3.1. Rationale and Objective

The International Agency for Research on Cancer had discussed and reported: a Global Effort which explained that cases of will double again by 2020, and will nearly triple by 2030. There were an estimated 12 million new cancer diagnoses and more than 7 million deaths worldwide this year. Kevin has summarized the data that the projected numbers for new diagnosis in 2030 may raise to 20 to 26 million and 13 to 17 million deaths (Kevin *et al.,* 2015). About 1% cancer incidence will be expected in countries like China, Russia, and India each year. Reason behind this hike in cancer incidence is tobacco use and higher-fat diets in less-developed countries.

Another report which was given in 2014 by the Centers for Disease Control and Prevention (CDC) revealed that cancer has moved forward cardiovascular disease as the leading cause of death in 22 states of America.Another new precognition from the International Agency for Research on Cancer has told anestimate of 27 million new cancer cases and 17 million cancer deaths by 2030. These numbers are almost double of the number of deaths found in 2007. Based on current trends of life style, new cancers which are to be diagnosed is expected to grow by 1% annually,so on deaths from the disease. Lifestyle factors such as smoking and obesity will act as the leading cause of cancer in poor and middle-income countries which will leave behind chronic infection as a cause of cancer.This is reflecting like poor nations will see biggest increases in cancer. There are two broad categories of drugs used to fight cancer. The first is cytotoxic (cell killing) and the second is cytostatics (cell stabilizing drugs).Forregrowth of the cells and formation of new cells, nucleic acid synthesis is mostimportantstep.For inhibiting the growth of cancer cells therefore we can target the nucleic acid synthesis and itsprecurcors in rapidly multiplying cells by cytotoxicdrugs.Especially in large solid tumors,lower percentage of cells are in

division than normal bone marrow,epithelial linings,reticuloendothelial(RE) system and gonads. So, these tissuesare particularly affected in a dose dependent manner by majority of drugs, though, there are differences in susceptibility to individual members.Cytotoxic drugs can be used to destroy tumors, boost the outcomes of surgery or radiotherapy, reduce metastases and alleviate cancer symptoms. Both categories lead to a reduction in the size of the tumor because cancer cells have such a high mortality rate that simply preventing them from dividing will lead to a reduction in the population. Drawbacks of these drugs are that they affect all dividing cells, including those of healthy tissue.Whereas cytostaticsare only useful forthe primary tumor and small tumors that have not been detected in tests. Advantage of cytostatics against cytotoxic is that sensitivity cancer cells whichoften divide markedly faster than normal cells,are more prone to death particularly by cytostatics. The effects on normal cells are less pronounced and healthy cells also recover faster.Type of tumor, its composition, and rate of development and proportion of cells in the distribution stage decides the effectiveness of chemotherapy.So, cytostaticsare given in as high-dose chemotherapy. This is used in treating leukemia, some lymphomas and brain tumors in children. At the same time, stem cell transplants are required as high-dose chemotherapy can completely destroy bone marrow. To reduce the population of above all the factors both categories lead to a reduction in the size of the tumor because cancer cells have such a high mortality rate that simply preventing them from dividing will lead to a reduction in the population. But none of these are “Cancer specific”, they all are simply “quickly dividing cell specific”. This is the explanation of the side effects associated with chemotherapy i.e. nausea, immunosuppression, ulceration and hair loss. Hence developed drug resistance and toxicity to most clinically used anticancer drugs is a matter of great concern and new anticancer agents need to be evolved with better

