# References


1. Hajdu, S.I., A note from history: landmarks in history of cancer, part 1. *Cancer*, **2011**. 117(5): p. 1097-1102.
2. Siegel, R.L., K.D. Miller, and A. Jemal, Cancer statistics, 2020. *CA: A Cancer Journal for Clinicians*, **2020**. 70(1): p. 7-30.
3. Lee, G.L. and A. Ramaswamy, Physical, psychological, social, and spiritual aspects of end-of-life trajectory among patients with advanced cancer: A phenomenological inquiry. *Death Studies* **2020**. 44(5): p. 292-302.
4. Singer, S., Psychosocial Impact of Cancer. *Recent Results in Cancer Research*, **2018**. 210: p. 1-11.
5. Desjardins, L.*, et al.*, Executive functions and social skills in pediatric brain tumor survivors. *Applied Neuropsychology: Child*, **2020**. 9(1): p. 83-91.
6. Walker, L.G.*, et al.*, Psychosocial aspects of cancer in the elderly. *European Journal of Surgical Oncology (EJSO)*, **1998**. 24(5): p. 375-378.
7. Ostrom, Q.T.*, et al.*, CBTRUS Statistical Report: Primary Brain and Other Central Nervous System Tumors Diagnosed in the United States in 2014-2018. *Neuro-oncology*, **2021**. 23(12 Suppl 2): p. iii1-iii105.
8. Bleeker, F.E., R.J. Molenaar, and S. Leenstra, Recent advances in the molecular understanding of glioblastoma. *Journal of Neurooncology*, **2012**. 108(1): p. 11-27.
9. Ostrom, Q.T.*, et al.*, CBTRUS Statistical Report: Primary Brain and Other Central Nervous System Tumors Diagnosed in the United States in 2015-2019. *Neuro-oncology*, **2022**. 24(Suppl 5): p. v1-v95.
10. Manrique-Guzmán, S., T. Herrada-Pineda, and F. Revilla-Pacheco, Surgical Management of Glioblastoma, in *Glioblastoma*, S. De Vleeschouwer, Editor. 2017, Codon Publications.: Brisbane (AU).
11. Katsigiannis, S.*, et al.*, Impact of time to initiation of radiotherapy on survival after resection of newly diagnosed glioblastoma. *Radiation Oncology*, **2019**. 14(1): p. 73.
12. Jia, J.L., B. Alshamsan, and T.L. Ng, Temozolomide Chronotherapy in Glioma: A Systematic Review. *Current Oncology*, **2023**. 30(2): p. 1893-1902.
13. Parasramka, S.*, et al.*, Procarbazine, lomustine and vincristine for recurrent high-grade glioma. *Cochrane Database of Systematic Reviews*, **2017**. 7(7): p. Cd011773.
14. Shergalis, A.*, et al.*, Current Challenges and Opportunities in Treating Glioblastoma. *Pharmacological Reviews*, **2018**. 70(3): p. 412-445.

15. Fernandes, G.F.d.S.*, et al.*, Recent advances in the discovery of small molecules targeting glioblastoma. *European Journal of Medicinal Chemistry*, **2019**. 164: p. 8-26.
16. McGranahan, T.*, et al.*, Current State of Immunotherapy for Treatment of Glioblastoma. *Current Treatment Options in Oncology*, **2019**. 20(3): p. 24.
17. Manikandan, C., A. Kaushik, and D. Sen, Viral vector: potential therapeutic for glioblastoma multiforme. *Cancer Gene Therapy*, **2020**. 27(5): p. 270-279.
18. Wong, E.T., E. Lok, and K.D. Swanson, Alternating Electric Fields Therapy for Malignant Gliomas: From Bench Observation to Clinical Reality. *Progress in Neurological Surgery*, **2018**. 32: p. 180-195.
19. Cramer, S.W. and C.C. Chen, Photodynamic Therapy for the Treatment of Glioblastoma. *Frontiers in Surgery*, **2019**. 6: p. 81.
20. Swartz, A.M.*, et al.*, Promising vaccines for treating glioblastoma. *Expert Opinions on Biological Therapy*, **2018**. 18(11): p. 1159-1170.
21. Pinheiro, R.*, et al.*, Targeting Gliomas: Can a New Alkylating Hybrid Compound Make a Difference? *ACS Chemical Neuroscience*, **2017**. 8(1): p. 50-59.
22. Sachkova, A.*, et al.*, Combined Applications of Repurposed Drugs and Their Detrimental Effects on Glioblastoma Cells. *Anticancer Research*, **2019**. 39(1): p. 207-214.
23. Fernandes, C.*, et al.*, Current Standards of Care in Glioblastoma Therapy, in *Glioblastoma*, S. De Vleeschouwer, Editor. 2017, Codon Publications.: Brisbane (AU).
24. Haar, C.P.*, et al.*, Drug resistance in glioblastoma: a mini review. *Neurochemical Research*, **2012**. 37(6): p. 1192-1200.
25. Zhang, X.*, et al.*, Chemoresistance caused by the microenvironment of glioblastoma and the corresponding solutions. *Biomedicine & Pharmacotherapy*, **2019**. 109: p. 39-46.
26. Delgado-Martín, B. and M.Á. Medina, Advances in the Knowledge of the Molecular Biology of Glioblastoma and Its Impact in Patient Diagnosis, Stratification, and Treatment. *Advanced Science*, **2020**. 7(9): p. 1902971.
27. Bahadur, S.*, et al.*, Current promising treatment strategy for glioblastoma multiform: A review. *Oncology Reviews*, **2019**. 13(2): p. 417.
28. Garnier, D.*, et al.*, Divergent evolution of temozolomide resistance in glioblastoma stem cells is reflected in extracellular vesicles and coupled with radiosensitization. *Neuro-oncology*, **2018**. 20(2): p. 236-248.
29. Bray, F.*, et al.*, Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*, **2024**. 74(3): p. 229-263.

30. Sun, Y.-S., *et al.*, Risk factors and preventions of breast cancer. *International Journal of Biological Sciences*, **2017**. 13(11): p. 1387.
31. Sharma, G.N., *et al.*, Various types and management of breast cancer: an overview. *Journal of Advanced Pharmaceutical Technology & Research*, **2010**. 1(2): p. 109-126.
32. Fragomeni, S.M., A. Sciallis, and J.S. Jeruss, Molecular Subtypes and Local-Regional Control of Breast Cancer. *Surgical Oncology Clinics of North America*, **2018**. 27(1): p. 95-120.
33. Trayes, K.P. and S.E. Cokenakes, Breast cancer treatment. *American Family Physician*, **2021**. 104(2): p. 171-178.
34. Nounou, M.I., *et al.*, Breast Cancer: Conventional Diagnosis and Treatment Modalities and Recent Patents and Technologies. *Breast Cancer (Auckland)*, **2015**. 9(Suppl 2): p. 17-34.
35. Jacobs, A.T., *et al.*, Targeted therapy for breast cancer: An overview of drug classes and outcomes. *Biochemical Pharmacology*, **2022**. 204: p. 115209.
36. Ortega, M.A., *et al.*, Signal Transduction Pathways in Breast Cancer: The Important Role of PI3K/Akt/mTOR. *Journal of Oncology*, **2020**. 2020: p. 9258396.
37. Dai, X., *et al.*, Breast Cancer Cell Line Classification and Its Relevance with Breast Tumor Subtyping. *Journal of Cancer*, **2017**. 8(16): p. 3131-3141.
38. Giakoumettis, D., A. Kritis, and N. Foroglou, C6 cell line: the gold standard in glioma research. *Hippokratia*, **2018**. 22(3): p. 105-112.
39. Sondka, Z., *et al.*, COSMIC: a curated database of somatic variants and clinical data for cancer. *Nucleic Acids Research*, **2024**. 52(D1): p. D1210-d1217.
40. Schrörs, B., *et al.*, Multi-Omics Characterization of the 4T1 Murine Mammary Gland Tumor Model. *Frontiers in Oncology*, **2020**. 10.
41. Simon, T., E. Jackson, and G. Giamas, Breaking through the glioblastoma micro-environment via extracellular vesicles. *Oncogene*, **2020**. 39(23): p. 4477-4490.
42. Habashy, K.J., *et al.*, Challenges in glioblastoma immunotherapy: mechanisms of resistance and therapeutic approaches to overcome them. *British Journal of Cancer*, **2022**. 127(6): p. 976-987.
43. Shikalov, A., I. Koman, and N.M. Kogan, Targeted Glioma Therapy-Clinical Trials and Future Directions. *Pharmaceutics*, **2024**. 16(1).
44. Anderson, B.O., *et al.*, The Global Breast Cancer Initiative: a strategic collaboration to strengthen health care for non-communicable diseases. *Lancet Oncology*, **2021**. 22(5): p. 578-581.

45. Barrios, C.H., Global challenges in breast cancer detection and treatment. *Breast*, **2022**. 62 Suppl 1(Suppl 1): p. S3-s6.
46. Tomiyama, A.*, et al.*, Protein Phosphatases-A Touchy Enemy in the Battle Against Glioblastomas: A Review. *Cancers (Basel)*, **2019**. 11(2).
47. Neel, B.G., H. Gu, and L. Pao, The 'Shp'ing news: SH2 domain-containing tyrosine phosphatases in cell signaling. *Trends in Biochemical Sciences*, **2003**. 28(6): p. 284-293.
48. Tautz, L. and E.A. Sergienko, High-throughput screening for protein tyrosine phosphatase activity modulators. *Methods in Molecular Biology*, **2013**. 1053: p. 223-240.
49. Rehman, A.U.*, et al.*, The Landscape of Protein Tyrosine Phosphatase (Shp2) and Cancer. *Current Pharmaceutical Design*, **2018**. 24(32): p. 3767-3777.
50. Chan, G., D. Kalaitzidis, and B.G. Neel, The tyrosine phosphatase Shp2 (PTPN11) in cancer. *Cancer and Metastasis Review*, **2008**. 27(2): p. 179-192.
51. Pandey, R., M. Saxena, and R. Kapur, Role of SHP2 in hematopoiesis and leukemogenesis. *Current Opinions in Hematology*, **2017**. 24(4): p. 307-313.
52. Shen, K.*, et al.*, Protein kinase structure and function analysis with chemical tools. *Biochimica et Biophysica Acta*, **2005**. 1754(1-2): p. 65-78.
53. Hof, P.*, et al.*, Crystal structure of the tyrosine phosphatase SHP-2. *Cell*, **1998**. 92(4): p. 441-450.
54. Köhn, M., Turn and Face the Strange: A New View on Phosphatases. *ACS Central Science*, **2020**. 6(4): p. 467-477.
55. Yang, F.*, et al.*, Gain-Of-Function E76K-Mutant SHP2 Promotes Cell Proliferation, Metastasis, And Tumor Growth In Glioblastoma Through Activation Of The ERK/CREB Pathway. *OncoTargets & Therapy*, **2019**. 12: p. 9435-9447.
56. Dance, M.*, et al.*, The molecular functions of Shp2 in the Ras/Mitogen-activated protein kinase (ERK1/2) pathway. *Cell Signalling*, **2008**. 20(3): p. 453-459.
57. Sturla, L.M.*, et al.*, Src homology domain-containing phosphatase 2 suppresses cellular senescence in glioblastoma. *British Journal of Cancer*, **2011**. 105(8): p. 1235-1243.
58. Roccograndi, L.*, et al.*, SHP2 regulates proliferation and tumorigenicity of glioma stem cells. *Journal of Neurooncology*, **2017**. 135(3): p. 487-496.
59. Hinshaw, D.C. and L.A. Shevde, The Tumor Microenvironment Innately Modulates Cancer Progression. *Cancer Research*, **2019**. 79(18): p. 4557-4566.
60. Furcht, C.M.*, et al.*, Multivariate signaling regulation by SHP2 differentially controls proliferation and therapeutic response in glioma cells. *Journal of Cell Science*, **2014**. 127(Pt 16): p. 3555-3567.

61. Zhan, Y., G.J. Counelis, and D.M. O'Rourke, The protein tyrosine phosphatase SHP-2 is required for EGFRvIII oncogenic transformation in human glioblastoma cells. *Experimental Cell Research*, **2009**. 315(14): p. 2343-2357.
62. Tomiyama, A. and K. Ichimura, Signal transduction pathways and resistance to targeted therapies in glioma. *Seminars in Cancer Biology*, **2019**. 58: p. 118-129.
63. Zhu, G.*, et al.*, Phase Separation of Disease-Associated SHP2 Mutants Underlies MAPK Hyperactivation. *Cell*, **2020**. 183(2): p. 490-502.e418.
64. Yuan, Y.*, et al.*, SHP2 promotes proliferation of breast cancer cells through regulating Cyclin D1 stability via the PI3K/AKT/GSK3β signaling pathway. *Cancer Biology & Medicine*, **2020**. 17(3): p. 707-725.
65. Aceto, N.*, et al.*, Tyrosine phosphatase SHP2 promotes breast cancer progression and maintains tumor-initiating cells via activation of key transcription factors and a positive feedback signaling loop. *Nature Medicine*, **2012**. 18(4): p. 529-537.
66. Sokolov, A.V.*, et al.*, Brain Cancer Drug Discovery: Clinical Trials, Drug Classes, Targets, and Combinatorial Therapies. *Pharmacological Reviews*, **2021**. 73(4): p. 1172-1203.
67. Gupta, S.K.*, et al.*, PARP Inhibitors for Sensitization of Alkylation Chemotherapy in Glioblastoma: Impact of Blood-Brain Barrier and Molecular Heterogeneity. *Frontiers in Oncology*, **2018**. 8: p. 670.
68. Yu, W.*, et al.*, O(6)-Methylguanine-DNA Methyltransferase (MGMT): Challenges and New Opportunities in Glioma Chemotherapy. *Frontiers in Oncology*, **2019**. 9: p. 1547.
69. Nelson, T.A. and J. Dietrich, Investigational treatment strategies in glioblastoma: progress made and barriers to success. *Expert Opinion on Investigational Drugs*, **2023**. 32(10): p. 921-930.
70. Lee, J.H. and A. Nan, Combination drug delivery approaches in metastatic breast cancer. *Journal of Drug Delivery*, **2012**. 2012: p. 915375.
71. Bansal, I., A.K. Pandey, and M. Ruwali, Small-molecule inhibitors of kinases in breast cancer therapy: recent advances, opportunities, and challenges. *Frontiers in Pharmacology*, **2023**. 14.
72. Haas-Kogan, D.A.*, et al.*, Everolimus for Children With Recurrent or Progressive Low-Grade Glioma: Results From the Phase II PNOC001 Trial. *Journal of Clinical Oncology*, **2024**. 42(4): p. 441-451.
73. François-Martin, H.*, et al.*, Long-Term Results with Everolimus in Advanced Hormone Receptor Positive Breast Cancer in a Multicenter National Real-World Observational Study. *Cancers (Basel)*, **2023**. 15(4).

74. Tripathi, R.K.P. and S.R. Ayyannan, Emerging chemical scaffolds with potential SHP2 phosphatase inhibitory capabilities – A comprehensive review. *Chemical Biology & Drug Design*, **2021**. 97(3): p. 721-773.
75. Song, Y.*, et al.*, Double-edged roles of protein tyrosine phosphatase SHP2 in cancer and its inhibitors in clinical trials. *Pharmacology & Therapeutics*, **2022**. 230: p. 107966.
76. Kong, J. and Y.-Q. Long, Recent advances in the discovery of protein tyrosine phosphatase SHP2 inhibitors. *RSC Medicinal Chemistry*, **2022**. 13(3): p. 246-257.
77. Mitra, R. and S.R. Ayyannan, Small-Molecule Inhibitors of Shp2 Phosphatase as Potential Chemotherapeutic Agents for Glioblastoma: A Minireview. *ChemMedChem*, **2021**. 16(5): p. 777-787.
78. Chen, Y.-N.P.*, et al.*, Allosteric inhibition of SHP2 phosphatase inhibits cancers driven by receptor tyrosine kinases. *Nature*, **2016**. 535: p. 148-152.
79. Ran, H.*, et al.*, Sticking It to Cancer with Molecular Glue for SHP2. *Cancer Cell*, **2016**. 30(2): p. 194-196.
80. LaMarche, M.J.*, et al.*, Identification of TNO155, an Allosteric SHP2 Inhibitor for the Treatment of Cancer. *Journal of Medicinal Chemistry*, **2020**. 63(22): p. 13578-13594.
81. Sun, Y.*, et al.*, Allosteric SHP2 Inhibitor, IACS-13909, Overcomes EGFR-Dependent and EGFR-Independent Resistance Mechanisms toward Osimertinib. *Cancer Research*, **2020**. 80(21): p. 4840-4853.
82. Taylor, A.M.*, et al.*, Identification of GDC-1971 (RLY-1971), a SHP2 Inhibitor Designed for the Treatment of Solid Tumors. *Journal of Medicinal Chemistry*, **2023**. 66(19): p. 13384-13399.
83. Drilon, A.*, et al.*, SHP2 Inhibition Sensitizes Diverse Oncogene-Addicted Solid Tumors to Re-treatment with Targeted Therapy. *Cancer Discovery*, **2023**. 13(8): p. 1789-1801.
84. Ou, S.I.*, et al.*, A12 The SHP2 Inhibitor RMC-4630 in Patients with KRAS-Mutant Non-Small Cell Lung Cancer: Preliminary Evaluation of a First-in-Man Phase 1 Clinical Trial. *Journal of Thoracic Oncology*, **2020**. 15(2): p. S15-S16.
85. Stice, J.P.*, et al.*, Abstract P207: BBP-398, a potent, small molecule inhibitor of SHP2, enhances the response of established NSCLC xenografts to KRASG12C and mutEGFR inhibitors. *Molecular Cancer Therapeutics*, **2021**. 20(12_Supplement): p. P207-P207.
86. Yang, X.*, et al.*, Discovery of thalidomide-based PROTAC small molecules as the highly efficient SHP2 degraders. *European Journal of Medicinal Chemistry*, **2021**. 218: p. 113341.
87. Zheng, M.*, et al.*, Novel PROTACs for degradation of SHP2 protein. *Bioorganic Chemistry*, **2021**. 110: p. 104788.

88. Chen, X., *et al.*, Discovery of a Novel Src Homology-2 Domain Containing Protein Tyrosine Phosphatase-2 (SHP2) and Cyclin-Dependent Kinase 4 (CDK4) Dual Inhibitor for the Treatment of Triple-Negative Breast Cancer. *Journal of Medicinal Chemistry*, **2022**. 65(9): p. 6729-6747.
89. Liu, M., *et al.*, Discovery of Novel Src Homology-2 Domain-Containing Phosphatase 2 and Histone Deacetylase Dual Inhibitors with Potent Antitumor Efficacy and Enhanced Antitumor Immunity. *Journal of Medicinal Chemistry*, **2022**. 65(18): p. 12200-12218.
90. Zhang, H., *et al.*, Combining EGFR Inhibitors With SHP2 Or LSD1 Inhibitors to Overcome Multidrug Resistance in Cancer. *Future Medicinal Chemistry*, **2022**. 14(8): p. 527-529.
91. Bobone, S., *et al.*, Targeting Oncogenic Src Homology 2 Domain-Containing Phosphatase 2 (SHP2) by Inhibiting Its Protein-Protein Interactions. *Journal of Medicinal Chemistry*, **2021**. 64(21): p. 15973-15990.
92. Tang, K., *et al.*, Medicinal chemistry strategies for the development of protein tyrosine phosphatase SHP2 inhibitors and PROTAC degraders. *European Journal of Medicinal Chemistry*, **2020**. 204: p. 112657.
93. Yuan, X., *et al.*, Recent Advances of SHP2 Inhibitors in Cancer Therapy: Current Development and Clinical Application. *Journal of Medicinal Chemistry*, **2020**. 63(20): p. 11368-11396.
94. Garcia Fortanet, J., *et al.*, Allosteric Inhibition of SHP2: Identification of a Potent, Selective, and Orally Efficacious Phosphatase Inhibitor. *Journal of Medicinal Chemistry*, **2016**. 59(17): p. 7773-7782.
95. Sang, Y., *et al.*, Targeting PDGFRα-activated glioblastoma through specific inhibition of SHP-2-mediated signaling. *Neuro-oncology*, **2019**. 21(11): p. 1423-1435.
96. Tannous, B.A. and C.E. Badr, An allosteric inhibitor of SHP2 effectively targets PDGFRα-driven glioblastoma. *Neuro-oncology*, **2019**. 21(11): p. 1348-1349.
97. Lu, L., *et al.*, Cryptotanshinone inhibits human glioma cell proliferation by suppressing STAT3 signaling. *Molecular and Cellular Biochemistry*, **2013**. 381(1-2): p. 273-282.
98. Lu, L., *et al.*, Cryptotanshinone inhibits human glioma cell proliferation in vitro and in vivo through SHP-2-dependent inhibition of STAT3 activation. *Cell Death Discovery*, **2017**. 8(5): p. e2767.
99. Lade, D.M. and Y.M. Agazie, Targeting SHP2 with an Active Site Inhibitor Blocks Signaling and Breast Cancer Cell Phenotypes. *ACS Bio & Med Chem Au*, **2023**. 3(5): p. 418-428.

