Chapter IV
CHAPTER IV

PROGESTERONE SCHIFF BASE CONJUGATES WITH HYDROXYPHTHAOQUINONES: SYNTHESIS, SPECTROSCOPY, MAGNETISM, EPR, CYCLIC VOLTAMMETRY, AND ANTIPROLIFERATIVE ACTIVITY AGAINST B16F10 MELANOMA CELLS

4.1 Introduction:

Melanoma is the eighth most commonly diagnosed cancers originating from the cells called melanocytes that produce the cellular pigment known as melanin, which is responsible for skin color. Some melanomas arise in normal skin while others arise in the pigmented skin (moles). The rise in the incidence of 1.5% of total deaths related to Melanoma is among the highest for all kinds of cancers world wise. Despite the attempts to prevent it, malignant melanoma accounted for 5% of malignancies in men and 4% in women in the USA for the year 2001 [1]. The incidence of primary cutaneous malignant melanoma has increased steadily over the past decade, which is possibly related to increased exposure to UV rays as a result of ozone depletion. This has prompted the use of sunscreen lotions with the skin protection factor (SPF) as precautionary measures.

Melanoma incidence is subject to large geographic and ethnic variations. An inverse correlation of melanoma incidence with latitude and with degree of skin pigmentation has been observed [2]. Cutaneous melanoma occurs predominantly in the fair skinned people who tan easily. Melanoma affects all age groups but is most commonly seen in patients between 30 and 60 years of age. Lifetime risk of melanoma in the US reached 1 in 87 in the year 2000 compared with only 1 in 15000 in 1935.
Melanoma remains a highly lethal disease and hence represents an enormous challenge for clinicians and drug design chemists.

Over the past 30 years there has been a change in the distribution and stage of melanoma found at diagnosis with an increasing number of thinner lesions (1mm thick) noted on presentation. Prognosis in malignant melanoma depends on tumor thickness, ulceration and nodal status [3-5]. Patients presenting with thicker lesions or regional nodal metastasis have a significantly poorer prognosis. It has been reported in many studies that the incidence of melanoma is more in men than in women [6]. In addition to the gender difference, the thickness of the lesion, anatomic location and the presence of ulceration in the primary lesion are more pronounced in men than in women. In majority of patients, melanoma does not recur locally and it is therefore presumed that the micrometastatic disease prior to surgical excision accounts for high relapse rates.

The very poor prognosis of melanoma once it has metastasized beyond the regional nodes, and its relative resistance to available chemotherapeutic agents has prompted a search for effective compounds having selective toxicity to melanomal cells.

4.2 Nature of disease:

Normally melanomas are observed in the skin (cutaneous) but they can also occur in the eye [7] (ocular or intraocular) and can metastasizes into other areas such as digestive tract, lymph nodes, liver, lungs or brain [8]. (Figure 4.1)

In men, melanoma is found in an area between the shoulders and hips or on the head and neck [9]. In women, melanoma often develops on the lower legs, between shoulders and hips, toenails under the fingernails on the palms or soles [10].
1. Nodular Melanoma

2. Melanoma of the foot

3. Cutaneous melanoma

4. Superficial melanoma of the ear

Figure 4.1: Types of melanoma

[15] and cadherins (E-cadherin and N-cadherin) respectively. Among these E-cadherin plays an important role in the development of melanoma. Tang et al. has demonstrated that E-cadherin is the major adhesion mediator between epidermal melanocytes and keratinocytes [16]. Melanocytes express E-cadherin whereas nevus cells and melanoma
(i) **Development of Melanoma:**

Approximately, two thirds of melanoma arises in the pre-existing benign nevi or moles of the skin [11]. Various signs are observable before or during the development of the disease which include: (i) The skin growth that increases in size and becomes irregular in shape, looks translucent, tans brown, black, red, pink or multicolored; (ii) Spot or growth that continues to itch, hurt, crust, erode or bleeds; (iii) An open sore that lasts for more than 4 weeks or heals and then reopens; (iv) A scaly or crusty bump that may cause pricking/tender sensation [12].

