenetrated network, which creates large interfacial areas between the polymers and electron acceptors (e.g. fullerene derivatives), thus leading to efficient photoinduced charge separation in a device. In order to capture a larger portion of solar energy, various types of low-bandgap polymers have been designed and investigated as a new donor structure in PSCs. Although recent advances in organic photovoltaics are largely based on conjugated organic polymers, a possible alternative approach to harvest sunlight for generating electrical power involves adding metals into organic-based polymers. This interest derives from the fact that incorporation of heavy metals into an organic framework can have a significant influence on their electronic and optical properties. Among these, metal-containing polymers stand out to be particularly interesting candidates in this area. Li Li a, Wing-Cheong et al reported the synthesis, characterization and photovoltaic properties of some donor-acceptor-based metal acetylide polymers containing electron-deficient 9,10-anthraquinone (figure 3.8). A class of soluble, solution-processable platinum(II) acetylide polymers functionalized with electron-deficient 9,10-anthraquinone spacer and their corresponding diplatinum model complexes were synthesized and characterized. The organometallic polymers exhibit good thermal stability and show low-energy broad absorption bands in the visible region. The effect of the presence of thiophene rings along
the polymer chain on the optical and photovoltaic properties of these metallated materials was examined. The low-bandgap polymer with thiophene, anthraquinone, thiophene (donor, acceptor, donor) fragment can serve as a good electron donor for fabricating bulk heterojunction polymer solar cells by blending with a methanofullerene electron acceptor. At the same donor:acceptor blend ratio of 1:4, the light-harvesting ability and solar cell efficiency notably increase when the anthraquinone ring is sandwiched by two thiophene units. Photoexcitation of such polymer solar cells results in a photoinduced electron transfer from the p-conjugated metallopolymer to [6,6]-phenyl C61-butyric acid methyl ester with power conversion efficiency up to w 0.35%. For safety concern, these metallopolymers were also tested for possible cytotoxicity and they do not show significant cytotoxic activity on human liver derived cells and skin keratinocytes at reasonable doses, rendering these functional materials safe to use in practical devices.

Discotic liquid crystals have been attracting growing interest not only because of fundamental importance as model systems for the study of charge and energy transport but also due their potential application in organic electronic devices. The 1,2,3,5,6,7-hexahydroxy-9,10-anthraquinone (28), commonly known as Rufigallol, (figure. 3.9) is one of the earliest systems reported to form columnar mesophases. Over the past 25 years, more than 100 discotic liquid crystals based on this core have been realized and studied for various physical properties\textsuperscript{45}. 
3.2 Importance of Present work;

N-Alkyl derivatives of anthraquinone-9,10-diones exemplified by drugs ametantrone(2) and mitoxantrone(1)\(^46,47,48\) are potent anti-cancer agents. Worldwide, cancer remains a leading cause of death. According to world Health Organiszation (WHO) of 58 million deaths in 2005, cancer accounted for 7.6 million (or 13%). Deaths from cancer in the world are projected to continue rising, with an estimated 9 million people dying from cancer in 2015 and 11.4 million dying in 2030. WHO also states that 40% of cancer can be prevented by a healthy diet, physical activity, and not using tobacco. The lung cancer is the most common cause of cancer death for men and women\(^49\).

Chemotheraphy is the treatment of diseases such as cancer using chemicals. Since 1960’s the development and use of drugs has significantly improved the prognosis for some types of cancer. Most types of cancer chemotherapy will consist of a number of different drugs, this is known as combination chemotherapy. Chemotherapy may be given in a variety of ways; intravenously, intramuscularly, orally, subcutaneously, intralesionally i.e directly into a cancerous area, intrathecally i.e. into the fluid around the spine, or Topically-medicine will be applied onto the skin. Since the drugs used to kill cancer cells also damage normal cells, the searching for antineoplastic agents with improved selectivity to malignant cells remains the central task for drug discovery and development \(^49,50\). According to the survey published in 1997 more than 315 drugs were under development in the United states for the treatment of cancer\(^51a-c\). According to the review \(^52\) of ca. 90 approved cancer drugs, more than 60% are of natural origin or modeled on natural products.

However, while the additional hydroxyl groups present at 5-and 8-positions of mitoxantrone lead to tenfold increase in its antineoplastic activity over ametantrone. This
beneficial enhancement is unfortunately countered by a ten fold increase in its cardiotoxicity. Mitoxantrone is drug of choice for the treatment of worsening relapsing-remitting multiple sclerosis (ms), secondary progressive MS. However, these clinical applications are limited due to the accumulative and irreversible cardiotoxicity. Recently, Mitoxantrone was found to induce a progressive increase in mitochondrial mass in the cancer cells but not in the cardiac cells.

