In silico and *in vitro* screening of potential repurposed drugs in nasopharyngeal carcinoma

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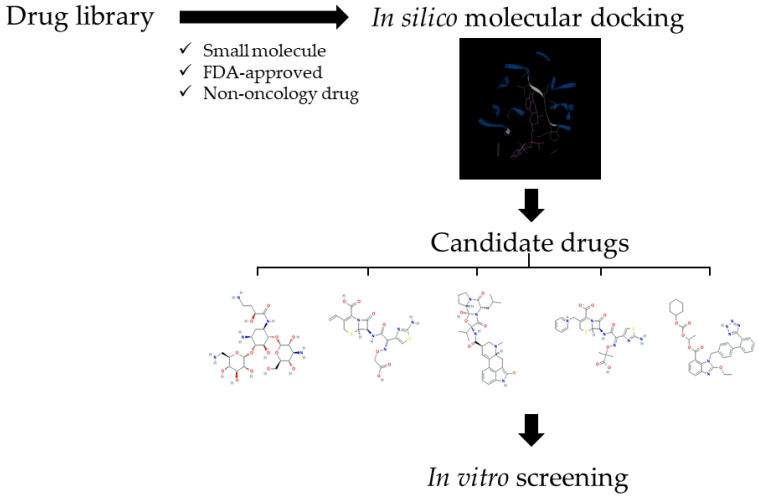
Abstract: Nasopharyngeal carcinoma (NPC) is a type of head and neck cancer. Standard treatments including radiotherapy and chemotherapy are effective only for the early stages. As NPC occurs in the silent painless area, causing no noticeable signs and symptoms therefore most NPC patients are detected in the advanced stages, causing treatment ineffectiveness. At present, targeted therapy is gaining popularity as it introduces less toxicity to normal cells and improves the patient's wellness. However, *de novo* cancer drug development is costly and time-consuming, therefore, drug repurposing could reduce the time and costs in development processes. Moreover, current technologies and accessible data allow rapid screening of drugs with known chemical structures via a computational approach. Thus, in this study, we applied a drug repurposing approach to screen potential drugs for NPC via *in silico* molecular docking by targeting serine–arginine protein kinase 1 (SRPK1), a key regulator in alternative splicing, followed by *in vitro* cell cytotoxicity screening on NPC cells. Here, we found among others that an anti-hypertensive drug, candesartan cilexetil, was cytotoxic to NPC cells.

Graphical abstract:



Keywords: Nasopharyngeal carcinoma; Drug repurposing; SRPK1; *in silico* molecular docking; Candesartan cilexetil.

1. Introduction

Nasopharyngeal carcinoma (NPC) is a type of head and neck cancer, arises from the nasopharynx epithelium. NPC is rare in other regions [1]. The worldwide incidence of NPC is 1 per 100,000 persons per year. However, NPC is prevalent in China and Southeast Asia including Thailand. The incidence rate of NPC in these regions is 25 - 30 per 100,000 persons per year [2]. Men are more likely to develop the disease than women with 2- to 3-fold. According to the National Cancer Institute (NCI) of Thailand, NPC is ranked 7th highest cancer found in Thai men in 2019 [3]. NPC is caused by several factors, including EBV infection, N-nitrosamine exposure, and genetic susceptibility. Nasendoscopy, biopsy, cell-free DNA, and EBV antigen in blood plasma are used in NPC diagnosis. The imaging study is also used in the diagnosis and staging for planning the treatment [1, 4].

Radiotherapy and chemotherapy are standard treatments for NPC. However, NPC occurs in the silent painless area which shows no distinct symptoms. Therefore, the NPC patients are diagnosed in advanced stages, which the treatment is less effective. Moreover, these treatments damage normal cells, leading to several side effects and patients' discomfort. In addition, after initial treatment, 15 - 58% of the patients exhibit recurrence, causing the patients to feel suffering from undergoing treatment again [5-8]. To reduce patients' discomfort and enhance the quality of life, targeted therapy is an interesting approach. It targets only cancer's specific pathways by using monoclonal antibodies or small molecule drugs that could reduce normal cell damage and side effects [9].

De novo drug development is a time-consuming and expensive process. The complete processes for a new drug takes at least 13 - 15 years and cost over $2 to $3 billion [10]. In recent years, the number of new drugs developed and entering preclinical testing, clinical trials, or clinical practice has gradually declined [11]. Furthermore, the number of successful drugs has not met the increasing knowledge and incidence, especially in cancer, which is a major health problem worldwide. While the incidence and mortality are increasing, however, only 5% of cancer drugs entering clinical trials Phase I are approved. Due to high investment in the development process, the cost of cancer drugs per one patient is high. Therefore, in poor and middle-income countries, a huge number of patients are unable to afford the costly cancer drug [12-14].