Based on the clinical and histopathological features, five steps of melanoma progression have been proposed which include the commonly acquired congenital nevus as the earliest proliferative melanocytic lesion and its development into the dysplastic nevus. This is followed by the aberrant differentiation of the radial growth phase (RPG), which is primary melanoma with acquired growth autoimmunity but not metastatic potential. The advanced stages of melanoma are the vertical growth phase (VPG), primary melanoma and the final stage, metastatic melanoma respectively.

Melanoma development involves processes that are determined collectively by various microenvironmental factors as well as intracellular communications between cells. The expression of a number of cell-surface molecules that mediate cell-cell interactions is associated with the melanoma development. These molecules include members of the immunoglobulin gene family such as Mel-CAM (melanocytic cell adhesion molecule) [13], ICAM-1 (intercellular cell adhesion molecule) [14]; integrins
melanoma cells show absence of E-cadherin molecule [17,18]. The loss of functional 
E-cadherin molecule appears to be one of the critical steps in the progression of 
melanoma leading to uncontrolled proliferation and progressive invasion [19]. Hence, 
cell-cell cross talk mediated by the cadherins and connexins results in a coordinated 
regulation of cell growth, differentiation, apoptosis and migration. Abnormal expression 
of a growth factor and its receptor along with the dysregulated intercellular 
communication leads to the tumor development and progression.

Normal melanocytes grown in vitro display little or no detectable production of 
growth factors and depend on the exogenous growth factors like fibroblast growth factor 
(FGF) and insulin for their proliferation. In contrast, malignant melanocytes obtained 
from the advanced stages of tumor progression synthesize a variety of growth factors and 
their receptors such as transforming growth factor (TGF), platelet derived growth factor 
(PDGF-A and PDGF-B), interleukin (IL-1), melanomaly growth stimulatory hormone, 
fibroblast growth factor (FGF), melanocyte growth factor, estrogen hormone receptor 
(ER) and progesterone hormone receptor (PR) [20] respectively. Among these the PR has 
a relevance to the drug design strategy used in the present work.

(ii) Melanogenesis:

The melanocyte is uniquely poised to carry out its primary function of delivering 
melanin, a polymeric pigment formed from successive oxidations of tyrosine, to 
keraatinocytes. Two general types of melanins are known, viz. black eumelanins formed 
by polymerization of dihydroxyindole precursors and the pheomelanins that are colored 
as a result of cysteine incorporation during oxidation [21].
In melanocytes, the tyrosinase enzyme catalyzes two successive reactions, viz. hydroxylation of tyrosine and oxidation of the product, L-dopa, respectively. It has been postulated that tyrosine may protect melanocytes from cytotoxic effects of superoxide anions. The product of dopa oxidation cyclizes to a 5,6-dihydroxyindole intermediate, which is highly reactive and upon further oxidation gives rise to eumelanin polymers by radical coupling pathway. In another reaction the covalent linkage of dopaquinone with cysteine has been found to yield incorporation of benzothiazine monomer into the pheomelanin polymer as shown in (Figure 4.2).

Figure 4.2: The pathway for biosynthesis of different classes of melanins
(iii) Redox regulation in melanoma:

It has been observed that nearly all pre-neoplasias in many organs have increased enzymatic or over-expressed antioxidant levels that are subsequently depressed after the cell undergoes transformation to become cancerous [22,23]. The levels of antioxidant enzymes such as MnSOD, catalase, glutathione peroxidase as well as those of non-enzymatic small molecular weight antioxidants such as (GSH), vitamin A, C and E respectively, are typically depressed in melanoma cells [24]. Bittinger, et al. have demonstrated that superoxide anion concentration is generally raised in melanoma cells [25]. Sasaki, et al. have shown that the level of ROS is elevated following an exogenous oxidant stress using chemiluminiscent probes by comparison with those under basal conditions using fluorescent molecular probes [26]. In these studies, the authors had exposed cells from various sources to oxidative stress, which resulted into generation of hydrogen peroxide by glucose/glucose oxidase system. All normal melanocytes were able to neutralize the peroxide stress, but melanoma cells showed dramatically less ability to neutralize the extracellular peroxide that resulted in a continuous buildup.

