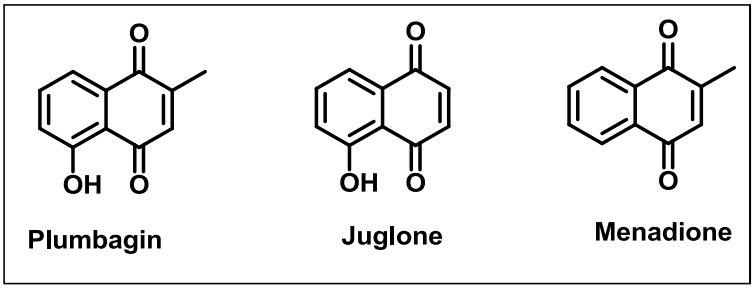
The thesis entitled "**Natural 1,4-naphthoquinone based New Chemical Entities: Synthesis, Anticancer and Anti-inflammatory Activities**" describes the chemical modifications of some highly potent 1,4-napthoquinone scaffolds such as juglone, plumbagin and menadione to synthesize a wide range of new chemical entities and their anticancer and anti-inflammatory activities. The results are presented in five chapters as detailed below.

Chapter I: **Natural 1,4-Naphthoquinones: Highly Useful Scaffolds for the Development of Potent Anti-Cancer Lead Molecules**

This chapter deals with the brief introduction of cancer, sources of natural products for anticancer therapy, quinones as anticancer agents and quinone based natural anticancer lead molecules.

Cancer is a generic term given to a group of related diseases that can affect any part of the body. There are over 200 types of cancers known till date. Cancer can be treated by different methods depending upon the nature and location in the body. The major types of cancer treatment are *surgery, radiation therapy, immunotherapy, targeted therapy, hormone therapy, chemotherapy.* Nature is an attractive source of new therapeutic compounds, because tremendous chemical diversity is found in millions of plants, animals, marine organisms and microorganisms. Molecules (called natural products) derived from these natural sources have played and continue to play dominant role in the discovery and development of potent drugs for the treatment of cancer. A total of 246 compounds were approved as anticancer drugs worldwide during the period 1940-2014. Significantly, over 60% of these approved anticancer drugs are derived from natural products or inspired by natural products.

Among the various classes of secondary metabolites, the quinone metabolites assume very high significance in view of their potent anticancer activities and are amenable for wide range of chemical modifications to make diverse New Chemical Entities (NCEs) with enhanced anticancer activities. Many clinically useful anticancer agents such as daunorubicin, doxorubicin, epirubine, idarubicin, mitoxantrone, amentantrone, pirarubicin and mitomycin contain quinone moiety. Recently, 1,4-naphthoquinones such as plumbagin, juglone and menadione have gained considerable attention due to their interesting biological activities including anticancer activity.



Plumbagin is mainly isolated from *Plumbago zeylanica* Linn, commonly known as Chitrak. *P.zeylanica* is one of the widely used plants in Indian traditional medicine (Ayurveda) and Traditional Chinese Medicine (TCM) for over 3000 years including cancer. Plumbagin is the active constituent of *P.zeylanica* and is responsible for its therapeutic applications. Plumbagin has been shown to exert wide range of biological activities including anticancer. Especially, plumbagin effectively inhibits the proliferation of various cancer cells *in vitro*. Plumbagin has been reported to exhibit anticancer activity by regulating different targets, which play an important role in apoptosis, autophagy and angiogenesis. The anticancer activity of plumbagin has also been investigated *in vivo* against different cancers. Plumbagin was even reported to show chemosensitization effects *in vitro*. It enhanced the sensitivity of cancer cells

to chemotherapeutic drugs such as paclitaxel in renal cancer, zolendronic acid in breast cancer.