100. Hartman, Z., W.J. Geldenhuys, and Y.M. Agazie, Novel Small-Molecule Inhibitor for the Oncogenic Tyrosine Phosphatase SHP2 with Anti-Breast Cancer Cell Effects. *ACS Omega*, **2020**. 5(39): p. 25113-25124.
101. Bhavana*, et al.*, Druggable targets of protein tyrosine phosphatase Family, viz. PTP1B, SHP2, Cdc25, and LMW-PTP: Current scenario on medicinal Attributes, and SAR insights. *Bioorganic Chemistry*, **2024**. 144: p. 107121.
102. Hu, Y.*, et al.*, 1,3,4-Thiadiazole: Synthesis, Reactions, and Applications in Medicinal, Agricultural, and Materials Chemistry. *Chemical Reviews*, **2014**. 114(10): p. 5572-5610.
103. Chaudhari, P.J.*, et al.*, Discovery and Anticancer Activity of Novel 1,3,4-Thiadiazole- and Aziridine-Based Indolin-2-ones via In Silico Design Followed by Supramolecular Green Synthesis. *ACS Omega*, **2022**. 7(20): p. 17270-17294.
104. Janowska, S., A. Paneth, and M. Wujec, Cytotoxic Properties of 1,3,4-Thiadiazole Derivatives—A Review. *Molecules*, **2020**. 25(18): p. 4309.
105. Matysiak, J.*, et al.*, Synthesis and antiproliferative activity of some 5-substituted 2-(2,4-dihydroxyphenyl)-1,3,4-thiadiazoles. *European Journal of Medicinal Chemistry*, **2006**. 41(4): p. 475-482.
106. Szeliga, M.*, et al.*, Design, synthesis and biological evaluation of novel 1,3,4-thiadiazole derivatives as anti-glioblastoma agents targeting the AKT pathway. *Bioorganic Chemistry*, **2020**. 105: p. 104362.
107. Lavecchia, A. and C. Di Giovanni, Virtual screening strategies in drug discovery: a critical review. *Current Medicinal Chemistry*, **2013**. 20(23): p. 2839-2860.
108. Kumar, S. and S.R. Ayyannan, Identification of new small molecule monoamine oxidase-B inhibitors through pharmacophore-based virtual screening, molecular docking and molecular dynamics simulation studies. *Journal of Biomolecular Structure and Dynamics*, **2023**. 41(14): p. 6789-6810.
109. Kumar, V.P.*, et al.*, Exploration of a library of piperonylic acid-derived hydrazones possessing variable aryl functionalities as potent dual cholinesterase and monoamine oxidase inhibitors. *Molecular Diversity*, **2023**. 27(6): p. 2465-2489.
110. Mitra, R., S. Kumar, and S.R. Ayyannan, Identification of new small molecule allosteric SHP2 inhibitor through pharmacophore-based virtual screening, molecular docking, molecular dynamics simulation studies, synthesis and in vitro evaluation. *Journal of Biomolecular Structure and Dynamics*: p. 1-20.
111. Szeliga, M., Thiadiazole derivatives as anticancer agents. *Pharmacological Reports*, **2020**. 72(5): p. 1079-1100.

112. Mitra, R., D. Singh, and S.R. Ayyannan, #150 Design, synthesis and evaluation of 5-substituted-2-amino-1,3,4-thiadiazole derivatives as anticancer agents. *Journal of Pharmaceutical Chemistry*, **2022**. 8(Supplement).
113. Hekal, M.H.*, et al.*, New 1,3,4-thiadiazoles as potential anticancer agents: pro-apoptotic, cell cycle arrest, molecular modelling, and ADMET profile. *RSC Advances*, **2023**. 13(23): p. 15810-15825.
114. Baranauskienė, L.*, et al.*, Inhibition and binding studies of carbonic anhydrase isozymes I, II and IX with benzimidazo[1,2-c][1,2,3]thiadiazole-7-sulphonamides. *Journal of. Enzyme Inhibition and Medicinal Chemistry*, **2010**. 25(6): p. 863-870.
115. Giordano, D.*, et al.*, Drug Design by Pharmacophore and Virtual Screening Approach. *Pharmaceuticals*, **2022**. 15(5): p. 646.
116. Gund, P., Three-Dimensional Pharmacophoric Pattern Searching, in *Progress in Molecular and Subcellular Biology*, F.E. Hahn*, et al.*, Editors. 1977, Springer Berlin Heidelberg: Berlin, Heidelberg. p. 117-143.
117. Ghemrawi, R.*, et al.*, The Discovery of Potent SHP2 Inhibitors with Anti-Proliferative Activity in Breast Cancer Cell Lines. *International Journal of Molecular Sciences*, **2022**. 23(8): p. 4468.
118. Liu, W.-S.*, et al.*, Structure based design of selective SHP2 inhibitors by De novo design, synthesis and biological evaluation. *Journal of Computer-Aided Molecular Design*, **2019**. 33(8): p. 759-774.
119. Berman, H., K. Henrick, and H. Nakamura, Announcing the worldwide Protein Data Bank. *Nature Structural & Molecular Biology*, **2003**. 10(12): p. 980-980.
120. Grygorenko, O.O., Enamine Ltd.: The Science and Business of Organic Chemistry and Beyond. *European Journal of Organic Chemistry*, **2021**. 2021(47): p. 6474-6477.
121. Molecular Operating Environment (MOE), 2024.06 Chemical Computing Group ULC, 910-1010 Sherbrooke St. W., Montreal, QC H3A 2R7, 2024.
122. Abraham, M.J.*, et al.*, GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers. *SoftwareX*, **2015**. 1-2: p. 19-25.
123. Gee, K.R.*, et al.*, Fluorogenic substrates based on fluorinated umbelliferones for continuous assays of phosphatases and beta-galactosidases. *Analytical Biochemistry*, **1999**. 273(1): p. 41-48.
124. Tripathi, R.K.P.*, et al.*, Design, synthesis, and pharmacological evaluation of 2-amino-5-nitrothiazole derived semicarbazones as dual inhibitors of monoamine oxidase and cholinesterase: effect of the size of aryl binding site. *Journal of Enzyme Inhibition and Medicinal Chemistry*, **2018**. 33(1): p. 37-57.

125. Baliyan, S.*, et al.*, Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitative Phytochemical Analysis of Ficus religiosa. *Molecules*, **2022**. 27(4): p. 1326.

126. Di, L.*, et al.*, High throughput artificial membrane permeability assay for blood–brain barrier. *European Journal of Medicinal Chemistry*, **2003**. 38(3): p. 223-232.

127. Rafehi, H.*, et al.*, Clonogenic assay: adherent cells. *Journal of Visualized Experiments*, **2011**(49).

128. Martinotti, S. and E. Ranzato, Scratch Wound Healing Assay. *Methods in Molecular Biology*, **2020**. 2109: p. 225-229.

129. Pfeffer, C.M. and A.T.K. Singh, Apoptosis: A Target for Anticancer Therapy. *International Journal of Molecular Sciences*, **2018**. 19(2).

130. Demchenko, A.P., Beyond annexin V: fluorescence response of cellular membranes to apoptosis. *Cytotechnology*, **2013**. 65(2): p. 157-172.

131. Kim, K.H. and J.M. Sederstrom, Assaying Cell Cycle Status Using Flow Cytometry. *Current Protocols in Molecular Biology*, **2015**. 111: p. 28.26.21-28.26.11.

132. OECD Test No. 423: Acute Oral toxicity - Acute Toxic Class Method. 2002. DOI: https://doi.org/10.1787/9789264071001-en.

133. Jain, D. and P.K. Basniwal, ICH guideline practice: application of validated RP-HPLC-DAD method for determination of tapentadol hydrochloride in dosage form. *Journal of Analytical Science and Technology*, **2013**. 4(1): p. 9.

134. Konsoula, R. and M. Jung, In vitro plasma stability, permeability and solubility of mercaptoacetamide histone deacetylase inhibitors. *International Journal of Pharmaceutics*, **2008**. 361(1-2): p. 19-25.

135. Zhang, Y.*, et al.*, PKSolver: An add-in program for pharmacokinetic and pharmacodynamic data analysis in Microsoft Excel. *Computer Methods and Programs in Biomedicine*, **2010**. 99(3): p. 306-314.

136. Wang, X.-C.*, et al.*, Synthesis of 2- (5- Substituted- 1,3,4- thiadiazolo- 2- ylimino)- 4- thiazolidinones under Microwave Irradiation. *Synthetic Communication*, **2008**. 38(6): p. 973-982.

137. Sabur, A.M. and M.A. Rdaiaan, Design, Synthesis and Biological Screening of New Benzimidazole Derivatives. *Journal of Physics: Conference Series*, **2021**. 1879(2): p. 022056.

138. Hansch, C., J.P. Björkroth, and A. Leo, Hydrophobicity and Central Nervous System Agents: On the Principle of Minimal Hydrophobicity in Drug Design. *Journal of Pharmaceutical Science*, **1987**. 76(9): p. 663-687.

139. Rosenau, C.P., *et al.*, Exposing the Origins of Irreproducibility in Fluorine NMR Spectroscopy. *Angewandte Chemie International Edition (English)*, **2018**. 57(30): p. 9528-9533.
140. Gee, K.R., *et al.*, Fluorogenic Substrates Based on Fluorinated Umbelliferones for Continuous Assays of Phosphatases and β-Galactosidases. *Analytical Biochemistry*, **1999**. 273(1): p. 41-48.
141. Wang, H.-H., *et al.*, Synthesis, molecular docking and evaluation of thiazolyl-pyrazoline derivatives containing benzodioxole as potential anticancer agents. *Bioorganic and Medicinal Chemistry*, **2013**. 21(2): p. 448-455.
142. Hawash, M., *et al.*, Synthesis and Biological Evaluation of Benzodioxole Derivatives as Potential Anticancer and Antioxidant agents. *Heterocyclic Communication*, **2020**. 26(1): p. 157-167.
143. Deshpande, S.R., *et al.*, Novel 3,4-Methylenedioxybenzene Scaffold Incorporated 1,3,5-Trisubstituted-2-pyrazolines: Synthesis, Characterization and Evaluation for Chemotherapeutic Activity. *Indian Journal of Pharmaceutical Science*, **2015**. 77(1): p. 24-33.
144. Mahmood, A., A. Villinger, and J. Iqbal, Therapeutic potentials and structure-activity relationship of 1,3-benzodioxole N-carbamothioyl carboxamide derivatives as selective and potent antagonists of P2X4 and P2X7 receptors. *European Journal of Medicinal Chemistry*, **2022**. 238: p. 114491.
145. Sammar, M., *et al.*, Correlation between cytotoxicity in cancer cells and free radical-scavenging activity: In vitro evaluation of 57 medicinal and edible plant extracts. *Oncology Letters*, **2019**. 18(6): p. 6563-6571.
146. Collins, A.R., Antioxidant intervention as a route to cancer prevention. *European Journal of Cancer*, **2005**. 41(13): p. 1923-1930.
147. Di, L., *et al.*, High throughput artificial membrane permeability assay for blood-brain barrier. *European Journal of Medicinal Chemistry*, **2003**. 38(3): p. 223-232.
148. Wang, F.-m., *et al.*, SHP-2 promoting migration and metastasis of MCF-7 with loss of E-cadherin, dephosphorylation of FAK and secretion of MMP-9 induced by IL-1 βin vivo andin vitro. *Breast Cancer Research and Treatment*, **2005**. 89(1): p. 5-14.
149. Zhan, Y., G.J. Counelis, and D.M. O'Rourke, The protein tyrosine phosphatase SHP-2 is required for EGFRvIII oncogenic transformation in human glioblastoma cells. *Experimental Cell Research*, **2009**. 315(14): p. 2343-2357.

150. Chitranshi, N., *et al.*, Loss of Shp2 Rescues BDNF/TrkB Signaling and Contributes to Improved Retinal Ganglion Cell Neuroprotection. *Molecular Therapy*, **2019**. 27(2): p. 424-441.
151. Nunes-Xavier, C.E., *et al.*, Protein Tyrosine Phosphatases in Neuroblastoma: Emerging Roles as Biomarkers and Therapeutic Targets. *Frontiers in Cell and Developmental Biology*, **2021**. 9: p. 811297.
152. D'Alessio, A., *et al.*, Shp2 in PC12 cells: NGF versus EGF signalling. *Cellular Signalling*, **2007**. 19(6): p. 1193-1200.
153. Hadari, Y.R., *et al.*, Binding of Shp2 Tyrosine Phosphatase to FRS2 Is Essential for Fibroblast Growth Factor-Induced PC12 Cell Differentiation. *Journal of Molecular & Cell Biology*, **1998**. 18(7): p. 3966-3973.
154. Chitranshi, N., *et al.*, PTPN11 induces endoplasmic stress and apoptosis in SH-SY5Y cells. *Neuroscience*, **2017**. 364: p. 175-189.
155. Katz, D., *et al.*, Increased efficiency for performing colony formation assays in 96-well plates: novel applications to combination therapies and high-throughput screening. *Biotechniques*, **2008**. 44(2): p. ix-xiv.
156. Paul, C.D., P. Mistriotis, and K. Konstantopoulos, Cancer cell motility: lessons from migration in confined spaces. *Nature Review Cancer*, **2017**. 17(2): p. 131-140.
157. Hartman, Z.R., M.D. Schaller, and Y.M. Agazie, The tyrosine phosphatase SHP2 regulates focal adhesion kinase to promote EGF-induced lamellipodia persistence and cell migration. *Molecular Cancer Research*, **2013**. 11(6): p. 651-664.
158. Pozarowski, P. and Z. Darzynkiewicz, Analysis of Cell Cycle by Flow Cytometry, in *Checkpoint Controls and Cancer: Volume 2: Activation and Regulation Protocols*, A.H. Schönthal, Editor. 2004, Humana Press: Totowa, NJ. p. 301-311.
159. Williams, B.R., *et al.*, Aneuploidy affects proliferation and spontaneous immortalization in mammalian cells. *Science*, **2008**. 322(5902): p. 703-709.
160. Weaver, B.A.A., *et al.*, Aneuploidy Acts Both Oncogenically and as a Tumor Suppressor. *Cancer Cell*, **2007**. 11(1): p. 25-36.
161. Gorrini, C., I.S. Harris, and T.W. Mak, Modulation of oxidative stress as an anticancer strategy. *Nature Review Drug Discovery*, **2013**. 12(12): p. 931-947.
162. Simon, H.U., A. Haj-Yehia, and F. Levi-Schaffer, Role of reactive oxygen species (ROS) in apoptosis induction. *Apoptosis*, **2000**. 5(5): p. 415-418.
163. Redza-Dutordoir, M. and D.A. Averill-Bates, Activation of apoptosis signalling pathways by reactive oxygen species. *Biochimica et Biophysica Acta*, **2016**. 1863(12): p. 2977-2992.

164. Nakamura, H. and K. Takada, Reactive oxygen species in cancer: Current findings and future directions. *Cancer Science*, **2021**. 112(10): p. 3945-3952.
165. Hoffman, W.P., D.K. Ness, and R.B. van Lier, Analysis of rodent growth data in toxicology studies. *Toxicological Sciences*, **2002**. 66(2): p. 313-319.
166. Todić, M.*, et al.*, Food and water consumption in assessment of acute oral toxicity of HEPALIP FORTE in rats. *Bosnian Journal of Basic Medical Sciences*, **2003**. 3(4): p. 47-53.
167. Piao, Y., Y. Liu, and X. Xie, Change Trends of Organ Weight Background Data in Sprague Dawley Rats at Different Ages. *Journal of Toxicologic Pathology*, **2013**. 26(1): p. 29-34.
168. Sellers, R.S.*, et al.*, Society of Toxicologic Pathology Position Paper: Organ Weight Recommendations for Toxicology Studies. *Toxicologic Pathology*, **2007**. 35(5): p. 751-755.
169. Majumdar, S. and S. Krishnamurthy, In vivo toxicological evaluation of barium-doped bioactive glass in rats. *Ceramics International*, **2022**. 48(22): p. 33288-33305.
170. Liyanagamage, D.S.N.K.*, et al.*, Acute and Subchronic Toxicity Profile of a Polyherbal Drug Used in Sri Lankan Traditional Medicine. *Evidence-Based Complementary and Alternative Medicine*, **2020**. 2020(1): p. 2189189.
171. Giannini, E.G., R. Testa, and V. Savarino, Liver enzyme alteration: a guide for clinicians. *Canadian Medical Association Journal*, **2005**. 172(3): p. 367-379.
172. Brana, I.*, et al.*, Initial results from a dose finding study of TNO155, a SHP2 inhibitor, in adults with advanced solid tumors. *Journal of Clinical Oncology*, **2021**. 39(15_suppl): p. 3005-3005.
173. Mitra, R.*, et al.*, Design, Synthesis and Evaluation of Thioacetamide-Tethered Thiadiazole-1,2,4-Triazole Hybrids as SHP2 Inhibitors for Cancer Therapy. *ChemistrySelect*, **2024**. 9(27): p. e202401602.
174. Biliz, Y.*, et al.*, Novel N-Acyl Hydrazone Compounds as Promising Anticancer Agents: Synthesis and Molecular Docking Studies. *ACS Omega*, **2023**. 8(22): p. 20073-20084.
175. Kassab, A.E., Anticancer agents incorporating the N-acylhydrazone scaffold: Progress from 2017 to present. *Archiv der Pharmazie (Weinheim)*, **2023**. 356(5): p. e2200548.
176. Sharma, P.C.*, et al.*, Hydrazone comprising compounds as promising anti-infective agents: chemistry and structure-property relationship. *Materials Today Chemistry*, **2020**. 18: p. 100349.
177. Thota, S.*, et al.*, N-Acylhydrazones as drugs. *Bioorganic & Medicinal Chemistry Letters*, **2018**. 28(17): p. 2797-2806.

178. Munir, R., *et al.*, Synthesis of Novel N-Acylhydrazones and Their C-N/N-N Bond Conformational Characterization by NMR Spectroscopy. *Molecules*, **2021**. 26(16): p. 4908.
179. Palla, G., *et al.*, Conformational behaviour and E/Z isomerization of N-acyl and N-aroylhydrazones. *Tetrahedron*, **1986**. 42(13): p. 3649-3654.
180. Syakaev, V.V., *et al.*, NMR study of conformation and isomerization of aryl- and heteroarylaldehyde 4-tert-butylphenoxyacetylhydrazones. *Journal of Molecular Structure*, **2006**. 788(1): p. 55-62.
181. Reis, D.C., *et al.*, Structural Studies and Investigation on the Activity of Imidazole-Derived Thiosemicarbazones and Hydrazones against Crop-Related Fungi. *Molecules*, **2013**. 18(10): p. 12645-12662.
182. Liberek, B., *et al.*, Thin-Layer chromatographic separation of the Z and E rotational isomers of α-N-nitroso-N-alkylamino acids. *Journal of Chromatography A*, **1974**. 95(2): p. 223-228.
183. Daina, A. and V. Zoete, A BOILED-Egg To Predict Gastrointestinal Absorption and Brain Penetration of Small Molecules. *ChemMedChem*, **2016**. 11(11): p. 1117-1121.

# Appendix

## A. Pharmacophore-based virtual screening

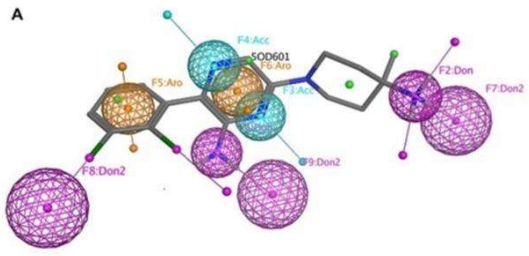
**Table A1.** Evaluation of the quality of SHP2 protein (PDB ID: 5EHR) by plot statistics in PROCHECK

| Parameters for evaluation | No. of residues | Score |
|---|---|---|
| Residues in most favoured regions [A,B,L] | 415 | 89.2% |
| Residues in additional allowed regions [a,b,l,p] | 44 | 10.1% |
| Residues in generously allowed regions [~a,~b,~l,~p] | 2 | 0.5% |
| Residues in disallowed regions | 1 | 0.2% |
| Number of non-glycine and non-proline residues | 436 | 100.00% |
| Number of end-residues (excl. Gly and Pro) | 8 | - |
| Number of glycine residues (shown as triangles)* | 35 | - |
| Number of proline residues | 19 | - |
| Total number of residues | 498 | - |

*Refer to the Figure A2 for details.

**A brief protocol for evaluation of protein through SAVES server**

The optimized protein structure of SHP2 (PDB ID: 5EHR) was uploaded to SAVES v6.0 web server (https://saves.mbi.ucla.edu/). Then protein was analyzed for different parameter (ERRAT, Verify 3D, PROVE, WHATCHEK, PROCHECK and CRYST). The Ramachandran plot from the results of PROCHECK was then downloaded. The graph of the Ramachandran plot is given in Figure A2.




B

| | | | | | | |
|---|---|---|---|---|---|---|
| F1: Don | 0 | | | | | |
| F2: Don | 4.8 | 0 | | | | |
| F3: Acc | 1.3 | 3.5 | 0 | | | |
| F4: Acc | 2.0 | 4.1 | 1.5 | 0 | | |
| F5: Aro | 2.0 | 6.0 | 2.3 | 2.3 | 0 | |
| F6: Aro | 1.5 | 2.7 | 0.8 | 0.8 | 2.3 | 0 |
| | F1: Don | F2: Don | F3: Acc | F4: Acc | F5: Aro | F6: Aro |

**Figure A1**. A) Pharmacophore model of co-crystallized **SHP099** showing the hypothetical spheres developed by MOE. Pink sphere = donor, blue sphere = acceptor, orange sphere = aromatic; B) The distance between the pharmacophoric features (in angstrom, Å) is presented in the distance matrix. Aro: Aromatic; don: donor; hyd: hydrophobic; acc: acceptor.