Thus, melanocytes were able to suppress the oxidant stress while the melanoma cells were unable to do so which prompted authors to suggest that under conditions of oxidative stress, melanin acts as a prooxidant in melanoma cells, generating superoxide anion that leads to an altered redox state with complex compensatory mechanisms [27].

The major redox-sensitive transcription factors in mammalian cells are NF-kB, AP-1 and thioredoxin. These factors are activated or inactivated depending upon the
increased or decreased levels of reactive oxygen species (ROS) within the tumor cell respectively. In case of melanoma cells, continuous high levels of ROS result in activation of NF-κB and activation of the downstream responses including activation of the anti-apoptotic proteins as shown in **(Figure 4.3)**

**Figure 4.3: Redox regulation in melanoma**

As pointed out earlier melanin itself can serve as an antioxidant or a prooxidant depending on its redox state, the presence of metal ions and state of melanin aggregation. Sarna, et al. [28] have shown that cellular melanin has the ability to mediate oxidative stress. Protective antioxidant action of melanin is found in its use by pathogenic black fungus and bacteria [29].
Melanins are capable of interacting with metal ions, which can influence the redox state (quinone/semiquinone/quinone) of melanins [30]. The deprotonation of the quinol form can promote binding of Zn$^{2+}$ ions, which stabilizes the EPR active semiquinone radical form of dihydroxyindoles [31]. Biologically relevant metals such as Fe and Cu may themselves be involved in specific redox activities with melanin and oxygen species [32]. For example, melanin initiated lipid peroxidation is accompanied by the presence of Fe ions and is thought to involve Fenton chemistry [33]. The dihydroxyindole intermediate was found to be pro-oxidant at low concentration relative to Fe because of the reduction of the metal by semiquinones produced by autoxidation [34].

(iv) Role of Sex Hormones in Melanoma:

Between 1973 to 1994 various investigators have conducted studies on Melanoma patients, which revealed that the incidence of the skin cancer in men rose by 154.4% Vs 90.2% for women. The mortality rates in the same period rose in men by 48.9% while by 21.4% in women respectively. The gender differences in melanoma led to an investigation of the effect of sex hormones in melanoma. Several studies reviewed for the presence of hormone receptors in melanoma cells showed that estrogen receptors (ER) were found in some of the biochemical and histochemical tests, but not in the immunohistocemical tests using monoclonal antibodies.

In one study, all the three methods detected the ER only with the biochemical method. These findings could be considered as false positive results and attributed to the binding of the hormone to a protein other than the ER or binding of the hormone to non-
melanomal cells or the existence of a new and antigenically different ER that cannot be detected by the specific monoclonal antibody where a change in the receptor region would result in a diminished response to the estrogens. Overall, estrogen receptors do exist on the melanoma cells, albeit low affinity receptors and not the type I high affinity receptors.

In view of the efficacy of the anti-estrogenic drug Tamoxifen in patients with ER positive breast cancers [35-38] many attempts have been made to achieve the same benefits in the melanoma patients. Tamoxifen was evaluated as a single agent in 12 studies [38] covering 213 patients with metastatic melanoma where response rate was only 7% in case of single agent chemotherapy while in combination therapy with dacarbazine, carmustine, and cisplatin it produced a synergistic effect [39].

It has been postulated that Tamoxifen may act through classical estrogen receptors that may activate insulin-like growth factor receptors, which is an important plasma membrane receptor of melanoma cells. Sex hormones are known to alter the autocrine and paracrine production of angiogenic factors, expression of adhesion molecules, activity of proteolytic enzymes and host immune responses to melanoma. Although overall survival rate was increased for patients receiving Tamoxifen, significant benefit was not observed either in single or multi-agent chemotherapy regimens for patients with metastatic melanoma.

