

TABLE OF CONTENTS

| S. No. | Title | Page No. |
|------------------|--------------------------------------------------------------|--------------|
| CHAPTER 1 | INTRODUCTION | 1-26 |
| 1.1. | General | 1-2 |
| 1.2. | Breast cancer | 2-3 |
| 1.3. | MAPK pathway | 3-6 |
| 1.4. | Targeting different members of MAPK pathway | 6 |
| 1.4.1. | KRAS inhibitors | 6-8 |
| 1.4.2. | Problems associated with KRAS inhibitors | 8 |
| 1.4.3. | B-RAF inhibitors | 8-10 |
| 1.4.4. | Problems associated with B-RAF inhibitors | 10-11 |
| 1.4.5. | MEK inhibitors | 11-13 |
| 1.4.6. | Problems associated with MEK inhibitors | 13 |
| 1.5. | Putative way to bypass resistance against MAPK pathway | 13-16 |
| 1.6. | Substituted pyrimidines as a source of diverse pharmacophore | 16-18 |
| 1.7. | Computer-aided drug design (CADD) | 18 |
| 1.7.1. | Scaffold hopping | 19-20 |
| 1.7.2. | Fragment based drug design (FBDD) | 20-21 |
| 1.7.3. | Molecular docking | 21-22 |
| 1.7.4. | Molecular dynamic simulations | 22-23 |
| 1.8. | Research issues/gaps | 23-24 |
| 1.9. | Research problem/problem formulation | 24-25 |
| 1.10. | Aim and objectives | 25 |
| 1.11. | Plan of work | 25-26 |
| CHAPTER 2 | REVIEW OF LITERATURE | 27-58 |
| 2.1. | Recent pyrimidine based anti-cancer agents | 27-3 |
| 2.2. | Pyrimidine derivatives as MAPK pathway inhibitors | 34-35 |
| 2.3. | Various small molecule heterocycles based ERK1/2 inhibitors | 35 |
| 2.3.1. | Thiazolidine-2,4-dione-based inhibitors | 35-37 |

| | | |
|------------------|---------------------------------------------------------------------------------------|--------------|
| 2.3.2. | Tetrahydropyridopyrimidine-based inhibitors | 37-38 |
| 2.3.3. | Coumarin-based inhibitors | 38-39 |
| 2.3.4. | Benzhydroxamate ester-based inhibitors | 39-40 |
| 2.3.5. | Pyridone-based inhibitors | 40-42 |
| 2.3.6. | Amino pyrazole-based inhibitors | 42-43 |
| 2.3.7. | Quinoline based inhibitors | 44-47 |
| 2.3.8. | Guanidino based inhibitors | 47-48 |
| 2.3.9. | Indole based inhibitors | 48-49 |
| 2.3.10. | Pyrrolidine based inhibitors | 49-50 |
| 2.3.11. | Indazole amide-based inhibitors | 50-51 |
| 2.3.12. | Imidazolinone-based inhibitors | 51-52 |
| 2.3.13. | Carbazole based inhibitors | 52-54 |
| 2.3.14. | Pyrimidine-pyrrololactam based inhibitors | 54 |
| 2.3.15. | Madecassic acid-based inhibitors | 54-55 |
| 2.3.16. | Benzothiophene based inhibitors | 55-56 |
| 2.4. | Pyrimidine based ERK inhibitors | 56-58 |
| CHAPTER 3 | MATERIAL AND METHODS | 59-81 |
| 3.1. | Molecular modeling | 59 |
| 3.1.1. | Softwares | 59-61 |
| 3.1.2. | Selection of ligand and protein | 61 |
| 3.1.3. | Scaffold hopping | 61-62 |
| 3.1.4. | Molecular docking | 62-63 |
| 3.1.5. | Fragment based drug discovery | 63-64 |
| 3.1.6. | Molecular dynamic simulations | 64-65 |
| 3.1.7. | MM-GBSA calculation | 65 |
| 3.1.8. | Density functional theory (DFT) analysis | 65-66 |
| 3.1.9. | Improving the top hit: Library generation and screening of derivatives of the top hit | 66-68 |
| 3.2. | Synthesis | 68 |
| 3.2.1. | Chemistry | 68 |
| 3.2.2. | General procedures for the synthesis of compounds pertaining to Formula-I | 68-69 |