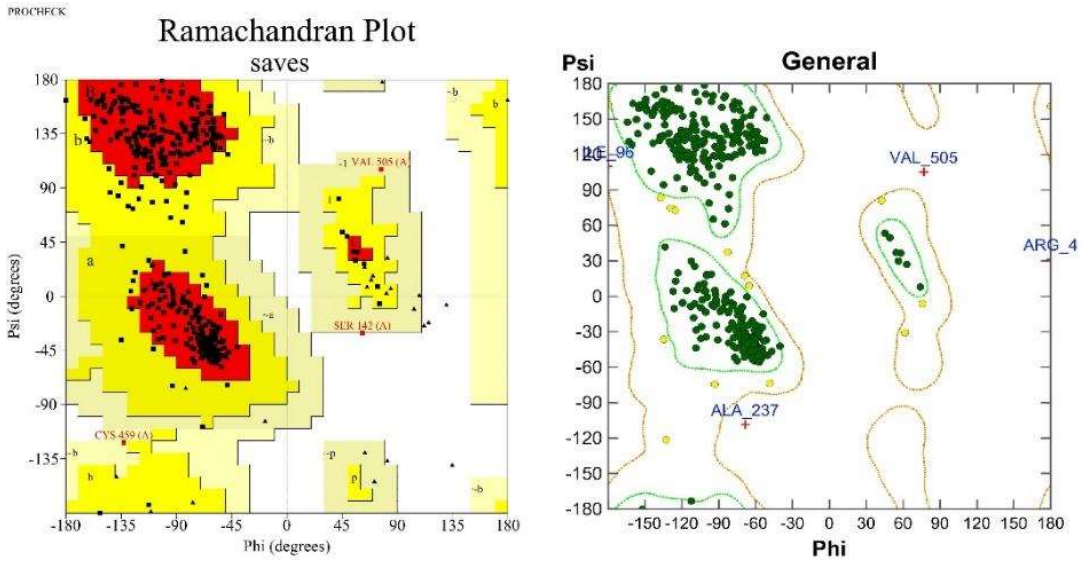



**Figure A2.** Ramachandran plot of SHP2 (PDB ID: 5EHR) obtained from PROCHECK (red & yellow) and phi-psi plot from MOE (black & white), respectively

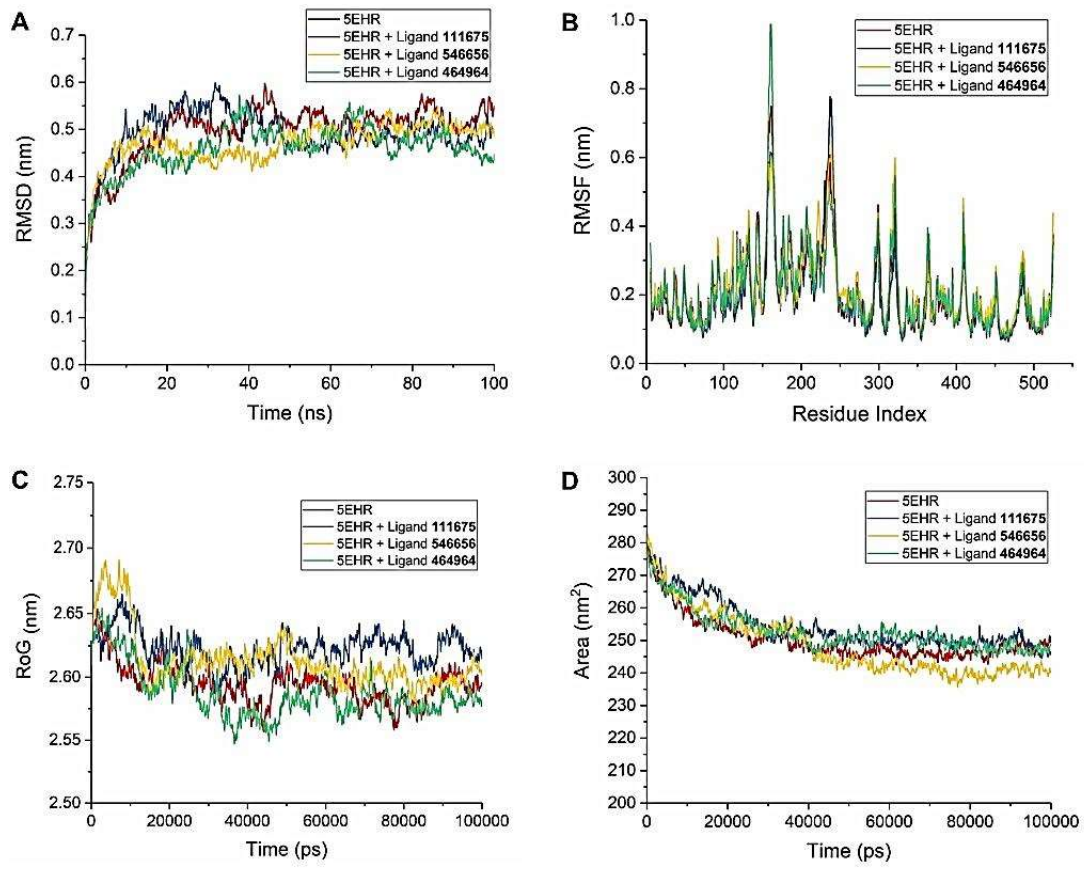



**Figure A3.** MD simulation results of most and least active hits; (A) RMSD of apoprotein and complex with ligands **111675**, **546656** and **464964**; (B) RMSF of apoprotein and complex with ligands **111675, 546656** and **464964**; (C) RoG of

apoprotein and complexes of ligand **111675**, **546656** and **464964;** (D) solvent accessible surface area of apoprotein and complexes of ligand **111675**, **546656** and **464964**.

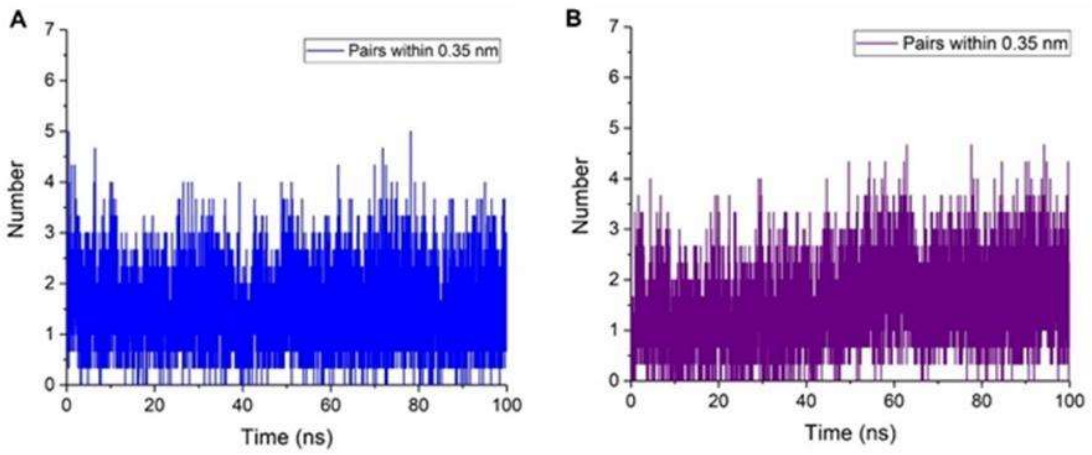



**Figure A4.** Hydrogen bonding analysis of 5EHR complexed with A) compound **111675** (shown in blue) and B) compound **546656** (shown in purple)

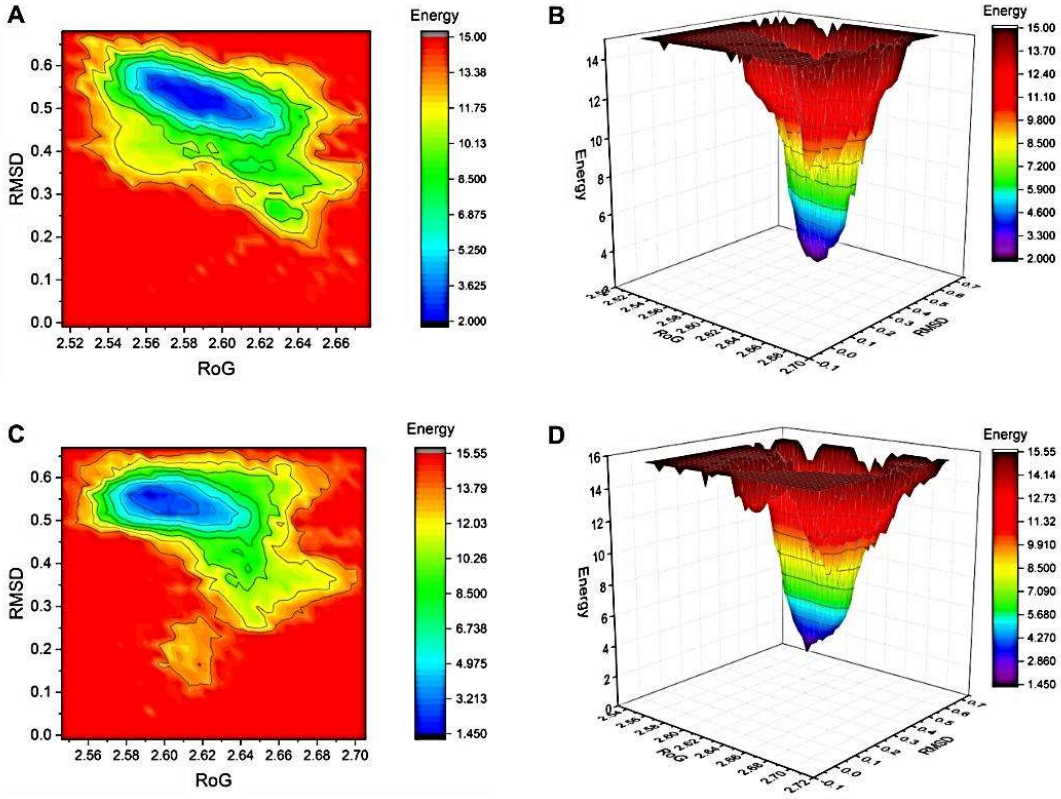



**Figure A5.** Free energy landscape (FEL) diagram of 5EHR apoprotein and its respective complexes; (A) 2D contour map of 5EHR; (B) funnel like 3D energy projection diagram of 5EHR; (C) 2D contour map of 5EHR and ligand **111675** complex; (D) funnel like 3D energy projection diagram of 5EHR and ligand **111675** complex