efficacy for the treatment and control of the disease. The national cancer institute had always played as a pioneer in the development of cancer treatment since last 80 years. Anticancer potential of quinone moiety can be dated back to 1974 through a mass screening program of National cancer institute, Bethesda, U.S.A. Quinoidal compounds from natural or synthetic origin represents the second largest class of anticancer agents. Doxorubicin is the first USFDA approved quinone based drug .In 1974 it was approved against the treatment of leukemia, breast cancer etc. In 1998 another drug that is Daunorubicin hydrochloride was another drug which was approved by USFDA for the treatment of leukemia. Many clinically important antitumor drugs containing quinone nuclei such as doxorubicin, Daunorubicin, mitomycin, mitoxantrone and saintopin were the drug of the era 1974-2002 and showed excellent anticancer activity for solid tumors. Naturally occurring quinones have captured human attention for thousands of years,initially by reason of their bright colors with possible uses as dyes (Pati*et al.,* 1947)Pigments of various colors later which were characterized as quinones have been isolated from high and lower plants,fungi as well as from animals.Crude preparations of plants,presently known to contain quinones as their active ingredients,were prescribed more than 4000 years ago as purgatives or drugs (Fieser*et al.,* 1995).

Since the mid 19th century,chemists have been studying the chemical properties of various quinones.The first synthesized and most common quinone, para benzoquinone was discovered in the late 1830's in Liebig's laboratory as the result of the oxidation of quinic acid with manganese dioxide and sulfuric acid. The various quinone scaffolds has been reported for the treatment of cancer. Especially, 1, 4-naphthoquinones are active quinone derivatives that are broadly utilized as crude materials in agrochemicals industries and pharmaceuticals (Fry *et al.,*1998). 1, 4-Naphthoquinone having two ketone chromophore which is essential for the biological activities from their capacity

to accept the electrons. Structure-activity relationship (SAR) exposed that cytotoxicity potency of 1,4-naphthoquinone is firmly connected with their electron accepting ability, which offers ascend to responsive oxygen species generation prompting DNA destruction and cell death (Salmon-Chemin*et al.,* 2001).Heterocyclic nucleuses are always in top for designing compound and have an area of prevalence in rational design of compounds. The piperazine scaffold has been regarded as a core and is frequently found in naturally active substances across a variety of different therapeutic drugs (Rathi*et al.,* 2016; Gurdal *et al.,*2015). The synthetic flexibility of piperazine scaffold led to the synthesis of a variety of its substituted analogues. Slight modification to the substitution pattern on the piperazine nucleus facilitates a recognizable difference in the medicinal potential of the resultant molecules (Njuguna, *et al.,* 2012). Piperazine core has two primary nitrogen atoms which exert the improvement in pharmacokinetic features of drug candidates because of their appropriate pKa. For this purpose, the characteristics of the piperazine template make this molecular subunit a useful and well-positioned system in the rational design of drugs (Rathi*et al.,* 2016).Among heterocyclic nucleus the oxadiazole chemistry has been developed extensively and is still developing. Most of the drugs are used clinically, which comprise oxadiazole moiety in association with various heterocyclic rings. Oxadiazoles are an important type of oxygen and nitrogen containing aromatic heterocyclic compounds, which possess desirable electronic and charge-transport properties and the various functional groups are easily introduced into the structurally rigid oxadiazole ring. These characteristics resulted in the extensive potential applications of oxadiazole based derivatives in the field of medicinal chemistry. Being concerned about the antitumor activity only, the characteristics of the oxadiazole template make this molecular subunit a useful and well-positioned system in the rational design of drugs (Bajaj *et al*., 2018;

El-Din *et al.,* 2015).Based on above rationale, in our present investigation, we attempted to design compounds through hybrid pharmacophore technology, by combining together two different pharmacophore having different therapeutic values (Naphthoquinone, piperazine, oxadiazole) to study the effect on anticancer activity of the designed compounds. *In-vitro* anticancer efficiency was determined in MCF-7(Human breast cancer), Hep-G_2 (Liver carcinoma) and Hela (Cervical carcinoma) cell lines. *In-vivo* anticancer activity of some potent compounds was determined in N-methyl-N-nitrosourea(MNU) induced breast cancer animal model.

Further to simulate their binding preference with target enzyme (EGFR Tyrosine kinase), molecular docking was conducted by using vlife software MDS package and *in-silico* pharmacokinetic properties were also predicted to check the drug likeness of the synthesized compounds.

