

TABLE OF CONTENTS

Acknowledgements	i
Table of Contents	iii
List of Figures	ix
List of Schemes	xiii
List of Tables	xv
List of Abbreviations	xvii
List of Symbols	xix
Preface	xxi
Chapter- 1 Introduction and Literature Review	1
1.1. Introduction	1
1.1.1. Overview of cancer – demography and epidemiology	1
1.1.2. Overview of glioblastoma multiforme (GBM) and breast cancer	3
1.1.2.1. Glioblastoma multiforme (GBM)	3
1.1.2.2. Breast cancer	5
1.1.2.3. Cell lines for GBM and breast cancer	8
1.1.2.4. Current challenges in the treatment of GBM and breast cancer – problem statement-I	9
1.1.3. Overview of SHP2 enzyme	11
1.1.3.1. Structure and functions of SHP2	11
1.1.3.2. Role of SHP2 in gliomagenesis and in breast cancer	14
1.1.3.3. Role of SHP2 in glioblastoma and breast cancer stem cells	16
1.2. Literature review	17
1.2.1. Natural and synthetic small molecules in GBM and breast cancer management	17
1.2.1.1. Natural and synthetic small-molecule drugs for GBM	17
1.2.1.2. Natural and synthetic small-molecule drugs for breast cancer	20
1.2.2. Natural small molecule inhibitors of SHP2	23
1.2.3. Synthetic small molecule inhibitors of SHP2	24
1.2.3.1. Development of small-molecule SHP2 inhibitors for GBM and breast cancer therapies	26
1.2.4. Opportunities and challenges in small-molecule SHP2 inhibitor discovery – problem statement–II	29
1.2.5. Overview of the 1,3,4-thiadiazole core scaffold	30
1.2.5.1. Chemistry of the 1,3,4-thiadiazole ring	30
1.2.5.2. Chemotherapeutic potential of 1,3,4-thiadiazole analogues	32

1.2.6. Summary	34
1.3. Rationale, objectives and plan of work	36
1.3.1. Rationale and design strategy	36
1.3.2. Objectives	38
1.3.3. Comprehensive plan of work	39
Chapter- 2 Thioacetamide-tethered 1,3,4-thiadiazole-1,2,4-triazole hybrids (STT Series)	41
2.1. Design rationale and plan of work	41
2.1.1. Design rationale	41
2.1.1.1. Lead-based library design	44
2.1.2. Plan of work	45
2.2. Experimental work	46
2.2.1. Pharmacophore-based virtual screening studies	46
2.2.1.1. Tools and datasets	47
2.2.1.2. Pharmacophore-based virtual screening protocol	47
2.2.2. Chemistry	48
2.2.2.1. Synthesis of compound 111675 (virtual lead SHP2 inhibitor)	48
2.2.2.2. Synthesis of compounds STT01-STT21	51
2.2.2.3. Physicochemical characterization	53
2.2.2.4. Spectral characterization	54
2.2.3. Biological studies	56
2.2.3.1. <i>In vitro</i> SHP2 enzyme inhibition and enzyme kinetics assay	56
2.2.3.2. <i>In vitro</i> DPPH assay for antioxidant property evaluation	58
2.2.3.3. <i>In vitro</i> blood-brain barrier permeability assay (PAMPA-BBB) of compound STT13	58
2.2.3.4. Cell-based assays	60
2.2.3.4.1. Cell conditioning and culture	60
2.2.3.4.2. <i>In vitro</i> antiproliferation study in cancer cell lines	60
2.2.3.4.3. Colony formation and scratch wound-healing assay of compound STT13	61
2.2.3.4.4. Annexin binding assay for detection of cellular apoptosis	62
2.2.3.4.5. Cell cycle analysis by flow cytometry	63
2.2.3.4.6. Reactive oxygen species (ROS) estimation by flow cytometry	64
2.2.3.4.7. Mitochondrial membrane potential (MMP) estimation by flow cytometry	64