Juglone is mainly present in *Juglandaceae* family (walnut family). It is found in significant amounts in *Juglans regia* in almost all plant parts such as roots, nuts, stem, branches and leaves. Juglone was reported to possess many biological properties such as anticancer, anti-proliferative, antibacterial, antifungal, antioxidant, antiviral and allelophatic. Juglone showed potent anticancer activity *in vitro* against various human cancer cell lines. The cytotoxicity of juglone is associated by regulating different targets. *In vivo* anticancer potential of juglone has also been evaluated in animal models against different cancers. In addition, juglone was also reported to exhibit radiosensitizing potential *in vitro* and *in vivo*.

Menadione is a simple 1,4-naphthoquinone analogue belonging to vitamin K family. Menadione is present in ferns such as *Asplenium indicum*, *Asplenium laciniatum* and in walnut husks of *Juglans.* Menadione exhibited significant *in vitro* anticancer activity against both rodent and multiple human cancers. Menadione was found to exhibit cytotoxicity by different mechanisms via regulating cellular signal factors. Significantly, recent clinical trial showed that menadione can be used for objective clinical responses in patients with advanced hepatocellular carcinoma. Further, menadione exhibited synergistic effect both *in vitro* and *in vivo* when combined with other chemotherapeutic drugs.

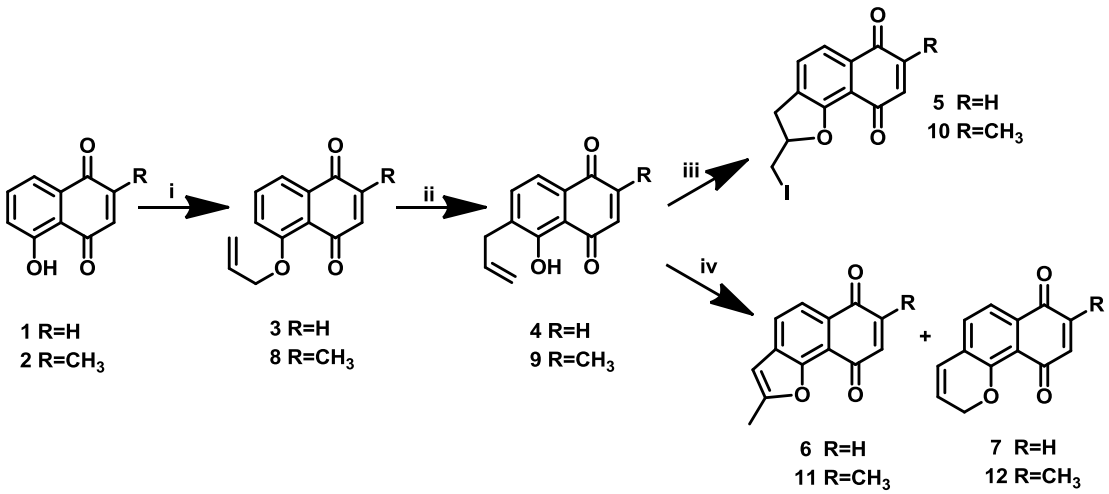
In view of the potent anticancer activity of the quinone metabolites, especially plumbagin, juglone and menadione both *in vitro* and *in vivo*, they can be considered as highly useful anticancer natural scaffolds for the development of a wide range of

diverse new chemical entities as highly potent and efficacious anticancer therapeutic agents.

<u>Chapter II:</u> Synthesis and Anticancer Activity of Some Novel Juglone Based 5,6-Fused 1,4-Naphthoquinone Hybrids

This chapter describes the synthesis of 5,6-fused hybrids of juglone and plumbagin and evaluation of their anticancer activity against seven human cancer cell lines. Further, the effect of potent compounds on cell cycle and apoptotic nature has been studied.