## B. Spectral data of selected synthesized compounds

### B1. Thioacetamide-tethered 1,3,4-thiadiazole-1,2,4-triazole hybrids (STT series)

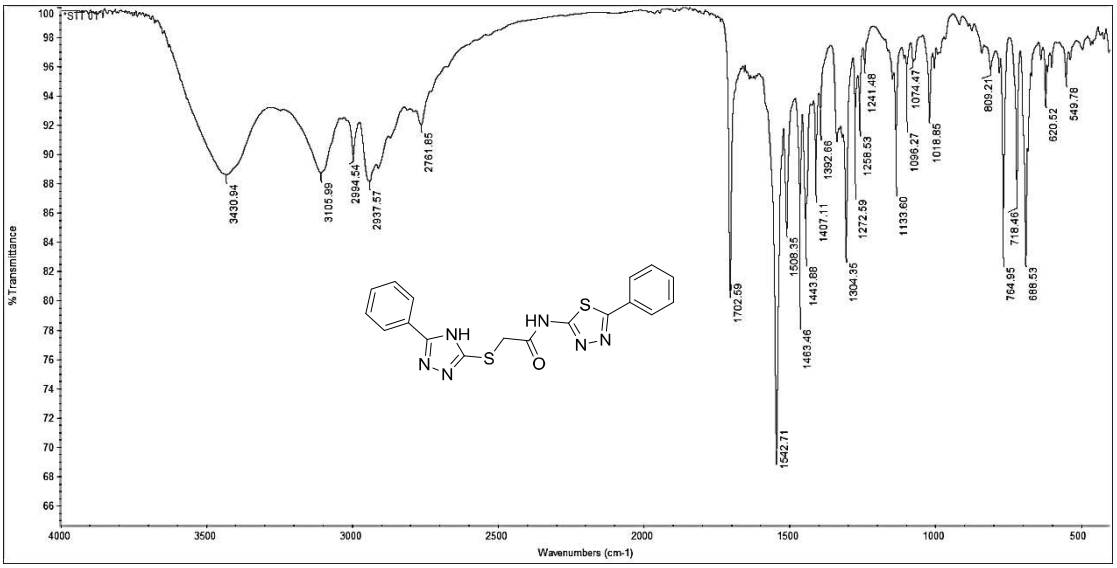



**Figure A6.** FTIR spectrum of compound **STT01**

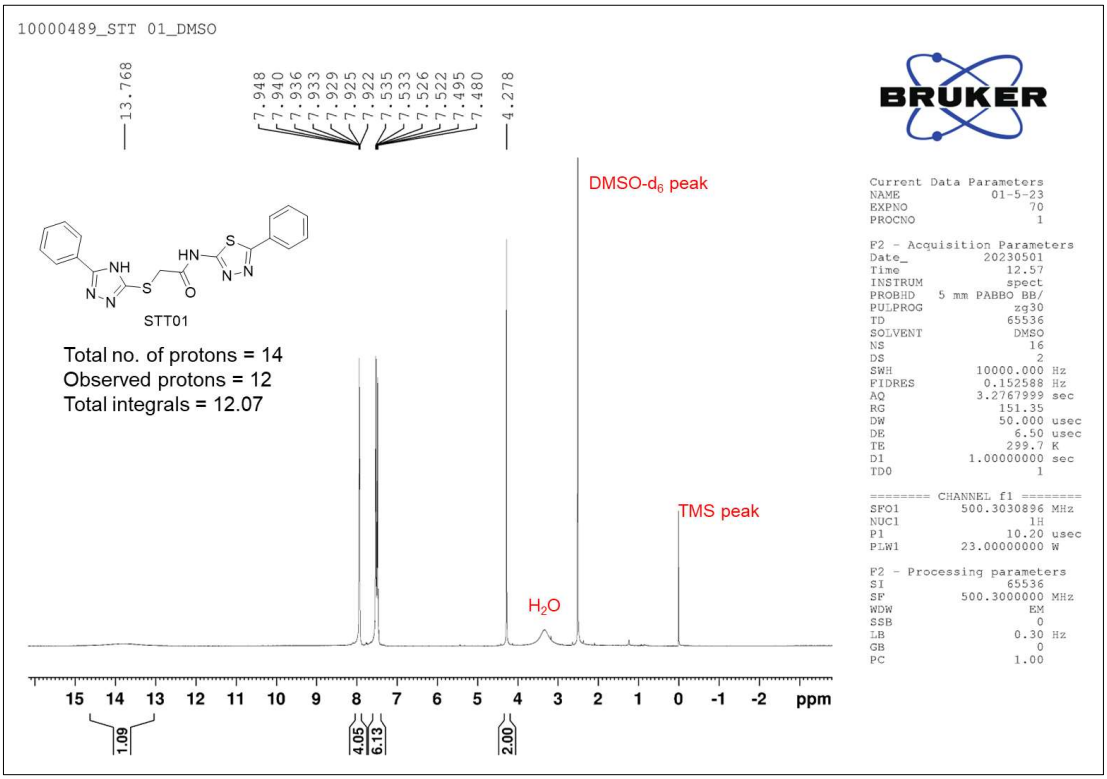



**Figure A7.** $^{1}$H NMR spectrum of compound **STT01**

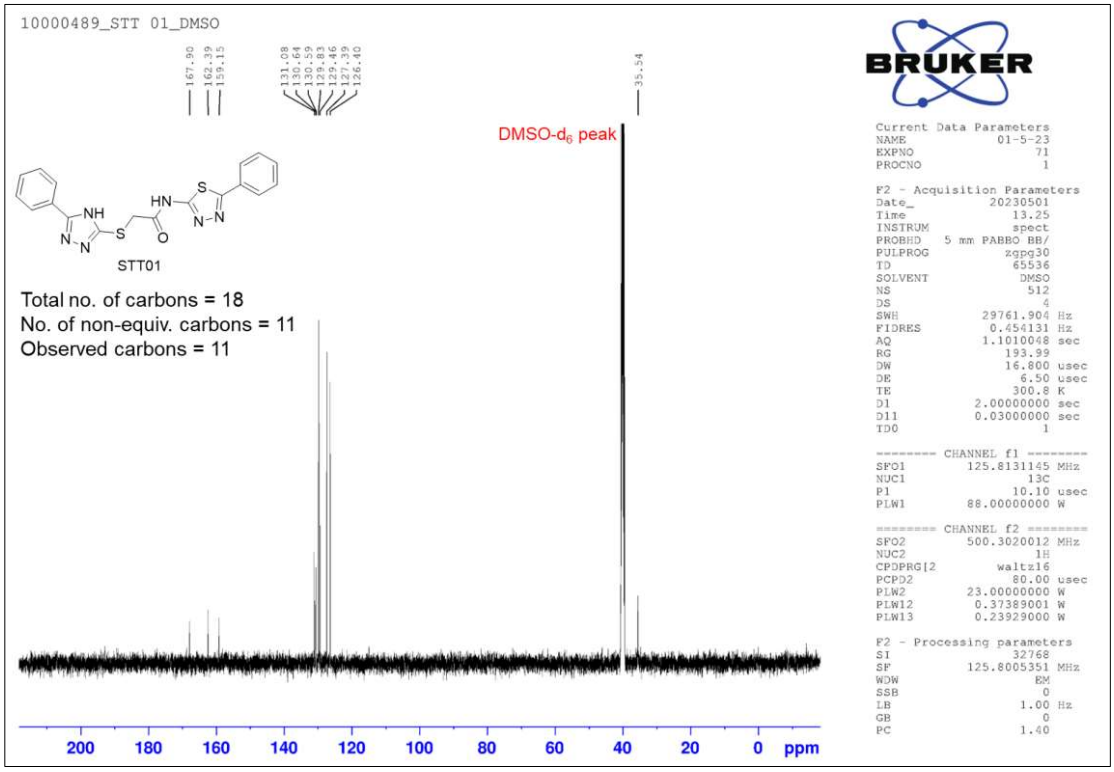



**Figure A8.** $^{13}$C NMR spectrum of compound **STT01**

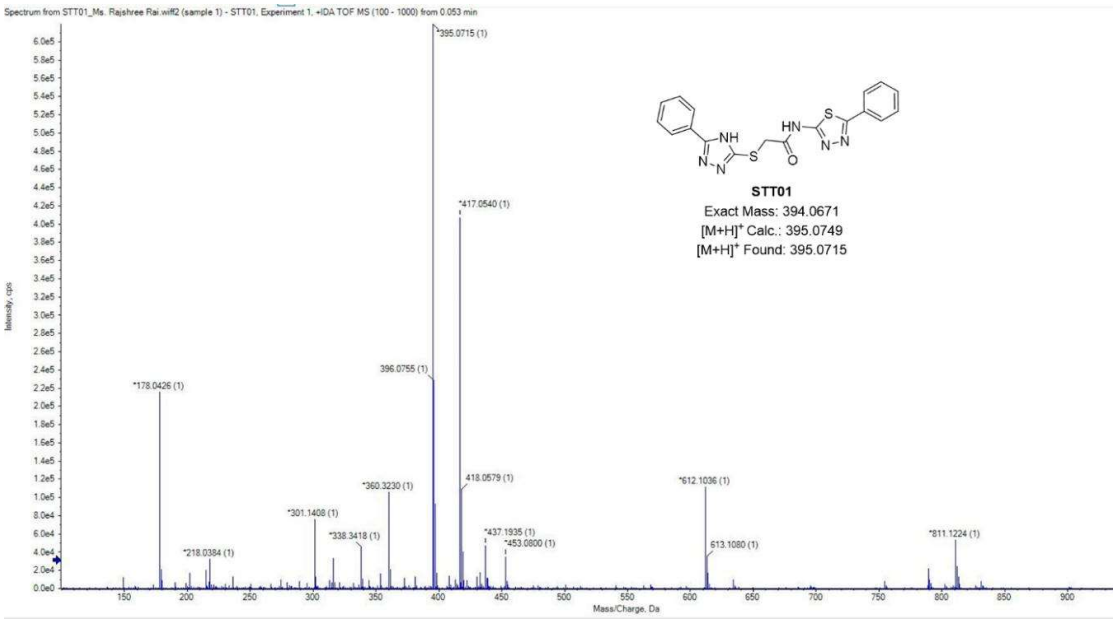



**Figure A9.** HRMS spectrum of compound **STT01**

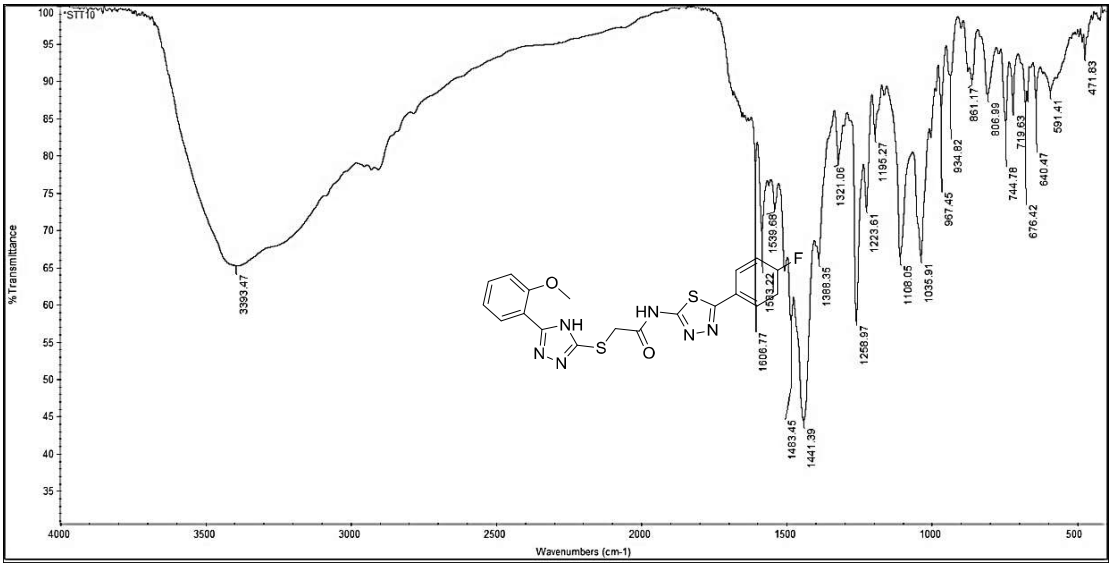



**Figure A10.** FTIR spectrum of compound **STT07**

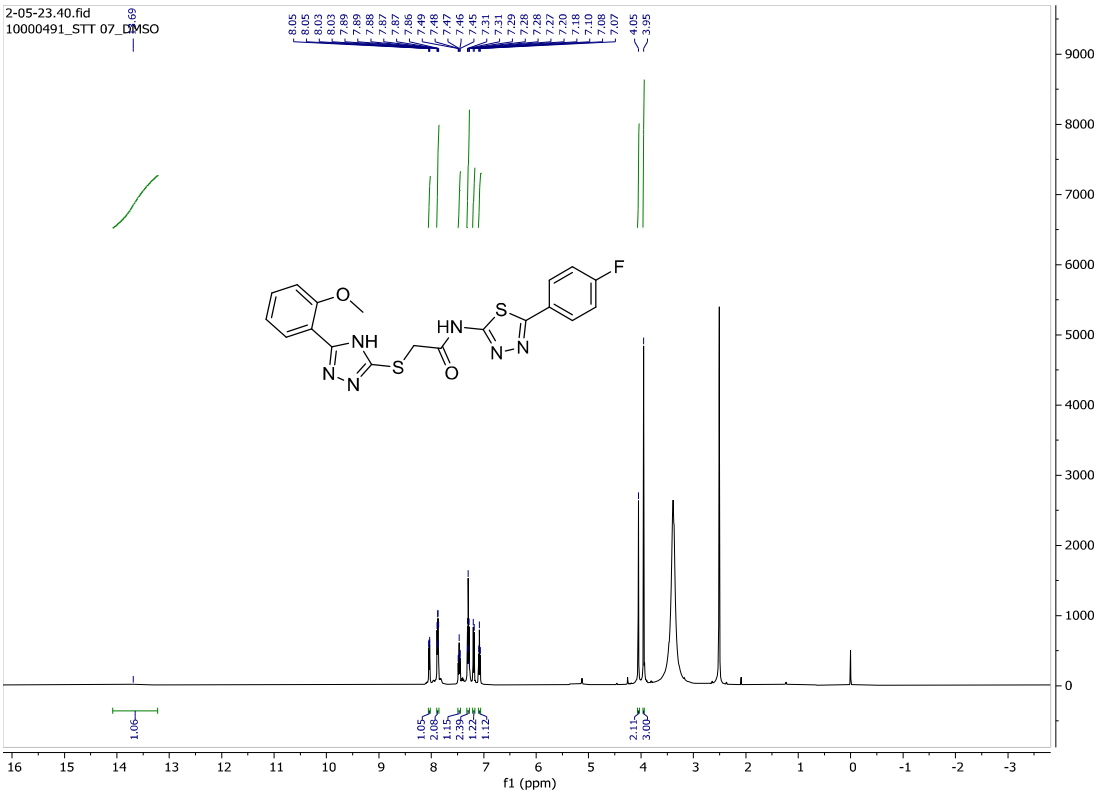



**Figure A11.** $^{1}$H NMR spectrum of compound **STT07**

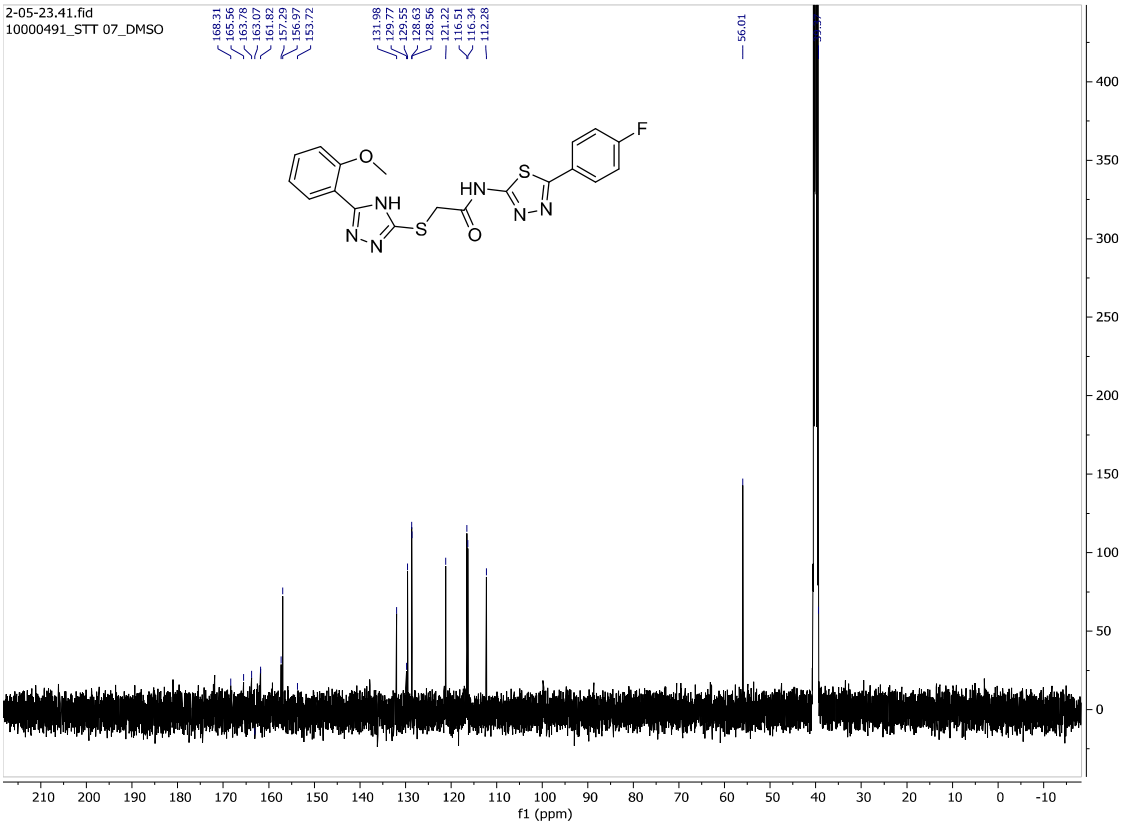



**Figure A12.** $^{13}$C NMR spectrum of compound **STT07**

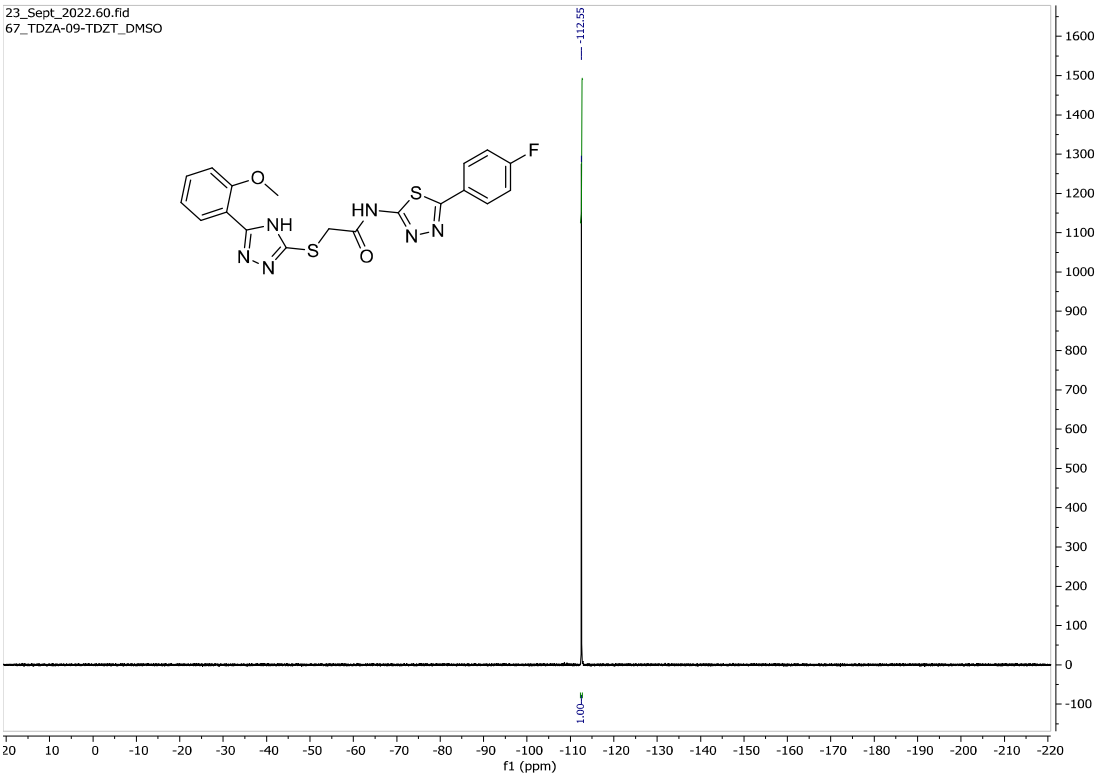



**Figure A13.** $^{19}$F NMR spectrum of compound **STT07**

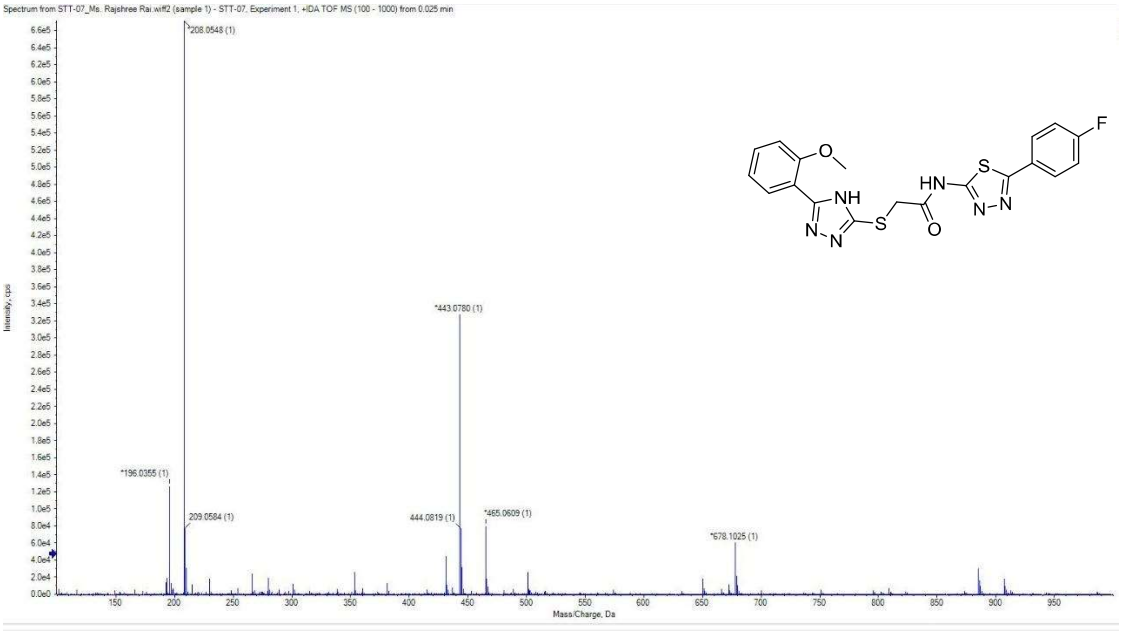



**Figure A14.** HRMS spectrum of compound **STT07**

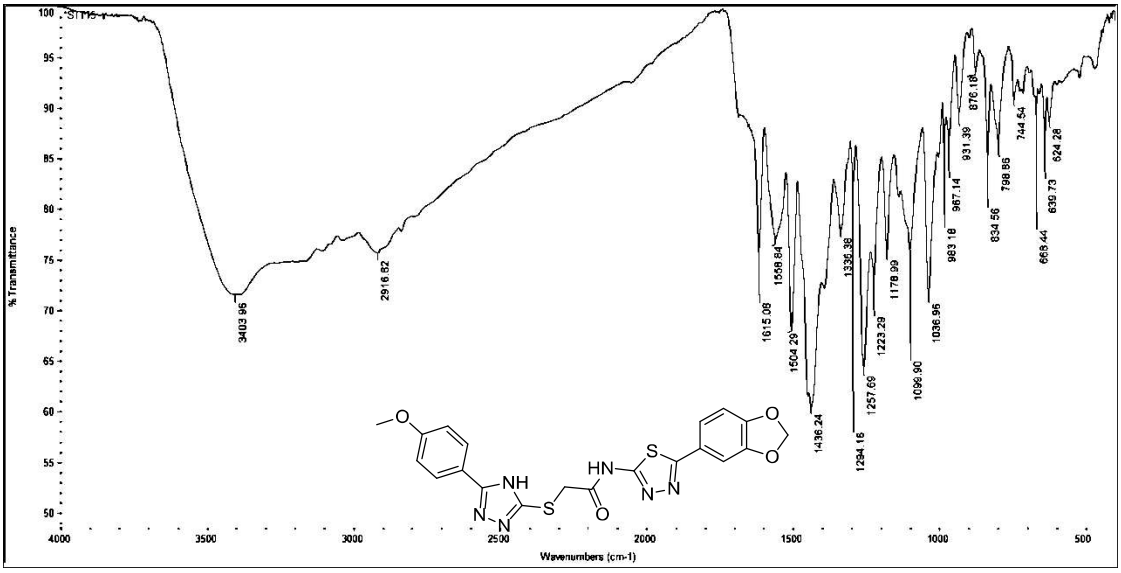



**Figure A15.** FTIR spectrum of compound **STT13**

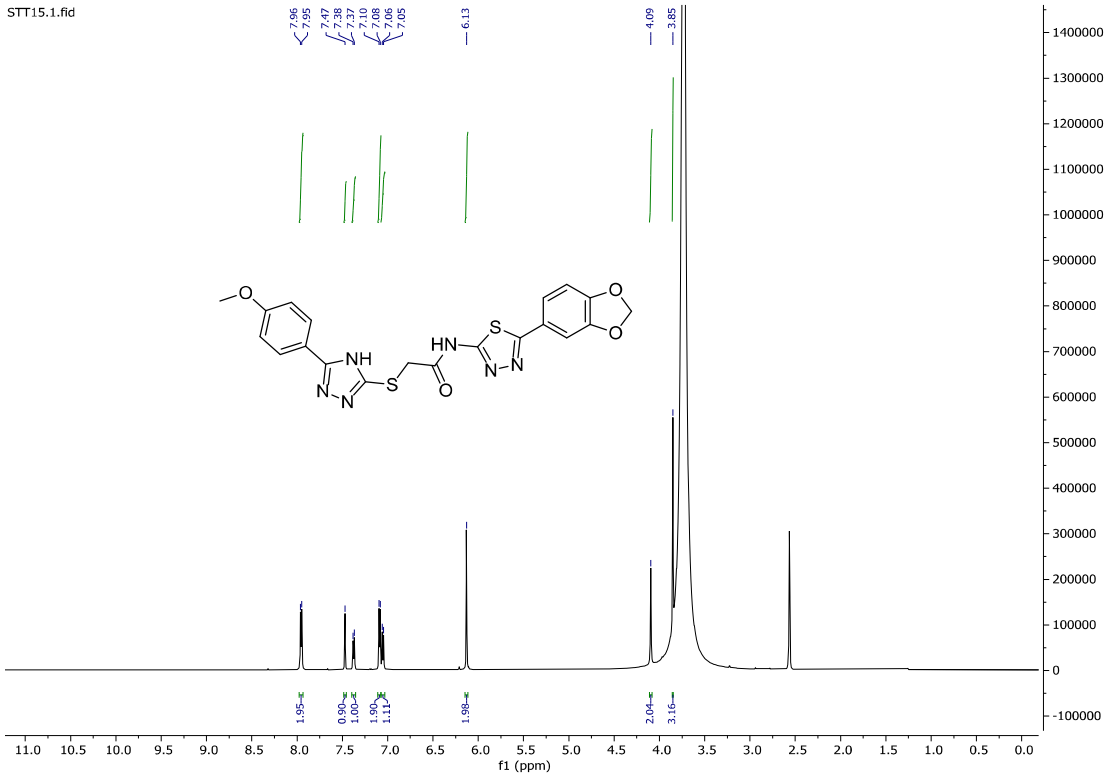



**Figure A16.** $^{1}$H NMR spectrum of compound **STT13**

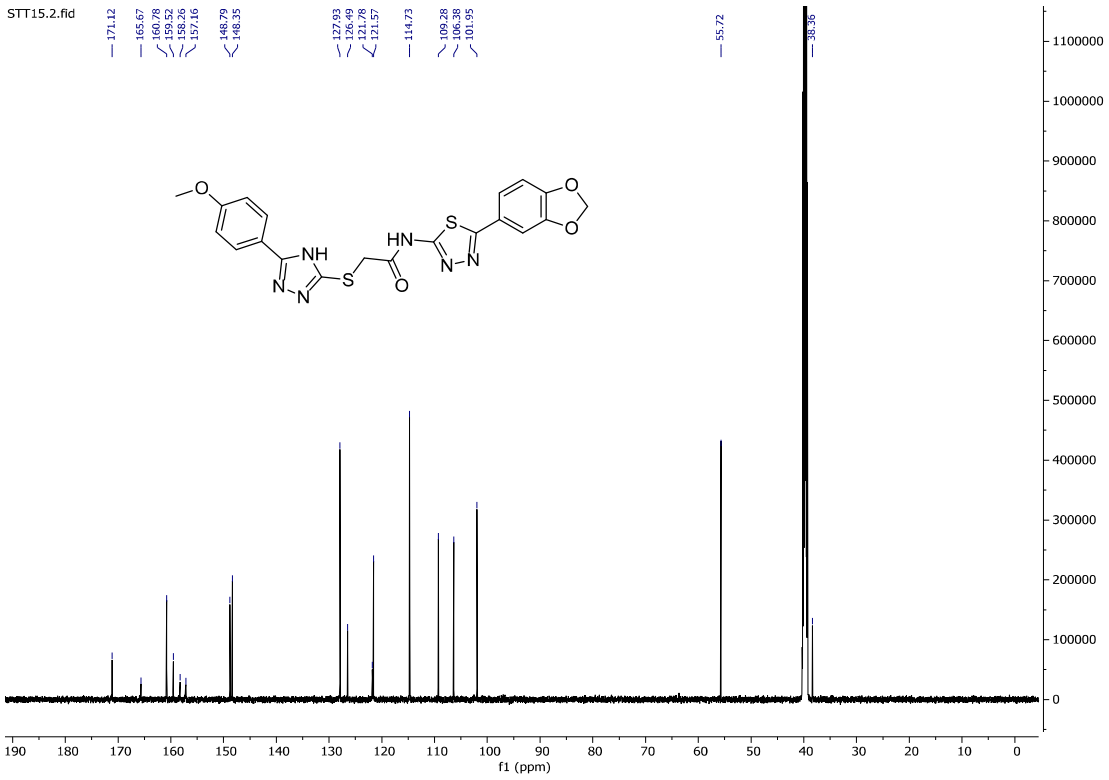



**Figure A17.** $^{13}$C NMR spectrum of compound **STT13**

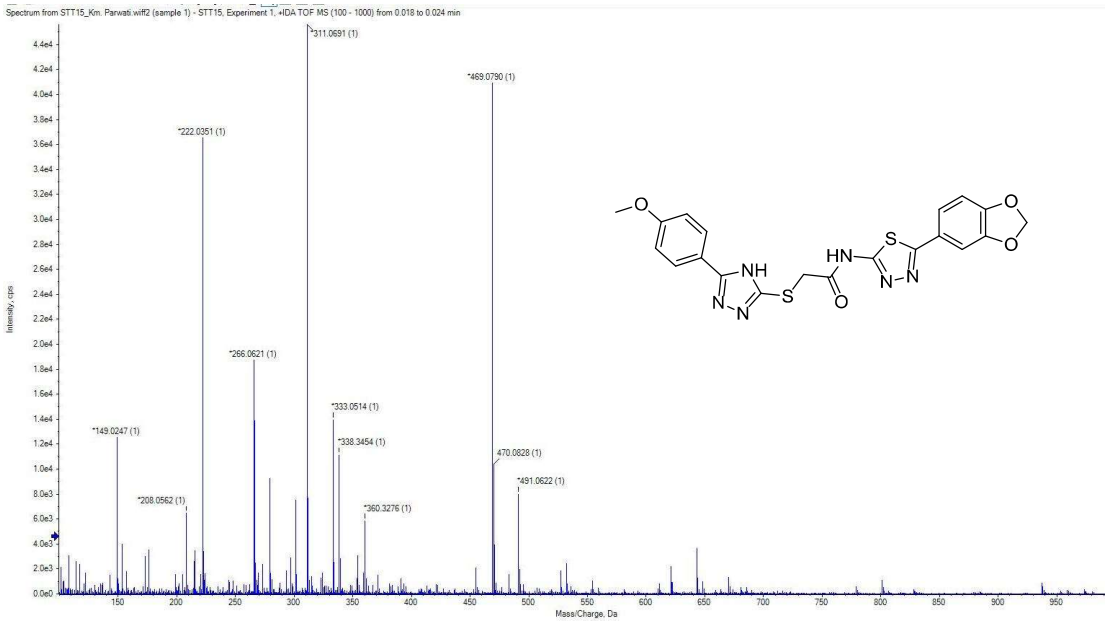



**Figure A18.** HRMS spectrum of compound **STT13**

## B2. 5-(Substituted phenyl)-1,3,4-thiadiazole-2-amine derived sulphur-linked acetamides (STS series)

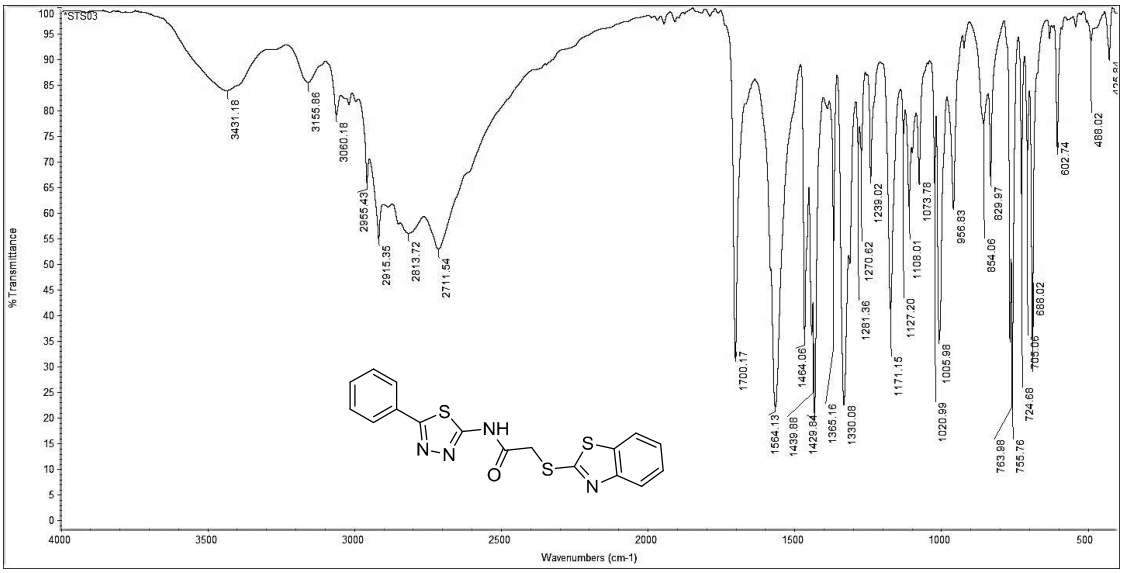



**Figure A19.** FTIR spectrum of compound **STS03**

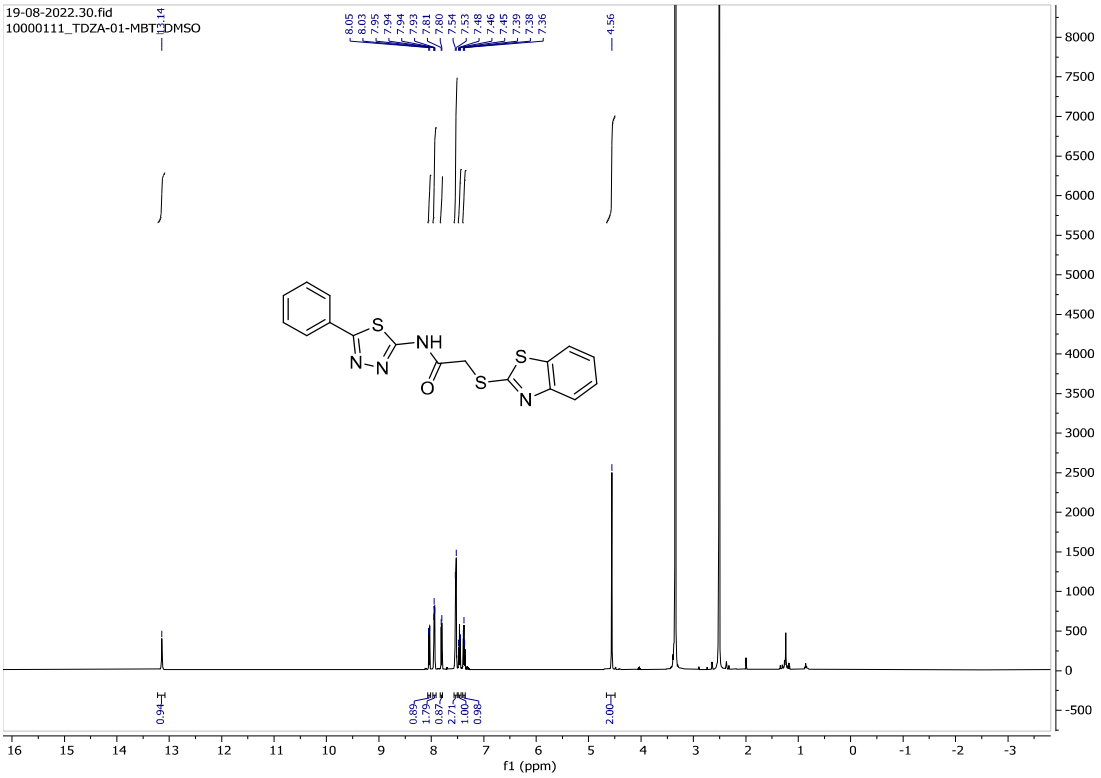



**Figure A20.** $^{1}$H NMR spectrum of compound **STS03**

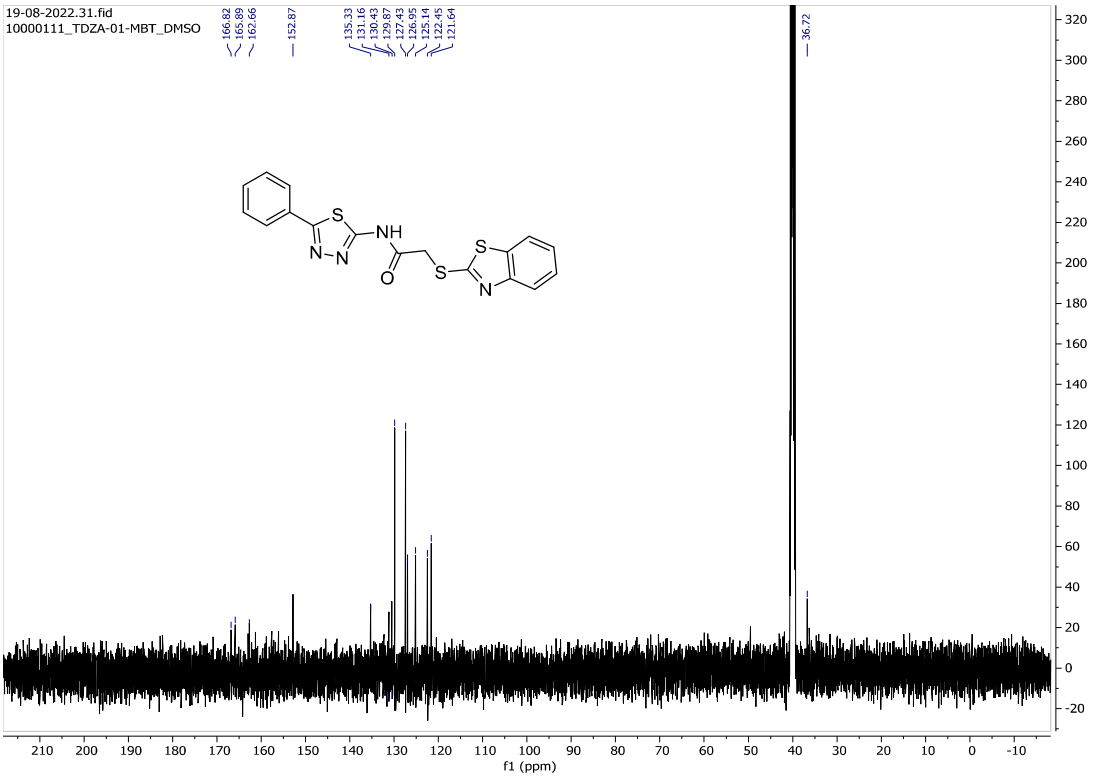



**Figure A21.** $^{13}$C NMR spectrum of compound **STS03**

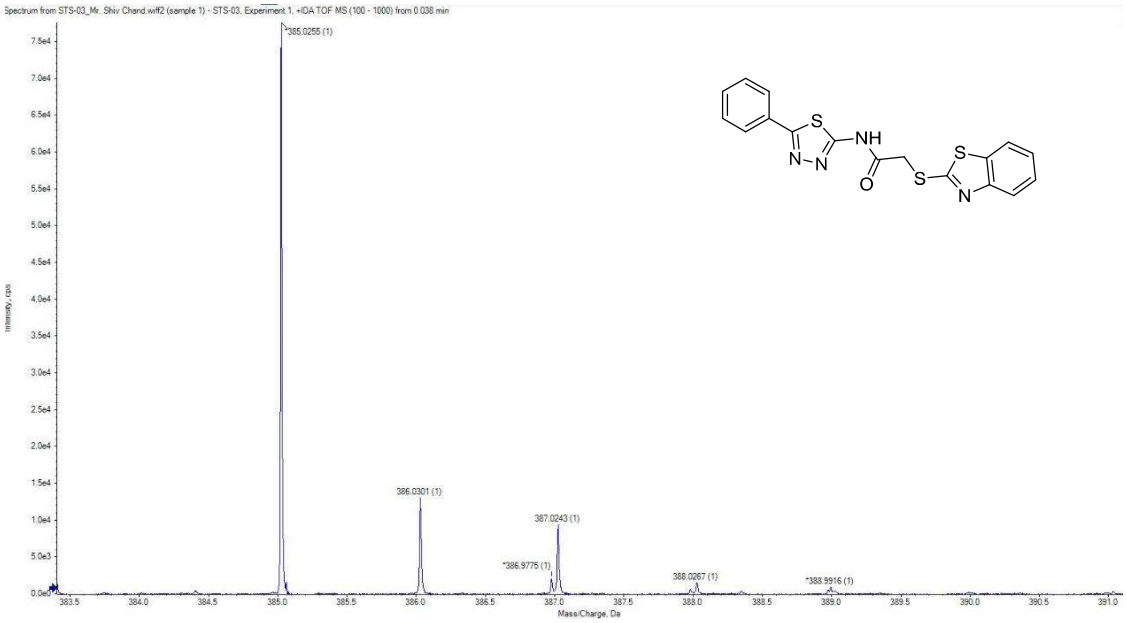



**Figure A22.** HRMS spectrum of compound **STS03**

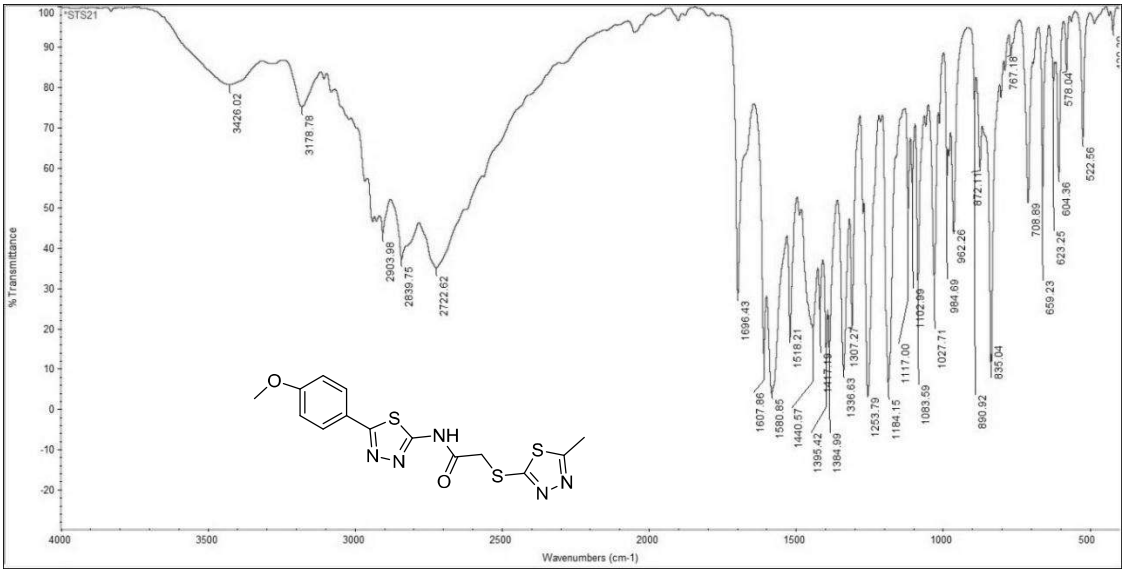



**Figure A23.** FTIR spectrum of compound **STS11**

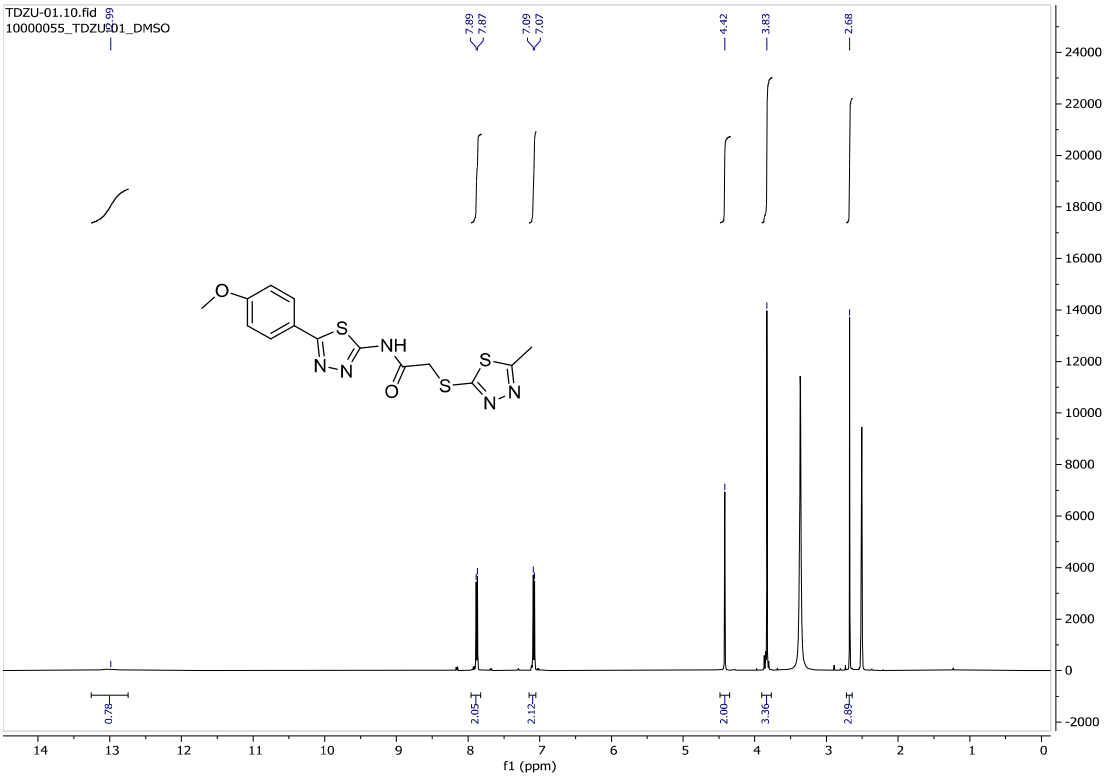



**Figure A24.** $^{1}$H NMR spectrum of compound **STS11**

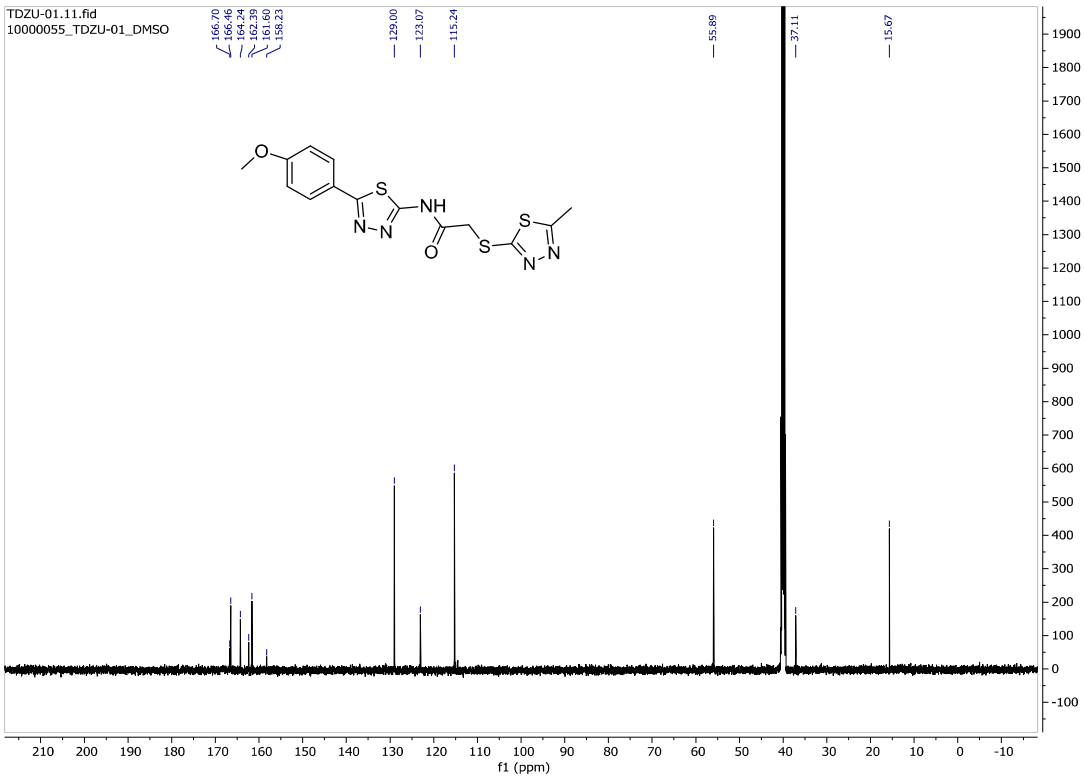