2.2.3.5. <i>In vivo</i> studies	65
2.2.3.5.1. <i>In vivo</i> acute oral toxicity evaluation of compound STT13	65
2.2.3.5.2. <i>In vivo</i> pharmacokinetic studies of compound STT13 in female Wistar rats	67
2.2.4. Computational studies	69
2.2.4.1. Molecular docking studies	70
2.2.4.2. Molecular dynamics simulation studies	71
2.2.4.3. Prediction of ADMET properties	71
2.2.4.4. Prediction of pharmacokinetic (PK) parameters	72
2.2.5. Statistical analysis	73
2.3. Results and discussion	73
2.3.1. Pharmacophore-based virtual screening (PBVS) studies	73
2.3.1.1. Identification of the virtual lead by rule-based screening, molecular docking and MD simulation	74
2.3.2. Chemistry	77
2.3.2.1. Synthesis of compound 111675 and designed final compounds STT01-STT21	77
2.3.2.2. Physicochemical characterization of final compounds	78
2.3.2.3. Spectral characterization	79
2.3.3. Biological studies	93
2.3.3.1. <i>In vitro</i> SHP2 inhibition and enzyme kinetics assay	93
2.3.3.2. <i>In vitro</i> antioxidant assay	99
2.3.3.3. <i>In vitro</i> blood-brain barrier permeability assay (PAMPA-BBB) of compound STT13	100
2.3.3.4. Cell-based study	101
2.3.3.4.1. Cell proliferation assay using MTT	101
2.3.3.4.2. Colony formation and scratch wound healing assay of compound STT13	104
2.3.3.4.3. Annexin binding assay for detection of cellular apoptosis	107
2.3.3.4.4. Cell cycle analysis by flow cytometry	107
2.3.3.4.5. Reactive oxygen species (ROS) estimation by flow cytometry	108
2.3.3.4.6. Mitochondrial membrane potential (MMP) estimation by flow cytometry	108
2.3.3.5. <i>In vivo</i> studies	111

	2.3.3.5.1. Acute oral toxicity study in female Wistar rats	111
	2.3.3.5.2. <i>In vivo</i> pharmacokinetic studies of compound STT13 in female Wistar rats	118
	2.3.4. Computational studies	124
	2.3.4.1. Molecular docking of compounds STT01-STT21 within the tunnel allosteric site of SHP2 (PDB ID: 5EHR)	124
	2.3.4.2. Molecular dynamics simulation studies for compound STT13	129
	2.3.4.3. Predicted ADMETox parameters	131
	2.4. Summary	133
Chapter- 3	Thioacetamide-linked 1,3,4-thiadiazole-2-amines (STS series)	137
	3.1. Design rationale and plan of work	137
	3.1.1. Design rationale	137
	3.1.2. Plan of work	139
	3.2. Experimental work	140
	3.2.1. Chemistry	140
	3.2.1.1. Synthesis of compounds STS01-STS25	140
	3.2.1.2. Physicochemical characterization	141
	3.2.1.3. Spectral characterization	141
	3.2.2. Biological studies	142
	3.2.2.1. <i>In vitro</i> SHP2 enzyme inhibition assay	142
	3.2.2.2. <i>In vitro</i> DPPH assay for antioxidant property evaluation	142
	3.2.2.3. <i>In vitro</i> blood-brain barrier permeability assay (PAMPA-BBB) of compound STS23	142
	3.2.2.4. Cell-based assays	142
	3.2.2.4.1. <i>In vitro</i> antiproliferation study in cancer cell lines	142
	3.2.2.4.2. Colony formation assay of compound STS09 and STS23	143
	3.2.2.5. <i>In vivo</i> studies	143
	3.2.2.5.1. <i>In vivo</i> acute oral toxicity evaluation of compound STS23	143
	3.2.3. Computational studies	143
	3.3. Results and discussion	144
	3.3.1. Chemistry	144
	3.3.1.1. Synthesis of final compounds STS01-STS25	144
	3.3.1.2. Physicochemical characterization of final compounds	144
	3.3.1.3. Spectral characterization	146
	3.3.2. Biological studies	158