This suggests the opportunities to look for novel anthraquinones with reduced cardiotoxicity. The planar tricyclic structure of anthraquinone is essential for interacting with DNA base pairs. Perhaps by introducing different side chains at 1- and 4-positions in anthraquinone skeleton with a variety of substituents, which may form additional interactions with the double-strand DNA (ds-DNA) or DNA-topoisomerase II (TOP II) cleavable complex to increase their binding affinities and selectivity.

3.3 Results and Discussion;

Synthesis of 1,8-Dihydroxy-4,5-diaminoanthraquinone (31) involves three steps starting from chrysazin (1,8-Dihydroxy anthraquinone) using Diethyl sulphate in DMF in presence of Potassium carbonate to obtain 1,8-Diethoxy anthraquinone (29) which when nitrated with nitrating mixture in presence of Boric acid yielded high pure 1,8-Diethoxy-4,5-dinitroanthraquine (30). This on treatment with Hydroiodic acid resulted not only in O-deethylation, but also in reduction of nitro groups with more than 95% yield of 4,5-Diaminochrysazin (31) as described in chapter 2. Treatment of (31) with sodium hydrosulftite in alkaline solution led to leuco-1,4,5,8-tetrahydroxyanthraquinone (32), this on condensation with appropriate amine in an inert gas atmosphere followed by oxidation of the resulting intermediate with oxygen gave 1,4-diamino substituted anthraquinones (33-37) as shown in Scheme 3.1.

All compounds were fully characterized by IR, NMR and Mass spectroscopy.
Scheme 3.1; reagents and conditions; (a) Diethyl sulphate, K₂CO₃, DMF, 100°C, (b) H₂SO₄, HNO₃, Boric acid (c) Hydroiodic acid, (d) Aq. N-buanol, Sodium hydrosulfite, 60°C, (e) beta-alanine, Amine(benzyl, cyclohexyl, cyclopentyl, cyclopropyl), ethanol, O₂, 50°C,
**Biological Activity; Anti-neoplastic:**

Compounds 33-37 subjected to in vitro testing using breast carcinoma MCF-7 & cervical cancer Hela cell lines for their cytotoxicity (Figure 3.1 and table 1) in comparison with mitoxantrone, it is observed that all compounds 33 – 37 exhibited very good inhibitory activity in both cell lines in comparison with mitoxantrone. While IC\textsubscript{50} values in MCF-7 Breast cancer cell line for all compounds ranged between 100 and 120 nM, but in Hela cervical cancer cell line still lower concentrations ranging 70 to 80 nM were effective. 1,4-bicyclohexylamino-5,8-dihydroxyanthraquinone (34) exhibited relatively better inhibitory activity while bisbeta-alanino-5,8-dihydroxyanthraquinone (36) showed
relatively less inhibitory activity against both cell lines. Relatively better inhibitory activity exhibited by compound (34) may be due to flexible nature of cyclohexyl moiety which undergoes different conformations under different conditions and also due to lipophilicity conferred to the molecule by the cyclohexyl group and which may increase the affinity for the cell membrane. Since these molecules showed very good in-vitro activity we plan to carry out in-vivo testing in future to find out the cardiotoxicity.

**Fig. 1**

**Fig.3.1:** Cytotoxic activity: MXPHK 1 (Compound 33); MXPHK 2 (Compound 34); MXPHK 3 (Compound 35); MXPHK 4 (Compound 36); MXPHK 5 (Compound 37);
Conclusion;

Synthesized five analogues of mitoxantrone starting from 4,5-Diaminochrysazin (31) in two steps. Treatment of (31) with sodium hydrosulfite in alkaline solution led to leuco-1,4,5,8-tetrahydroxyanthraquinone (32), this on condensation with appropriate amine (benzylamine, cyclohexyl amine, cyclopentyl amine, beta-alanino and cyclopentylamine) in an inert gas atmosphere followed by oxidation of the resulting intermediate with oxygen gave 1,4-diamino substituted anthraquinones (33-37). Compounds 33-37 subjected to in vitro testing using breast carcinoma MCF-7 & cervical cancer Hela cell lines for their cytotoxicity in comparison with mitoxantrone. It is observed that all compounds 33 – 37 exhibited very good inhibitory activity in both cell lines in comparison with mitoxantrone, 1,4-bis(cyclohexylamino)-5,8-dihydroxyanthraquinone (34) exhibited relatively better inhibitory activity while bisbeta-alanino-5,8-dihydroxyanthraquinone (36) showed relatively less inhibitory activity against both cell lines,
3.4 Experimental;

3.4.1 General methods/instrument

Chemicals and reagents of laboratory grade were obtained from local dealers and were used without further purification IR spectra were recorded on Nicolet avatar 320 FT-IR spectrometer, \(^1\)H and \(^{13}\)C NMR spectra were recorded in CDCl\(_3\) / DMSO-d\(_6\) at 200MHz on Bruker A G spectrometer Chemical shifts are reported in \(\delta\) units down field from TMS as internal standard Mass spectra were recorded using GC-MS-QP2010S (direct probe) and Q-TOF micro\(^{TM}\) AMPSMAX10/6A system.