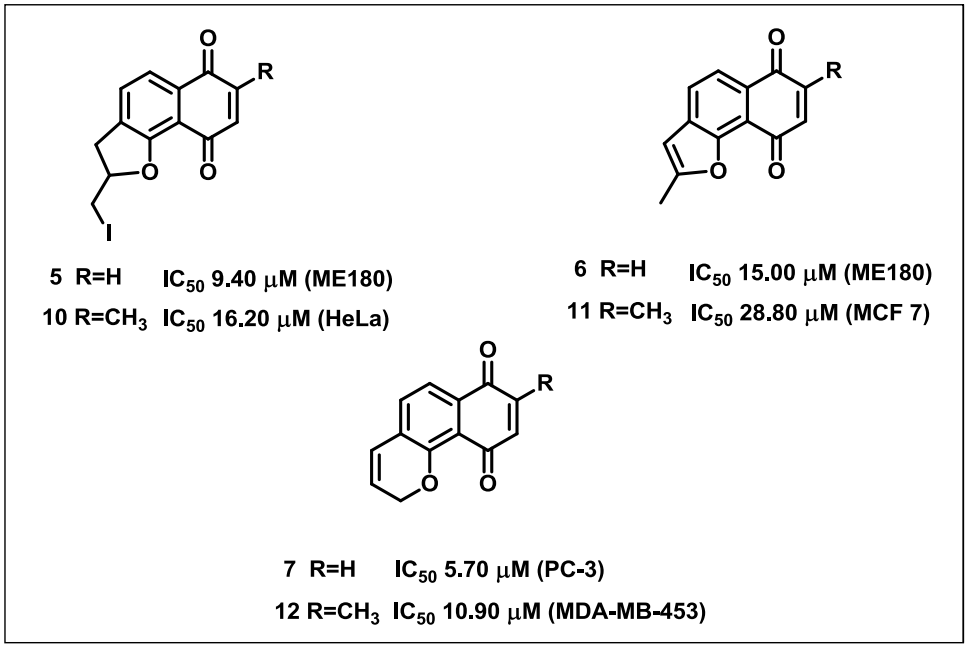
The present work is aimed to synthesize some novel cyclic ethers such as benzofuran or benzopyran-quinone hybrid compounds by utilizing phenolic hydroxyl group and keeping the quinone moiety intact as it is the key function responsible for the bioactivity of both the scaffolds (**Scheme 1**).



Scheme 1 Reagents and conditions: (i) allyl bromide, Ag_2O, CH_2Cl_2, rt, 20 h; (ii) diphenyl ether, 160 ^oC, 24 h; (iii) I_2, $SnCl_4$, CH_2Cl_2, 12 h; (iv) $PdCl_2$, $Cu(OAc)_2.H_2O$, LiCl, DMF, rt, 3 h

The *in vitro* cytotoxicity of the synthesized compounds **(3–12)** of juglone and plumbagin was studied using ME-180 & HeLa (cervix), MCF-7, MDA-MB-453 & MDA-MB-231 (breast), PC-3 (prostate) and HT-29 (colon) cancer cell lines by employing MTT assay. The results of *in vitro* anticancer activity indicated that hybrids **5**, **7** and **12** were more potent than the standard etoposide and parent compounds against MCF-7, PC-3 and MDA-MB-453 cancer cell lines respectively. The mechanism studies showed that the anticancer activity of benzopyran hybrids **7** and **12** could be attributed to the induction of cell cycle arrest and apoptosis in cancer cell lines. Fused dihydrobenzofuran or benzopyran moiety seems to be important for enhanced antiproliferative activity of juglone and plumbagin.