	3.3.2.1. <i>In vitro</i> SHP2 inhibition assay	158
	3.3.2.2. <i>In vitro</i> antioxidant assay	162
	3.3.2.3. <i>In vitro</i> blood-brain barrier permeability assay (PAMPA-BBB) of compound STS23	163
	3.3.2.4. Cell-based assays	164
	3.3.2.4.1. Cell proliferation assay using MTT	164
	3.3.2.4.2. Colony formation assay of compound STS09 and STS23	169
	3.3.2.5. <i>In vivo</i> studies	169
	3.3.2.5.1. Acute oral toxicity study in female Wistar rats	169
	3.3.3. Computational studies	174
	3.3.3.1. Molecular docking of compounds STS01-STS25 within the tunnel allosteric site of SHP2 (PDB ID: 5EHR)	174
	3.3.3.2. Molecular dynamics simulation studies for compound STS23	177
	3.3.3.3. Predicted ADMETox parameters	178
	3.4. Summary	180
Chapter- 4	S-Acetohydrazones of 1,3,4-thiadiazole-2-thiol (TEH Series)	183
	4.1. Design rationale and plan of work	183
	4.1.1. Design rationale	183
	4.1.2. Plan of work	185
	4.2. Experimental work	185
	4.2.1. Chemistry	185
	4.2.1.1. Synthesis of compounds TEH01-TEH21	185
	4.2.1.2. Physicochemical characterization	187
	4.2.1.3. Spectral characterization	188
	4.2.2. Biological studies	188
	4.2.2.1. <i>In vitro</i> SHP2 enzyme inhibition assay	188
	4.2.2.2. Cell-based assays	188
	4.2.2.2.1. <i>In vitro</i> antiproliferation study in cancer cell lines	188
	4.2.2.2.2. Colony formation assay and scratch wound-healing assay of compounds TEH06 and TEH19	189
	4.2.2.3. <i>In vivo</i> studies	189
	4.2.2.3.1. <i>In vivo</i> acute oral toxicity evaluation of compound TEH06	189
	4.2.3. Computational studies	189
	4.3. Results and discussion	191
	4.3.1. Chemistry	191
	4.3.1.1. Synthesis of final compounds TEH01-TEH21	191

4.3.1.2. Physicochemical characterization of final compounds	191
4.3.1.3. Spectral characterization	192
4.3.2. Biological studies	208
4.3.2.1. <i>In vitro</i> SHP2 inhibition assay	208
4.3.2.2. Cell-based study	212
4.3.2.2.1. Cell proliferation assay using MTT	212
4.3.2.2.2. Colony formation and scratch wound healing assay of compounds TEH06 and TEH19	215
4.3.2.3. <i>In vivo</i> studies	216
4.3.2.3.1. Acute oral toxicity study in female albino mice	216
4.3.3. Computational studies	223
4.3.3.1. Molecular docking of compounds TEH01-TEH21 within the tunnel allosteric site of SHP2 (PDB ID: 5EHR)	223
4.3.3.2. Molecular dynamics simulation studies for compound TEH06	227
4.3.3.3. Predicted ADMETox parameters	229
4.4. Summary	234
Chapter- 5 Concluding remarks and future perspectives	237
5.1. Conclusions	237
5.2. Future perspectives	241
References	243
Appendix	A1-A27
List of Publications	A29