It has been shown that Quercetin, a beta-diketone compound of natural occurrence, is capable of inhibiting melanoma cell growth by interacting with type II estrogen-binding sites situated on melanoma cells [40].
4.3 Therapy for Melanoma:

A dilemma that traditionally faces the cancer chemist is the incorporation of tumor selectivity into design of the drug. Melanoma cells possess certain features that distinguish them from melanocytes hence rendering them amenable to selective chemotherapy. For example, melanocytes possess a unique phenotype containing enzyme, viz. tyrosinase, a polyphenol oxidase, which catalyzes the oxidation of tyrosine to levo-dopa to dihydroindole and ultimately to the biopigment melanin. This enzyme is active in both melanocytes as well as in melanoma cells but is expressed in larger amounts in the latter. Hence, the tyrosine enzyme represents a unique biochemical target for the design of antimelanomal drugs [41].

Since melanocytes serve a non-essential role in the host, the potential cytotoxic drugs should be able to discriminate between melanocytic cell and non-melanocytic cells rather than normal and malignant cells. Melanoma cells are responsive to a specific polypeptide hormone (MSH), which causes an increase in pigmentation, and decrease in growth, marking a difference between melanocytes and non-melanocytes.

Finally, improvements in the understanding of the prognosis and staging of melanoma has allowed better categorization of patients based on their risk of developing metastatic disease and hence permitting the development of logical strategies using surgery, radiation and adjuvant therapies with toxicity profiles that are appropriately based on the level of rise of recurrence.
(i) Surgery:

The principal treatment modality for primary cutaneous melanoma is surgery. Early diagnosis results in 90% cure rates of primary melanoma. The tumor thickness is the single variable that most accurately determines therapy and progress. The local control of primary melanoma requires wide excision of the tumor or biopsy at the site down to the deep fasia with a margin of normal appearing skin. Limited excisions are associated with local recurrence rates in the range of 30-60% [42]. Surgical excision of metastasis to regional lymph nodes is potential curative therapy. Clinical staging of the regional nodes is mainly based upon physical examination. Any palpable nodes that are >1-1.5 cm in diameter or very hard or fixed to adjacent structures are considered to be involved in metastatic melanoma and are removed along with the lesion during surgery to prevent recurrence of the disease.

(ii) Radiation therapy:

The role of adjuvant therapy in the management of melanoma remains unclear. It is rarely employed for definitive treatment of a primary melanoma site. The main role of adjuvant radiation is the treatment of the nodal basin after resection of regionally advanced melanoma. This is supported by only one clinical randomized trial of adjuvant radiation in cutaneous melanoma, which has produced no benefits to treated patients [43]. Several non-randomized studies have suggested that post-operative radiation to the neck or axilla after radical lymph node dissection decreases regional recurrence rates in node-positive patients [44-46]. In a case-study review, Shen et al. [47] observed that the low
incidence of cervical recurrence after regional lymph node dissection did not justify the routine use of postoperative radiation. The most effective radiation schedules involve large doses per fraction and are usually combined along with adjuvant interferon therapy. The optimal timing for such an integration of two therapies is yet to be determined.

(iii) Chemotherapy:

Cancer Chemotherapy can be either systemic treatment where cytotoxic drugs act on the cancer cells all over the body or the localized treatment such as infusion and perfusion where cytotoxic drugs are injected into the limb or the area affected by melanoma. In patients with early stages of melanoma where metastases of melanoma cells in the blood stream occur either in skin, subcutaneous tissues, distant lymph nodes and lung respectively, the minor surgery under local anesthesia is found to be more effective than in case of the patients with visceral (liver, bone, brain) metastases. The treatment of the latter type is carried out by chemotherapy followed by radiotherapy. Various other treatments are being developed such as Adoptive Immunotherapy using interleukin-2 (IL-2) and lymphokine activated killer cells (LAK) to stimulate the immune system in an attempt to mediate the tumor regression [48].