Potent molecules

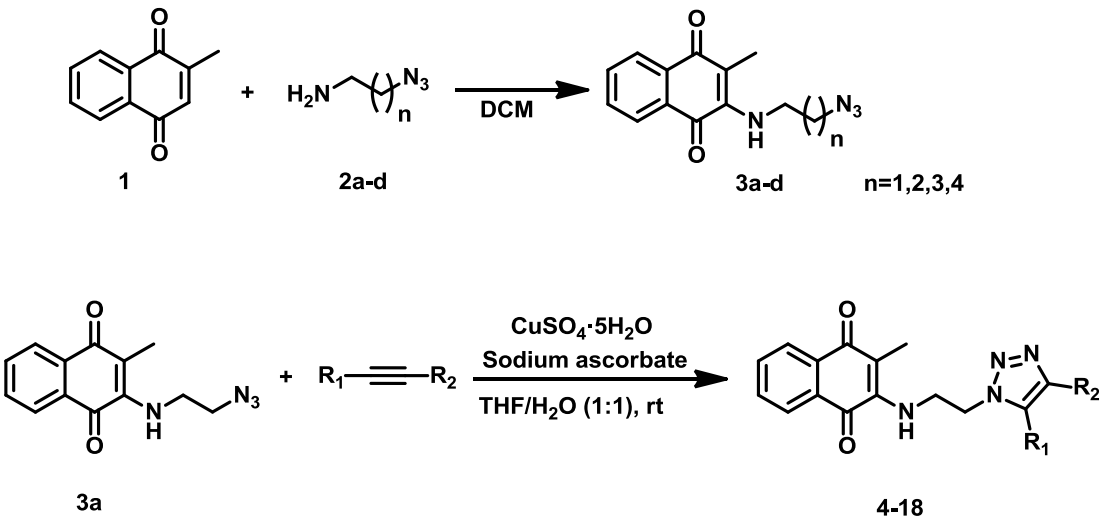


<u>Chapter III:</u> Novel Menadione Hybrids: Synthesis, Anticancer Activity and Cell Based Studies

This chapter illustrates the design, synthesis of novel menadione based 1,2,3-triazole hybrids and their anticancer activity against five cancer cell lines. Further cell based

studies have been carried to evaluate the potent compound for its apoptotic inducing nature.

The objective of the present work is to synthesize menadione based 1,2,3-triazole hybrids by employing copper catalyzed azide-alkyne cycloaddition (CuAAC) by considering the fact that combination of two pharmacophores in a single molecule (molecular hybridization) is a versatile tool for drug discovery and development (**Scheme 2**).