| | | |
|------------------|----------------------------------------------------------------------------------------------------------------------------|---------------|
| 3.2.2.1. | Synthesis of (<i>E</i>)-1-(4-bromophenyl)-3-(4-substitutedphenyl)prop-2-en-1-one (3) | 70 |
| 3.2.2.2. | Synthesis of 4-(4-bromophenyl)-6-(4-substitutedphenyl)pyrimidin-2-amine (5) | 71 |
| 3.2.2.3. | Synthesis of final compounds pertaining to Formula-I (8a-8o) | 72 |
| 3.2.3. | General procedures for the synthesis of compounds pertaining to Formula-II | 73-74 |
| 3.2.3.1. | Synthesis of final compounds pertaining to Formula-II (10a-10o) | 74-76 |
| 3.3. | Biological evaluation | 76 |
| 3.3.1. | Enzymatic assay | 76—77 |
| 3.3.1.1. | Procedure for enzymatic assay | 77 |
| 3.3.1.2. | Analysis of data | 78 |
| 3.3.2. | Anti-proliferative assay | 78 |
| 3.3.2.1. | Cell lines under study | 78-80 |
| 3.3.2.2. | Culturing of the cell lines | 80 |
| 3.3.2.3. | Cytotoxicity assay (MTT) | 80-81 |
| 3.3.2.4. | Procedure | 81 |
| CHAPTER 4 | RESULTS AND DISCUSSION | 82-165 |
| 4.1. | Molecular modeling analysis | 82 |
| 4.1.1. | Virtual screening of ERK2 inhibitors <i>via</i> hybrid scaffold hopping–FBDD approach | 82-98 |
| 4.1.2. | Improving binding affinity of the virtually screened hit (Ligand 8) <i>via</i> optimizing substituents (Formula-I) | 99-109 |
| 4.1.3. | Exploring effect of ring size increase on binding affinity of hit (Ligand 8) derivatives (Formula-II) | 109-117 |
| 4.2. | Results of synthesis | 117-118 |
| 4.2.1. | List of synthesized compounds | 118-120 |
| 4.2.2. | Characterization of compounds pertaining to Formula-I (8a-8o) | 121-123 |
| 4.2.3. | Compound sheets | 123 |

| | | |
|------------------|---------------------------------------------------------------------------------------------------------------|---------|
| 4.2.3.1. | 3-(4-(4-bromophenyl)-6-(4-chlorophenyl)pyrimidin-2-yl)-2-(4-chlorophenyl)thiazolidin-4-one (8a) | 123-124 |
| 4.2.3.2. | 3-(4-(4-bromophenyl)-6-(4-chlorophenyl)pyrimidin-2-yl)-2-(2-chlorophenyl)thiazolidin-4-one (8b) | 124 |
| 4.2.3.3. | 3-(4-(4-bromophenyl)-6-(4-chlorophenyl)pyrimidin-2-yl)-2-(2-methoxyphenyl)thiazolidin-4-one (8c) | 125 |
| 4.2.3.4. | 3-(4-(4-bromophenyl)-6-(4-chlorophenyl)pyrimidin-2-yl)-2-(4-hydroxyphenyl)thiazolidin-4-one (8d) | 126 |
| 4.2.3.5. | 3-(4-(4-bromophenyl)-6-(4-chlorophenyl)pyrimidin-2-yl)-2-(2,5-dimethoxyphenyl)thiazolidin-4-one (8e) | 127 |
| 4.2.3.6. | 3-(4-(4-bromophenyl)-6-(4-chlorophenyl)pyrimidin-2-yl)-2-(2,4-dimethoxyphenyl)thiazolidin-4-one (8f) | 128 |
| 4.2.3.7. | 3-(4-(4-bromophenyl)-6-(4-chlorophenyl)pyrimidin-2-yl)-2-(4-nitrophenyl)thiazolidin-4-one (8g) | 129 |
| 4.2.3.8. | 3-(4-(4-bromophenyl)-6-(4-chlorophenyl)pyrimidin-2-yl)-2-phenylthiazolidin-4-one (8h) | 130 |
| 4.2.3.9. | 3-(4-(4-bromophenyl)-6-(4-chlorophenyl)pyrimidin-2-yl)-2-(2-nitrophenyl)thiazolidin-4-one (8i) | 131 |
| 4.2.3.10. | 3-(4-(4-bromophenyl)-6-(4-methoxyphenyl)pyrimidin-2-yl)-2-(4-methoxyphenyl)thiazolidin-4-one (8j) | 132 |
| 4.2.3.11. | 3-(4-(4-bromophenyl)-6-(4-methoxyphenyl)pyrimidin-2-yl)-2-(4-chlorophenyl)thiazolidin-4-one (8k) | 133 |