**Figure A25.** $^{13}$C NMR spectrum of compound **STS11**

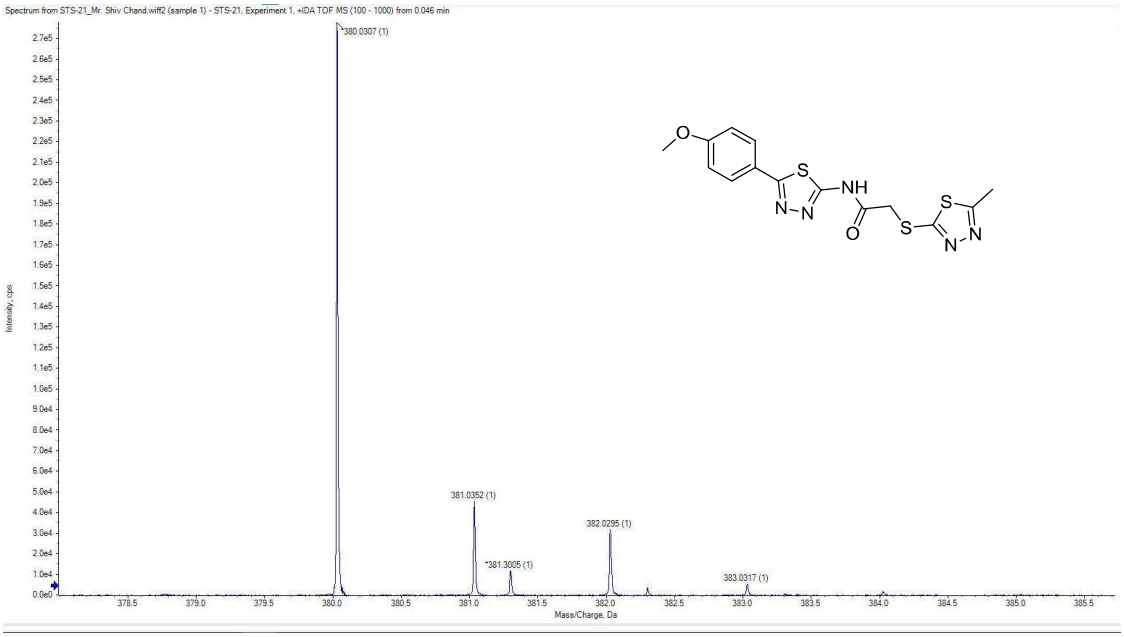



**Figure A26.** HRMS spectrum of compound **STS11**

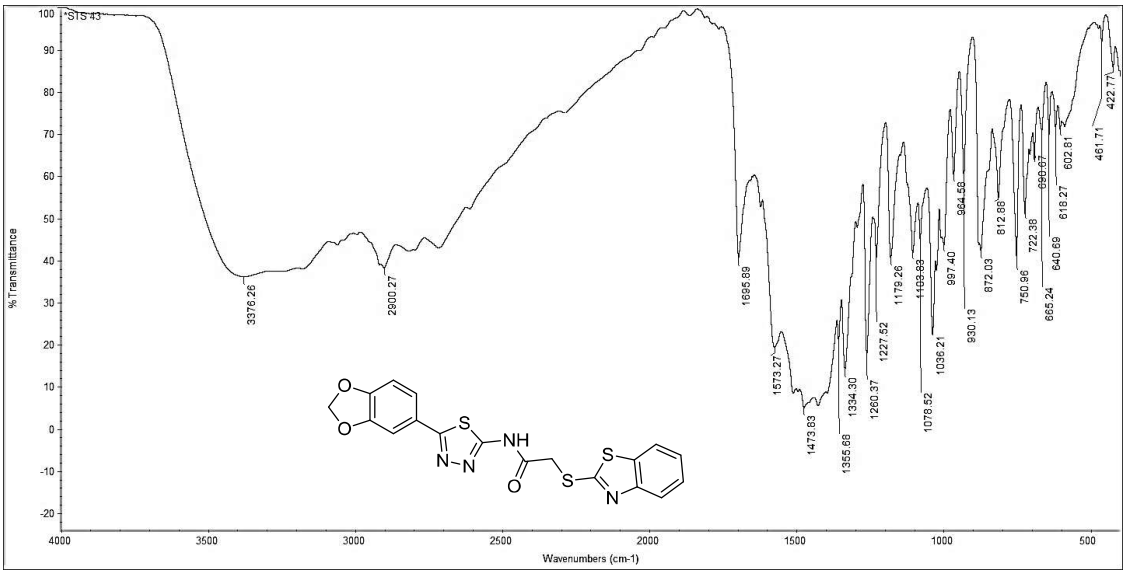



**Figure A27.** FTIR spectrum of compound **STS23**

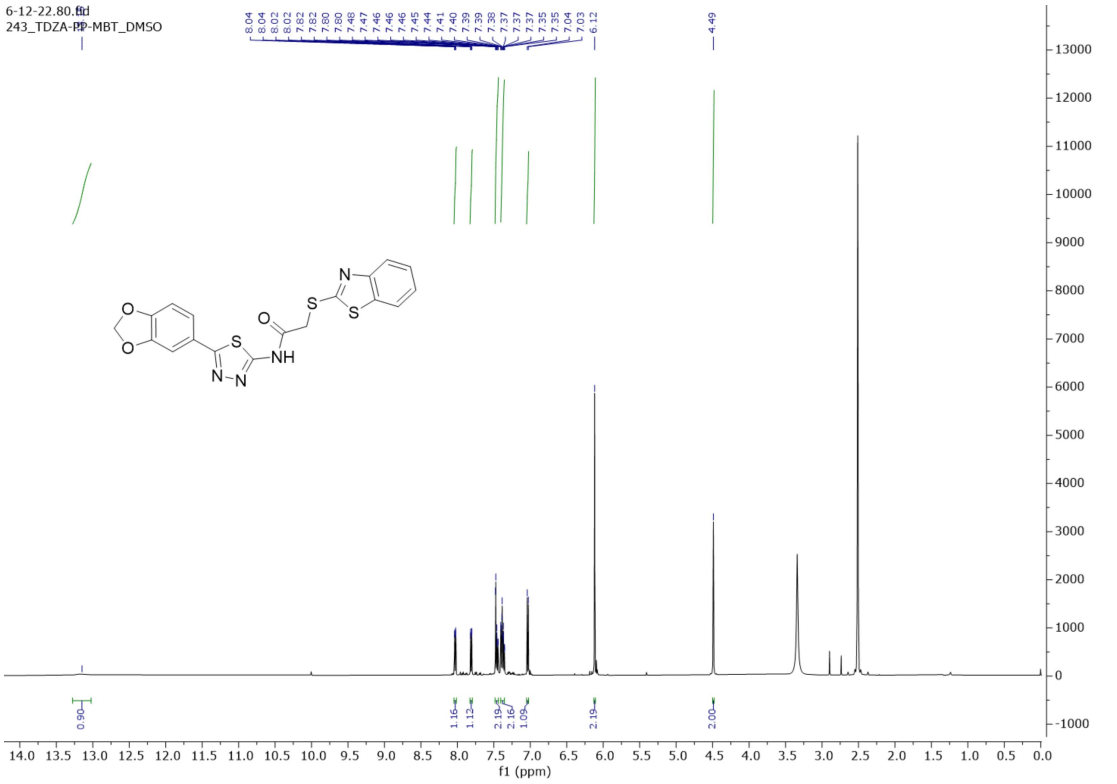



**Figure A28.** $^{1}$H NMR spectrum of compound **STS23**

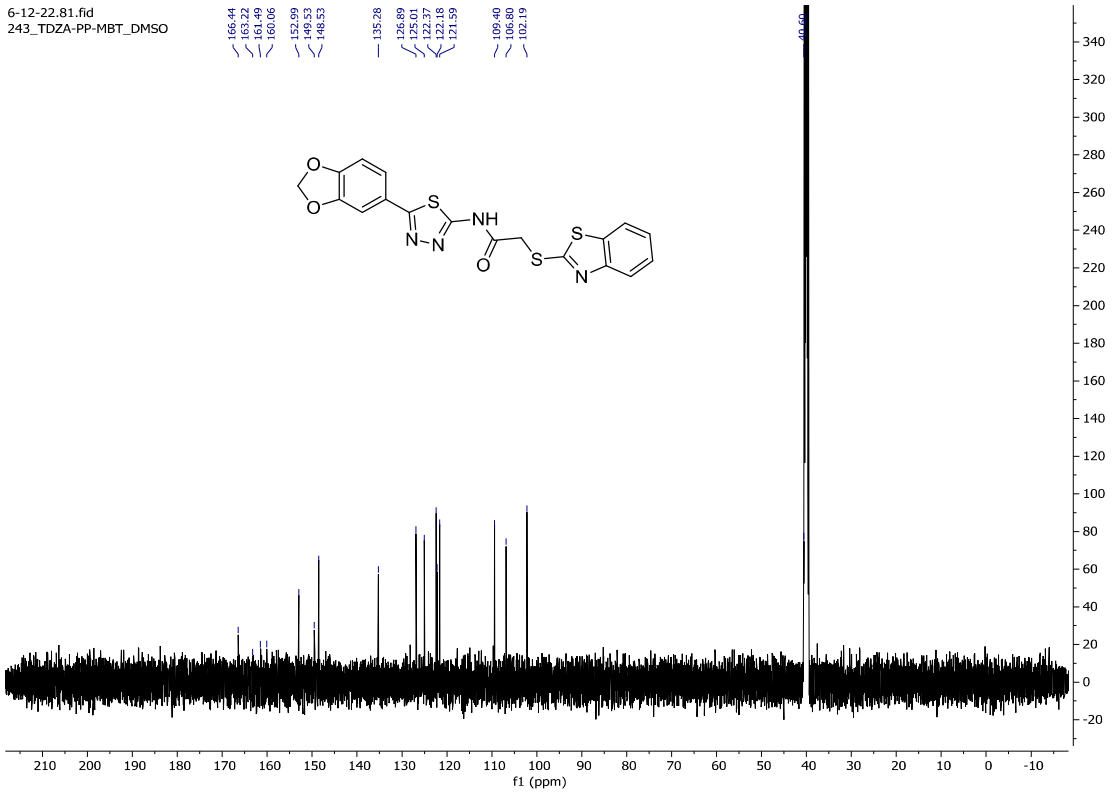



**Figure A29.** $^{13}$C NMR spectrum of compound **STS23**

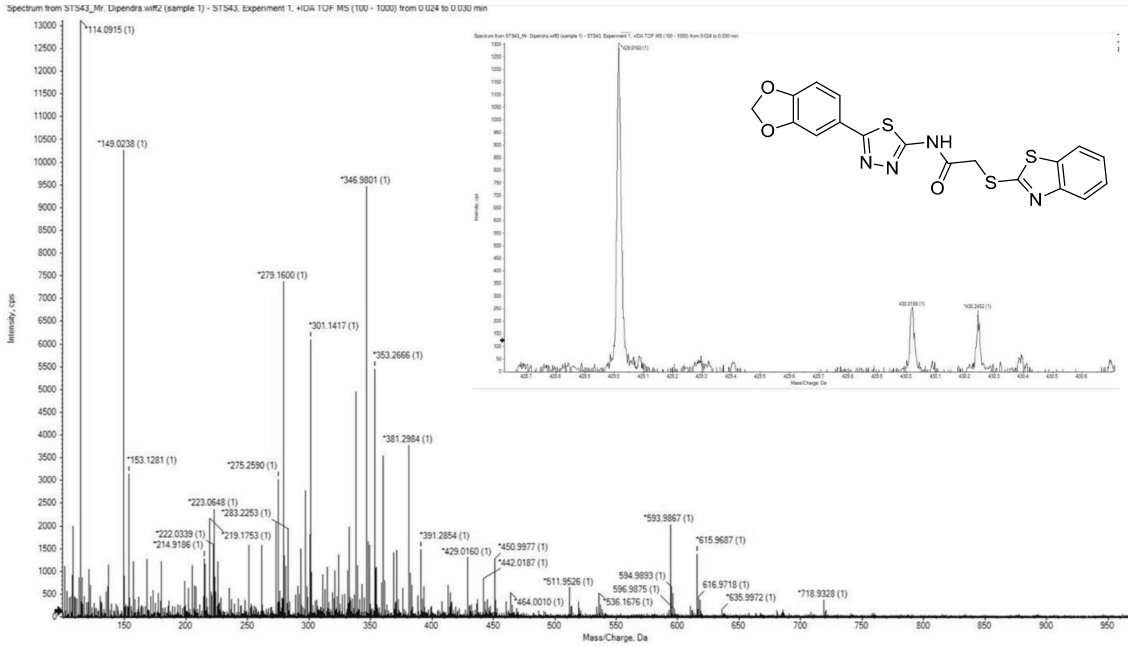



**Figure A30.** HRMS spectrum of compound **STS23**

## B3. *S*-Acetohydrazones of 5-methyl-1,3,4-thiadiazole-2-thiol (TEH series)

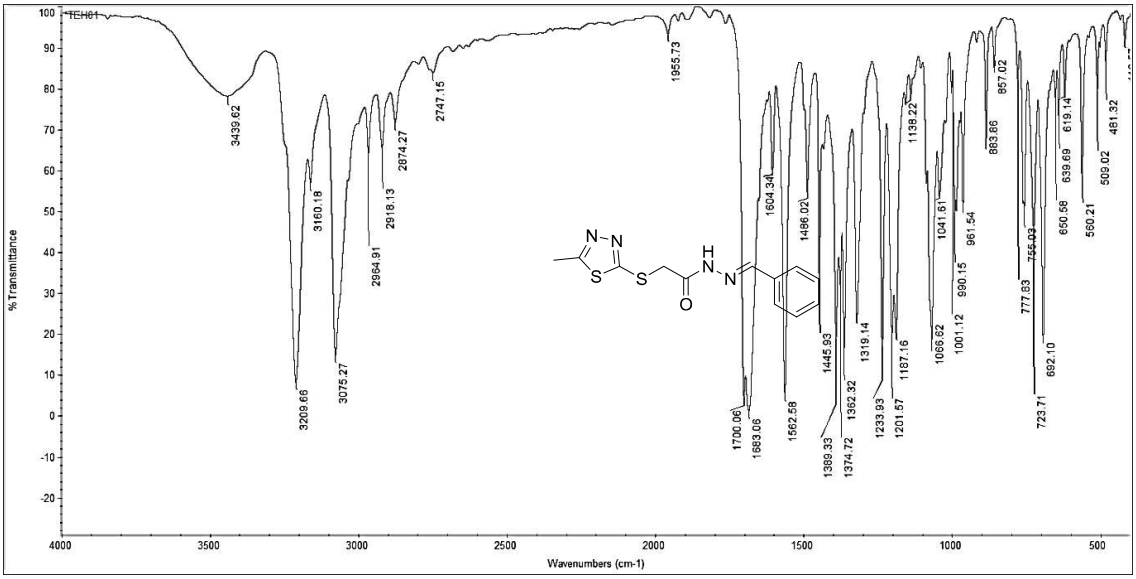



**Figure A31.** FTIR spectrum of compound **TEH01**

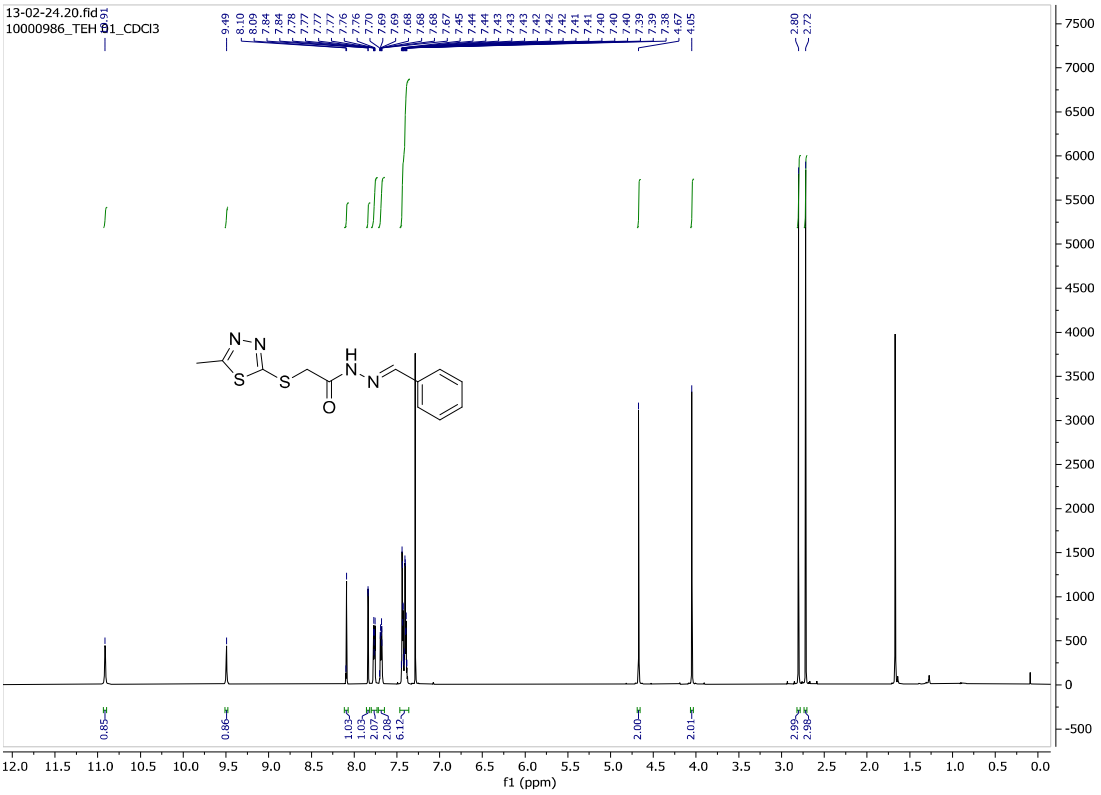



**Figure A32.** $^{1}$H NMR spectrum of compound **TEH01**

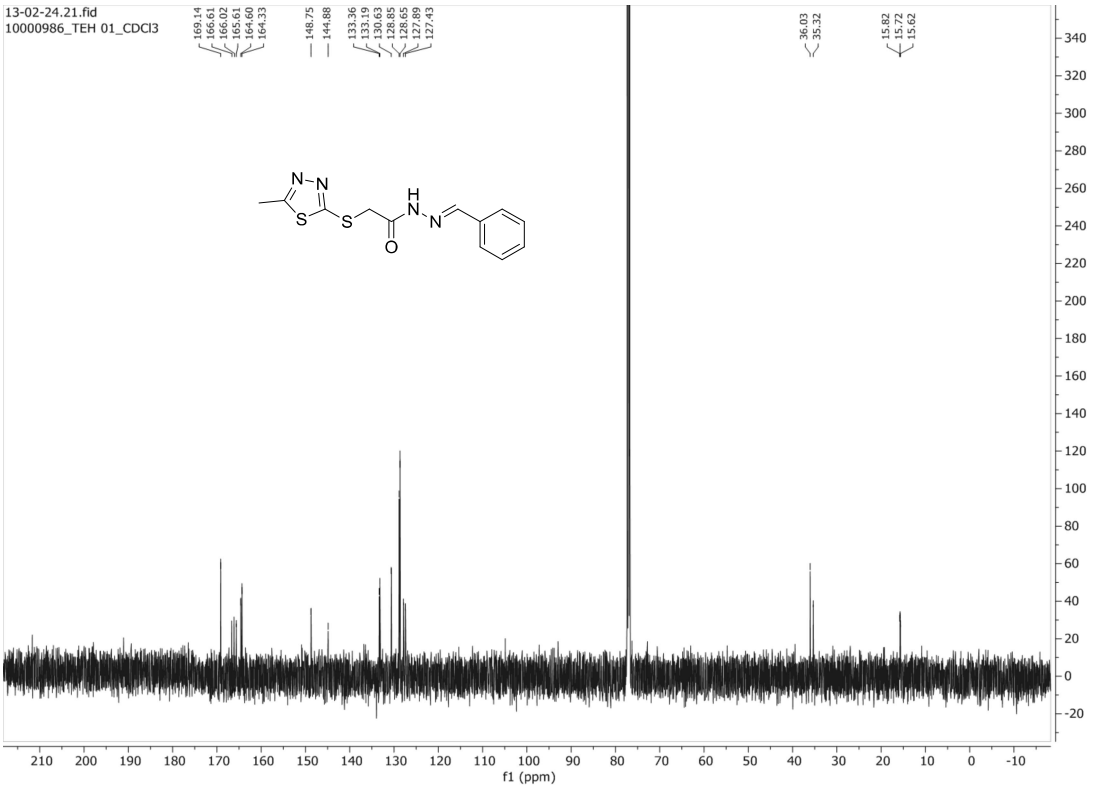



**Figure A33.** $^{13}$C NMR spectrum of compound **TEH01**

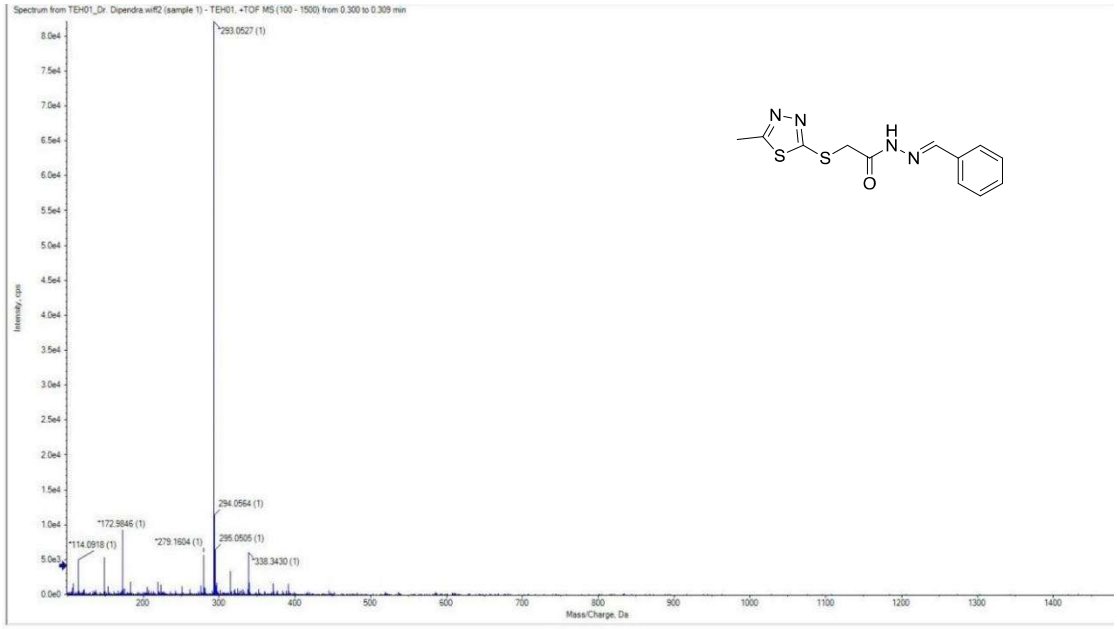



**Figure A34.** HRMS spectrum of compound **TEH01**

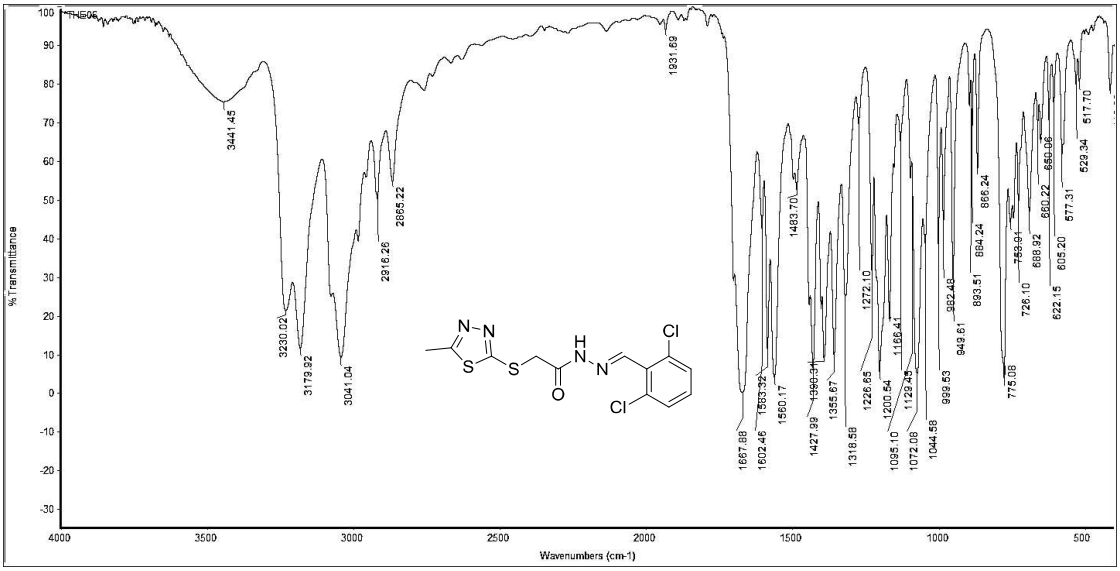



**Figure A35.** FTIR spectrum of compound **TEH06**

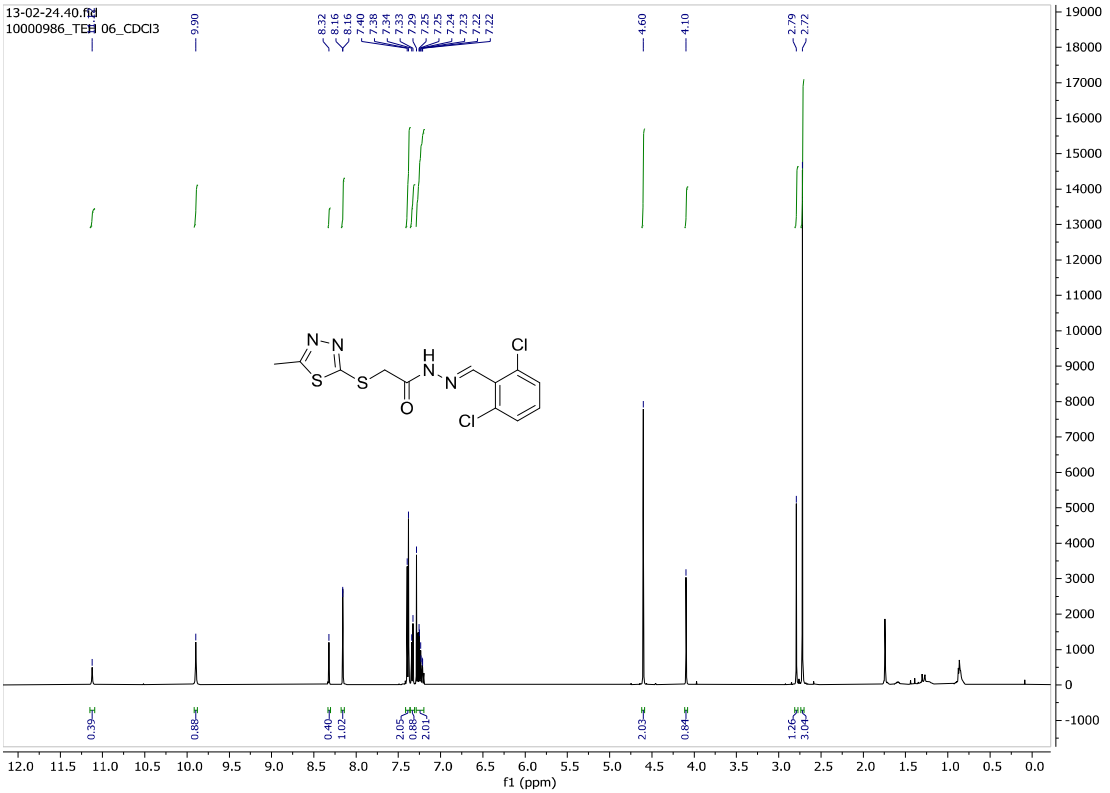



**Figure A36.** $^{1}$H NMR spectrum of compound **TEH06**

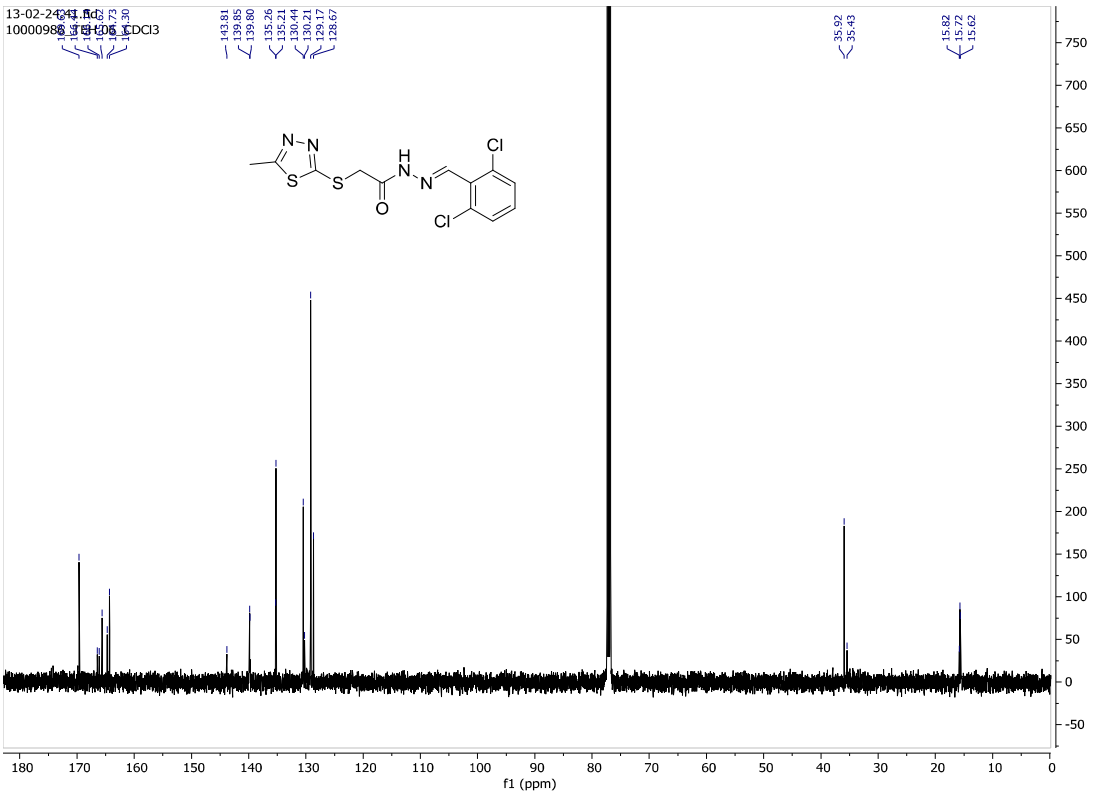



**Figure A37.** $^{13}$C NMR spectrum of compound **TEH06**

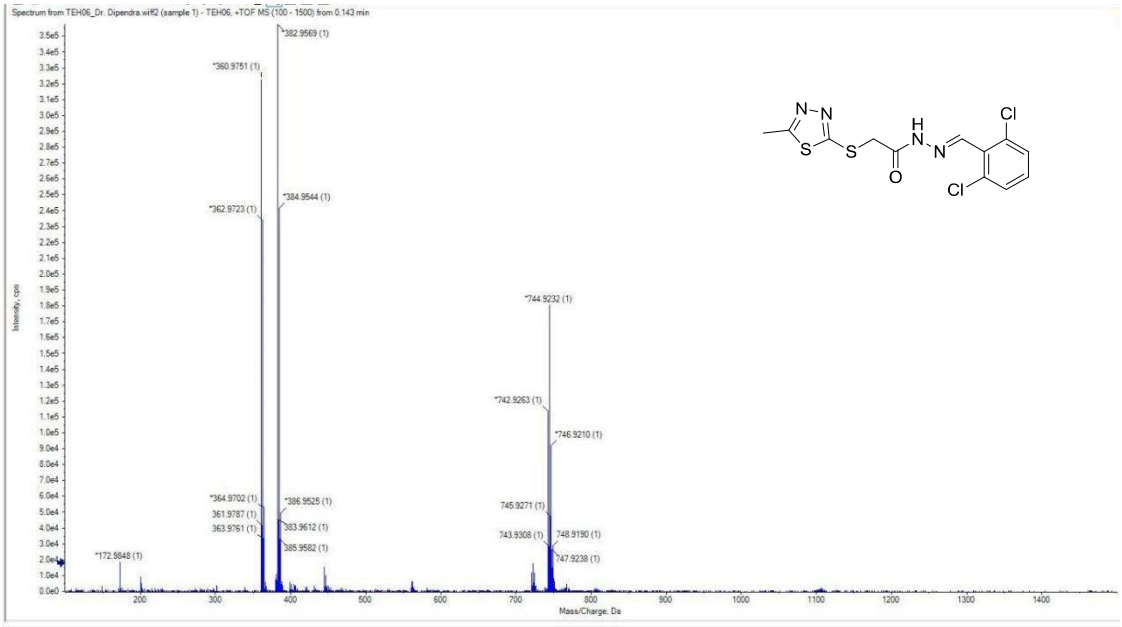



**Figure A38.** HRMS spectrum of compound **TEH06**

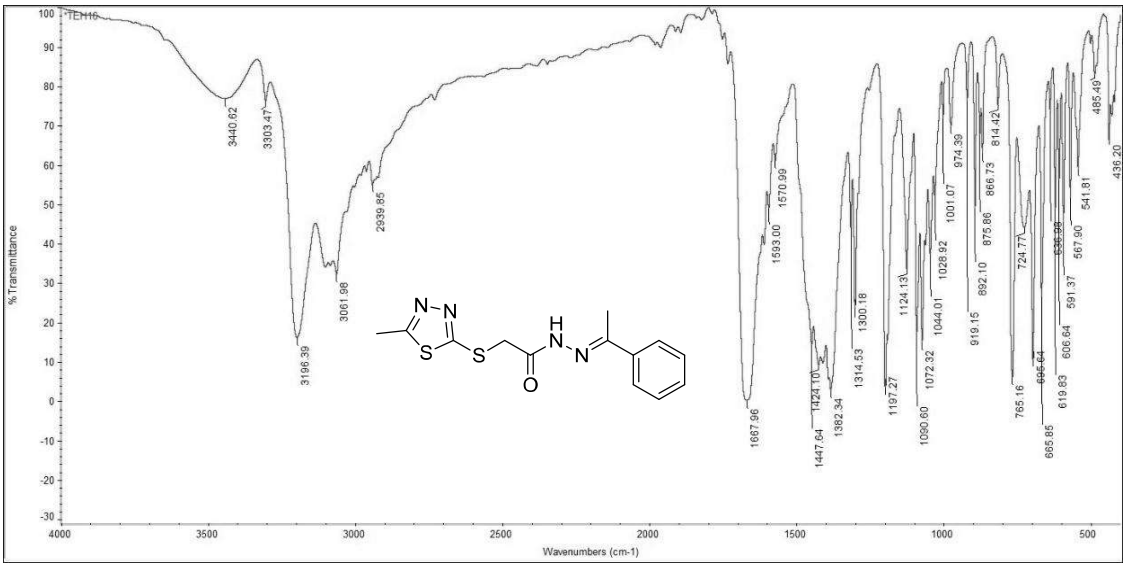



**Figure A39.** FTIR spectrum of compound **TEH16**

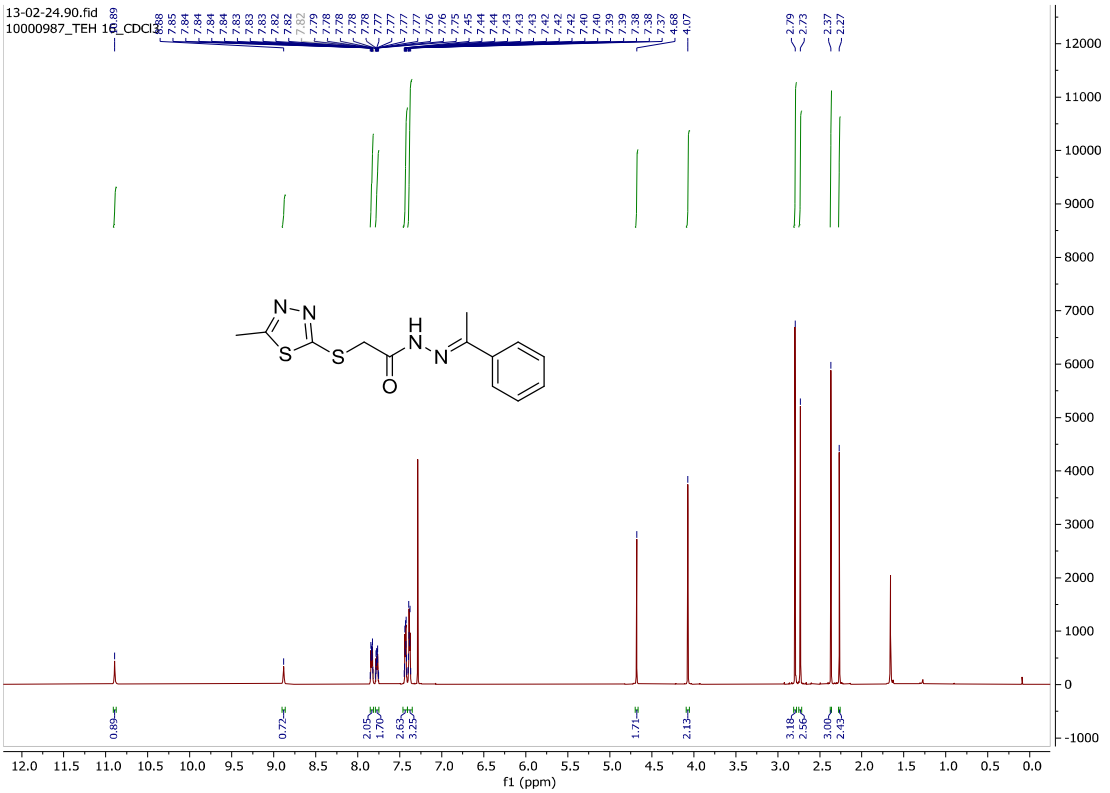



**Figure A40.** $^{1}$H NMR spectrum of compound **TEH16**

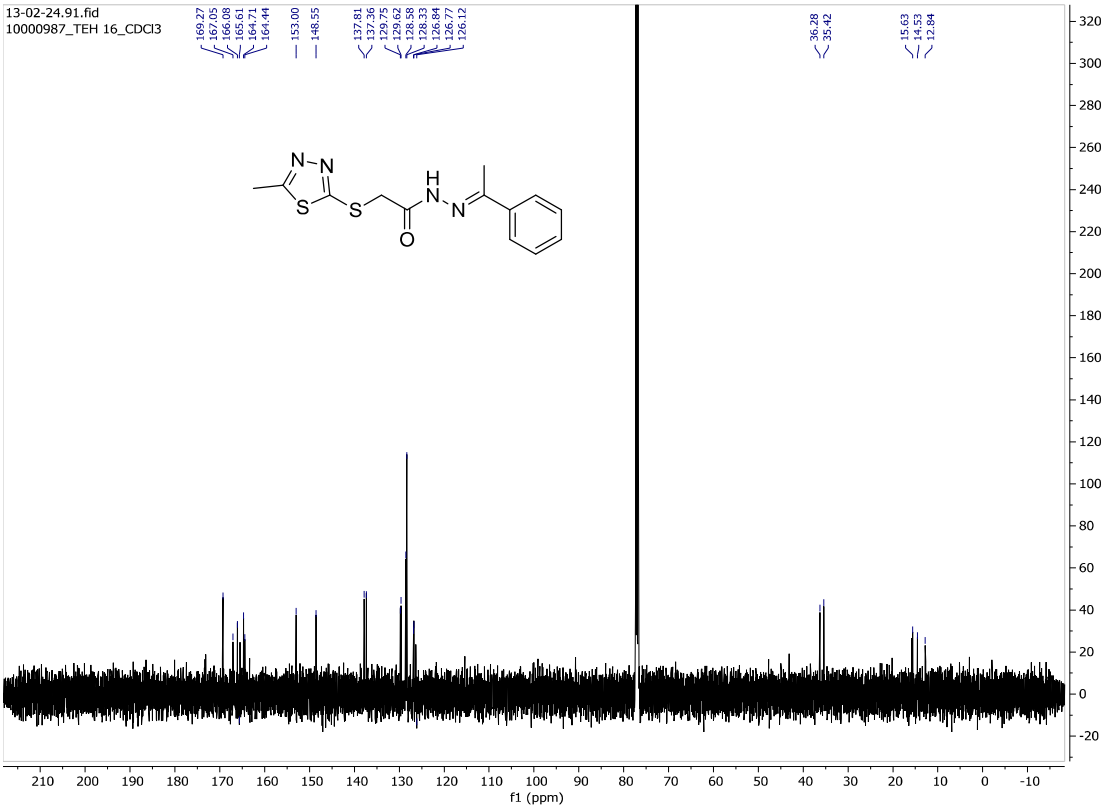



**Figure A41.** $^{13}$C NMR spectrum of compound **TEH16**

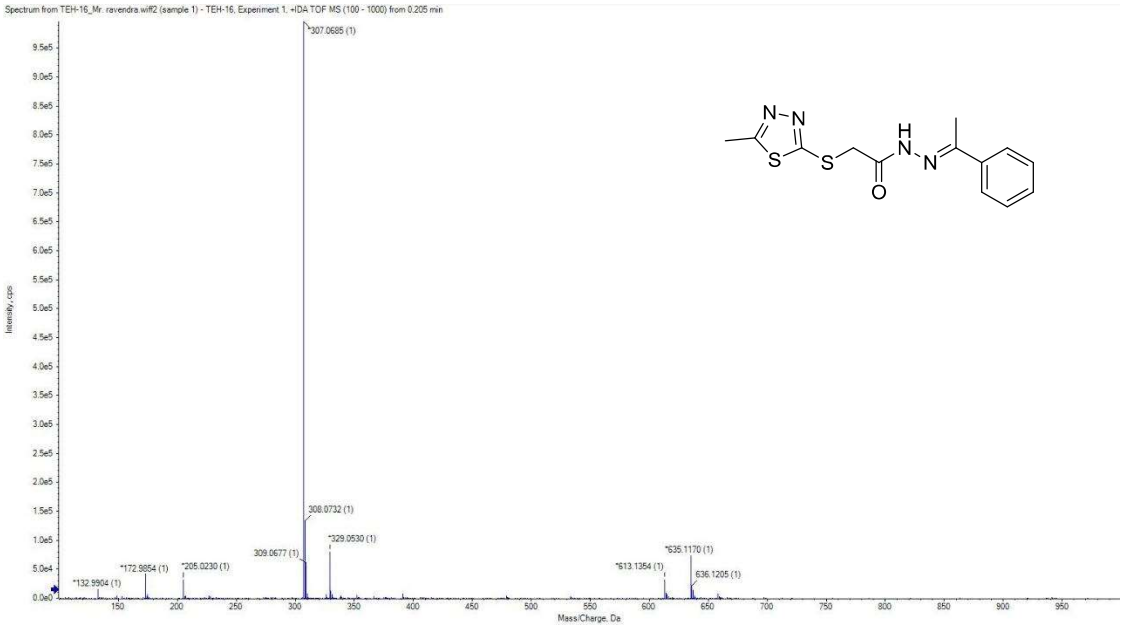



**Figure A42.** HRMS spectrum of compound **TEH16**

## C. UV-based LogP determination

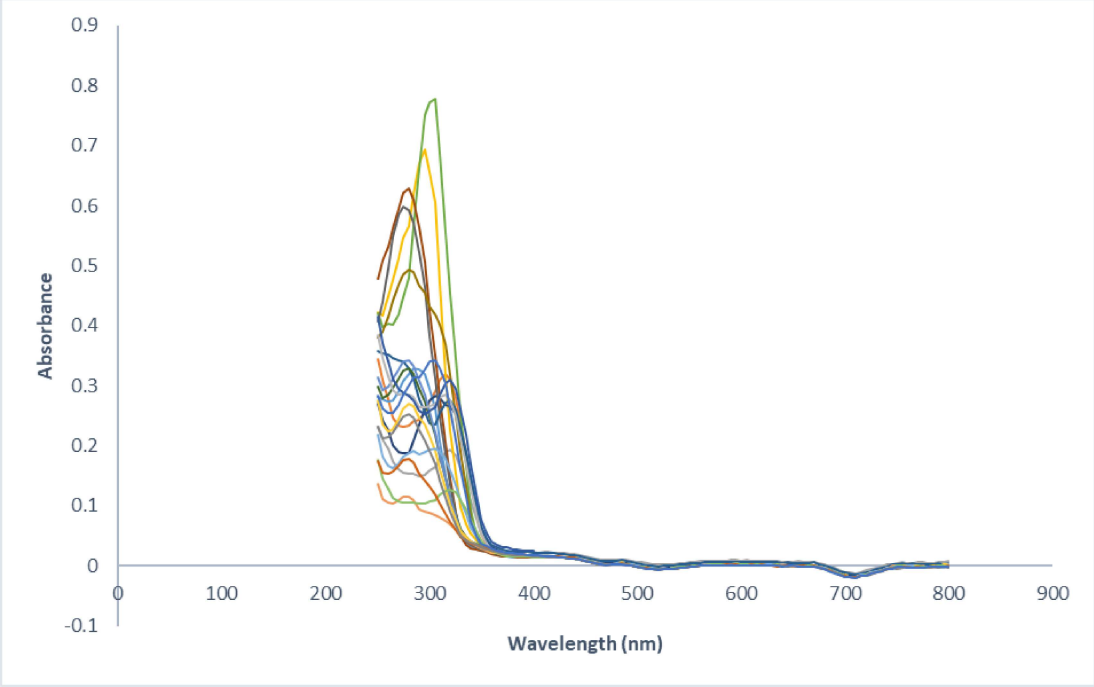



**Figure A43.** UV $\lambda_{max}$ spectra of **STT series** of compounds

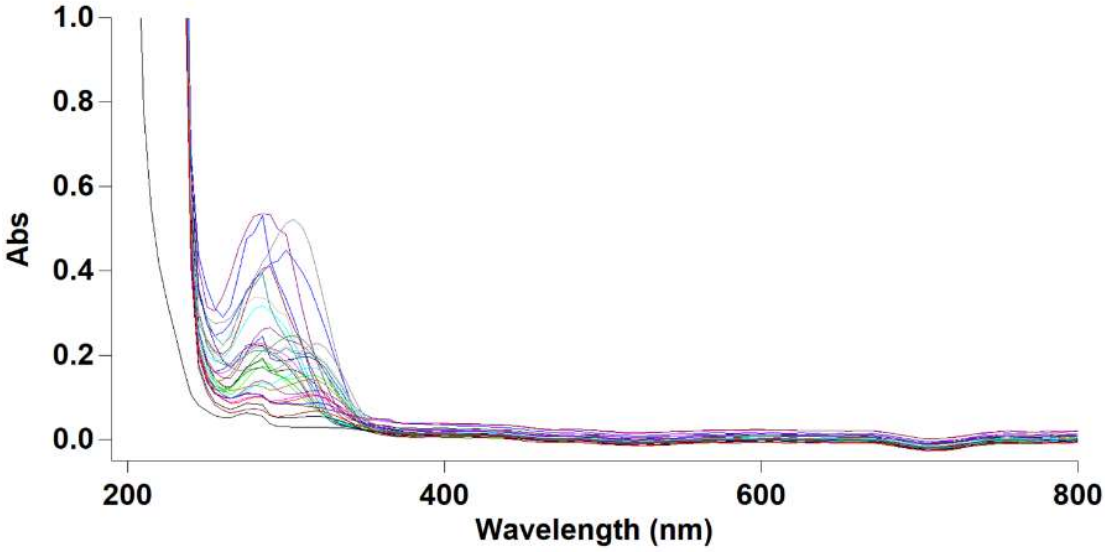



**Figure A44.** UV $\lambda_{max}$ spectra of **STS series** of compounds

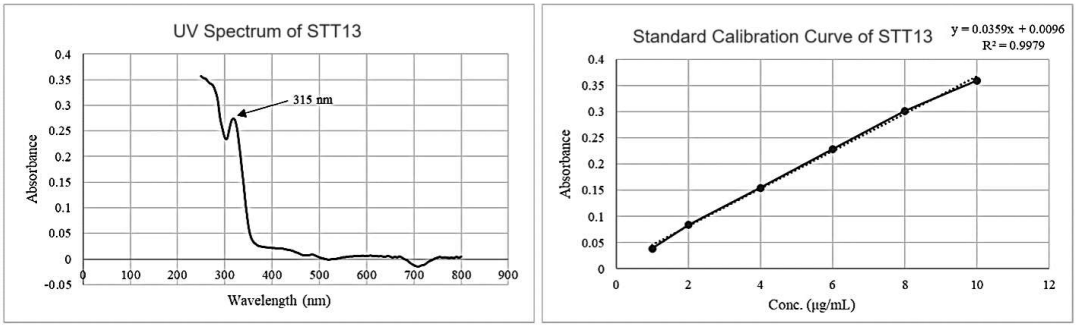