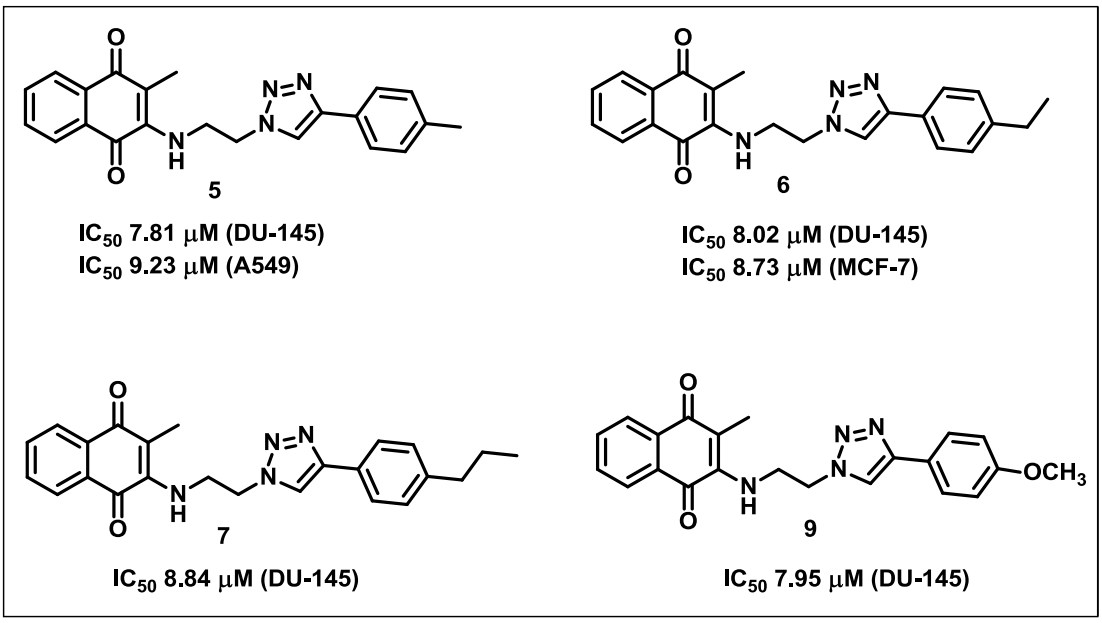


Scheme 2

These synthesized analogues were evaluated for their anticancer activity against a panel of five cancer cell lines, namely MCF-7 (breast), A549 (lung), Hela (cervical), DU-145 (prostate) and B-16 (mouse melanoma) by employing MTT assay. The results of *in vitro* cytotoxicity indicated that the hybrids **5, 6** and **9** were more potent than the standard tamoxifen against A549 cell line. Compounds **5**, **6**, **7**, **9**, **12**, **13** were more potent than the standard tamoxifen against DU-145 cell line and compounds **5, 12, 13** and **18** were more potent than the standard tamoxifen against Hela cell line. More interestingly, compound **6** was more potent than the standard

tamoxifen and parent menadione against MCF-7 cell line. The in depth biological studies showed that the anticancer activity of the hybrid **6** could be attributed to the induction of cell cycle arrest and apoptosis in human breast (MCF-7) cancer cell line.

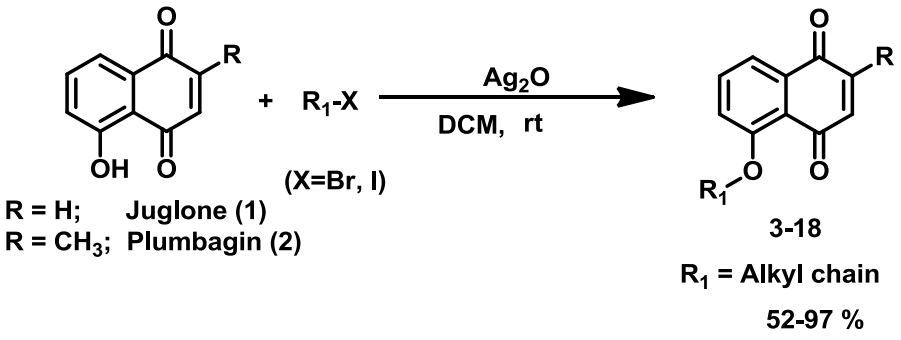
Potent molecules



<u>Chapter IV:</u> Synthesis, Anticancer and Anti-inflammatory Activities of 5-*O*-Alkyl ethers of Juglone and Plumbagin

This chapter deals with the synthesis of 5-*O*-alkyl ethers of juglone and plumbagin and their anticancer and anti-inflammatory activities. Apoptotic inducing nature and effect on cell cycle of potent compounds have been studied.

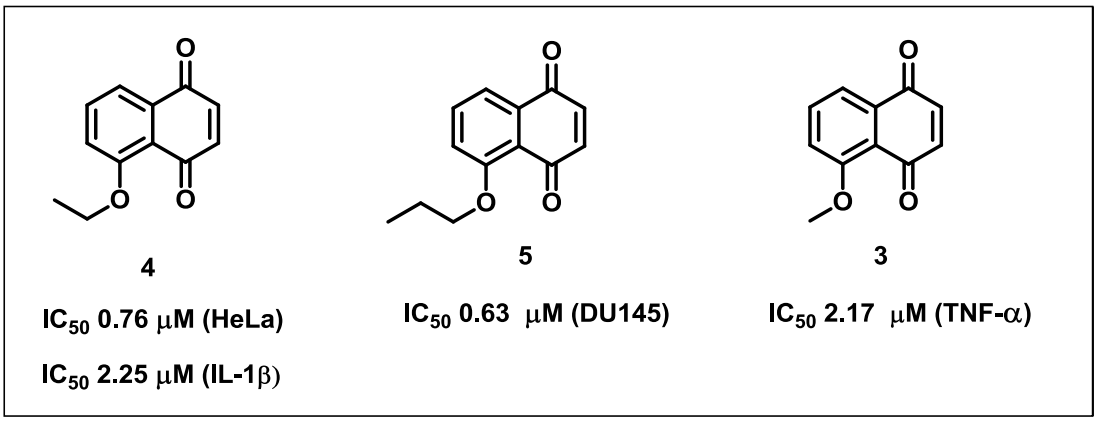
In this chapter, novel acyclic ethers of juglone and plumbagin were synthesized by converting the phenolic hydroxyl group at C-5 position based on the fact that cyclic ethers enhanced the anticancer activity (**Scheme 3**).



