CHAPTER 5

Concluding Remarks and Future Perspectives

5. Concluding remarks and future perspectives

5.1. Conclusions

The Src homology-2 (SH2) domain-containing phosphatase-2 (SHP2), also known as PTPN11, is the first proto-oncogenic phosphatase and is a key mediator in carcinogenesis regulating nearly all the signalling pathways through a kind of "master control". Small-molecule inhibitors targeting several allosteric sites and an orthosteric (active) site have been developed and reported. The allosteric inhibitors, the prototype being **SHP099** (IC_{50} = 0.070 μM), mostly bind to the interface formed by the N-SH2, C-SH2 and the PTP domains to stabilize SHP2 in its 'closed' state. These inhibitor molecules target some of the carcinomas in which SHP2 is overexpressed and few of them are in various phases of clinical trial. Nevertheless, there are no SHP2 inhibitory molecules that have been unequivocally translated into drugs against cancer. Direct orthosteric inhibition has proven challenging due to the polar nature of the catalytic PTP site and considerable sequence homology between the catalytic domains of different members of the PTPN subfamily like SHP1 or PTP1B leading to off-target effects.

With the above problem statement, we envisaged to develop new potent heterocyclic scaffolds as SHP2 inhibitors and evaluating them for their targeted biochemical and pharmacological effect (**Figure 5.1**). Firstly, pharmacophore-based virtual screening of Enamine Advanced Database, an online bioactive library of more than 55,0000 potential anticancer molecules against an anti-SHP2 pharmacophore model built by creating hypothetical space regions on **SHP099** by MOE software to identify 37 hit molecules which were subsequently filtered using rules-based filtering techniques like PreADMET, PAINS-Remover to get 35 virtual hits. Based upon the binding affinity of the molecules towards SHP2 (by molecular docking and MD simulation results), the topmost molecule **111675** [(2-((5-(5-chloro-2-methoxyphenyl)-4*H*-1,2,4-triazol-3-yl)thio)acetyl)-D-tryptophanate] (SHP2 $K_i = 0.118$ μM) was synthesized, characterized and evaluated for SHP2 inhibition which unveiled its moderately potent SHP2 inhibitory activity ($IC_{50} = 0.878 \pm 0.008$ μM). Unfortunately, compound **111675** did not possess strong antiproliferation activity against MCF-7 cells ($GI_{50} > 1000$ μM). Thus, to improve the pharmacological outcome, rational and systematic optimizations were done on the molecule **111675** to design, synthesize and evaluate three novel azacyclic scaffolds having the common 1,3,4-thiadiazole core along with diverse active linkers and variable centres.

The first series i.e., **STT series** is essentially a hybrid of 1,3,4-thiadiazole and 1,2,4-triazole rings tethered by a thioacetamide linker. A set of 21 homologues were designed, synthesized, characterized by NMR, FTIR & HRMS and evaluated for *in vitro* SHP2 inhibitory activity where compound **STT13** (*N*-(5-(benzo[*d*][1,3]dioxol-5-yl)-1,3,4-thiadiazol-2-yl)-2-((5-(4-methoxyphenyl)-4*H*-1,2,4-triazol-3-yl)thio)acetamide) emerged as the most potent SHP2 inhibitor ($IC_{50} = 0.318 \pm 0.001$ μM) inhibiting the enzyme in a mixed to non-competitive manner indicating a possible allosteric mode of inhibition. A SAR study was done from the IC_{50} data and the corresponding structure of the **STT** homologues. *In silico* studies revealed that the lead inhibitor strongly binds to the tunnel allosteric site of SHP2. Compound **STT13** possessed moderate antioxidant activity as determined by a DPPH assay (DPPH $IC_{50} = 37.12$ μM) and an effective *in vitro* BBB permeability value of $(4.461 \pm 0.327) \times 10^{-6}$ cm/s indicating potential to cross the BBB. Further, cytotoxicity studies revealed that compound **STT13** caused death of SHP2-driven MCF-7 (GI_{50} = 37.02 ± 0.25 μM), U87MG (GI_{50} = 68.69 ± 0.21 μM), PC12 (GI_{50} = 99.83 ± 0.04 μM) & SH-SY5Y cells (GI_{50} = 23.72 ± 0.89 μM) in a dose-dependent manner and inhibited MCF-7 cell colony formation and migration. Flow cytometric analysis showed that it exerted its antiproliferative effect on U87MG cells by inducing early apoptosis (Q1 & Q2 phase) and inhibiting cell cycle progression at the G1 & S phase. Compound **STT13** was shown to increase oxidative stress in the U87MG cells by promoting ROS generation and loss of mitochondrial integrity. It displayed no systemic, haematological, histopathological or neurobehavioral toxicity in adult female Wistar rats on oral administration with an LD_{50} > 2000 mg/kg BW (acc. to OECD 423). RP-HPLC based *in vivo* pharmacokinetic study in rats revealed a large half-life of elimination ($t_{1/2}$ = 88.72 h) and a C_{max} of 0.59 μM achieved at T_{max} of 2 h indicating rapid and significant oral adsorption of compound **STT13**.