| | | |
|------------------|-------------------------------------------------------------------------------------------------------------------|---------|
| 4.2.3.12. | 3-(4-(4-bromophenyl)-6-(4-methoxyphenyl)pyrimidin-2-yl)-2-(2-nitrophenyl)thiazolidin-4-one (8l) | 134 |
| 4.2.3.13. | 3-(4-(4-bromophenyl)-6-(4-methoxyphenyl)pyrimidin-2-yl)-2-(2-methoxyphenyl) thiazolidin-4-one (8m) | 135 |
| 4.2.3.14. | 3-(4-(4-bromophenyl)-6-(4-methoxyphenyl)pyrimidin-2-yl)-2-(2-chlorophenyl)thiazolidin-4-one (8n) | 136 |
| 4.2.3.15. | 3-(4-(4-bromophenyl)-6-(4-methoxyphenyl)pyrimidin-2-yl)-2-(4-hydroxyphenyl)thiazolidin-4-one (8o) | 137 |
| 4.2.4. | Characterization of compounds pertaining to Formula-II (10a-10o) | 137-140 |
| 4.2.5. | Compound sheets | 140 |
| 4.2.5.1. | 3-(4-(4-bromophenyl)-6-(4-chlorophenyl)pyrimidin-2-yl)-2-(4-chlorophenyl)-1,3-thiazinan-4-one (10a) | 140-141 |
| 4.2.5.2. | 3-(4-(4-bromophenyl)-6-(4-chlorophenyl)pyrimidin-2-yl)-2-(2-chlorophenyl)-1,3-thiazinan-4-one (10b) | 141 |
| 4.2.5.3. | 3-(4-(4-bromophenyl)-6-(4-chlorophenyl)pyrimidin-2-yl)-2-(2-methoxyphenyl)-1,3-thiazinan-4-one (10c) | 142 |
| 4.2.5.4. | 3-(4-(4-bromophenyl)-6-(4-chlorophenyl)pyrimidin-2-yl)-2-(4-hydroxyphenyl)-1,3-thiazinan-4-one (10d) | 143 |
| 4.2.5.5. | 3-(4-(4-bromophenyl)-6-(4-chlorophenyl)pyrimidin-2-yl)-2-(2,5-dimethoxyphenyl)-1,3-thiazinan-4-one (10e) | 144 |
| 4.2.5.6. | 3-(4-(4-bromophenyl)-6-(4-chlorophenyl)pyrimidin-2-yl)-2-(2,4-dimethoxyphenyl)-1,3-thiazinan-4-one (10f) | 145 |

| | | |
|------------------|----------------------------------------------------------------------------------------------------------------|---------|
| 4.2.5.7. | 3-(4-(4-bromophenyl)-6-(4-chlorophenyl)pyrimidin-2-yl)-2-(4-nitrophenyl)-1,3-thiazinan-4-one (10g) | 146 |
| 4.2.5.8. | 3-(4-(4-bromophenyl)-6-(4-chlorophenyl)pyrimidin-2-yl)-2-phenyl-1,3-thiazinan-4-one (10h) | 147 |
| 4.2.5.9. | 3-(4-(4-bromophenyl)-6-(4-chlorophenyl)pyrimidin-2-yl)-2-(2-nitrophenyl)-1,3-thiazinan-4-one (10i) | 147-148 |
| 4.2.5.10. | 3-(4-(4-bromophenyl)-6-(4-methoxyphenyl)pyrimidin-2-yl)-2-(4-methoxyphenyl)-1,3-thiazinan-4-one (10j) | 148-149 |
| 4.2.5.11. | 3-(4-(4-bromophenyl)-6-(4-methoxyphenyl)pyrimidin-2-yl)-2-(4-chlorophenyl)-1,3-thiazinan-4-one (10k) | 149-150 |
| 4.2.5.12. | 3-(4-(4-bromophenyl)-6-(4-methoxyphenyl)pyrimidin-2-yl)-2-(2-nitrophenyl)-1,3-thiazinan-4-one (10l) | 150-151 |
| 4.2.5.13. | 3-(4-(4-bromophenyl)-6-(4-methoxyphenyl)pyrimidin-2-yl)-2-(2-methoxyphenyl)-1,3-thiazinan-4-one (10m) | 151-152 |
| 4.2.5.14. | 3-(4-(4-bromophenyl)-6-(4-methoxyphenyl)pyrimidin-2-yl)-2-(2-chlorophenyl)-1,3-thiazinan-4-one (10n) | 152-153 |
| 4.2.5.15. | 3-(4-(4-bromophenyl)-6-(4-methoxyphenyl)pyrimidin-2-yl)-2-(4-hydroxyphenyl)-1,3-thiazinan-4-one (10o) | 153-154 |
| 4.3. | Biological evaluation | 154 |
| 4.3.1. | Enzymatic activity | 154-157 |
| 4.3.2. | Anti-cancer activity | 157-161 |
| 4.3.3. | Normal cell toxicity | 161-162 |
| 4.4. | Structural activity relationship study (SAR) | 162 |
| 4.4.1. | For Formula I compounds (thiazolidinone- | 162-164 |

| | | |
|------------------|----------------------------------------------------------------|----------------|
| | pyrimidine derivatives) | |
| 4.4.2. | For Formula II compounds (thiazinanone-pyrimidine derivatives) | 14-165 |
| CHAPTER 5 | SUMMARY AND CONCLUSION | 166-170 |
| | REFERENCES | 171-190 |
| | APPENDIX | 191-223 |