In cases of advanced inoperable melanoma, the combined chemotherapy was carried out using cisplatin, vinblastine, interleukin-2 (IL-2) and interferon alpha (IFN-alpha). However, this treatment was associated with severe toxicities including intense myelosuppression, infections, IL-2 induced constitutional toxicity and hypotension. The treatment of advanced cutaneous melanoma remains disappointing
since use of single cytotoxic drug such as fotemustine or temozolomide produce response rate less than 20%. Finally, it should be noted that malignant melanoma is refractory to most single chemopreventive agents and even combination therapies are without any promising results.

a. Single agent Chemotherapy:

For the treatment of systemic melanoma wherein metastases have occurred at few places like skin, subcutaneous tissues, lymph node and lung, dacarbazine (DTIC, 5-(3,3-dimethy-1-triazenyl)-1 H imidazole-4-carboxamide)(1) works with a response rate of 20%.

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**Dacarbazine(1)**

It, however, fails to respond in case of metastasis in brain, liver and bone respectively [49]. The cytotoxic action of DTIC is believed to be related to DNA alkylation at O-6 position of the guanine residue. Various analogs of dacarbazine have been synthesized to improve the response rate, which include 1-(4-carboxyphenyl)-3-3dimethyltriazene (CB10-277) and temezolomide, which is a monomethyl pro-drug form of DTIC.

Nitrosoureas such as carmustine (2) and lomustine (3) have also been used
against melanoma [50,51]. However, the response rate of these compounds is not very encouraging (< 25%) and they fail to exhibit activity against brain metastasis in spite of having excellent liposoluble property. Fotemustine(4), the new analogue, shows good potential and has activity against cerebral metastasis [52].

Platinum complexes such as cisplatin and carboplatin exhibit which have response rates of 10% to 25% when used against melanoma compared to the overall response rates of around 14 % by vinca alkaloids [53,54]. The most selective inhibitory action against melanoma is exerted by betulinic acid (5), which is extracted from the bark of birch.
trees (betulaceae family). The compound shows cytotoxic effects against melanoma cells by inhibiting the cell cycle at G0/G1 stage and inducing apoptosis [55]. The discovery of betulinic acid as a selective inhibitor inducing apoptosis is an important addition to the list of effective therapeutic agents against melanoma. Interestingly the action of betulinic acid can be blocked by progesterone, which is suggestive of some hormonal role in melanoma.

b. Combined Chemotherapy:

To overcome the ineffectiveness of single agent chemotherapy, many agents have been used in combination to improve the response rates. One of these combinations include therapeutic agents such as DTIC, nitrosourea, vincristine and chlorpromazine which show response rates of 17-22% whereas the other combination includes cisplatin, bleomycin, vinblastine and chlorpromazine which the response rate of 34-47% respectively. The introduction of Tamoxifen, which is a hormone receptor antagonist modulating the sensitivity of tumor cells to cisplatin via inhibitory effects on calcium channels has been found to improve the immune response and overall survival rates upto 50%. This shows that modulation of hormone receptors may provide an interesting way for targeting melanomal growths. In fact this forms the basis of the work described in the present chapter.
4.4 Nature and scope of present investigation:

The work described in the present chapter is based on two premises:

1. Possible presence of estrogen receptors in melanoma cells allowing the use of estrogen ligands as carrier species for transporting cytotoxic agents to the melanoma cells, and

2. Lowered antioxidant defense of melanoma cells making them vulnerable to agents capable of generating oxidative stress intracellularly. The synthetic strategy used by us is to combine the estrogenic scaffold with the redox active naphthoquinone ligands of natural occurrence employing ethylenediamine linker as shown in Scheme 4.1.

![Scheme 4.1](image-url)
The quinone ligands linked in this manner exhibit facile redox cycling, which can effectively generate oxidative stress triggering apoptosis in melanoma cells.