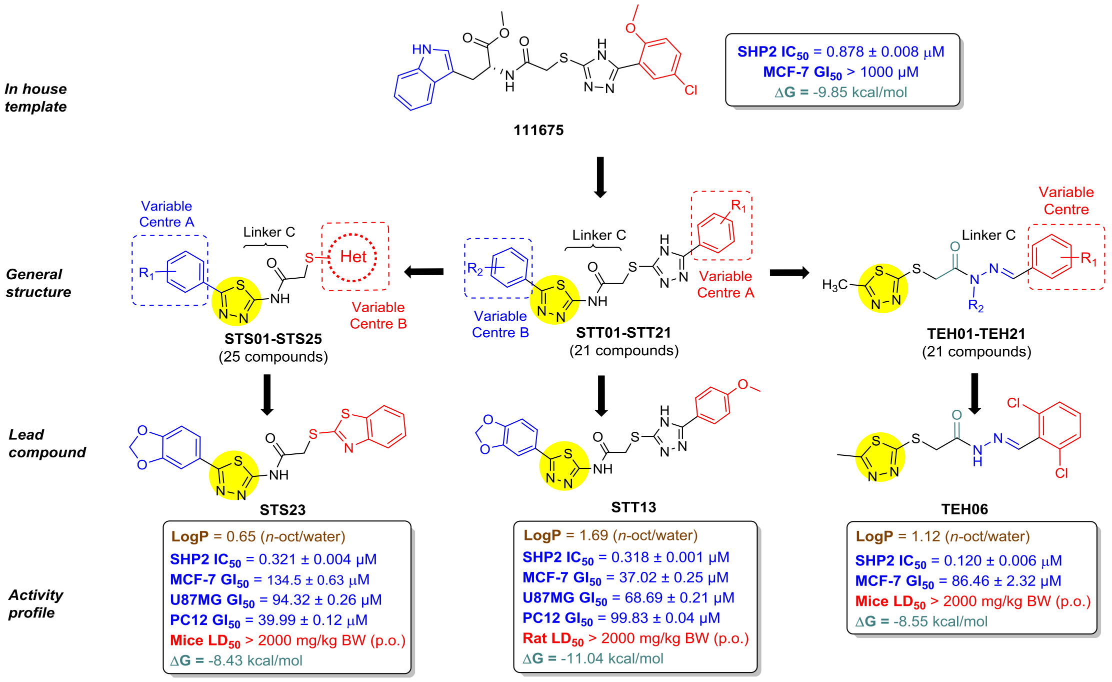

Figure 5.1. Conclusive summary of the overall outcome of the research

The second series is a library of 1,3,4-thiadiazole-2-amine derived thioacetamide derivatives (**STS series**), a total of 25 compounds which were likewise synthesized, characterized and evaluated for SHP2 inhibition. Compound **STS23** (*N*-(5-(benzo[*d*][1,3]dioxol-5-yl)-1,3,4-thiadiazol-2-yl)-2-(benzo[*d*]thiazol-2-ylthio)acetamide) was the *in vitro* lead SHP2 inhibitor with an IC_{50} of 0.321 ± 0.004 μM. SAR study indicated that the benzothiazole derivatives were particularly potent against SHP2. Cellular assays of compound **STS23** on MCF-7 cells revealed moderately potent antiproliferative, anti-survival and antimigratory effects. The molecule was found to be non-toxic up to a single oral dose of 2000 mg/kg BW in rats. Improvement via lead simplification and 'scaffold hopping' of the **STT** chemotype resulted in the development of the third series i.e., **TEH** series which consists of a library of 21 *S*-acetohydrazones of 5-methyl-1,3,4-thiadiazole-2-thio bearing a substituted benzylidene moiety and were synthesized & characterized via spectroscopic techniques. The synthesized compounds were evaluated for their anti-SHP2 potential and compound **TEH06** ((*E*)-*N*'-(2,6-dichlorobenzylidene)-2-((5-methyl-1,3,4-thiadiazol-2-yl)thio)acetohydrazide) emerged as the most potent SHP2 inhibitor (IC_{50} = 0.120 ± 0.006 μM). Post-screening molecular docking and MD simulations revealed stabilizing interactions of the molecule with SHP2. Cellular assays of compound **TEH06** on MCF-7 cells revealed moderately potent antiproliferative, anti-survival and antimigratory effects. The molecule was found to be non-toxic up to a single oral dose of 2000 mg/kg BW in adult female albino mice.

In conclusion, we applied pharmacophore-based virtual screening and ligand-guided lead optimization approaches to identify and develop novel heterocyclic pharmacophoric scaffolds based on the 1,3,4-thiadiazole nucleus as small-molecule SHP2 inhibitors with noticeable *in vitro* anticancer efficacy and in vivo safety. The research highlights the potential of ligand based virtual screening and rational scaffold design techniques in accelerating drug discovery for breast cancer and GBM by aiding in the identification of potential targeted inhibitors, designing small-molecule ligands and evaluating their desired pharmacological property.

5.2. Future perspectives

Further work on the abovementioned research is envisaged and are as follows:

The developed scaffolds can be further optimized by rational ligand modification techniques to enhance their SHP2 inhibitory activity to obtain nanomolar inhibition.

The allosteric-ness and selectivity of the developed scaffolds need to be experimentally determined through direct methods like using truncated SHP2 constructs and using paralogues for *in vitro* assays.

Pilot studies on *in vivo* efficacy of the lead compound(s) as anticancer agents can be performed in murine or rat-derived xenograft models for breast cancer and GBM.