**Figure A45.** UV spectrum (at a concentration of 10 µg/mL in HPLC grade methanol) and standard calibration curve (at $\lambda_{max}$ = 315 nm) of compound **STT13**

## D. Cellular assays for estimation of antiproliferation activity

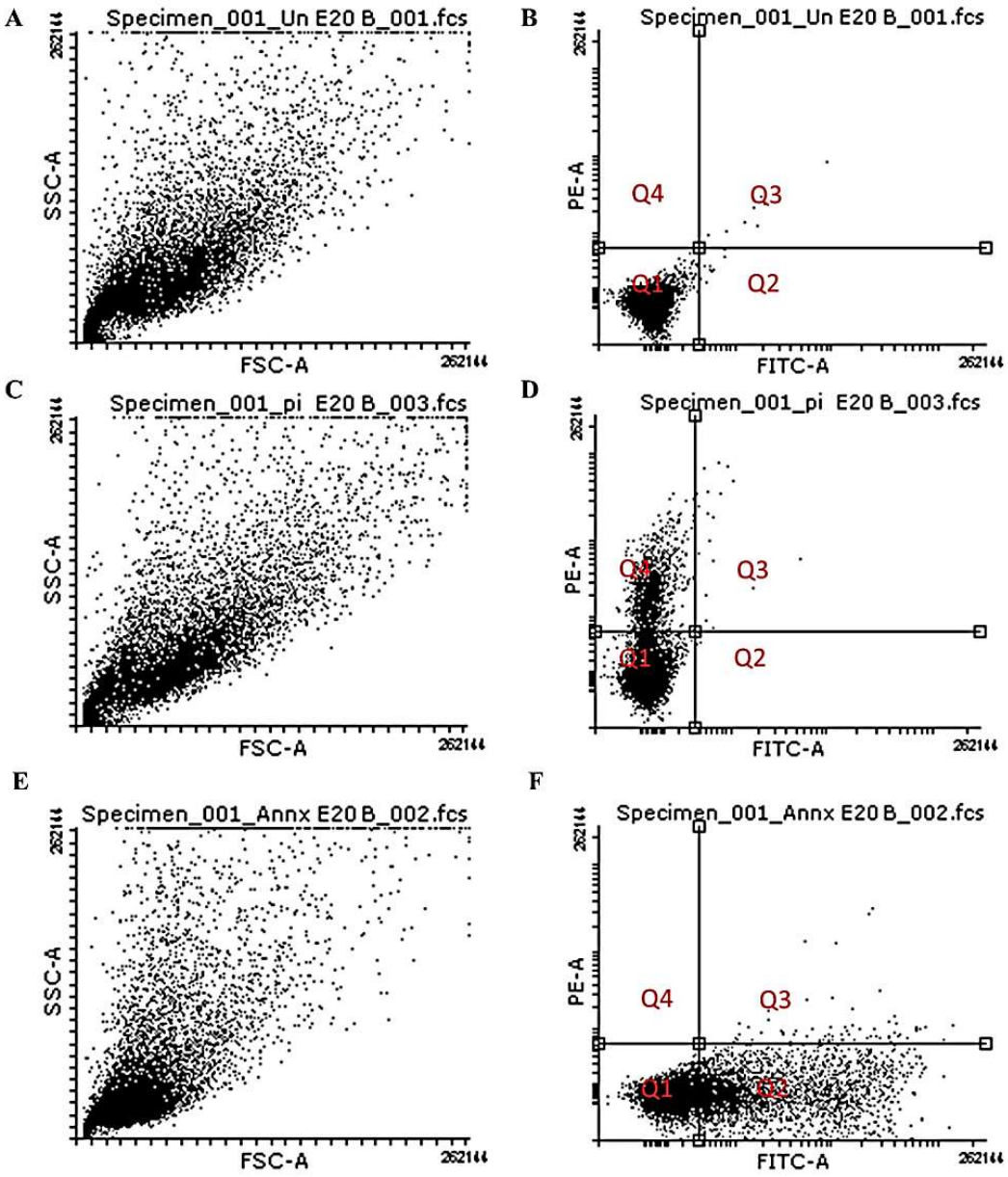



**Figure A46.** Flow cytometric analysis of apoptosis of U87MG cells. A) Cell scatter plot indicating side scatter (SSC) parameter versus forward scatter (FSC) parameter and B) propidium iodide (PI) fluorescence versus Annexin V-FITC fluorescence dot plot for unstained U87MG cells showing live (Q1), early apoptotic (Q2), late apoptotic (Q3) and necrotic (Q4) cell populations. C) Cell scatter plot indicating side scatter (SSC) parameter versus forward scatter (FSC) parameter and D) propidium iodide (PI) fluorescence versus Annexin V-FITC fluorescence dot plot for U87MG cells treated with only PI. E) Cell scatter plot indicating side scatter (SSC) parameter versus forward scatter (FSC) parameter and F) propidium iodide (PI) fluorescence versus Annexin V-FITC fluorescence dot plot for U87MG cells treated with only Annexin-FITC.

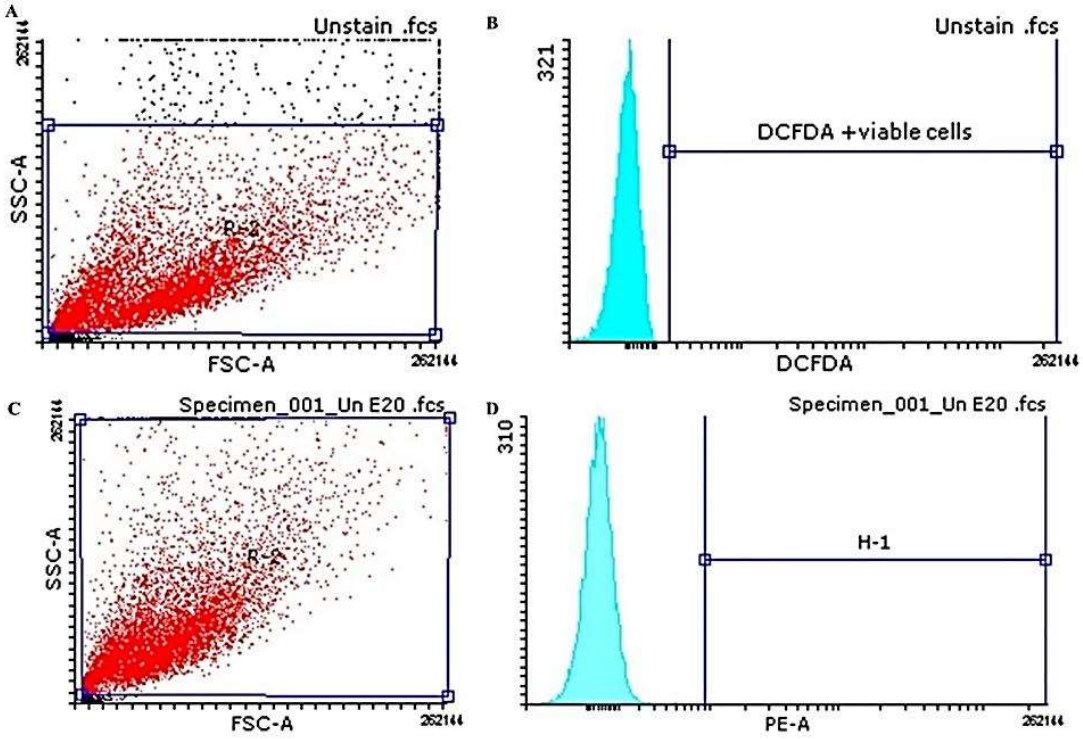
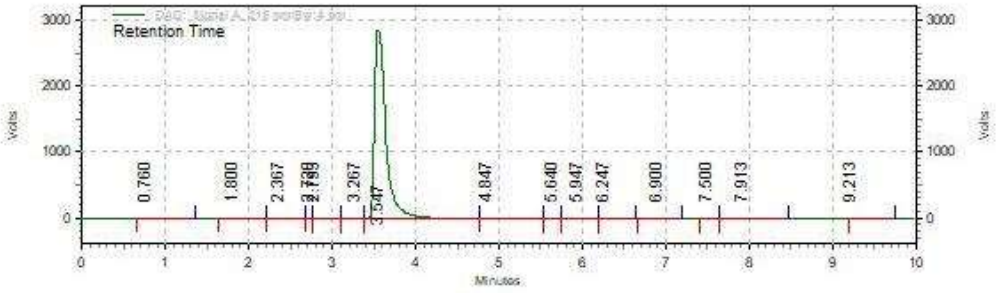



**Figure A47.** Flow cytometric estimation of reactive oxygen species (ROS) and mitochondrial membrane potential (MMP) of U87MG cells. A) Cell scatter plot indicating side scatter (SSC) parameter versus forward scatter (FSC) parameter and B) histogram of unstained U87MG cells showing the cell counts in Y-axis versus the $H_2$DCFDA intensity in X-axis. C) Cell scatter plot indicating side scatter (SSC) parameter versus forward scatter (FSC) parameter and D) histogram of unstained U87MG cells showing the cell counts in Y-axis versus the J-aggregate intensity in X-axis.

## E. RP-HPLC Method Development and Validation for compound STT13

**Area %Report**

Data File: C:\Users\IIT-BHU\Desktop\STT15_10_1.rslt\STT15_10_1.dat
Method: C:\Enterprise\Projects\Method\Rangan.met
Acquired: 19-04-24 14:30:49 (GMT +05:30)
Printed: 19-04-24 14:41:33 (GMT +05:30)




**DAD: Signal A, 215 nm/Bw:4 nm**
**Results**

| Retention Time | Area | Area % | Height | Height % |