Scheme 3

In total, sixteen novel 5-*O*-alkyl ether analogues of juglone and plumbagin were synthesized. These analogues along with their parent scaffolds were screened for their anticancer and anti-inflammatory activity. The results of *in vitro* cytotoxicity revealed that 5-*O*-alkyl ether analogues (**3-10**) of juglone displayed highly potent anticancer activity than parent juglone against all the tested cell lines except MDA-MB-231, A549. Compounds **3-8** exhibited highly significant cytotoxic activity (< 1 μM) against DU145 cell line. Compound **5** displayed highest cytotoxicity with IC_{50} 0.63 μM against DU145 cell line. Compound **4** displayed highest cytotoxicity with IC_{50} 0.76 μM against HeLa cell line. The in depth biological studies showed anticancer activity of compounds **4** and **5** could be attributed to induction of cell cycle arrest and apoptosis in cancer cell lines. In addition to anticancer activity, 5-*O*-alkyl ethers of juglone also displayed potent anti-inflammatory activity. Compound **3** displayed the highest TNF-α inhibitory activity (IC_{50} 2.17 μM) and compound **4** exhibited the highest IL-1β inhibitory potential (IC_{50} 2.25 μM).

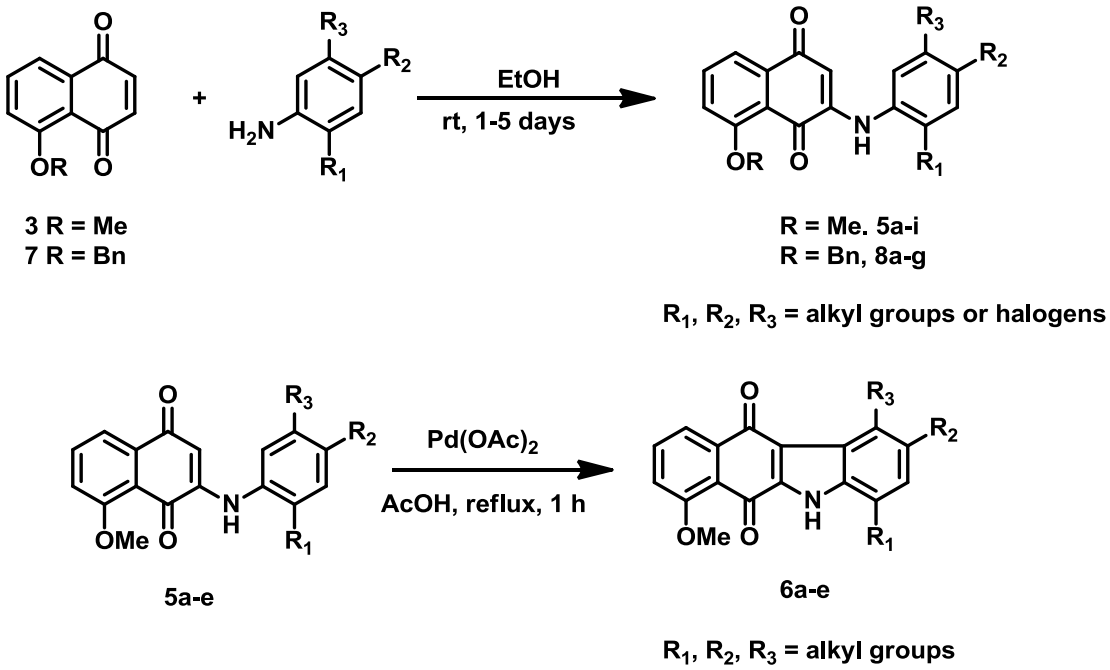
Potent molecules



<u>Chapter V:</u> Synthesis, Anticancer and Anti-inflammatory Activities of Anilino-quinone and Carbazole-quinone Based Hybrids of Juglone

This chapter describes the synthesis anilino-quinone based and carbazole-quinone based analogues of juglone, their anticancer activity against seven human cancer cell lines and anti-inflammatory activity. Further, the effect of potent compound's apoptotic nature has been studied.

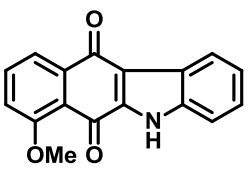
