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REVITALIZING OLD DRUGS: UNCOVERING POTENTIAL DENGUE ANTIRIVALS THROUGH *IN SILICO* TARGET PREDICTION AND *IN VITRO* VALIDATION

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ABSTRACT

Dengue virus (DENV) infection is a rising health concern worldwide. Despite the alarming situation, there is no effective antiviral for DENV infections. With the increasing number of NS3 viral inhibitors developed for other diseases, the development of drugs targeting dengue NS3 protein is an interesting venture. Thus, this study is set forth to identify potential DENV inhibitors by building a prediction model of NS3 dengue antiviral. Initially, the models were built using bioactivity data of 62,354 compounds using ligand-based (L-B), and proteochemometric (PCM) modelling approaches. For the L-B approach, a Random Forest (RF) one-vs-one classification model was utilized while the PCM model employed the Parzen-Rosenblatt Windows (PRW) algorithm. Subsequently, the validated predictive models were used to screen marketed drugs, and *in vitro* assays were conducted to validate the drug's viral inhibitory potential. Finally, the interactions that are responsible for the observed *in vitro* results were validated using molecular docking. The *in silico* studies revealed that both L-B and PCM models performed well in the internal and external validations. However, the L-B model showed better accuracy in the external validation, in terms of its sensitivity (0.671). Although none of the drugs were better than ribavirin (positive control), all selected drugs were able to moderately inhibit the viral activities *in vitro* at the highest concentration tested with zileuton (47% - 2mM), trimethadione (36% - 20mM) and linalool (54% - 2 mM). In the protease assay, zileuton showed comparable results with linalool when tested against NS3 protease with a reduction of activity at 17.89% and 18.42%, respectively. Based on these findings, two compounds were developed from the combination of the selected drugs, which are ziltri (zileuton and trimethadione) and zilool (zileuton and linalool). The molecular docking study showed that all drugs and compounds were able to achieve binding affinity of >-4.1 kcal/mol with ziltri showing the highest affinity at -7.7 kcal/mol surpassing the control, panduratin A. The occupation of both S1 and S2 subpockets of NS2B-NS3 may be essential and a reason for the lower binding energy shown by the proposed compounds compared to the screened drugs. Despite this interesting observation, the study also possesses inherent limitations. The utilization of surrogate data in the training set becomes necessary due to the scarcity of bioactivity data. Consequently, the differences in species may affect the predictions, due to the extrapolation of the cross–species activity is uncertain. Additionally, the drug filtering parameters prior to the prediction process, may require reconsideration as the ligand needs to be able to fit into the broad active site of NS3 protease. Notwithstanding these constraints, the current study demonstrated that the discovery of five lead compounds for DENV inhibitors can be achieved through a combination of *in silico* modelling, and *in vitro* validation

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CHAPTER 1
INTRODUCTION

1.1 Research Background

Dengue virus (DENV) infection is a rising health concern worldwide. The World Health Organization (WHO) reported that the prevalence of dengue infection has increased dramatically in the past decades[1], where approximately 390 million people were infected with DENV, with 96 million infections reported each year[2].

To date, the DENV antiviral drug is still absent in the market despite the success seen in Hepatitis C Virus (HCV) and human immunodeficiency virus (HIV)[3]. The complexity of the dengue disease itself, characterized by its acute and self-limiting nature in most cases, adds to this challenge. Moreover, developing an effective antiviral demands activity against all 4 serotypes of DENV, heightening the difficulty. Furthermore, the absence of in vivo assessments using animal models might contribute to the dearth of clinically approved antivirals for DENV[4].

Numerous investigations of Flavivirus have been conducted to find potential drug targets such as proteins involved in events such as endocytosis, viral fusion to the host membrane, viral transcription, and the release of progeny viruses from the host cell[5]. Among these proteins, the NS3 protein is an interesting target for potential antiviral as this protein is vital for viral replication.

To date, there are more than 65 compounds tested *in vitro* targeting the dengue NS3 protease as reported in the ChEMBL database[6] (https://www.ebi.ac.uk/chembl/). Despite the numbers and laborious efforts of finding the potential NS3 inhibitor, however, only a few compounds are being developed further. Several reasons have been highlighted including poor pharmacokinetic properties, limited and insufficient potency of the pan-serotype activity, and adverse effects in animals[7]. Due to the low rate of successful drug production, many pharmaceutical companies have shifted their interest to drug reprofiling or repositioning instead of developing drugs *de novo*. Drug repositioning is an attractive approach and has advantages over traditional drug development in terms of cost, time, and safety profile[8]. By using similar approach, Pu et al.,[9] discovered the anti-DENV features of sunitinib and erlotinib where both drugs are currently used to treat several cancers.