|---|---|---|---|---|
| 0.760 | 34597 | 0.06 | 1188 | 0.02 |
| 1.800 | 37560 | 0.06 | 1941 | 0.03 |
| 2.367 | 52157 | 0.09 | 3020 | 0.05 |
| 2.720 | 12212 | 0.02 | 4257 | 0.07 |
| 2.793 | 14963 | 0.03 | 2353 | 0.04 |
| 3.267 | 10431 | 0.02 | 854 | 0.01 |
| 3.547 | 58325831 | 99.22 | 6023279 | 99.54 |
| 4.847 | 190349 | 0.32 | 8635 | 0.14 |
| 5.640 | 5916 | 0.01 | 528 | 0.01 |
| 5.947 | 15030 | 0.03 | 895 | 0.01 |
| 6.247 | 5267 | 0.01 | 359 | 0.01 |
| 6.900 | 16511 | 0.03 | 1131 | 0.02 |
| 7.500 | 2898 | 0.00 | 288 | 0.00 |
| 7.913 | 55492 | 0.09 | 2602 | 0.04 |
| 9.213 | 2213 | 0.00 | 56 | 0.00 |
| Totals | 58781427 | 100.00 | 6051386 | 100.00 |

**Figure A48.** HPLC chromatogram of compound **STT13** at a concentration of 10 µM

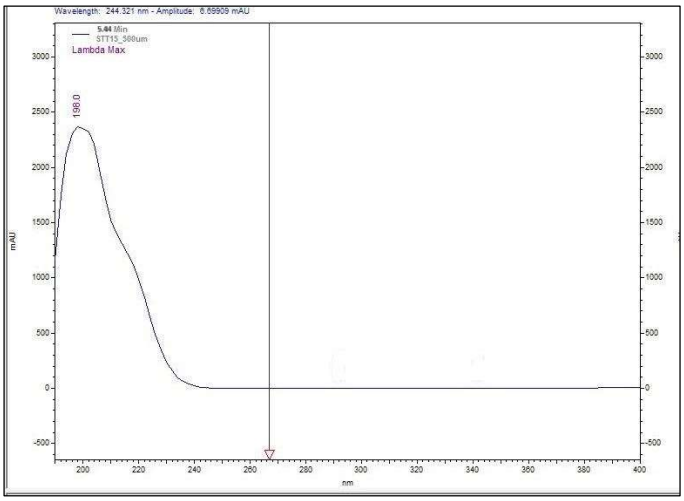



**Figure A49.** Spectrum of compound **STT13** in 100% acetonitrile at 5.44 min

**Table A2.** System Parameters for RP-HPLC Method of compound **STT13**

| System Parameters | STT13 | Formulae |
|---|---|---|
| Average % Purity (AUC upto LOD) | 98.54 ± 1.63 | |
| Retention time ($t_R$) [min] | 5.440 | |
| Dead time ($t_0$) [DMSO marker] [min] | 0.960 | |
| Adjusted retention time (t) [min] | 4.48 | $t = t_R - t_0$ |
| Retention factor (k) | 4.666 | $k = (t_R - t_0)/t_0$ |
| Volumetric flow rate ($F_R$) [mL/min] | 0.7 | $V = F_R \times t_R$ |
| Retention volume (V) [mL] | 3.808 | $S = W_{0.05H}/2f$ |
| Peak width (W) [min] | 0.50 | $N = 16(t_R/W)^2$ |
| Tailing (or, Symmetry) factor (S) | 0.98 | $H = L/N$ |
| Theoretical plates (N) | 2002 | |
| HETP (H) [mm] | 8.01 | |

$W_{0.05H}$ = Peak width at 5% peak height above peak baseline, *f* = length where vertical plumb line from the peak summit intersects a horizontal line at 5% peak height above peak baseline, LOD: limit of detection. HETP: height equivalent of a theoretical plate.

**Table A3.** System suitability parameters for RP-HPLC method developed for compound **STT13** (n=6)

| System Suitability Parameters | Value obtained | SD | %RSD | Acceptance Criteria |
|---|---|---|---|---|
| Retention (or, Capacity) factor (k) | 4.620 | 0.091 | 1.954 | $k \geq 2.0$ |