3.4.2 Preparation of leuco-1, 4, 5, 8-tetrahydroxyanthraquinone (32);

To a sodium hydroxide aqueous solution (10% 1L) containing n-butanol (50ml) was added 4,5-diaminochrysazin(31)[preparation as described in chapter 2](38.3g, 142mmol) with stirring and the resulting dark-blue suspension was de-aerated by stirring for 15min with a stream of N\(_2\) which was bubbled through it. Sodium hydrosulfite(22.5g, 123mmol) was gradually added with stirring, while the reaction mixture was heated and maintained at 60\(^0\)c for 30 min. Then the reaction mass was cooled to room temperature, neutralized with HCl(4N) and allowed to stand. The resulting precipitate was collected by filtration, washed with water and dried in vaccuo at 50\(^0\)c to give leuco-1,4,5,8-tetrahydroxyanthraquinone(32) 35g, 90% as a brown flake, MP.230-235\(^0\)c (decomposition); \(^1\)H NMR (DMSO-d\(_6\)); \(\delta\) 3.0 (s, 4H), 7.15 (s, 2H), 9.8 (s, OH), GC-MS (DI); 274 (M\(^+\))

3.4.3 General procedure for the preparation of final compounds (33-37);

Condensation of leuco-1, 4, 5, 8-tetrahydroxyanthraquinone (32) with large excess of appropriate Amine in ethanol in an inert gas atmosphere followed by oxidation of resulting intermediate with oxygen at 50\(^0\)c for 15hrs gave 1,4-disubstituted anthraquinone. These compounds were purified by subjecting to silica column chromatography.
3.4.3.1. 1-benzylamino-4, 5, 8-trihydroxyanthraquinone-9,10-dione(33);

Using compound 32(1g, 3.64mmol) and benzylamine (5g, 46.7mmol) as starting materials the Title compound 33 was obtained as a blue-Brown solid (0.3g, yield=23%); MP194°C (decomposition); IR (K Br); 2900, 2800,1569, 1496, 1454, 1392, 1350, 1172, 1087, 968, 790, 744, 698, 551, 466cm⁻¹; ¹H NMR (CDCl₃); δ 4.64(d, 2H), 7.16-7.27(m, 9H), 10.34(b, 1H), 12.39(s, 1H), 13.01(s,1H), 13.32(s, 1H); ¹³C NMR(CDCl₃, 50MHz); δ 46.9, 124.2, 126.3, 126.8, 127.6, 128.9, 129.0,156.8, 183; HRMS: 362.2043(M+H)

3.4.3.2. 1, 4-bis (cyclohexylamino)-5, 8-dihydroxyanthraquinone-9,10-dione(34);

Using compound 32 (1g, 3.64mmol) and cyclohexylamine(5g, 50mmol) as starting materials the compound 34 was obtained as a blue-Brown solid (1.0g, yield=63%); MP 220°C (decomposition); IR (K Br); 2927, 2854, 1566, 1446, 1392, 1149, 1080, 956, 825, 729, 675, 551, 482, 428cm⁻¹; ¹H NMR (CDCl₃, 200MHz); δ 1.35(m,20H), 3.8 (m, 2H), 7.1(s,2H), 7.26(s, 2H), 10.8(b, 2H), 13.8(s, 2H); ¹³C NMR (CDCl₃, 50MHz); δ 24.3, 25.4, 33.2, 50.9, 108.6, 115.5, 124.3, 129.2, 145.7, 155.2, 184.6; HRMS :434.320(M⁺)

3.4.3.3. 1, 4-bis (cyclopentylamino)-5, 8-dihydroxyanthraquinone-9, 10-dione(35);

Using compound 32 (1g, 3.64mmol) and cyclopentylamine(5g, 58.8mmol) as starting materials the Title compound 35 was obtained as a blue-Brown solid (0.3g, yield=20%); MP 240°C (decomposition); IR (K Br); 2954, 2866, 1604, 1558, 1500, 1450, 1396, 1353, 1164, 1080, 972, 825, 663, 547, 458cm⁻¹; ¹H NMR (CDCl₃, 200MHz); δ 1.58(m,16H), 4.1(m,2H), 7.1(s, 2H), 7.26(s, 2H), 10.8(b, 2H), 13.8(s, 2H); ¹³C NMR (CDCl₃, 50MHz); δ 24.5, 34.5, 54.4, 109.2, 115.9, 124.9, 125, 146.5, 155.7, 185.2; HRMS(ES⁺) = 406.2104(M⁺)