LIST OF FIGURES

Fig. No.	Title	Page No.
Figure 1.1.	Age-standardized incidence and mortality rate of different cancers in India	1
Figure 1.2.	Different types of malignant and non-malignant cancers of the brain and CNS	2
Figure 1.3.	Pie-chart showing number of new cancer cases in 2022, in India for females of all ages.	6
Figure 1.4.	Cellular heterogeneity of the GBM microenvironment	9
Figure 1.5.	Protein tyrosine phosphatase (PTP) superfamily	11
Figure 1.6.	SHP2 molecular catalytic mechanism.	12
Figure 1.7.	Structure of SHP2.	13
Figure 1.8.	Role of SHP2 as a molecular mediator in GBM and breast cancer.	15
Figure 1.9.	Major pathways modulated by natural substances (shown alongside) in GBM	17
Figure 1.10.	Alkylating agents approved for GBM therapy.	18
Figure 1.11.	Targeted enzyme inhibitors for brain tumors and breast cancer.	19
Figure 1.12.	Different natural and synthetic small-molecule SHP2 inhibitory scaffolds	23
Figure 1.13.	Different allosteric and orthosteric site of SHP2 and their inhibitory scaffolds	24
Figure 1.14.	Selected synthetic small-molecule SHP2 inhibitors in clinical trial	26
Figure 1.15.	Various fragmental analogues of SHP099 and cryptotanshinone	27
Figure 1.16.	Few SHP2 inhibitors clinically proven effective in breast cancer	28
Figure 1.17.	Comparison between the paralogues of SHP2	29
Figure 1.18.	Canonical forms of 1,3,4-thiadiazole ring system	31
Figure 1.19.	Anticancer mechanism of 1,3,4-thiadiazole derivatives	34
Figure 1.20.	Schematic representation for plan of work	39
Figure 2.1.	Design rationale of compounds STT01-STT21	43
Figure 2.2.	Skeletal formulae of rationally designed compounds (STT01-STT21)	44
Figure 2.3.	Plan of work for this series of compounds	46
Figure 2.4.	A) Allosteric activation of SHP2. B) Dephosphorylation of DiFMUP to DiFMU	56

Figure 2.5.	Results of pharmacophore-based virtual screening	74
Figure 2.6.	3D & 2D binding mode of ligands 111675 and SHP099 .	76
Figure 2.7.	Structure-activity relationship (SAR) of the synthesized compounds STT01-STT21	95
Figure 2.8.	Enzyme kinetics graphs on the mode of fl-SHP2 inhibition by compound STT13 .	98
Figure 2.9.	Percent free radical scavenging (% FRS) activity	99
Figure 2.10.	In vitro cytotoxicity of compound STT13 on MCF-7, PC12, U87MG and SH-SY5Y cells.	103
Figure 2.11.	Colony formation and anti-migration assay of MCF-7 cells by compound STT13	106
Figure 2.12.	Flow cytometric study of apoptosis and cell cycle of U87MG cells.	109
Figure 2.13.	Flow cytometric estimation of ROS and MMP of U87MG cells.	110
Figure 2.14.	Acute oral toxicity study of compound STT13 in adult female Wistar rats.	113
Figure 2.15.	Acute oral toxicity of compound STT13 on highly perfused organs of female Wistar rats.	117
Figure 2.16.	Effect of compound STT13 on CBC of female Wistar rats	118
Figure 2.17.	Calibration curve of compound STT13 in rat plasma spiked samples	119
Figure 2.18.	Natural logarithm of % compound STT13 remaining vs. incubation time graph	120
Figure 2.19.	Plasma concentration (μM) of compound STT13 vs. time curve	122
Figure 2.20.	Superimposed image of compounds STT01-STT21 with SHP099 within SHP2	124
Figure 2.21.	3D orientation image of compounds STT20 & STT13 with SHP099 within SHP2	127
Figure 2.22.	2D interaction map of compounds STT20 & STT13 with residues of SHP2	128
Figure 2.23.	Protein-ligand contact diagrams of compound STT13 with SHP2.	130
Figure 2.24.	Summary of key outcomes of the current series of compounds STT01-STT21	134
Figure 3.1.	Design rationale of compounds STS01-STS25	137
Figure 3.2.	Skeletal formulae of rationally designed compounds (STS01-STS25)	138
Figure 3.3.	Schematic workflow for plan of work	139