| Tailing (or, Symmetry) factor (S) | 0.98 | 0.002 | 0.178 | $S \leq 2.0$ |
| Theoretical plates (N) | 2002 | 19.112 | 0.954 | $N \geq 2000$ |
| HETP (H) [mm] | 8.01 | -- | -- | -- |
| Retention time ($t_R$) [min] | 5.44 | 0.043 | 0.792 | RSD ≤ 1.0% |
| Peak purity | 1 | 0.000 | 0.000 | RSD ≤ 1.0% |

# LIST OF PUBLICATIONS

1. **Mitra, R**., and Ayyannan, S. R. (**2021**). Small-Molecule Inhibitors of Shp2 Phosphatase as Potential Chemotherapeutic Agents for Glioblastoma: A Minireview. *ChemMedChem*, 16(5), 777-787. doi: 10.1002/cmdc.202000706.

2. **Mitra, R**., and Ayyannan, S. R. (**2022**). Role of Lysine-specific Demethylase 1 and Its Small Molecule Inhibitors in Glioblastoma Multiforme Therapy. *Anticancer Agents Med .Chem.*, 22(18), 3062-3085. doi: 10.2174/1871520622666 220421092414.

3. **Mitra, R**., Singh, D., and Ayyannan, S. R. (**2022**). Design, synthesis and evaluation of 5-substituted-2-amino-1,3,4-thiadiazole derivatives as anticancer agents. *J. Pharm. Chem., 8*(Supplement). 150. Retrieved from https://pubs.vensel.org/index.php/jphchem/article/view/247.

4. **Mitra, R.,** Kumar, S., and Ayyannan, S. R. (**2023**). Identification of new small molecule allosteric SHP2 inhibitor through pharmacophore-based virtual screening, molecular docking, molecular dynamics simulation studies, synthesis and in vitro evaluation. *J. Biomol. Struct. Dyn.*, 1-20. doi: 10.1080/07391102.2023.2291733.

5. **Mitra, R**., Kumar, S., Chaudhuri, A., Agrawal, A. K., and Ayyannan, S. R. (**2024**). Design, Synthesis and Evaluation of Thioacetamide-Tethered Thiadiazole-1,2,4-Triazole Hybrids as SHP2 Inhibitors for Cancer Therapy. *ChemSelect, 9*(27), e202401602. doi: 10.1002/slct.202401602.

6. **Mitra, R**., Kumar, S., Chaudhuri, A., Majumdar, S., Agrawal, A. K., Sairam, K., Ayyannan, S. R. (**2024**). *S*-Acetohydrazones of 5-Methyl-1,3,4-thiadiazole-2-thiol: A New Class of SHP2 Inhibitory Scaffold Possessing Potential Anti-Breast Cancer Activity. (*In communication*)

7. **Mitra, R**., Kumar, S., Chaudhuri, A., Agrawal, A. K., and Ayyannan, S. R. A HETEROCYCLIC THIOACETAMIDE DERIVATIVE AND A METHOD OF SYNTHESIS THEREOF. (2024). Indian Patent Application No. 202411046345.