Drug repurposing is an alternative strategy for drug development. It is the use of the approved drug for another indication than it was originally invented for [15]. For instance, thalidomide was used as an antiemetic in pregnancy. It was banned from the market due to its side effects. However, in 2006, U.S. Food and Drug Administration (FDA) approved it for newly diagnosed multiple myeloma treatment together with dexamethasone [16]. Drug repurposing is a short circuit for drug development. Due to its strategy of using an existing drug, drug profile and toxicity were studied in the drug development of its prescription. Therefore, the time and cost of the development process are reduced. This helps in speeding up treatment and reducing the financial concerns of patients. Moreover, current technologies and a wealth of accessible data make it is possible to plan the trials, so the risk of failure is likely to be low. About 30% of approved drugs by the FDA are repurposed drugs. It also allows rapid screening and candidate compound selection via a computational approach [11, 14, 17].

Thus, we used a drug repurposing approach to screen potential drugs for NPC treatment by targeting serine-arginine protein kinase 1 (SRPK1) via *in silico* molecular docking. SRPK1 is one of the key regulators in alternative splicing. Aberrant in alternative splicing could facilitate cancer to acquire oncogenic properties [18, 19]. From *in silico* molecular docking against SRPK1, the candidate drugs were obtained and cell cytotoxicity on NPC was tested. Candesartan cilexetil was found to have cytotoxicity to NPC cells. It is prescribed as an angiotensin II receptor blocker which is used to treat high blood pressure [20].

2. Materials and Methods

2.1 In silico molecular docking

All drugs in this study were retrieved from DrugBank database (https://go.drugbank.com). They are small molecule drugs with molecular weights of 300-1200 Da, FDA-approved and non-oncology drugs. iGEMDOCK was used to perform the molecular docking against the target protein, SRPK1. The corresponding PDB ID of the SRPK1 is 4WUA.

2.2. In vitro screening

2.2.1. Cell culture

CNE1, an NPC cell line, was used in the screening. CNE1 was cultured in Roswell Park Memorial Institute (RPMI) medium containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37°C in 5% CO_2 humidified incubator.

2.2.2.Cell viability assay

To test the cell cytotoxicity of candidate drugs on CNE1, a cell viability assay was performed. CNE1 was treated with different concentrations of candidate drugs including 0, 1, 10, and 50 μM for 48 hours in the condition described previously. Cell viability was obtained using Cell Counting Kit-8 (CCK-8).

3. Results

3.1. In silico molecular docking

Table 1 shows the list of candidate drugs and their binding energy towards SRPK1. Molecular structures of the candidate drugs are shown in **Figure 1**. Binding energy indicates the stability of binding between ligand and target protein. Amikacin and Candesartan cilexetil showed binding energies relatively lower than the other drug candidates. The docked structure of amikacin for SRPK1 is shown in **Error! Reference source not found.**

Table 1. List of candidate drugs obtained from *in silico* molecular docking and their prescriptions

Drugs	Binding energy (kcal/mol)	Prescriptions
Amikacin	-121.696	Antibiotics
Candesartan cilexetil	-120.591	Anti-hypertension
Bromocriptine	-107.763	Hyperprolactinemia
Cefixime	-107.538	Antibiotics
Ceftazidime	-100.153	Antibiotics

3.2. Cell viability after treated with candidate drugs

After the CNE1 was treated with candidate drugs for 48 hours, the percentage of cell viability showed a significant decrease in a dose-dependent manner when the cell was treated with candesartan cilexetil (**Figure 3**). Furthermore, it also gave a similar percentage of cell viability as the positive control, SRPKIN-1, an SRPK1-specific inhibitor. No significant difference in the percentage of cell viability was observed for other candidate drugs.

4. Discussion

Here, we attempted to identify potential repurposed drugs targeting SRPK1 for NPC treatment. In this study, amikacin, and candesartan cilexetil exhibited the rather low binding energies, indicating that it was bound to the protein spontaneously without consuming energy. Amikacin is an antibiotic belonging to an aminoglycoside group for treatment of a broad-spectrum bacterial infection. It is also used in treating a gram-negative bacterial infection in cancer patients [21]. It has been demonstrated that amikacin could inhibit

migration and invasion of triple-negative breast cancer cells MDA-MB-231 [22]. However, amikacin did not show cytotoxicity on NPC in our experimental conditions. Furthermore, candesartan cilexetil, an oral drug used for hypertension management, revealed cytotoxicity to NPC cells comparable to the SRPK1-specific inhibitor. Candesartan cilexetil is an angiotensin II subtype 1 receptor (AT_1R) antagonist that blocks the binding of angiotensin II. It belongs to the angiotensin receptor blockers (ARBs) class [20]. Previous studies indicated that other drugs in ARBs class, including valsartan and losartan, also exhibited antitumor effects on NPC. They inhibited cell proliferation and angiogenesis via apoptosis induction mediated by the PI3K/AKT signaling pathway [23, 24]. CNE1 that was used in this study also expressed AT_1R. Therefore, candesartan cilexetil might have the same target as valsartan and losartan. The relevance of this compounds and SRPK1 and the corresponding pathways should be further explored.