Figure 3.4.	Structure-activity relationship (SAR) of the synthesized compounds STT01-STT21 .	160
Figure 3.5.	Percent free radical scavenging (% FRS) activity	163
Figure 3.6.	In vitro cytotoxicity of compound STS23 on MCF-7, PC12, U87MG and SH-SY5Y cells	168
Figure 3.7.	Inhibition of colony formation of MCF-7 cells by compounds STS23 and STS09 .	169
Figure 3.8.	Acute oral toxicity study of compound STS23 in adult female albino mice	171
Figure 3.9.	Effect of compound STS23 on CBC of female albino mice	172
Figure 3.10.	Acute oral toxicity of compound STS23 on highly perfused organs of female Wistar rats.	173
Figure 3.11.	3D superimposition image of STS series of compounds in SHP2 (PDB ID: 5EHR)	175
Figure 3.12.	3D & 2D orientation image of compounds STS23 & STS24	176
Figure 3.13.	Protein-ligand contact diagrams of compound STS23 with SHP2 (PDB ID: 5EHR).	178
Figure 3.14.	Summary of findings for the current series STS01-STS25	181
Figure 4.1.	Design rationale of compounds TEH01-TEH21	183
Figure 4.2.	Skeletal formulae of rationally designed compounds (TEH01-TEH21)	184
Figure 4.3.	Schematic workflow for plan of work	185
Figure 4.4.	Possible rotational isomers in synthesized compounds of the TEH series	194
Figure 4.5.	Representative 500 MHz ¹ H NMR spectra of compound TEH06 in CDCl ₃	195
Figure 4.6.	Few salient SAR points and the skeletal formula of compound TEH06 .	210
Figure 4.7.	In vitro cytotoxicity of compound TEH06 on MCF-7 cells	214
Figure 4.8.	Colony formation and anti-migration assay of MCF-7 cells by compound TEH06	315
Figure 4.9.	Acute oral toxicity study of compound TEH06 in adult female albino mice	219
Figure 4.10.	Schematic representation of the track plot of compound TEH06	220
Figure 4.11.	Acute oral toxicity of compound TEH06 on highly perfused organs of female albino mice	221

Figure 4.12.	Effect of compound TEH06 on CBC of female albino mice	222
Figure 4.13.	Superimposed image of compounds TEH01-TEH21 with SHP099 within SHP2	223
Figure 4.14.	3D & 2D orientation image of compounds TEH10 & TEH06 with SHP099 within SHP2	226
Figure 4.15.	Protein-ligand contact diagrams of compound TEH06 with SHP2 (PDB ID: 5EHR).	228
Figure 4.16.	BOILED-egg model of few clinical SHP2 inhibitors & compounds TEH01-TEH21 .	232
Figure 4.17.	Summary of all findings for the current series TEH01-TEH21	235
Figure 5.1.	Conclusive summary of the overall outcome of the research of the current thesis	239

LIST OF SCHEMES

Scheme No.	Title	Page No.
Scheme 1.1.	Mechanism of cyclization of thiosemicarbazate salt	32
Scheme 2.1.	Synthetic scheme for compound 111675	49
Scheme 2.2.	Synthetic scheme final compounds STT01-STT21	51
Scheme 3.1.	Synthetic scheme for compounds STS01-STS25	140
Scheme 4.1.	Synthesis of compounds TEH01-TEH21 .	186