4.5: Experimental

4.5.1 Materials:

All chemicals used in the synthesis of the ligand and quinone conjugates were of AR grade while the solvents were distilled prior to their use. Progesterone (6) (Sigma Chemicals), Ethylenediamine, Juglone (8), Plumbagin (9) were used as supplied.

a. Synthesis of Progesterone ethylenediamine Schiff base:

For the synthesis ethylenediamine ligand, progesterone acetate (PA) and ethylene diamine (EN) were added to methanolic solution in 1:1 molar ratio. The resulting mixture was heated at 40 °C on water bath with constant heating for 8 h during which the color of the mixture changed from colorless to dark brown. After completion of the reaction (monitored by silica gel TLC) the solvent was stripped off on the rotavapor the precipitate obtained was further washed with cold water and dried in vacuum over anhydrous CaCl₂.

b. Synthesis of hydroxynaphthoquinone conjugate of Progesterone Schiff base:

*Juglone (5-hydroxy-1,4-naphthoquinone) conjugate*

The juglone conjugate was prepared by refluxing the methanolic solution of the Schiff base and juglone together in 1:1 molar ratio for 4hrs. The precipitate of the complex was washed and dried under vacuum.
Plumbagin (5-hydroxy, 2-methyl-1,4-naphthoquinone) conjugate

The plumbagin conjugate of progesterone Schiff base was prepared by refluxing the methanolic solutions of the ligand and plumbagin in 1:1 molar ratio for 4 hrs. The precipitate of the conjugate was washed and dried under vacuum.

4.6: Results and Discussion:

The condensation reaction of ethylenediamine (EN) with progesterone (PR) in 1:1 stiochiometry in DMSO solvent yields a dark brown Schiff base compound. This was further reacted with Juglone (5-hydroxy-1, 4-naphtoquinone) or Plumbagin (2-methyl-5-hydroxy-1, 4-naphtoquinone) in stoichiometric amounts resulting in dark brown precipitates which were filtered and washed several times with cold ethanol to remove the quinonoidal residues. Satisfactory analyses were obtained for all compounds as indicated in Table 4.1.

Table 4.1: Analytical data on hydroxynaphthoquinone conjugates of Progesterone Schiff base

<table>
<thead>
<tr>
<th>Compound</th>
<th>Molecular weight</th>
<th>Color</th>
<th>C%</th>
<th>H%</th>
<th>N%</th>
</tr>
</thead>
<tbody>
<tr>
<td>PREN</td>
<td>354</td>
<td>Brown</td>
<td>76.86</td>
<td>9.42</td>
<td>6.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(77.46)*</td>
<td>(9.60)</td>
<td>(7.9)</td>
</tr>
<tr>
<td>PREN-J</td>
<td>528</td>
<td>Dark brown</td>
<td>74.10</td>
<td>7.50</td>
<td>5.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(75.00)</td>
<td>(7.57)</td>
<td>(5.30)</td>
</tr>
<tr>
<td>PREN-P</td>
<td>542</td>
<td>Dark brown</td>
<td>74.35</td>
<td>7.70</td>
<td>5.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(75.27)</td>
<td>(7.74)</td>
<td>(5.10)</td>
</tr>
</tbody>
</table>

*Values in parentheses represent the calculated values.