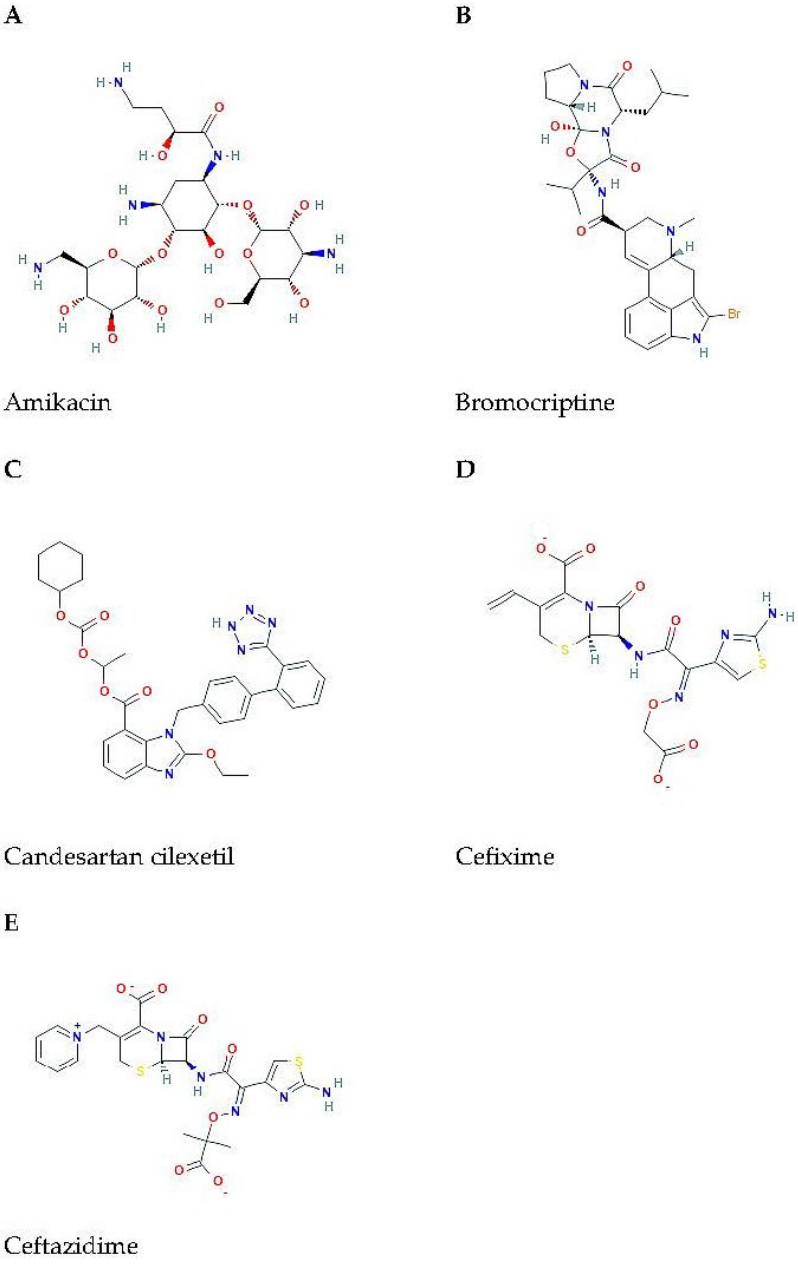


Figure 1. Molecular structures of candidate drugs (obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/))