LIST OF TABLES

Table No.	Title	Page No.
Table 1.1.	FDA approved chemotherapeutic drugs for different gliomas	18
Table 1.2.	Major classes of small-molecule enzyme inhibitors in clinical trial for GBM	20
Table 1.3.	FDA approved chemotherapeutic drugs for breast cancer	21
Table 2.1.	PreADMET predicted ADME properties of compounds 111675 and SHP099	75
Table 2.2.	PreADMET predicted toxicity parameters of compounds 111675 and SHP099	75
Table 2.3.	Drug-likeness parameters of compounds 111675 and SHP099	75
Table 2.4.	Binding affinity, binding energy and K_i values for 111675 and SHP099	76
Table 2.5.	Physicochemical characterization data of compounds STT01-STT21	78
Table 2.6.	<i>In vitro</i> enzyme inhibition results of compounds 111675 and STT01-STT21	93
Table 2.7.	Antioxidant activity of compounds STT05 , STT13 and STT21 by DPPH assay	100
Table 2.8.	PAMPA-BBB assay data	101
Table 2.9.	Growth inhibition data (GI_{50}) of tested compounds	102
Table 2.10.	Colony formation and wound closure data of compound STT13	107
Table 2.11.	Behavioural analysis of compound STT13 in female Wistar rats	114
Table 2.12.	Regression statistics of compound STT13	119
Table 2.13.	Plasma stability data for compound STT13 in rat plasma	120
Table 2.14.	Plasma concentration of compound STT13 at different time points (h)	121
Table 2.15.	PK parameters after oral administration of compound STT13	122
Table 2.16.	Molecular docking data of compounds STT01-STT21	126
Table 2.17.	Comparison of experimental and computational SHP2 inhibitory data	126
Table 2.18.	Predicted ADME properties of compounds STT01-STT21	132

Table 2.19. Predicted toxicological properties of compounds STT01-STT21	133
Table 3.1. Physicochemical characterization data of compounds STS01-STS25	145
Table 3.2. <i>In vitro</i> enzyme inhibition results of compounds STS01-STS25	159
Table 3.3. Antioxidant activity of compounds of STS Series by DPPH assay	163
Table 3.4. PAMPA-BBB assay data	164
Table 3.5. Growth inhibition data (GI ₅₀) of tested compounds of STS series	167
Table 3.6. Behavioural analysis of compound STS23 in female Wistar rats.	170
Table 3.7. Molecular docking data of compounds STS01-STS25	175
Table 3.8. Predicted ADME properties of compounds STT01-STT21	179
Table 3.9. Predicted toxicological properties of compounds STS01-STS25	180
Table 4.1. Physicochemical characterization data of compounds TEH01-TEH21	192
Table 4.2. Chemical shifts of duplicated ¹ H NMR signals of compounds TEH01-TEH21	197
Table 4.3. <i>In vitro</i> SHP2 inhibition data of compounds TEH01-TEH21	209
Table 4.4. MCF-7 cell growth inhibition data of compounds TEH01-TEH21	213
Table 4.5. Behavioural analysis of compound TEH06 in female Wistar rats	217
Table 4.6. Molecular docking data of compounds TEH01-TEH21	224
Table 4.7. Predicted ADME properties of compounds TEH01-TEH21	230
Table 4.8. Predicted toxicological properties of compounds TEH01-TEH21	231
Table 4.9. Predicted PK and bioavailability parameters of compound TEH06	233

LIST OF ABBREVIATIONS

Abbreviations	Full forms
Akt	Protein kinase b
BBB	Blood-brain barrier
BCSC	Breast cancer stem cell
C-SH2	C-terminal Src homology-2 domain
DiFMUP	6,8-Difluoro-4-methylumbelliferyl phosphate
DGC	Differentiated glioma cells
DNMT1	DNA (cytosine-5)-methyltransferase 1
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
EGFR ν III	Epidermal growth factor receptor variant III
ER	Estrogen receptor
ERK	Extracellular signal-regulated/activated kinase
HER2	Human epidermal growth factor receptor 2
GBM	Glioblastoma multiforme
GOF	Gain-of-function
GRB2	Growth factor receptor-bound protein 2
GSC	Glioma stem cells/GBM stem-like cells
GSH	Glutathione
HIF1 α	Hypoxia inducible factor 1 alpha
HIF1 α i	HIF1 α inhibitors
JAK	Janus kinase
MAPK	Mitogen-activated protein kinase
MEK	mAPK/ERK kinase
MOE	Molecular Operating Environment
mTOR	Mammalian target of rapamycin
N-SH2	N-Terminal Src homology-2 domain
p21 ^{Cip1}	Cyclin-dependent kinase interacting protein 1
p53	Tumor protein 53