The infrared spectra of PR shows strong absorptions at 2930 and 2850 cm\(^{-1}\) due to CH stretches of the C-17 acetyl group and the olefinic CH of the ring A
respectively [Fig. 4.4]. Upon Schiff base formation an additional strong band can be seen at 3280 cm$^{-1}$ due to hydrogen bonded NH group of the side chain. The addition of PREN in the quinonoidal nucleus of Juglone (PRENJG) and plumbagin (PRENPL) results in many overlapping absorptions due carbonyl and imino chromophores. These are observed in the region 1690-1610 cm$^{-1}$. The $\nu$ C-O stretch of the naphthoquinone ligands is slightly shifted on the lower energy side (from 1235 cm to 1228 cm in Juglone) due to electron donation from the Schiff base.
Figure 4.4a: Infrared spectra of a) PR, b) PREN, c) PRENJ, d) PRENP.
Figure 4.4b: Infrared spectra of a) PR, b) PREN, c) PRENJ, d) PRENP.
The electronic spectra of the three compounds in DMSO solvent are shown in Fig. 4.5, which confirms the addition of naphthoquinone fragments to the progesterone Schiff base. The electronic spectrum of non-conjugated juglone and plumbagin individually is shown in the dotted lines. Thus, the additional peaks at 400 nm (PRENJ) and 420 nm (PRENP) represent the quinonoidal absorptions.
Figure 4.5: Electronic spectra of Progesterone and its hydroxynaphthoquinone conjugates
Finally, the electrochemical profiles of the compounds were examined in DMSO solvent using tetraethyl ammonium perchlorate as a supporting electrolyte with platinum as the working electrode and Ag/AgCl as a reference electrode. The voltammetric trace of plumbagin alone (in dotted lines) exhibits two reversible redox couples at −0.54 v and −1.56 v respectively. The first redox peak corresponds to the reduction of the naphthoquinone to naphthosemiquinone anion while the second one corresponds to the reduction of naphthoquinone to naphthohydroquinone, the dianion of the ligand (Figure 4.6). Upon conjugation with the progesterone Schiff base the first peak is slightly shifted (Δ=0.25 v) to more positive potential indicating more facile reduction to semiquinone species. This would lead to generation of more pronounced intracellular oxidative stress which may lead to the more pronounced inhibition of cell proliferation thereby leading to extensive cellular death.

The quinone ↔ semiquinone couple in case PRENPL compound (in inset) is reversible based on the scan rate dependence and correlating with the current generated. Interestingly the second catholic peak corresponding to the conversion into hydroquinone species is transformed into an irreversible couple.
Figure 4.6: Cyclic voltammogram of Progesterone Schiff base and its plumbagin conjugate.
4.7: Antiproliferative activity against B16F10 melanoma cells:

All synthesized compounds were evaluated for their antiproliferative properties. It is clear that the parent Schiff base (PREN) has no antiproliferative activity shown by MTT assay, (Figure 4.7). On the other hand incorporation of naphthoquinone moieties into PREN leads to potentially active species. Among the two-quinone conjugates the PRENPL has a superior antiproliferative action with $IC_{50}$ value of 6 $\mu$M while for the PRENJG it is 7.2 $\mu$M respectively. The pronounced antiproliferative effect observed in case of PRENP is probably due to its Quinone $\Leftrightarrow$ Semiquinone redox couple.

Figure 4.7: Trypan blue assay of progesterone ethylene-dimine derivative and its hydroxynaphthoquinone conjugates tested against B16 F10 melanoma cells. L1= PREN, L2= PRENJ and L3= PRENP.
The phase contrast microscopy images after treatment of melanoma cells at the IC$_{50}$ values of the two conjugates are shown in (Figure 4.8). The cells in the control sample are proliferative in the normal mode while those treated with the test compounds are exhibiting shrinking of the nuclei characteristic of apoptotic death. The effect is particularly severe in case of PRENP conjugate. This may be due to oxidative stress produced by this conjugate as a result of the facile quinone $\Leftrightarrow$ semiquinone redox couple.

Figure 4.8: Phase contrast microscopy on B16F10 melanoma cells tested against Progesterone and its hydroxynaphthoquinone conjugates.
4.7: Conclusions:

In the earlier chapter we had described metal conjugates of progesterone Schiff base where metal redox was found to influence the antiproliferative activities of the ligands. Since the basis of such influence was postulated to be the intracellular oxidative stress generated by those compounds, in the present work we have examined the possibility of generating similar phenomenon by employing redox active quinonoidal conjugates. The result obtained presently lends support to the suggestions that modulation of oxidative stress can form biochemical basis for designing selective anticancer agents in future.
References:


52. C. Jacquillet, D. Khayat, P. Banzet, Cancer, 66(1990), 1873.