3.4.3.4. 1, 4-bis (beta-alanino-5, 8-dihydroxyanthraquinone-9, 10-dione (36);

Using compound 32 (1g, 3.64mmol) and beta-alanine(5g, 56.1mmol) as starting materials the Title compound 36 was obtained as a blue-Brown solid (0.3g, yield=20%); MP >280°C; IR (K Br); 3400, 2900, 2657, 2333, 1743, 1693, 1608, 1562, 1454, 1350, 1172, 1076, 972, 825, 628, 547, 470 cm⁻¹; ¹H NMR (DMSO-d₆, 200MHz); δ 2.65(t, 4H),
3.69(t, 4H), 7.13(s, 2H), 7.53(s, 2H), 10.57(b, 2H), 13.46(s, 2H); $^{13}$C NMR (DMSO-$d_6$, 50MHz); $\delta$ 34.6, 38.7, 107.7, 115.7, 124.7, 125.5, 147.0, 154.9, 173.1, 183.7; HRMS (ES$^+$)= 414.2769(M$^+$).

3.4.3.5. 1, 4-bis (cyclopropylamino)-5, 8-dihydroxyanthraquinone-9, 10-dione (37);

Using compound 32 (1g, 3.64mmol) and cyclopropylamine(5g, 87.8mmol) as starting materials The compound 37 was obtained as a blue-Broun solid (0.3g, yield=20%); MP >280°C; IR (K Br); 2900, 2800, 1608, 1569, 1454, 1404, 1342, 1299, 1211, 1164, 1022, 960, 806, 628, 474 cm$^{-1}$; $^1$H NMR (CDCl$_3$, 200MHz); $\delta$ 0.83(m, 8H), 2.5(m, 2H), 7.13(s, 2H), 7.72(s, 2H), 10.2(b, 2H), 13.4(s, 2H); $^{13}$C NMR (CDCl$_3$, 50MHz); $\delta$ 7.9, 24.3, 29.6, 110, 115, 124, 125, 147, 155, 186; HRMS(ES$^+$)= 373.1162(M$^+$ Na).

3.4.4 Biological activity;

3.4.4.1 In vitro growth inhibition assay;

The cells were maintained in Dulbecco’s Modified Eaglés Medium (Sigma-Aldrich Inc., USA) supplemented with 10% fetal bovine serum (Sigma-Aldrich Inc., USA) in a CO$_2$ incubator. The cytotoxicity of the compounds was measured by MTT assay$^{57}$. The cells were planted in a 96-well plate at the density of 10,000 cells per well (Hela) and 10,000 cells per well (MCF-7). After 24 hours, the cells were treated with different concentrations of analogues of mitoxantrone (25 nM to 400 nM). The cells were further incubated for 24 hours. The cytotoxicity was measured by adding 5mg/ml of MTT (Sigma-Aldrich Inc., USA) TO each well and incubated for another three hours. The purple formazan crystals were dissolved by adding 100μl of DMSO to each well. The absorbance was read at 570 nm in a spectrophotometer (spectra Max 340). The cell death was calculated as follows;

$$\text{Cell death} = 100 - \left[\frac{\text{test absorbance}}{\text{control absorbance}} \times 100\right]$$

The test result is expressed as concentration of a test compound which inhibits the cell growth by 50% (IC$_{50}$).
Table 1: Cytotoxic activity of compounds 33-37 on HeLa cell line (cervical epithelial adenocarcinoma cell line) and MCF-7 (Breast carcinoma cell line).

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 (nM) ± SD</th>
<th>*HeLa</th>
<th>IC50 (nM) ± SD</th>
<th>*MCF-7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitoxantrone</td>
<td>59.36 ± 5.24</td>
<td></td>
<td>101.16±18.49</td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>73.82 ± 5.23</td>
<td></td>
<td>107.93±3.9</td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>73.7 ± 0.89</td>
<td></td>
<td>105.78±5.18</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>75.66 ±13.61</td>
<td></td>
<td>112.65±5.69</td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>79.51±7.28</td>
<td></td>
<td>115.2±10.53</td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>76.88±3.49</td>
<td></td>
<td>104.48±7.86</td>
<td></td>
</tr>
</tbody>
</table>

* Values are average of three independent experiments ± standard deviation, conducted in triplicate for each concentration.
3.5 REFERENCES;


45. Sandeepkumar.; *Phase Transitions.** **2008**, *81*(1), 113-118.