## 6.1. Summary and Conclusion

It is a known fact that serendipity has played a pivotal role in the discovery of many drugs used today. Indeed the serendipitous discoveries of antitumor potential of quinoidal compounds, natural and synthetic quinones are distinguished as anticancer moieties. Prototype of these quinone compounds enabled medicinal chemists to modify and yield better antineoplastic agents with improved therapeutic profiles. Cancer is usually caused due to complex interactions of factors include aging, tobacco, sun exposure, radiation exposure, chemicals and other substances, some viruses and bacteria, certain hormones, family history of cancer, alcohol, poor diet , lack of physical activity or being overweight. Therefore the discovery of novel anticancer drugs is still a big challenge for medicinal chemists.

We attempted to design and synthesize two different series of 1, 4–naphthoquinone analogs using hybrid pharmacophore approach. Purity of these designed and synthesized compounds were ascertained by melting point and $R_f$ value (TLC Analysis).The chemical structures of 1,4–naphthoquinone analogs were established by spectroscopic FT-IR,$^{1}$H NMR,$^{13}$C NMR, Mass function and elemental analysis.

The *in-vitro* anticancer activity of synthesized compounds was evaluated against three human cancer cell lines MCF-7 (Human breast carcinoma), Hep-$G_2$ (liver carcinoma) and HeLa (cervical carcinoma) using MTT assay. Further *in-silico* study was performed on tyrosine kinase (EGFR TK) to get an insight about binding of compounds with the active site of target enzyme. The pharmacokinetic properties of synthesized compounds were also predicted by software vLife MDS to observe the *in-silico* ADME profile.

In **Series-1**, a total of nineteen piperazine substituted 1,4–naphthoquinone derivatives (MB1-MB-19) were designed by hybrid pharmacophore approach, where different substituted piperazines were tethered to acid chloride derivative of 3-((3-methyl-1,4-

dioxo-1,4-dihydronaphthalen-2-yl)thio) propanoic acid (compound-3).The Structural characterization by spectroscopic techniques FT-IR, $^{1}H$ NMR, $^{13}C$ NMR, Mass and elemental analysis substantiated the hypothesis of reaction. The results of the anti-proliferative screening by MTT assay revealed that compounds **MB-9** and **MB-18** are utmost powerful anticancer agents. Furthermore, in the enzyme inhibition assay compounds **MB-9 (**$IC_{50}$ = 1.80 ± 0.06 μM) and **MB-18 (**$IC_{50}$ = 2.03 ± 0.07 μM) showed the good promising inhibitory activity against EGFR tyrosine kinase with and in comparison to standard drug imatinib with $IC_{50}$ = 3.54 ± 0.11μM. Both of these compounds are found to act in a dose dependent manner. Based on these results, compound **MB-9** and **MB-18** were selected for further *in-vivo* study.

The *in-vivo* study was further preceded in MNU induced breast cancer animal model. $LD_{50}$ was determined for the selected compounds and were calculated according to log-probit method. 50% of animal death was observed at the dose of 400 mg/kg. The compound MB-9 treated group at the dose of 5mg/kg in 10% DMSO through intraperitonial route has significantly reduced the growth of cancer cells.The *in-silico* docking studies substantiated the findings of wet lab where **MB-9** showed highest docking score and explained a significant binding with the active site of the enzyme EGFR tyrosine kinase. (PDB ID: 2GS6). The drug likeliness of **MB-9** was confirmed by Qikprop analysis using Schrodinger software. In continuation of the idea of hybrid pharmacophore approach and pursuance of newer and safer agents as EGFR tyrosine kinase inhibitors, the heterocyclic substituted piperazines were replaced with substituted 1,3,4 Oxadiazoles nucleuses for the **Series-2**. The starting material in series-2 *i.e.* 3-((3-methyl-1, 4-dioxo-1, 4-dihydronaphthalen-2-yl) thio) propanoyl chloride (compound-4) was tethered to different substituted 1, 3, 4 Oxadiazoles nucleuses to get 1, 3, 4- Oxadiazoles substituted 1, 4-naphthoquinone derivatives **(MB-20– MB-33).**

The structure elucidation and purity of all the derivatives were supported by FT-IR, $^{1}H$ NMR, $^{13}C$ NMR, Mass spectra and elemental analysis. Initially for the anti-proliferative screening, MTT assay (a colorimetric assay) was conducted and found that Compound **MB-24** and **MB-32** were utmost powerful anticancer agents. Additionally **MB-24** was sorted with $IC_{50}$ (9.30 ± 0.14 μM) against MCF-7 cancer cell line which showed better inhibition than **MB-9 (**15.63 ± 0.47 μM**)** and **MB-18 (**16.96 ± 0.21 μM**)**. This outcome can be concluded by exploration of the binding of the heterocyclic 1, 3,4-oxadiazole nucleus **(MB-24)** within the active site of EGFR Tyrosine kinase enzyme through *in-silico* molecular docking study that substantiated the *in-vivo* EGFR tyrosine kinase inhibition. The compound **MB-24** showed the inhibitory regression of cancer cell growth with $IC_{50}$ = 1.53 ± 0.05 μM in comparison to standard drug imatinib with $IC_{50}$ = 3.54 ± 0.11 μM which confirmed inhibition of EGFR-TK. A molecular docking study was performed to verify the binding modes and consensual interactions of compound **MB-24** with the active site residues of EGFR-tyrosine kinase. Docking results with a highest dock score of (-67.04) which is comparable to standard drug imatinib (dock score = -62.92) explained a significant binding interaction with the active site of the enzyme (PDB ID: 2GS6) with high binding affinity for EGFR. Compound **MB-24** was evaluated for drug –likeliness characteristics using the QikProp module of Schrodinger software and the result was found to be comparable with standard drug imatinib.

The *in-vivo* anticancer activity of compound MB-24, MB-32 (Series-2) was studied on MNU induced breast cancer animal model. $LD_{50}$ was determined for the selected compounds and were calculated according to log-probit method. 50% of animal death was observed at the dose of 400 mg/kg. Compound MB-24 and MB-32 treated group at the dose of 5mg/kg in 10% DMSO through intraperitonial route has significantly reduced the growth of the cancer cells.

To examine the cytotoxicity of compounds whether present in a dose dependent manner, it was found that at the dose of 10 mg/kg compound MB-24 achieved maximum effect of tumor growth volume reduction w.r.t. 5 mg/kg and 20 mg/kg dose. Therefore, these findings suggested that the rational design of 1,3,4- Oxadiazoles substituted 1,4-naphthoquinones as a hopeful potential anticancer agent for auxiliary expansion in cancer therapy.

Based on the structure activity relationship only we have preceded further.**MB-9** and **MB-18** shows structurally that they have halogen atom and methoxy groups respectively as substituents in the phenyl ring attached to the piperazine ring, which are further enhancing their interactions, uplifting their potential. Similarly, presence of methoxy and methyl group substitutions in the phenyl ring attached to the oxadiazole ring respectively in **MB-24** and **MB-32**, contributes towards interactions with receptor binding site. To bring this study to clinical testing needs further exploration for their toxicity profiles. We know toxicological manifestations of 1, 4-naphthoquinone includes decreased number of erythrocytes in the blood, decreased sulfhydryl compounds and hemoglobin levels in the blood, hepato toxicity, and depressed phagocytic activity of leukocytes in a long term use. So, it can be further explored for their toxicity profiles and making these compounds suitable for preclinical study.