PAINS	Pan assay interference
PARP	Poly-ADP ribose polymerase
PBVS	Pharmacophore based virtual screening
PCa	Prostate cancer
PKD1	Phosphoinositide-dependent kinase-1
PI3K	Phosphoinositide-3-Kinase
PR	Progesterone receptor
PTEN	Phosphatase and tensin homolog
pTyr	Phosphotyrosines
PTP	Protein tyrosine phosphatase
PTPN	Non-receptor protein tyrosine phosphatase
PUMA	p53 upregulated modulator of apoptosis
RAF	Rapidly accelerated fibrosarcoma kinase
RAS	Rat sarcoma small GTPase
RMSD	Root mean square deviation
RTK	Receptor tyrosine kinase
ROS	Reactive oxygen species
SHP2	Src homology-2 (SH2) domain-containing phosphatase-2
STAT3	Signal transducer and activator of transcription 3
TME	Tumor microenvironment
TMZ	Temozolomide
TNBC	Triple negative breast cancer
TPC	Tumor propagating cells
USFDA	United States Food and Drug Administration
VEGF	Vascular endothelial growth factor

LIST OF SYMBOLS

Symbol	Meaning
α	Alpha
β	Beta
Δ	Delta
λ	Lambda
$^{\circ}\text{C}$	Degree celsius
\AA	Angstrom
mg	milligram
μg	Microgram
μM	Micromolar
mM	Millimolar
mL	Millilitre
μL	Microlitre
h	Hour
nm	Nanometer
ppm	Parts per million
rpm	Revolutions per minute
kcal	Kilocalories
MHz	Megahertz
J	Coupling constant
d	Doublet
t	Triplet
m	Multiplet
dd	Doublet of doublet
m/z	Mass-to-charge ratio
%	Percent
pH	Potential of hydrogen
<	Less than
>	More than
\pm	Plus, or minus

PREFACE

The Src homology-2 (SH2) domain-containing phosphatase-2 (SHP2) is the first proto-oncogenic protein tyrosine phosphatase (PTP) mediating carcinogenesis by regulating nearly all the signalling pathways through a kind of “master control”. Several allosteric sites and an orthosteric site have been identified in SHP2. Yet, there is a dearth of clinically approved SHP2 inhibitors due to inherent challenges like the polar nature of the catalytic site and sequence homology between catalytic domains of PTPN paralogues. We have applied pharmacophore-based virtual screening and ligand-guided lead optimization approaches to identify and develop novel heterocyclic pharmacophoric scaffolds bearing 1,3,4-thiadiazole core as small-molecule SHP2 inhibitors and evaluated them pharmacologically.

The present study is divided into five chapters and are as follows.

Chapter 1 initiates a comprehensive exploration of glioblastoma multiforme (GBM) and breast cancer, encompassing their background, pathophysiology, and the current therapeutic landscape. It also deals with SHP2 as a target of GBM and breast cancer and an exhaustive literature review on reported small-molecule SHP2 inhibitory scaffolds and the research objectives.

Chapter 2 deals with virtual screening-guided design, synthesis, and biological evaluation of thioacetamide tethered thiadiazole-1,2,4-triazole hybrids (**STT series**)

Chapter 3 deals with the design, synthesis, and biological evaluation of 5-(substituted phenyl)-1,3,4-thiadiazole-2-amine derived sulphur-linked acetamides (**STS series**)

Chapter 4 deals with the design, synthesis, characterization and biological evaluation of S-acetohydrazones of 5-methyl-1,3,4-thiadiazole-2-thiol (**TEH series**)

Chapter 5 presents the concluding remarks and future prospective

An appendix of additional supporting information, spectral data of representative compounds, and a list of publications from the course of the Ph.D. work are included.