The objective of the present work is to synthesize R-phenylamino-*O*-methyl juglone analogues, carbazole-quinone analogues and R-phenylamino-*O*-benzyl juglone analogues (**Scheme 4**). Their anticancer potentiality was evaluaed against a panel of seven human cancer cell lines namely lung (A549), glioblastoma (U87MG), cervical (HeLa, SiHa), prostate (PC3), breast (MDA-MB-231) and colon cancer (HT-29) by employing MTT assay.



Scheme 4

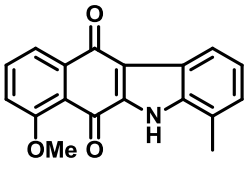
Compound **6a** exhibited significant cytotoxicity with IC_{50}: 10.49 μM against HeLa cell line. Compound **6b** displayed promising cytotoxicity with IC_{50}: 19.60 μM and 20.82 μM against U87MG and MDA-MB-231 cell lines respectively. Compound **6c** displayed significant cytotoxicity with IC_{50}: 18.70 μM against HeLa cell line. The in depth biological studies showed anticancer activity of compound **6a** could be attributed to induction of apoptosis in HeLa cancer cell lines. Anilino-*O*-methyl juglone analogues **5c**, **5h** and benzyl ether of juglone (**7**) displayed significant anti-inflammatory activity. Especially, compound **5h** displayed the highest IL-1β inhibitory activity with IC_{50}: 0.66 μM.

Potent molecules



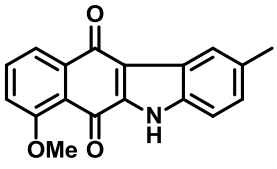
6a

IC_{50} 10.49 μM (HeLa)



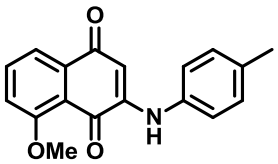
6b

IC_{50} 19.60 μM (U87MG)
IC_{50} 20.82 μM (MDA-MB-231)



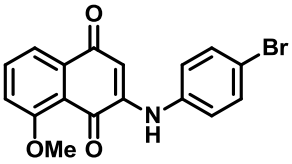
6c

IC_{50} 18.70 μM (HeLa)



5c

IC_{50} 2.57 μM (TNF-α)
IC_{50} 2.98 μM (IL-1β)



5h

IC_{50} 6.72 μM (TNF-α)
IC_{50} 0.66 μM (IL-1β)

In conclusion, sixty eight New Chemical Entities of natural 1,4-naphthoquinone metabolites such as juglone, plumbagin and menadione have been synthesized and evaluated for their anticancer and anti-inflammatory activities. Twenty four compounds have been identified as the most potent ones, which can serve as lead molecules for further development.