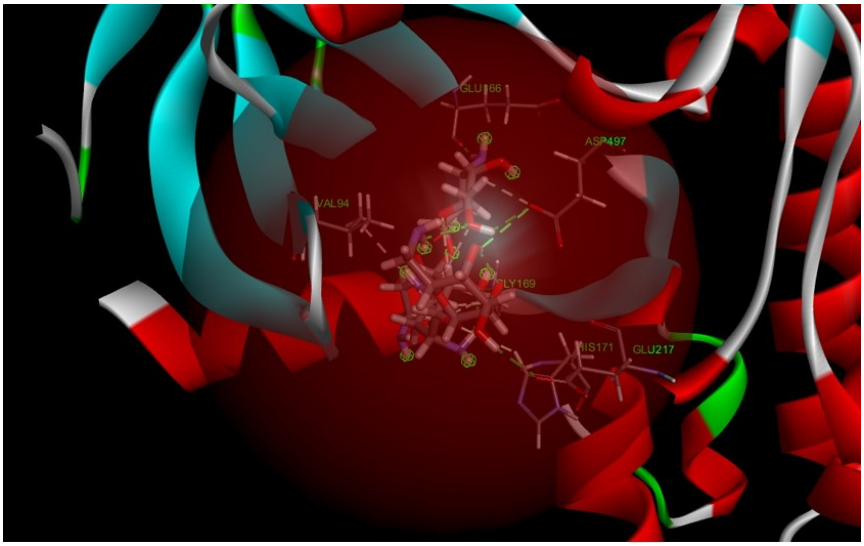


Figure 2. Docked structure of amikacin in SRPK1 binding pocket

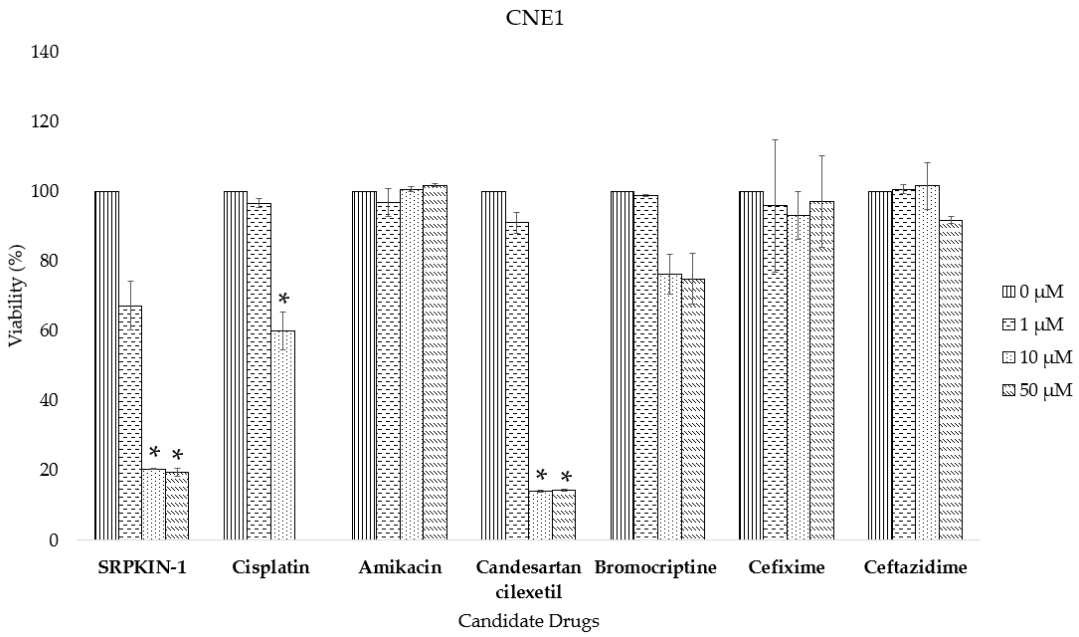


Figure 3. Percentage viability of CNE1 after treating with candidate drugs ($p < 0.05$)

5. Conclusions

Potential repurposed drugs were screened through *in silico* molecular docking and *in vitro* cell cytotoxicity on NPC cells. Candesartan cilexetil exhibited cytotoxicity on an NPC cell line, CNE1. It might be a promising drug for NPC treatment. However, the effects of this compound on other cancer phenotypes including proliferation, migration, invasion, and cell apoptosis in NPC cells must be investigated. Moreover, the target of this compound must be further validated. Our work also demonstrated that drug repurposing is a promising strategy for cancer drug development.

Author Contributions: Conceptualization, T.J..; methodology, N.P., P.P., C.N., K.V., T.R.; investigation, N.P..; resources, T.R., T.S., and T.J.; writing—original draft preparation, N.P.; writing—review and editing, T.R., T.S., and T.J.; supervision, T.J.; project administration, T.J.; funding acquisition, T.J. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by National Research Council of Thailand (NRCT) and Mahidol University (NRCT5-RSA63015-12).

Acknowledgments: N.P. is a recipient of Scholarship for Young Scientists, Faculty of Science, Mahidol University. P.P. is a recipient of Science Scholarship of Thailand. C.N. is a recipient of Development and Promotion of Science and Technology Talents Project.

Conflicts of Interest: The authors declare no conflict of interest.

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