**Abstract form**

Discovery and Biological Evaluation of a Novel Inhibitor for Human Breast Cancer Treatment

Cheng-Chiao Huang1,2,3,#, Chia-Ming Hsu3,#, Kai-Cheng Hsu1,3,4,5,6, Tony Eight Lin1,3, Shih-Chung Yen7, Min-Wu Chao8,9,10,\*, Shiow-Lin Pan1,3,4,5,6,\*

1 Ph.D. Program for Cancer Molecular Biology and Drug Discovery, College of Medical Science and Technology, Taipei Medical University and Academia Sinica, Taipei, Taiwan

2 Division of General Surgery, Department of Surgery, Taipei Medical University Hospital, Taipei, Taiwan

3 Graduate Institute of Cancer Biology and Drug Discovery, College of Medical Science and Technology, Taipei Medical University, Taipei, Taiwan

4 Ph.D. Program in Drug Discovery and Development Industry, College of Pharmacy, Taipei Medical University, Taipei, Taiwan

5 TMU Research Center of Cancer Translational Medicine, Taipei Medical University, Taipei, Taiwan

6 TMU Research Center of Drug Discovery, Taipei Medical University, Taipei, Taiwan

7 Warshel Institute for Computational Biology, The Chinese University of Hong Kong (Shenzhen), Shenzhen, Guangdong, China

8 School of Medicine, College of Medicine, National Sun Yat-sen University, Kaohsiung, Taiwan

9 Institute of Biopharmaceutical Sciences, College of Medicine, National Sun Yat-sen University, Kaohsiung, Taiwan

10 The Doctoral Program of Clinical and Experimental Medicine, College of Medicine, National Sun Yat-sen University, Kaohsiung, Taiwan

#These authors contributed equally to this work.

\*Correspondence.

**Purpose**

Triple-negative breast cancer (TNBC) remains a significant challenge in the medical field, posing a considerable gap in treatment options due to the high incidence of recurrence and low survival rates. The dysregulation of RNA splicing mechanisms frequently catalyzes tumor formation across different cancers. In this regard, research has highlighted the pivotal role of CLK2, an oncogenic kinase involved in RNA splicing, in the context of breast cancer.

**Materials and Methods**

In this study, we employed a structure-based virtual screening (SBVS) approach to pinpoint promising candidates capable of inhibiting CLK2, thereby introducing novel chemical entities with potential for combating TNBC. This methodological strategy aims to expand the repertoire of therapeutic options available to address the unique challenges posed by TNBC.

**Results**

Compound 670551 emerged as a noteworthy discovery in our investigation, showcasing its potential as a novel inhibitor of CLK2 with a 50% inhibitory concentration (IC50) value of 619.7 nM. Its remarkable selectivity for targeting CLK2 is particularly notable amidst a spectrum of protein kinases. Significantly, the functional attributes of Compound 670551 were elucidated through rigorous experimentation, revealing its pronounced impact on the viability and proliferation of breast cancer cells. Specifically, our cell-based assays underscored its efficacy in downregulating crucial RNA splicing proteins such as SRSF4 and SRSF6, consequently instigating cellular apoptosis pathways.

**Conclusion**

In this study, we unveil a groundbreaking discovery: a novel CLK2 inhibitor that holds immense promise as a prospective therapeutic avenue for advancing the treatment of TNBC. This revelation marks a significant step forward in the quest for innovative TNBC therapies, offering renewed hope for patients and physicians.