3.2. Designof 1, 4-Naphthoquinone Derivatives

Antitumor potential of quinoidal compounds prompted us to continue our investigation towards design and synthesis of 1,4-naphthoquinone derivatives by Hybrid pharmacophore approach.

MB-1 to **MB-19**

Series-1: Designed compounds were synthesized by reacting acid chloride product of 3-((3-methyl-1, 4-dioxo-1, 4-dihydro naphthalen-2-yl) thio) propanoic acid (3) with different substituted piperazine to study the effect on biological activity after tethering the two moieties (**Table 3.1**).

Table 3.1: Design of Piperazine substituted 1, 4-Naphthoquinone derivatives

Compound	R	Compound code	R
MB-1	H	**MB-11**	4-FC_6H_5-
MB-2	C_6H_5-	**MB-12**	4-$CF_3C_6H_5$-
MB-3	$(C_6H_5)_2CH_2$-	**MB-13**	3-FC_6H_5-
MB-4	$C_6H_5CH_2$-	**MB-14**	4-$OCH_3C_6H_5$-
MB-5	C_5H_4N-	**MB-15**	2-$OCH_3C_6H_5$-
MB-6	2-$CH_3C_6H_5$-	**MB-16**	4-$NO_2C_6H_5$-
MB-7	2-ClC_6H_5-	**MB-17**	2,3-$(CH_3)_2C_6H_5$-
MB-8	2,3-ClC_6H_5-	**MB-18**	2,3-$(OCH_3)_2$-4-BrC_6H_5-
MB-9	4-ClC_6H_5-	**MB-19**	2-$NO_2C_6H_5$-
MB-10	2-FC_6H_5-		

MB-20 to **MB-33**

Series-2: Designed compounds were synthesized by reacting acid chloride product of 3-((3-methyl-1, 4-dioxo-1, 4-dihydro naphthalen-2-yl) thio) propanoic acid (3) with different substituted oxadiazole nucleus to study the effect on biological activity after tethering the two moieties (**Table 3.2**).

Table 3.2: Design of Oxadiazole substituted 1, 4 Naphthoquinone derivatives

Compound	R	Compound	R
MB-20	CH_3-	**MB-27**	2,4-$(OCH_3)_2C_6H_5$-
MB-21	CH_2CH_3-	**MB-28**	4-Cl- C_6H_5-
MB-22	$CH_2CH_2CH_3$-	**MB-29**	2-ClC C_6H_5-
MB-23	C_6H_5-	**MB-30**	2,3-$(Cl)_2C_6H_5$-
MB-24	4-$OCH_3C_6H_5$-	**MB-31**	4-CH_3 C_6H_5-
MB-25	2-$OCH_3C_6H_5$-	**MB-32**	2,5-$(CH_3)_2C_6H_5$-
MB-26	3-$OCH_3C_6H_5$-	**MB-33**	4-OH-3-$CH_3C_6H_5$-

3.3. Plan of Work

Figure 3.1: Schematic representation of plan of work

3.4. Design Synthesis of:

- Series-1: Derivatives of 2-methyl-3-((3-oxo-3-(piperazin-1-yl) propyl) thio) naphthalene-1, 4-dione
- Series-2: Derivatives of N-(1,3,4-oxadiazol-2-yl)-3-((3-methyl-1,4-dioxo-1,4-dihydronaphthalen-2-yl)thio)propanamide

3.5. Characterization of Synthesized Compounds

- Physiological characterization including solubility, melting point and TLC analysis (R_f value) etc.
- Structural confirmation of synthesized compounds by FT-IR, ^{1}H-NMR and ^{13}C-NMR, mass and elemental (CHN) analysis.

3.6.*In-Silico* Study

Docking analysis by vLife MDS 4.6 software and in silico pharmacokinetic predictionwith QikProp of Schrodinger Maestro.

3.7 Biological Evaluation

- *In-vitro* anticancer activity by MTT assay
- *In-vitro* enzyme inhibition assay (EGFR tyrosine kinase)
- *In-vivo* anticancer activity in N-methyl-N-nitrosourea (MNU) induced breast cancer, animal model.